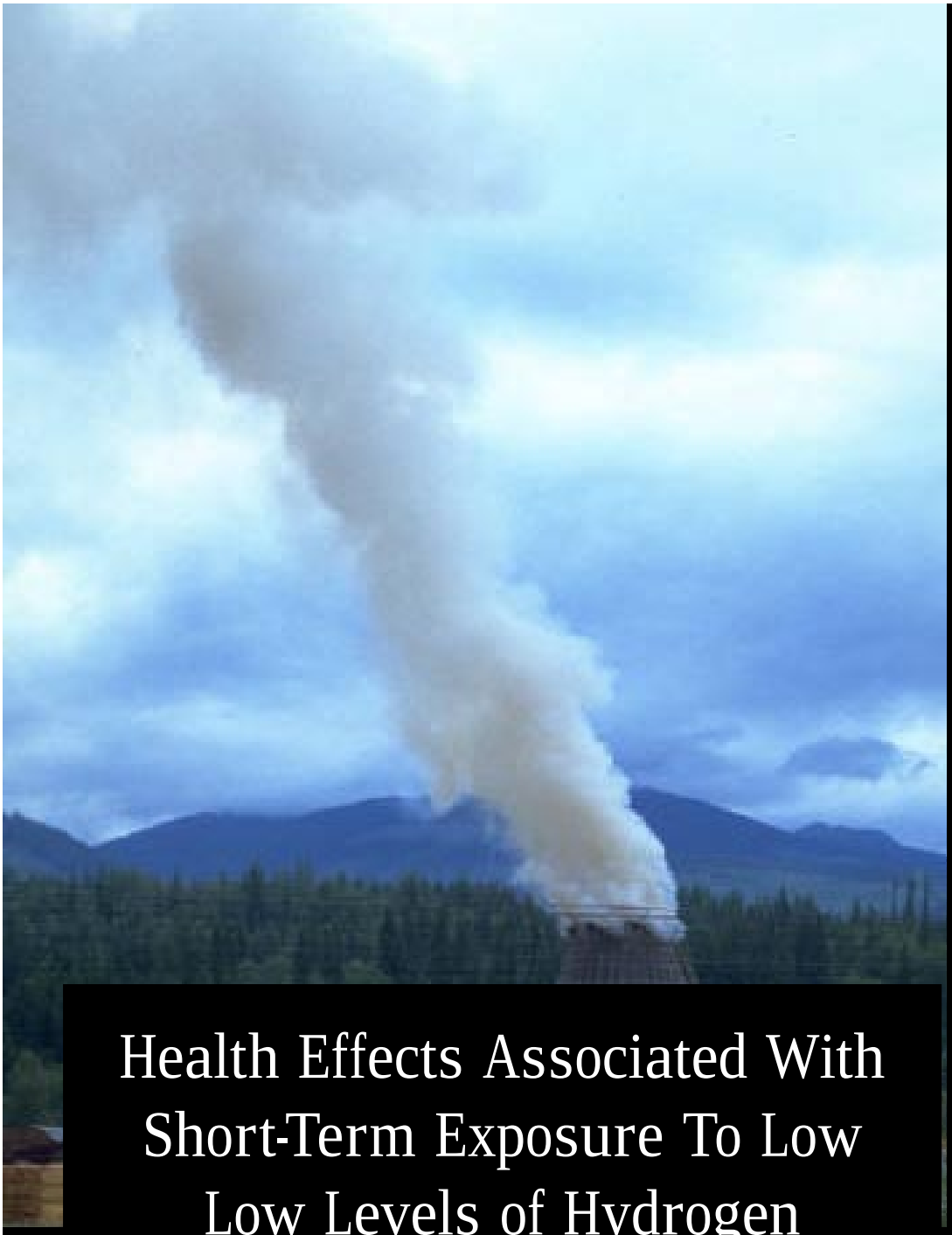

REPORT

Health Effects Associated With Short-Term Exposure To Low Low Levels of Hydrogen Sulphide (H_2S)

- A Technical Review -

Alberta HEALTH AND WELLNESS

Health Surveillance

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Prepared for:

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EXECUTIVE SUMMARY

Health Effects Associated with Short-term Exposure to Low levels of Hydrogen Sulphide (H_2S) – A Technical Review[1]

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations - Final Report.* The report contained a series of recommendations aimed, in part, to increase awareness of sour gas and its potential impacts on public health.

In response to *Recommendation 9*[2], Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). Cantox Environmental Inc. was retained by AH&W to perform the review. The review focused on the health effects information on short-term exposures to H_2S at concentrations in the range of 0 to 100 ppm. A principal aim of the work was to update the conclusions reached earlier by the Ad Hoc Committee on H_2S Toxicity (Alberta Health, 1988).

The review was performed under the auspices of an Expert Panel, with members drawn from Alberta Environment, regional health authorities, industry, and other stakeholders. Terms of Reference for the work were developed over a six-month period, after which a detailed and comprehensive review of the available scientific literature was completed. The Terms of Reference specified that the review was to focus on the health effects of short-term exposures to low levels of H_2S on normal, healthy individuals. Thus, issues surrounding "hypersusceptible" individuals were not directly addressed. Similarly, health effects that might occur secondary to the odour of H_2S were not specifically addressed.

A set of eligibility criteria was developed to properly focus the review on studies found in the published scientific literature that satisfied the Terms of Reference. The eligibility criteria allowed for the systematic selection of studies based on both technical and practical considerations. Technical demands included the need for the studies to have tested for health effects within the concentration range of interest (*i.e.*, 0 to 100 ppm) for a prescribed duration (*i.e.*, up to 30 days) using the most appropriate mode of exposure (*i.e.*, inhalation). Practical requirements included the need for the studies to have been published in the English language in peer-reviewed scientific publications that were readily available.

A set of 45 studies met the eligibility criteria for review. The studies included clinical studies involving controlled exposures of volunteer human subjects, non-clinical investigations involving controlled exposures of test animals, and case-control studies involving evaluation of individuals exposed to H_2S as a result of accidental releases. Non-clinical studies dominated the set, with close to 75 percent of the 45 studies involving animal testing.

The review included a critical assessment of the overall technical quality of each study based on consideration of experimental design, conduct and reporting, as well as grading of the level of confidence that could be assigned to the study findings and conclusions. A pre-defined set of "quality" criteria was developed to ensure that assessment and grading were performed objectively and consistently.

The assessment of technical quality revealed a number of common weaknesses in experimental design, execution and reporting. Only 15 percent of the studies achieved a "high" confidence index ranking. Close to 50 percent of the studies were

[1] Report prepared by Donald B. Davies, Ph.D., DABT and Susan E. Haggarty, B.Sc. (Cantox Environmental Inc.) for Alberta Health and Wellness, July 2002.

[2] Recommendation 9 reads as follows: The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed as soon as practical due to its urgency. (Alberta Health and Wellness has committed to lead the review for H_2S).

assigned a "low" confidence index ranking. Common weaknesses included failure to follow conventional testing protocols, failure to follow Good Laboratory Practice (GLP) guidelines, use of a single exposure concentration only, use of a single sex only, use of too few test subjects, lack of routine measures of toxicity, and inadequate descriptions of exposure conditions. In addition, a large number of the studies were directed at understanding of the mechanism of action of H_2S as opposed to identifying health effects *per se.*

Apart from gauging the technical quality of each study, the review included critical assessment of the toxicological significance and clinical relevance of the study findings. The findings were summarized and a weight-of-evidence approach was used to assess the meaning and relevance of the overall health effects information. Emphasis was given to studies assigned a "moderate" or "high" confidence index ranking for this purpose. Clinical and non-clinical findings were differentiated.

The review made no attempt to extrapolate the results from the animal testing to the human condition.

The following conclusions emerged from the review:

A. Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations could not be firmly established.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

B. Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting. The toxicological significance and clinical relevance of the responses was not readily apparent.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects remained unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. The signs and symptoms that did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations

from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al*., 1940). In a second older study conducted by Mitchell and Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

No indications of significant effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed before, during and after gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species were not found.

TECHNICAL SUMMARY

Health Effects Associated with Short-term Exposure to Low levels of Hydrogen Sulphide (H_2S) – A Technical Review[3]

Background

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations - Final Report.* The report contained a series of recommendations aimed at increasing awareness of sour gas and its potential impacts on public health, improving the regulatory system surrounding sour gas, and fostering better communication between all affected stakeholders on matters relating to sour gas.

In response to *Recommendation 9*[4], Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). In keeping with the mandate of the Provincial Advisory Committee, the focus of the review was to be on the health effects of "acute" or short-term exposure to H_2S. By way of direction, the Advisory Committee referred to the *"table of effects of H_2S"* that had been prepared by AHEW on the basis of conclusions reached by the Ad Hoc Committee on H_2S Toxicity following an earlier review of the literature (Alberta Health, 1988). The Provincial Advisory Committee urged that a revised table be developed as soon as possible.

In fulfilling its commitment, Alberta Health and Wellness retained Cantox Environmental Inc. ("the consultant") to perform the review. Consistent with the direction provided in *Recommendation 9*[4], the review was conducted under the auspices of an Expert Panel, with members drawn from Alberta Environment, regional health authorities, industry, and other stakeholders. The Expert Panel was charged with:

- Providing advice and direction in relation to the overall scope and nature of the work.
- Assisting in the development of the exact Terms of Reference for the work.
- Helping to identify scientific literature pertaining to the health effects of H_2S.
- Assisting in the development of objective criteria for assessing the technical quality of the scientific literature.
- Providing technical advice to assist in the grading and interpretation of the scientific literature.
- Critiquing progress and draft reports prepared by the consultant.

Terms of Reference

Terms of Reference for the work were developed over a six-month period, after which a detailed and comprehensive review of the available scientific literature was completed. The final Terms of Reference were:

- The review was to focus on the health effects following short-term exposure. The term *"short-term"* was to include exposures of both an acute and sub-acute variety, to capture exposures lasting a few hours to a few days.
- The review was to focus on health effects following 'low dose' exposures. The term *"low dose"* was to include concentrations of H_2S up to and including 100 ppm.
- The review was to focus on health effects *per se*. Although a formal definition of *"health effects"* was not adopted by the Expert Panel,

[3] Report prepared by Donald B. Davies, Ph.D., DABT and Susan E. Haggarty, B.Sc. (Cantox Environmental Inc.) for Alberta Health and Wellness, June 2002.

[4] Recommendation 9 reads as follows: *The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed as soon as practical due to its urgency. (Alberta Health and Wellness has committed to lead the review for H_2S).*

the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*

- The review was to give special consideration to the potential health effects of H_2S on the developing fetus in light of the potential increased sensitivity of this life-stage to the harmful effects of chemicals.
- The review was to focus on scientific studies involving exposures to H_2S via inhalation in order to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (*e.g.*, oral, dermal, injection) were to be excluded from review.
- The review was to be limited to information found in peer-reviewed scientific publications.
- The review was to focus on full-length, primary scientific papers describing original work, rather than on review articles or abstracts.
- The review was to include consideration of information from clinical investigations involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory, and "population" studies involving exposures following routine or accidental releases of H_2S into the environment.
- The review was to include a critical assessment of the technical quality of each scientific paper based on consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

It was determined that the review of the health effects information would not specifically address the potential physiological or psychological responses that might be elicited by unpleasant odours, such as the characteristic "rotten egg" smell of H_2S. However, at the same time, it was determined that the review should carefully document studies for which odour complaints were recorded, including descriptions of any signs and symptoms observed that may have been related to the odour of H_2S.

Similarly, the Expert Panel agreed that emphasis would be directed at assessing the effects of exposure to H_2S on normal populations, but information which emerged during the review which related to the issue of "hypersusceptibility" also would be captured. The need to consider such information respected the fact that a significant portion of the general population may have pre-existing health problems, such as cardiovascular or respiratory disease, that may increase susceptibility to H_2S.

Methods

A comprehensive search of the scientific literature was performed to identify studies that complied with the Terms of Reference. A staged search strategy was employed, with primary reliance placed on electronic databases accessed through the DIALOG Information Retrieval Service. Secondary search methods were used to confirm the completeness and adequacy of the DIALOG search, and included manual searching of literature citations found in review articles and personal communication with the members of the Expert Panel and other specialists in the field. The various papers identified through the search were retrieved and then reviewed against a set of pre-determined "eligibility criteria" that were fashioned around the Terms of Reference for the work. The eligibility criteria required that the exposure conditions used in the studies be adequately documented and consistent with the Terms of Reference vis-à-vis exposure concentration tested (*i.e.*, 0 to 100 ppm), exposure duration (*i.e.*, up to 30 days), mode of exposure (*i.e.*, inhalation) and other applicable qualifiers.

A set of 45 studies met the eligibility criteria for review. The studies included clinical studies involving controlled exposures of volunteer human subjects, non-clinical investigations involving controlled exposures of test animals, and case-control studies involving evaluation of individuals exposed to H_2S as a result of

accidental releases. Non-clinical studies dominated the set (75%). Very few clinical studies (n=8) or case-control studies (n=4) satisfying the eligibility criteria were identified.

Each of the 45 studies was subjected to independent review by two members of the consultant's team. The review proceeded in two stages. The first stage included critical assessment of the overall technical quality of the study based on consideration of experimental design, conduct and reporting, as well as grading of the level of confidence that could be assigned to the study findings and conclusions. A pre-defined set of "quality" criteria was developed to ensure that assessment and grading were performed objectively and consistently. The quality criteria were based largely on the recommendations of leading scientific authorities for the proper design, execution and reporting of health effects studies. The authorities included the Organization for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (USEPA). Each study was graded or ranked (*e.g.*, high, moderate, low) according to how well the quality criteria were satisfied.

The second stage of the review involved critical interpretation of the findings from each study, and assessment of the toxicological significance and clinical relevance of any observed responses. The collective findings from all studies were then summarized and a weight-of-evidence approach was used to assess the meaning and relevance of the overall health effects information.

The review of the 45 studies that met the eligibility criteria proceeded with a clear understanding of the need for objectivity, consistency and scientific rigor. Emphasis was assigned to those studies that achieved a "confidence index ranking" of moderate or high. Studies assigned a low ranking received less attention, but were included for completeness. The case-control studies consistently received "low" confidence index rankings owing to inadequate exposure characterization, possible subject bias, inconsistent reporting of findings and generalized weaknesses in experimental design, conduct and reporting. Accordingly, very little attention was given to the findings and conclusions from these studies.

Findings

The assessment of technical quality revealed a number of weaknesses in experimental design, conduct and/or reporting. Only 15 percent of the studies achieved a "high" confidence index ranking. Close to 50 percent of the studies were assigned a "low" rating. The common weaknesses included:

- Failure to follow conventional testing protocols. (Only 20 percent of the studies followed protocols that matched or approached those recommended by leading scientific authorities).
- Failure to follow Good Laboratory Practice (GLP) guidelines. (Reference to GLP was found in one study only).
- Use of a single exposure concentration only. (Close to 50 percent of the studies involved testing with a single concentration of H_2S, thereby precluding assessment of the dose-responsiveness of any observed health effects, and preventing any meaningful conclusions as to whether or not any responses were treatment related).
- Use of a single sex only. (Forty percent of the studies involved testing with one gender, thereby limiting the ease of extrapolation of any findings to the general population).
- Use of too few test subjects. (Many studies used only limited numbers of test animals or human subjects, thereby rendering interpretation of the significance of any observed health effects difficult).
- Lack of routine measures of toxicity. (Most studies failed to include measurement of routine toxicity endpoints, such as signs and symptoms, body weights and pathology. The lack of this information added to the difficulty in interpreting the biological significance of any observed changes).

- Inadequate descriptions of exposure conditions. (In many instances, information respecting exposure chambers and gas delivery systems was limited, and often target exposure concentrations were not confirmed analytically. This raised concerns with respect to the reliability of the study findings).

In addition, many of the studies were directed more at understanding the mechanism of action of H_2S, especially at the cellular level, than at identifying health effects *per se*. In this regard, the primary focus of close to 70 percent of the studies was to examine "biochemical" or "metabolic" changes in response to H_2S exposure (*e.g*. changes in enzyme activities, changes in lipid, carbohydrate and/or protein metabolism). Although the basis for the conduct of these studies was often explained and justified by the study investigators, the toxicological significance and clinical relevance of the observed changes were not readily apparent in most instances.

The health effects information obtained from the studies was organized and summarized on a system-by-system basis, consistent with the approach used by the Ad Hoc Committee on H_2S Toxicity in 1988. Much of the information referred to effects of H_2S on the nervous system and respiratory system. This was not unexpected given the signs and symptoms commonly reported following H_2S poisoning (*i.e*., symptoms consistent with nervous system involvement) and the portal of entry of the inhaled gas (*i.e*., via the respiratory tract). A significant amount of information dealt with effects on the reproductive system following exposures to H_2S before, during and/or after pregnancy. Effects on "metabolic" systems, especially shifts from aerobic to anaerobic metabolism, were also commonly studied. Information on most other organ systems, including the skin, liver, kidney, GI tract, hematopoietic and immunologic systems, was found to be generally lacking. Principal findings are highlighted below.

Mortality

No deaths were recorded in any of the clinical or case-control studies reviewed.

In terms of animal testing, with the exception of a single study of moderate quality (Weedon *et al.*, 1940) no deaths were reported following short-term exposures to H_2S at concentrations up to 100 ppm. The exception involved the exposure of rats and mice to H_2S at concentrations up to 1000 ppm for 16 hours, with the discovery of deaths following exposure to 63 ppm for as little as one hour. In a second older study, conducted by Mitchell and Yant (1925), deaths were reported among rats, guinea pigs and dogs following prolonged exposures to 100 to 140 ppm; however, the reliability of the information was judged to be highly suspect. The weight-of-evidence suggests that death is very unlikely following short-term exposure to H_2S at concentrations less than 100 ppm.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

Signs and Symptoms

No subjective complaints were voiced by young, healthy male and female subjects following controlled exposures to H_2S at concentrations up to 10 ppm for 15 to 30 minutes. Exposures were via a specially-fitted mouthpiece, thereby precluding any responses related to eye irritation or the odour of H_2S. Exposures were performed under conditions of moderate to strenuous exercise, thereby exaggerating the amount of H_2S inhaled relative to resting conditions.

In a separate study, complaints of unpleasant odour, and dryness of the throat, with or without headache were recorded among individuals with mild to moderate asthma following "whole-body" exposure to 2 ppm of H_2S for 30 minutes under controlled conditions.

Signs and symptoms among test animals following single or repeated exposures at concentrations up to 100 ppm varied from

"nothing" through hyperactivity and lethargy to unconsciousness depending on the species and exposure conditions. Signs were often transient and resolved quickly.

Eye

Complaints of eye irritation were recorded in only one of the clinical studies reviewed. The complaints were registered following brief exposure to 100 to 150 ppm of H_2S. The study was an older investigation, performed in 1925.

Fewer than 10 percent of the non-clinical studies reported signs of eye irritation, even at concentrations as high as 100 ppm. Most responses were mild and non-descript (*e.g.*, preening of the eyes and facial area, "washing" of the face). In no instance was the irritation confirmed through *in situ* ophthalmoscopic examination or post-mortem pathological evaluation.

The findings contradict those found by the Ad Hoc Committee on H_2S Toxicity in 1988. The Ad Hoc Committee reported that the eye *"is very sensitive to the irritant action of H_2S"* and *"irreversible eye tissue damage can occur at 20 ppm H_2S …"*. The discrepancy in findings was traced to a reliance on review articles by the Ad Hoc Committee in which unsubstantiated opinions regarding the sensitivity of the eye to H_2S were propagated.

Respiratory System

Clinical studies involving exposure of young, healthy individuals to H_2S at concentrations up to 10 ppm for 15 to 30 minutes while performing mild to strenuous exercise revealed no airway symptoms nor changes in pulmonary function. Exposure of individuals with mild to moderate asthma to 2 ppm of H_2S for 30 minutes produced mixed results, with 20 percent of the subjects (*i.e.*, 2 of 10) showing changes in pulmonary compliance consistent with bronchial obstruction. Airway symptoms were absent in all subjects.

Effects of short-term exposure to H_2S on the respiratory system noted in animal tests were confined to modest changes in the architecture of the lining of the respiratory tract among rats exposed to 80 ppm. The changes were generally transient, with recovery and/or repair of the tissues noted within a few days post-exposure. The lining of the nasal passages was most affected, consistent with the fact that rats are obligate nasal breathers.

Nervous System

Signs and symptoms consistent with nervous system involvement were generally absent from the clinical studies. Apart from complaints of headache among 3 of 10 individuals with mild to moderate asthma following exposure to 2 ppm of H_2S for 30 minutes, no other indications of nervous system involvement were reported.

With respect to the non-clinical studies, signs and symptoms suggestive of nervous system involvement varied by study, and ranged from "nothing" to agitation, hyperactivity, lethargy, drowsiness or unconsciousness. In many cases, the symptoms were transient, and disappeared during the exposure period or shortly thereafter.

A number of "biochemical" and functional changes within the brain and nervous tissues of rats, mice or guinea pigs were reported following short-term exposure to H_2S in the concentration range of interest (*i.e.*, 0 to 100 ppm). The changes included shifts in lipid, protein and RNA content, altered enzyme activities, and irregularities in EEG activity. The biological significance and clinical relevance of these changes were not readily apparent. In most instances, the findings originated from studies assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and/or reporting.

Reproductive System

No eligible studies on the effects of short-term exposure to low levels of H_2S on human

reproductive performance and outcome were identified.

No reliable evidence to suggest that H_2S is a reproductive toxin or teratogen emerged from the non-clinical studies. In the most definitive study of this type, rats exposed to H_2S at concentrations up to 80 ppm before, during and after gestation showed no changes in reproductive performance indices (*e.g.*, fecundity, fertility, gestation time, number of implantations, number of resorptions, litter size). No structural changes were observed among the fetuses. Growth and development of the offspring were normal, as judged by evaluation of developmental landmarks and behavioral testing.

Several studies reported "biochemical" changes within the developing nervous tissues of neonates following *in utero* exposure to H_2S at concentrations ranging from 20 to 75 ppm. The changes, which consisted of possible shifts in neurotransmitter levels as well as alterations in protein and lipid metabolism, were of unknown biological significance.

Olfactory System

In terms of clinical studies, responses involving the olfactory system were limited to complaints of an *"unpleasant smell"* from individuals with mild to moderate asthma during exposure to 2 ppm of H_2S for 30 minutes. The smell was transient, with the subjects becoming accustomed to the odour within a few minutes after the start of exposure.

In non-clinical studies, rats exposed to H_2S at concentrations up to 100 ppm showed no structural changes in the architecture of the nasal olfactory epithelium; however, cytochrome oxidase residing within the olfactory epithelial tissues was inhibited at 80 ppm. The latter finding might account for the sensitivity of the olfactory neurons, and possibly provides an explanation for the olfactory paralysis reported to occur at higher concentrations of H_2S (*i.e.*, 150 to 200 ppm).

Metabolic Systems

Clinical studies involving the exposure of young, healthy and "aerobically fit" male and female subjects to H_2S at concentrations up to 10 ppm for up to 30 minutes demonstrated a possible shift from aerobic to anaerobic metabolism, as evidenced by increased oxygen uptake, elevated blood lactate levels and/or changes in the activities of certain enzymes involved in the tricarboxylic acid (TCA) cycle. The shift was modest, and was not evident among the female subjects. Nevertheless, it was consistent with expectations given the mechanism of action of H_2S (*i.e.*, inhibition of cytochrome oxidase). The clinical relevance of the observed changes was judged to be questionable given the modest nature of the responses and the fact that shifts to anaerobic metabolism occur regularly in response to increased tissue oxygen and energy demands.

Skin and Integumentary System, Cardiovascular System, Kidney, Liver, Gastrointestinal System, Hematopoietic System and Immunological System

Little information was found in the published literature concerning the effects of short-term exposure to H_2S at concentrations in the 0 to 100 ppm range on these systems. Any information that was available was generally non-descript and/or non-specific. No evidence emerged to suggest that these systems were especially responsive to H_2S, but this needs to be interpreted with caution in light of the limited published information that was found.

Conclusions

Conclusions reached concerning the health effects of short-term exposures to low levels of H_2S were separated to distinguish between the evidence gathered from clinical versus non-clinical studies. The conclusions are presented below.

Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations could not be firmly established.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting. The toxicological significance and clinical relevance of the responses was not readily apparent.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects remained unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms that did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al*., 1940). In a second older study conducted by Mitchell and Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is remote.

No indications of significant effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed before, during and after gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species were not found.

It must be emphasized that no attempt was made in the present review to extrapolate the findings from the animal studies to the human condition. Specifically, no attempt was made to adjust the findings to account for differences between species in factors such as pulmonary dynamics

that would act to determine the "toxic load" of H_2S received in each of the studies reviewed. Similarly, no attempt was made to extrapolate the findings from the clinical and case-control studies to the general population. Thus, caution must be exercised in the interpretation and use of the conclusions.

I. BACKGROUND

In December 2000, the Provincial Advisory Committee on Public Safety and Sour Gas released its final report entitled *Findings and Recommendations-Final Report.* The report contains a series of recommendations aimed at increasing awareness of sour gas and its potential impacts on public health, improving the regulatory system surrounding sour gas, and fostering better communication between all affected stakeholders on matters relating to sour gas.

Among the 87 recommendations contained in the report, several relate to the need to increase awareness of the potential health hazards of sour gas *and* to ensure that regulatory standards reflect current understanding of the health effects information on sour gas and its constituents. Two of these recommendations were central to the present work. They were:

9. The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry, and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed, as soon as practical due to its urgency.

59 The EUB work with Alberta Health and Wellness, regional health authorities, and other stakeholders to develop clear requirements and evacuation criteria to address the hazard of SO_2 as a result of ignition.

In response to *Recommendation 9,* Alberta Health and Wellness (AH&W) committed to completing a comprehensive review of the health effects information currently available for hydrogen sulphide (H_2S). At the same time, AH&W agreed to accept responsibility for performing a similar review for sulphur dioxide (SO_2), in partial fulfillment of *Recommendation 59.* In keeping with the mandate of the Provincial Advisory Committee, the focus of the reviews was to be on the health effects of "acute" or short-term exposures to H_2S and SO_2. By way of direction, the Advisory Committee referred to the *"table of effects of H_2S"* prepared by the Ad Hoc Committee on H_2S Toxicity in 1988 (Alberta Health, 1988) as a possible starting point for the review, and urged that a revised table be developed as soon as possible.

In fulfilling its commitments, Alberta Health and Wellness elected to retain Cantox Environmental Inc. ("the consultant") of Calgary, AB to perform the reviews of the health effects information. On the recommendation of the consultant, AH&W commissioned an Expert Panel to assist in defining the scope and nature of the work and to provide technical advice for the performance of the reviews. The Expert Panel consisted of members drawn from Alberta Environment, regional health authorities, industry and other interested stakeholders. In order to ensure that the performance of the work took advantage of different perspectives and expertise surrounding the potential health hazards of sour gas.

This report focuses on the review of the health effects information currently available on H_2S. A separate report will be prepared on the health effects of SO_2. In order to maintain some degree of continuity, this report references the earlier report prepared by the Ad Hoc Committee on H_2S Toxicity on behalf of Alberta Health and Wellness (1988). A similar format has been followed to ease review and interpretation of the findings and conclusions.

The work commenced in October 2001 with the inaugural meeting of the Expert Panel. Terms of Reference were finalized late in March 2002. The review of the health effects information began in April 2002. The work was completed in July 2002 with the issuance of this final report summarizing the health effects information.

II. INTRODUCTION

At the request of Alberta Health and Wellness (AH&W), a comprehensive review of the currently available scientific literature on the health effects of hydrogen sulphide (H_2S) was undertaken. The review focused specifically on the health effects following short-term exposures to low concentrations of H_2S. To ensure full understanding of the nature and scope of the review, the following definitions were developed:

short-term, *adj.* **1**. extending over a few hours or a few days. **2.** encompassing acute and sub-acute events lasting up to 30 days.

low concentrations, *n, pl.* concentrations ranging from 0 to 100 parts-per-million (ppm) in air.

health effect, *n.* an undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.

Cantox Environmental ("the consultant") was retained by Alberta Health and Wellness to complete the review. In keeping with the need expressed by the Provincial Advisory Committee on Public Safety and Sour Gas to work with Alberta Environment, regional health authorities, industry and other stakeholders, an "Expert Panel" was commissioned to provide advice and direction regarding the performance of the work.

The members of the Panel were[5]:

Dr. Randy Angle
(Alberta Environment)

Dr. Nicholas Bayliss
(Alberta Health and Wellness)

Dr. Donald Davies ("consultant")
(Cantox Environmental Inc.)

Dr. Stephan Gabos
(Alberta Health and Wellness)

Dr. Brent Friesen
(Calgary Health Region)

Mr. Geoffrey Granville
(Shell Canada Ltd.)

Dr. Paul Hasselback
(Chinook Health Region)

Mr. Alex MacKenzie
(Alberta Health and Wellness)

Dr. Ingrid Vicas
(Calgary Health Region – Alberta Poison Centre)

The specific roles performed by the Expert Panel were:

- To provide advice and direction in relation to the overall scope and nature of the work.
- To assist in the development of the exact Terms of Reference for the work.
- To assist in identifying scientific literature pertaining to the health effects of H_2S.
- To assist in the development of criteria for gauging the technical quality of the scientific literature.
- To provide technical advice, as needed, to assist the consultant in the grading and interpretation of the literature.
- To audit the work performed by the consultant to ensure objectivity, consistency and rigour in the grading and interpretation of the scientific literature.
- To review and comment on progress reports developed by the consultant.

The Panel met on seven occasions during the course of the work, in October 2001, January 2002, February 2002, March 2002, May 2002, early June 2002 and late June 2002.

Much discussion surrounded the Terms of Reference for the work to ensure a clear

[5] The full titles and affiliations of the Panel members can be found in Appendix 1.

understanding on the part of the consultant and Panel members as to the exact scope and nature of the review. As indicated already, it was determined that the review would focus on the health effects associated with short-term exposures to low concentrations of H_2S. The exact Terms of Reference are outlined in Chapter III.

In completing the review, a comprehensive search of published scientific literature was performed in order to identify, as best possible, all available scientific papers dealing with the health effects of short-term exposure to H_2S. Eligibility criteria were developed to narrow the list of studies to those satisfying the Terms of Reference. Studies meeting the eligibility criteria were then subjected to a detailed and thorough review, including an assessment of the technical quality of the work based on consideration of experimental design, conduct and reporting. Interpretation of the biological significance and clinical relevance of the findings from each study formed part of the review. The studies covered a broad spectrum of investigations and included non-clinical studies involving exposure of test animals under controlled experimental conditions, clinical studies with volunteer human subjects exposed under controlled conditions, and case-control studies involving exposure of humans as a result of accidental releases of H_2S. In conducting the review, the consultant relied on technical expertise as well as professional judgement to arrive at conclusions. A deliberate and conscious attempt was made to ensure that the review was performed with full respect for the need for objectivity, consistency and care in the interpretation of the data.

III. TERMS OF REFERENCE

Work aimed at developing the Terms of Reference for the review began in October 2001. The final Terms of Reference were reached in March 2002. During the course of this period, the Expert Panel met on four occasions to debate the exact Terms of Reference to be followed. Agreement was reached on the following terms:

- The review was to focus on the health effects following short-term exposure. The term *"short-term"* was to include exposures of both an acute and subacute variety, to capture exposures lasting a few hours to a few days. The subacute category was further defined to include exposures extending up to 30 days.
- The review was to focus on health effects following 'low dose' exposures. The term *"low dose"* was to include concentrations of H_2S up to and including 100 ppm. Effects observed at higher concentrations were to be included at the discretion of the consultant, subject to agreement by the Expert Panel.
- The review was to focus on health effects *per se*. Although a formal definition of *"health effects"* was not adopted by the Expert Panel, the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*
- The review was to give special consideration to the potential health effects of H_2S on the developing fetus in light of the potential increased sensitivity of this life-stage to the harmful effects of chemicals, and the concerns often expressed during pregnancy over chemical exposures.
- The review was to be limited to peer-reviewed scientific publications. Preference was to be given to English-language journals, with specific foreign-language papers to be reviewed at the discretion of the Expert Panel.
- The review was to include *all* currently and readily available journal articles, with a strict need to avoid possible journal and/or sponsor bias.
- The review was to focus on scientific studies involving exposures to H_2S via inhalation to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (*e.g.*, oral, dermal, injection) were to be excluded from review.
- The review was to focus on full-length primary scientific papers describing original work rather than on review articles and abstracts.
- The review was to include consideration of clinical investigations involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory, and "population" studies involving exposures following routine or accidental releases of H_2S into the environment.
- The review was to include a critical assessment of the technical quality of each scientific paper based on consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

Further discussion surrounded the need for each scientific paper to be subjected to independent review by two members of the consultant's team to ensure objectivity, consistency and fairness in judging the technical quality of the scientific studies, and consistency and rigour in the interpretation of findings and conclusions.

Additional discussion centered on the need for independent audit of the consultant's work, with agreement reached that the members of the Expert Panel would perform separate reviews of selected scientific papers to ensure that the consultant's findings fairly represented the strengths and weaknesses of each reviewed document.

Much discussion was devoted to distinguishing between *"science"* and *"policy"* as they related to the performance of the work, with the understanding that linkages invariably exist between the two elements. It was agreed that the review of the health effects information would be strictly a scientific exercise in which the quality of the information would be assessed against a set of technical criteria and the scientific meaning of the study findings would be presented, without consideration of the public health implications. Interpretation of the biological significance and clinical relevance of the findings would be allowed, but not interpretation of the significance of the findings for the purposes of setting policy for the protection of public health.

The question of the need to assess the possible health implications associated with the odour of H_2S was discussed at length. It was acknowledged that concern over the odour of H_2S represents a valid and important issue given the reported physiological and/or psychological responses that can be elicited through the sense of smell, the very low odour threshold for H_2S, and the number of odour complaints that historically have been registered in Alberta in relation to sour gas operations. Much discussion centered on whether or not the detection of an unpleasant odour in and of itself constitutes a health effect *per se*. Differing opinions were expressed in this regard. Some Panel members held that an odour, be it pleasant or unpleasant, simply represents a response to a stimulus, and as such, even an unpleasant odour does not qualify as an undesirable or harmful effect. Other Panel members argued that, although the primary response to an odour may not qualify as an adverse effect, the secondary responses that the odour might trigger could prove to be undesirable or harmful. Despite the differing opinions, it was agreed that the review of the health effects information would not specifically address the potential physiological or psychological responses that might be elicited by unpleasant odors, such as the characteristic "rotten egg" smell of H_2S. However, at the same time, it was determined that the review should carefully document studies for which odour complaints were recorded, including a description of any signs and symptoms that emerged which may have been related to the unpleasant smell of H_2S.

Some discussion centered over the need to clarify issues surrounding the effects of exposure to low levels of H_2S on "hypersusceptible" individuals[6], based on reports suggesting that certain individuals may be especially responsive to H_2S owing to pre-existing medical conditions. Agreement was reached that emphasis would be directed at assessing the effects of exposure to H_2S on normal populations, but information which emerged during the review which related to the issue of hypersusceptibility, as defined by the Illinois Institute for Environmental Quality (IIEQ, 1974), also would be captured. The need to consider such information respected the fact that a significant portion of the general population may have pre-existing health problems, such as cardiovascular or respiratory disease, that may increase susceptibility to H_2S.

In addition, some discussion focused on the need to consider reports of health effects following intermittent "peak" exposures to H_2S occurring against a chronic low-level "background" of exposure, as might occur for individuals living downwind of industrial (*e.g.*, pulp mills) or agricultural (*e.g.*, feedlots) sources. Agreement was reached that this type of exposure scenario did not satisfy the definition of "short-term" adopted for the purposes of the present review.

Finally, some discussion revolved around the need to include the effects of short-term exposures to low concentrations of H_2S on livestock as part of the review. It was agreed that the review would focus only on the potential effects on humans since work relating to the effects on livestock would be addressed through a separate initiative (*i.e.*, the Western Inter-provincial Scientific Studies Association – WISSA- initiative). It was understood that the

[6] The term "hypersusceptible" was used in the context of the description provided by the Ad Hoc Committee on H_2S Toxicity (Alberta health, 1988) and referred to individuals who might be at increased risk due to pre-existing medical conditions that could be aggravated by exposure to H_2S. The term was not used to describe individuals who may be intrinsically more sensitive to chemicals in general and might respond to lower concentrations of H_2S than the general population.

potential effects on humans would be assessed through consideration of clinical, non-clinical, and case-control studies involving short-term exposures to low levels of H_2S. With respect to the non-clinical studies, emphasis was necessarily to be given to tests performed with conventional laboratory animal species, such as rats, mice and guinea pigs.

In completing the work, some reliance on professional judgement was needed in order to grade the technical quality of the studies and to interpret the biological significance and clinical relevance of the findings and conclusions. The consultant was cautioned by the Expert Panel of the absolute need for objectivity, the need for independent appraisal, and the need to limit opinion.

IV. METHODS

The work proceeded in stages beginning with a comprehensive search of the published scientific literature to identify studies in which the health effects of short-term exposures to low concentrations of H_2S were investigated. The scientific papers describing the studies were then retrieved and catalogued. Each study was subsequently subjected to a detailed review, which first encompassed an evaluation of the technical quality of the investigation based on consideration of experimental design, conduct and reporting. A set of "quality" criteria was developed for each of the various study types (*i.e.*, clinical, non-clinical, and case-control) to permit objective and consistent judgement of technical strengths and weaknesses. The criteria were based on the recommendations of leading scientific authorities for the proper design, execution and reporting of health effects studies. A grading system was established and, on the basis of the initial evaluation of technical quality, each study was assigned a confidence index ranking (*e.g.*, low, moderate or high) that reflected the level of confidence that could be assigned to the findings and conclusions. A detailed assessment of the observations recorded from each study was then performed, with emphasis placed on those studies which achieved a moderate or high grade. The information from each study was summarized and captured in a "document review form" developed specifically for this work (see Appendices 5 and 6). The form contained details concerning study design features, observations, authors' conclusions, and strengths and weaknesses vis-à-vis technical quality. Interpretation of the biological significance and clinical relevance of the study findings also formed part of the review. A description of the various stages comprising the work follows.

A. Search Strategy

As indicated earlier, the first stage of work involved a comprehensive search of the published scientific literature to identify, as best possible, all studies dealing with the health effects associated with short-term exposure to low concentrations of H_2S. Three search methods were used:

- On-line electronic databases
- Citation sourcing
- Personal communication

A brief description of each method is found below.

On-line electronic databases

A series of search terms was developed, matching the overall Terms of Reference for the work and satisfying a set of eligibility criteria that were developed for studies to be included in the review. The eligibility criteria are shown below. Search terms were broadened at the discretion of the consultant to ensure that the search was complete. Possible bias in the selection of terms was avoided by the involvement of 3 individuals in the final choice of terms. A full listing of the search terms can be found in Appendix 2.

Eligibility Criteria[1]	
Study classification	Primary research (*i.e.*, papers describing original work)
Study type	All (*i.e.*, clinical, non-clinical, case-control, *etc.*)
Exposure duration[2]	0 to 30 days (*i.e.*, acute and sub-acute)
Exposure concentration[2]	0 to 100 ppm of H_2S
Exposure route	Inhalation
Publication type	Full-length peer-reviewed publications
Other	English-language
Additions	Reproductive and developmental toxicity studies

[1] The eligibility criteria for the selection of studies to be reviewed reflected the Terms of Reference for the work, and were determined collectively by the members of the Expert Panel.

[2] Studies for which exposure duration and/or exposure concentration(s) were not specified were deemed to be ineligible and were excluded from review.

Searching was performed using the DIALOG Information Retrieval Service. The following electronic databases were accessed:

- BIOSIS PREVIEWS (1993 to 2002)
- ENVIRONMENTAL BIBLIOGRAPHY (1966 to 2002)
- ENVIROLINE (1975 to 2002)
- LIFE SCIENCES COLLECTION (1982 to 2002)
- MEDLINE (1966 to 2002)
- POLLUTION ABSTRACTS (1970 to 2002)
- TOXFILE (1966 to 2002)

Study titles and/or abstracts were downloaded and then reviewed against the Terms of Reference for the work. Studies bearing titles or abstracts that clearly met the eligibility criteria were retrieved and collated. For cases in which the match between the study title and Terms of Reference was equivocal or not readily obvious, the study was ordered and then accepted or rejected following a preliminary review.

Citation sourcing

Readily available review articles on the health effects of H_2S were retrieved and the bibliographies were manually searched for citations matching the eligibility criteria for the review. The search was completed, in part, to confirm the adequacy and completeness of the electronic search. Very few new studies were identified. A complete listing of the bibliographies that were reviewed is provided in Appendix 3.

Personal communication

A full listing of the studies meeting the eligibility criteria was provided to each member of the Expert Panel, with a request to review the list for completeness and accuracy based on his/her personal knowledge and/or records of studies on the health effects of H_2S.

Based on the three search methods, a total of 45 studies satisfying the eligibility criteria were identified, retrieved and subjected to detailed review.

B. H_2S Database

As indicated earlier, the Terms of Reference for the work evolved over a six-month period, during which time the eligibility criteria for the studies to be included in the review shifted as new information became available. In order to accommodate the shifts, and out of respect for the large body of information that exists on the health effects of H_2S, a computerized bibliographic database was developed to allow for the orderly sorting and classification of the published studies identified through the search strategy. A bibliographic form was created which captured pertinent information respecting study identification, source, type, design features *etc.* (see Appendix 4). A separate form was completed for each study and stored in the database. The database was designed to permit the entire complement of studies to be searched against the eligibility criteria. As a result, shifts in the criteria as the Terms of Reference for the review evolved were easily accommodated. More than 600 studies were entered into the database. A search against the final set of eligibility criteria pinpointed the 45 studies that were subjected to detailed review. A CD version of the database can be made available upon request. Requests should be directed to Alberta Health and Wellness.

C. Quality Criteria

Each of the 45 studies meeting the final set of eligibility criteria was subjected to a detailed review and evaluation. As part of the review, the overall technical quality of the study was assessed based on consideration of experimental design, conduct and reporting. This step of the review was deemed to be important since it had a direct bearing on the level of confidence that could be assigned to the study findings and conclusions. A set of quality criteria was developed for each study type (*i.e.*, non-clinical, clinical, case-

control) on the basis of the recommendations of leading scientific and regulatory authorities for the proper design, execution and reporting of health effects studies (Clarke and Oxman, 2001; OECD,1993; USEPA, 1998). The recommendations were captured in a series of "check-lists" organized in a Q&A format, against which the studies were compared. Full copies of the check-lists can be found in Appendix 5. A sample of the Q&A format is shown opposite.

Judgement of the technical quality of the studies against a pre-defined set of quality criteria that reflected the opinions of leading experts helped to ensure that the reviews proceeded in an objective, consistent and fair manner. A grade was assigned to each study depending on the outcome of the review. Grading necessarily involved weighing the strengths and weaknesses of the study, using the appropriate check-list for guidance. Grades were expressed as a "Confidence Index Ranking" to indicate the overall level of confidence that could be given to the findings and conclusions. The rankings included:

- *High* – Signifying that the study meets or exceeds the recommended guidelines, with no serious weaknesses in experimental design, conduct or reporting. Procedures are well-described and results are properly disclosed to permit meaningful interpretation. Study validity is obvious. Confidence in the findings and conclusions is high.
- *Moderate* – Signifying that the study generally subscribes to the recommended guidelines, but minor deficiencies in design, conduct or reporting detract from the interpretation of the results. Study validity is evident, but not obvious. Careful attention to detail in describing procedures and presenting results may be lacking. Confidence in the findings and conclusions is somewhat restrained, but not weak.
- *Low* – Signifying that the study fails to meet the recommended guidelines and serious weaknesses in design, conduct and reporting are evident. Significant departures from the recommended guidelines may be present. Sufficient detail is lacking to permit meaningful interpretation of results. Study validity is questionable. Confidence in the findings and conclusions is low.

A great deal of care was taken in the assignment of confidence index rankings out of respect for the need to ensure objectivity, consistency and fairness. The ranking relied on the consultant's technical expertise, familiarity with the recommended guidelines, and professional judgement.

Sample Check-list

Non-clinical study – Exposure conditions:

- ☐ Was a full description (manufacturer, purity, lot no.) of the reagents (including H_2S) used in the trial provided?
- ☐ Was there a gradient of exposure levels?
  Guideline: Three exposure levels in addition to control.
- ☐ Were the chosen exposure levels appropriate to investigate the primary objective?
- ☐ Were previous preliminary trials conducted to identify the range of appropriate exposure levels?
- ☐ Were both nominal and actual exposure concentrations recorded?
- ☐ Was the duration of exposure appropriate to investigate the primary objective?
- ☐ Was the duration of exposure precisely defined?
- ☐ If exposure chambers were employed, were animals exposed during the equilibration period?
  Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.
- ☐ Was the mode of administration appropriate?
- ☐ Was the frequency of administration appropriate to investigate the primary objective?
- ☐ Was evidence provided to justify the dosing regime?
- ☐ Were exposure concentrations, chamber airflow, temperature and humidity monitored on a regular basis?

D. Review Process

Each study was subjected to independent review by at least two members of the consultant's team. The primary reviewers were:

Dr. Donald B. Davies, Ph.D., DABT
Vice-President, Scientific Programs

Ms. Susan E. Haggarty, B.Sc.
Environmental Risk Analyst

In the event of disagreement between the two reviewers over the interpretation of the study findings or the assignment of the confidence index ranking, a third reviewer was engaged and any differences were resolved through discussion among all three individuals. Disagreement was rare. In virtually all cases, the confidence index ranking was obvious.

The review began with the assessment of the technical quality of the studies, and then proceeded to the interpretation of the study findings and conclusions. Emphasis was given to those studies which achieved a moderate or high confidence index ranking. On the advice of the Expert Panel, studies that received a "low" ranking were still considered in the overall interpretation of the health effects information but these studies received less emphasis. The detailed review of the studies commenced in early April 2002, and was completed by July 2002.

V. SUMMARY OF HEALTH EFFECTS

A. Overview

The Terms of Reference for the work were to identify and assess the health effects associated with short-term exposure to H_2S at concentrations up to 100 ppm. As indicated earlier, "short-term" exposure referred to exposures of an acute or subacute nature, extending from a few hours up to 30 days. Special consideration also was given to the potential effects of H_2S on the developing fetus, in which case, exposures necessarily extended over several weeks to capture the gestation and post-natal periods. Although emphasis was directed at exposure concentrations in the 0 to 100 ppm range, observations from studies involving exposures to graded concentrations within *and* above the range were recorded in order to provide a more comprehensive survey of potential health effects.

Each study satisfying the eligibility criteria described earlier (Chapter IV) was subjected to a detailed review. The review encompassed the following:

- Determination of study objective(s);
- Review of principal findings and conclusions;
- Review of strengths/weaknesses in study design, conduct and reporting;
- Ranking or "scoring" of the study on the basis of the level of confidence that could be assigned to the findings and conclusions; and
- Assessment of the toxicological significance and clinical relevance of the study findings.

The ranking of the studies relied on a pre-determined set of "quality criteria" (Chapter IV) that were based largely on the recommendations of several leading scientific authorities for the proper design, execution and reporting of clinical and non-clinical studies (*i.e.*, Clarke and Oxman, 2001; OECD, 1993; USEPA, 1998). Ranking was completed with full respect for the need for consistency, objectivity and fairness, and followed a weight-of-evidence approach in which study strengths and weaknesses were carefully measured. Each study was subjected to two independent reviews. A written summary capturing the highlights of each study, complete with a "Confidence Index Ranking", was prepared. The full set of summaries can be found in Appendix 6.

Readers are strongly encouraged to review the study summaries found in Appendix 6 to obtain detailed information with respect to the findings and conclusions from each study, as well as details concerning study design, conduct and reporting.

A compilation and summary of the reported health effects associated with short-term, "low dose" exposures to H_2S is presented below. The effects are arranged on a system-by-system basis and are distinguished by study type (*i.e.*, clinical, non-clinical, case-control). Emphasis was placed on those studies which achieved a "Confidence Index Ranking" of "high" or "moderate", signifying that the study satisfied most quality criteria and showed no serious deficiencies in design, conduct and reporting. Less emphasis was given to studies assigned a "low" rating since little confidence could be placed in the findings and conclusions. A listing of "positive" findings from the various studies (*i.e.*, evidence of health effects), together with the corresponding exposure concentrations is attached (see Figure 1). A companion listing of "negative" findings (*i.e.*, absence of health effects) is captured in Figure 2. Both figures have been included to permit more meaningful interpretation of the findings from a weight-of-evidence perspective. Two separate figures (Figures 3 and 4) listing the principal findings relating to reproductive and developmental toxicity also are included. The level of confidence assigned to each set of findings is indicated. It must be emphasized that the information contained in each of these figures should *not* be considered in isolation. Rather each entry in the figures should be reviewed in combination with the corresponding written summary of the study, as well as the information that follows to ensure that the biological significance and clinical relevance of the

finding(s) are understood. Otherwise, the entries could be easily misconstrued.

It also must be emphasized that, although the information respecting the health effects associated with short-term exposure to H_2S is distinguished by study type (*i.e.,* clinical, non-clinical and case-control), no attempt has been made to extrapolate the findings from the animal studies to the human condition. Such an exercise would require careful consideration of the "toxic load" received by each test specie under the various exposure conditions studied. "Toxic load" is the mass (or flux) of H_2S delivered to the target site(s), which is a function of concentration and time, and differs across exposed species for physiological reasons. The determination of "toxic load" would necessarily depend upon:

- Exposure concentration(s).
- Duration of exposure.
- Specie-specific pulmonary dynamics (*e.g.,* breathing patterns, breathing rates).
- Differences in pharmacokinetic profiles of H_2S between species (*e.g.,* absorption, distribution, metabolism and excretion patterns).
- Architectural variations in the respiratory tract of different species that could affect the deposition pattern and ultimate disposition of any inhaled H_2S.
- Relative life-span of each test specie, particularly for studies involving repeated exposures extending over several days.
- Other specie variations.

Thus, care should be taken in the interpretation of the health effects information, particularly with respect to extrapolation of the findings to the general human population.

B. General Comments

The review of health effects information has been organized on a system-by-system basis, consistent with the approach used earlier (Alberta Health, 1988). For ease of review, studies were segregated by study type (*i.e.*, non-clinical, clinical and case-control). Similarly, given the extensive amount of information that was available for some systems, the information is arranged according to the nature of the effects (*i.e.,* signs and symptoms, functional changes, structural changes, biochemical changes). A total of 45 studies met the eligibility criteria and were subjected to detailed review and assessment. Some general comments concerning the study "sample" follow:

- Non-clinical investigations involving controlled exposure of test animals comprised the greatest number of studies (75%). Most of the remaining studies involved clinical trials with volunteer human subjects exposed to H_2S under controlled conditions (15%). Only four "case-control" studies were identified and reviewed. The preponderance of non-clinical tests presents the challenge of extrapolating the findings to the human condition, and heightens the difficulty of assessing the public health implications of the results.
- Very few studies achieved a "high" confidence index ranking (15%), in which the experimental design, conduct and reporting were well-described and in good agreement with the recommendations found in testing guidelines. Approximately 40% of the studies achieved a "moderate" rating, signifying some weaknesses that undermined confidence in the findings and conclusions. The remaining studies (45%) were found to be deficient, with findings and conclusions that could not be supported owing to serious limitations in experimental design, conduct and/or reporting. These studies were assigned a "low" rating. It should be noted that the preponderance of "low" scores was not the product of overly strict quality criteria, but rather reflected a general lack of attention to detail in the studies and a failure to subscribe to standard operating procedures and conventional testing protocols. Common deficiencies which hindered the interpretation of study findings are shown on the following page.

Common Deficiencies of "Low" Rated Studies

Clinical:

- Limited number of subjects
- Lack of reliable exposure estimates

Non-clinical:

- Failure to follow conventional testing protocols
- Use of too few test animals
- Use of a single sex only
- Inadequate details concerning animal housing, husbandry, and handling
- Use of a single exposure concentration only
- Inadequate description of exposure conditions
- Failure to confirm target exposure concentration(s)
- Failure to include conventional measures of toxicity

Case-control:

- Limited number of test subjects
- Unreliable exposure estimates
- Possible selection bias in subject recruitment
- Excessive lag time between exposure and testing
- Reliance on self-administered questionnaires
- Failure to adequately control for obvious confounding variables (*i.e.*, exposure to other chemicals)
- Reporting inconsistencies

- Only a single study evidently was performed under Good Laboratory Practice (GLP) conditions, a set of operating principles first introduced in the early 1980's as a means to enhance the reliability and reproducibility of experimental results from non-clinical toxicity studies (USEPA, 1989).The lack of attention to GLP in all of the remaining studies casts some doubt on the reliability of the findings.
- Of the non-clinical studies reviewed, very few (20%) followed a conventional study design, such as those recommended by the Organization for Economic Cooperation and Development (OECD) or the United States Environmental Protection Agency (USEPA) (OECD, 1993; USEPA, 1998). As a result, standard measures of toxicity (*e.g.,* body weights, signs and symptoms, gross pathology) were routinely omitted. This hindered interpretation of the findings as well as the comparison of results across different studies. Moreover, it limited interpretation of the biological significance of certain findings (*e.g.,* changes in biochemical indices) since symptomatic and/or pathological correlates of the changes could not be determined.
- Approximately 50% of the studies were not intended to examine the health effects *per se* of H_2S. Rather, the primary objective was often to advance understanding of the mechanism of action of H_2S through the study of various physiologic, metabolic and/or biochemical indices. In many cases, the toxicological significance and clinical relevance of the observed changes in these indices was not readily apparent. In most instances, routine measures of toxicity were not included in the overall experimental design, thereby precluding assessment of the significance or relevance of the changes through consideration of symptomatic or pathological correlates.
- A sizeable number of the studies (45%) used only a single exposure concentration of H_2S. Accordingly, the dose-responsiveness of any findings could not be assessed, thereby hindering determination as to whether or not the effects were treatment related.
- Approximately 40% of the studies involved testing using a single sex only, with males predominating. In no case was justification for the use of the one sex only provided. This deficiency extended to both the clinical and non-clinical trials. A further 30% of the studies failed to specify which sex was tested (*i.e*., male, female or both). Both types of deficiency hindered interpretation of the significance and relevance of the study findings, and added to the difficulty of extrapolating the results to the general population.
- The study 'sample' showed significant differences in the amount of information available on the effects of short-term exposures to H_2S across different organ systems. As might be expected given its mechanism of action and the portal of entry of inhaled H_2S, a number of studies were identified in which effects on the nervous system and respiratory system were examined. However, effects on many of the

remaining systems were found to have received little attention. Very few, if any, studies were available describing the effects of short-term exposures to H_2S on the liver, kidney, gastrointestinal tract, skin, hematopoietic, and immunological systems. Moreover, the majority of these studies received a "low" confidence index ranking, thereby precluding meaningful assessment of the toxicological significance and/or clinical relevance of the findings. Of the remaining studies, most provided only non-specific information that was of limited use in assessing system-specific responses.

The overall impression of the study "sample" was that weaknesses exist in the information currently available on the health effects associated with short-term, "low-dose" exposure to H_2S. In the majority of studies reviewed, conventional testing protocols were ignored and basic design elements were missing (*e.g.*, testing of graded exposure concentrations in both sexes, measurement of routine toxicity indices). Moreover, much of the information is of unknown or questionable toxicological significance as a result of an emphasis on monitoring biochemical and/or metabolic indices (*e.g.*, disturbances in enzyme activities, lipid or protein content of tissues, neurotransmitters levels, ECG or EEG tracings, serum mineral content) instead of standard toxicological endpoints. The clinical relevance of many of the findings also is difficult to assess. In many cases, the study conclusions were judged to be tenuous.

A summary of the health effects information follows. Emphasis is given to studies which were assigned a confidence index ranking of "high" or "moderate". Reference is made to studies which achieved a "low" rating only for completeness. For ease of interpretation, the 'Confidence Index Ranking' for each study is indicated in the text according to Study ID number using the following color and symbol scheme:

Confidence Index Ranking	
High or high-to-moderate	▲
Moderate-to-high, moderate or moderate-to-low	◆
Low or low-to-moderate	●

Summary remarks are included at the end of each section to assist in the interpretation of the various study findings.

C. Mortality

Clinical studies
All clinical studies involving human subjects were free of deaths, as would be expected given the low exposure concentrations used ($\leq$ 30 ppm) and the administrative controls in place.

Non-clinical studies
Mortality was rarely observed following inhalation exposure to H_2S at concentrations up to 100 ppm. However, Weedon *et al.* (1940) did report deaths among rats and mice following "whole body" exposure to 63 ppm of H_2S for up to 16 hours under controlled conditions. The mice were more markedly affected than the rats, with 4 of 4 mice dying within as little as one hour after onset of exposure, and 1 of 8 rats dying within 16 hours. All remaining rats survived. Gross findings recorded at necropsy for the animals dying on test revealed hemorrhagic infiltration of the lungs and congestion of the brain, liver and/or kidneys consistent with generalized systemic toxicity. No deaths occurred in rats and mice exposed to 16 ppm of H_2S in the same study. At 250 ppm, 3 of 8 rats and 4 of 4 mice were found dead within several hours. At 1000 ppm, all animals died within one hour of exposure. The study was assigned a confidence index ranking of "moderate", with no serious weaknesses noted in experimental design, conduct and reporting. However, the study was "dated", and the exposure chamber, gas delivery system, and monitoring equipment were very basic compared to present-day standards (Study ID ◆ 316).

In a second older study, Mitchell and Yant (1925) reported deaths among rats exposed "continuously" to 100 to 140 ppm of H_2S for 18 to 48 hours. The incidence of deaths could not be determined. No deaths were recorded following continuous exposure of rats to 35 to 65 ppm of H_2S for up to 100 hours. In the same study, 1 of 2 guinea pigs and 1 of 2 dogs were found dead following continuous exposure to 103 ppm of H_2S for 18 to 48 hours and 8 to 16 hours, respectively.

All guinea pigs survived exposures to 35 to 65 ppm of H_2S. At higher concentrations (*i.e.*, >100 ppm), a higher incidence of mortality and/or shorter time to death was recorded for all three species tested. Canary birds were found to be especially sensitive to H_2S, with deaths recorded within 8 to 18 hours following continuous exposure to 35 to 65 ppm. The study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting. Accordingly, caution must be exercised in the interpretation of the significance of the findings (Study ID ● 444).

No other studies reported deaths in the 0 to 100 ppm range, including studies using mice (Elovaara *et al.*, 1978 – Study ID ● 155; Savolainen *et al.*, 1980 – Study ID ● 280). Deaths were reported at exposure concentrations of 300 to 500 ppm and greater in several animal species including rats (Lopez *et al.*, 1986 – Study ID ◆ 466; Khan *et al.*, 1990 – Study ID ◆ 210; Struve *et al.*, 2001 – Study ID ◆ 296), pigs and rabbits (O'Donoghue, 1961 – Study ID ● 255).

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

Case-control studies
No deaths were reported in any of the case-control studies reviewed.

Summary:
Based on the weight-of-evidence, the likelihood of mortality occurring in response to short-term exposure to H_2S at concentrations up to 100 ppm is remote.

Clinical studies:
No deaths were reported in any of the clinical studies reviewed.

Non-clinical studies:
Deaths were rare following short-term exposures to low levels of H_2S. Deaths of rats and mice were recorded by Weedon et al. (1940) following exposure to 63 ppm of H_2S from as little as one hour to up to 16 hours. Deaths of rats, guinea pigs and dogs were reported by Mitchell and Yant (1925) following continuous exposures to 100 to 140 ppm of H_2S for at least 8 hours. No other deaths occurred in any of the remaining studies reviewed at concentrations up to 100 ppm, even when exposures extended over several hours per day for several days. The significance of these findings is discussed in Chapter VI (Other Considerations).

Case-control studies:
No deaths were reported in any of the case-control studies reviewed.

D. Signs and Symptoms

Although the routine observation of test subjects and/or test animals is a standard requirement of most clinical and non-clinical testing guidelines, a significant number of the studies reviewed contained no reference or description of signs and symptoms of toxicity either during or following exposure. As a result, information on signs and symptoms following short-term exposure to low levels of H_2S is somewhat limited.

Clinical studies
In most clinical trials involving exposure of human subjects to H_2S under controlled conditions at concentrations in the 0 to 10 ppm range, no complaints or symptoms of discomfort were reported.

In a series of studies aimed at elucidating the effects of H_2S on various pulmonary, metabolic and biochemical markers of aerobic versus anaerobic metabolism, Bhambhani *et al.* (1991; 1994; 1996a; 1996b; 1997) reported no complaints of discomfort, headache, nausea, sore throat or undue fatigue among male and female subjects during or following exposure to H_2S at concentrations up to 10 ppm for up to 30 minutes. Exposures were via a specially-fitted mouthpiece. The subjects performed moderate to strenuous exercise throughout the exposure period. All subjects were in good health and aerobically fit (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121, Study ID ▲ 122).

Similarly, Kangas and Savolainen (1987) reported no symptoms among test subjects following

"whole-body" exposure to H_2S at concentrations up to 30 ppm for 30 to 45 minutes under controlled conditions (Study ID ● 207). However, confidence in the study was rated "low" due to serious weaknesses in reporting.

In contrast, Jappinen *et al*. (1990) reported than asthmatic subjects complained of an unpleasant odour, dryness of the nose and throat and/or headache during and after "whole body" exposure to 2 ppm of H_2S for 30 minutes. The former symptoms were generally transient and appeared only at the start of the exposure. None of the subjects suffered from severe asthma (Study ID ◆ 202).

In a study to investigate possible airway inflammation in response to exposure to swine dust, Larsson *et al.* (1994) monitored the general health status of healthy volunteers after working in a swine containment building for up to 5 hours. Measurements taken within the building revealed the presence of low concentrations of H_2S (<0.05 ppm). Subjects complained of headaches, malaise, nausea and/or drowsiness. However, the symptoms were not ascribed to H_2S given the low concentrations involved (Study ID ● 662).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S complained of eye irritation and respiratory discomfort within 2 to 15 minutes of exposure. The symptoms progressed to pain in the eyes, throat irritation, coughing, and excessive salivation with continued exposure for up to 4 hours. Loss of the sense of smell also was recorded. The authors described the tests as *"preliminary"*. The study was assigned a "low" confidence index ranking (Study ID ● 444).

It is noteworthy that any influence of odour on symptom reporting was effectively eliminated in the series of investigations by Bhambhani *et al.* through breathing exclusively via the mouthpiece.

Non-clinical studies

Signs of toxicity recorded following controlled exposures of test animals to H_2S at concentrations ranging from 0 to 100 ppm varied from no obvious symptoms, through initial hyperactivity, to loss of consciousness. For instance, Khan *et al.* (1990) reported no "marked" symptoms of toxicity among male rats exposed to 10, 50 or 200 ppm of H_2S for four hours. Regrettably, the term "marked" was not defined (Study ID ◆ 210). Similarly, Kosmider *et al.* (1967) found no observable evidence of toxicity among rabbits of both sexes exposed to 71 ppm of H_2S repeatedly for 30 minutes each day for five consecutive days (Study ID ◆ 223). Lopez *et al*. (1987, 1988) reported no signs or symptoms among male rats following a single exposure to 10 or 200 ppm H_2S for four hours (Study ID ◆ 231 and Study ID ◆ 233).

In contrast, several studies revealed systematic responses, typically with involvement of the respiratory and central nervous systems. Common signs included initial restlessness or agitation, changing to drowsiness, lethargy and fatigue, with or without laboured breathing and evidence of mild to moderate respiratory distress. The affected species included mice (Elovaara *et al.*, 1978 – Study ID ● 155; Savolainen *et al.*, 1980 – Study ID ● 280; Weedon *et al.*, 1940 – Study ID ◆ 316), rats (Lopez *et al*., 1986 – Study ID ◆ 466; Weedon *et al*., 1940- Study ID ◆ 316), rabbits (Kosmider *et al*., 1971 – Study ID ● 595), guinea pigs (Haider *et al.*, 1980 – Study ID ● 179; Mitchell and Yant, 1925 – Study ID ● 444) and dogs (Mitchell and Yant, 1925 - Study ID ● 444). Exposure concentrations ranged from 20 to 100 ppm, delivered on an acute (*i.e.*, up to 16 hours) or repeated subacute (*i.e.*, once daily for up to 14 days) basis. The most marked signs of intoxication were reported by Kosmider *et al.* (1967) in a study involving exposure of rabbits to 71 ppm of H_2S for 1.5 hours. The animals reportedly lost consciousness (Study ID ● 223). The confidence index ranking assigned to this study was "low" in light of serious limitations in experimental design, conduct and reporting.

Signs and symptoms monitored as part of certain non-clinical studies included body weight, body weight gain, and feed consumption. Changes in these parameters can represent direct toxicity, or alternatively, they may simply reflect non-specific stress, whereupon the animal may refuse feed, thereby slowing growth. In a few instances, body weight losses or reduced body weight gains were reported following short-term exposure to

H_2S. Specifically, Lopez *et al.* (1986) reported an initial modest weight loss (*i.e.*, < 5%) among male rats following exposure to 40 ppm of H_2S for six hours. Growth was restored within 18 hours post-exposure (Study ID ◆ 466). Curtis *et al.* (1975) observed modest to mild reductions in body weight gain (*i.e.* 5-10 %) among pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416). As part of a reproductive and developmental toxicity study, Dorman *et al.* (2000) reported reduced feed intake, accompanied by a modest reduction in body weight gain (*i.e.*, 5-6%) among male and female rats during the first week of exposure to 80 ppm of H_2S for six hours per day as part of a two-week pre-mating period. Normal feed intake resumed by the second week of the study (Study ID ▲ 149).

Case-control studies

Case-control studies consistently revealed a number of subjective complaints allegedly caused by exposure to H_2S among the exposed cohorts. Symptoms were of both an immediate and latent variety.

Kilburn (1997) reported that subjects exposed for "hours" to H_2S at concentrations ranging from 1 to 50 ppm complained of a variety of symptoms at the time of exposure. The symptoms included loss of strength, headache, excessive fatigue, chest pain and awareness of an unpleasant odour. Latent symptoms reported by the same subjects two to six years after the exposure varied from eye, respiratory and gastrointestinal disorders (*e.g.*, eye irritation, throat irritation, dry cough, shortness of breath, indigestion, nausea, loss of appetite) through sleep disturbances and altered states of mood, to memory loss and loss of concentration (Study ID ● 216).

In a subsequent study, Kilburn (1999) reported that subjects exposed to 1 to 40 ppm of H_2S for up to one week as a result of emissions associated with a refinery explosion and fire complained of altered moods when questioned more than three years after the incident (Study ID ● 217).

As part of an inquiry into the events surrounding the "blow out" of a sour gas well near Cynthia, Alberta (*i.e.*, the Lodgepole blow-out), witnesses from the area recalled experiencing a variety of symptoms, including headache, eye irritation, nausea, loss of appetite, and diarrhea, together with a host of upper and lower respiratory tract symptoms, such as sore throat, nasal irritation, nosebleeds and shortness of breath, which they alleged were linked to H_2S exposure (Study ID ● 327).

In each of these three case studies, a "low" confidence index ranking was assigned owing to serious deficiencies in experimental design, conduct and reporting. The most notable deficiencies included lack of reliable exposure estimates, excessive lag time between exposure and the evaluation of subjects, inconsistent and possibly biased reporting of findings, possible bias in the selection of subjects, and failure to control for obvious confounding variables (*i.e.*, exposures to other chemicals).

As part of a retrospective analysis completed by Alberta Social Services and Community Health (1983) of the nature and frequency of complaints received from residents living both near and distant from the site of the "Lodgepole" sour gas well blow-out, a number of signs and symptoms were identified that appeared to correlate with concentrations of H_2S measured at several locations within the surrounding area. A total of 31 different health-related symptoms were recorded, the most common of which were "feeling sick", headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, vomiting, stomach cramps and skin allergies. Complaints of an unpleasant odour were very frequent. Peak concentrations of H_2S measured in the area ranged from 0.3 to 5.5 ppm, with mean daily average concentrations ranging from 0.01 to 0.4 ppm. Exposures extended over 2 two-week periods, during which H_2S was released directly into the atmosphere. The study was assigned a "low-to-moderate" confidence index ranking owing to several weaknesses in design, conduct and reporting. Weaknesses included lack of reliable exposure estimates, possible subject bias, possible information bias, and exclusive reliance on self-reported symptoms (Study ID ● 424).

Summary:
It is regrettable that for most of the studies

reviewed either routine observation of the subjects or test animals was not performed or the results were not reported. Based on the findings from the remaining studies, with emphasis necessarily given to the observations from those investigations which achieved a "moderate" or "high" confidence index ranking, it would appear that adverse signs and symptoms are unlikely to occur among healthy young adults as a result of short-term exposure to H_2S at concentrations up to 10 ppm.

Clinical studies:
The series of studies by Bhambhani et al. revealed that short-term exposure to concentrations up to 10 ppm can be tolerated without complaint by young, healthy and "fit" individuals, even when inhaling the gas directly while performing moderate to strenuous exercise. The lack of symptoms among the subjects was especially revealing since they were aware in advance of the clinical signs that might be experienced. However, possible symptoms related to the irritant properties of the gas cannot be dismissed since the subjects breathed through a mouthpiece. Some asthmatics or individuals with respiratory disorders might respond at lower concentrations, as suggested by the work of Jappinen et al. (1990). In that case, the symptoms were mild and transient; however severe asthmatics were excluded from the study, the concentration tested was low (i.e., 2 ppm) and the duration of exposure was short (i.e., 30 minutes).

Non-clinical studies:
With progressively higher concentrations, the likelihood of adverse clinical symptoms will mount, based largely on the findings from animal studies. However, no consistent evidence was presented to suggest that serious, irreversible symptoms might occur at concentrations up to 100 ppm. The animals generally recovered quickly from any signs of clinical discomfort or stress.

Case-control studies:
Case-control studies consistently revealed a number of subjective complaints allegedly caused by exposure to H_2S among the exposed cohorts. However, these studies received "low" confidence index rankings owing to serious weaknesses in experimental design, conduct and/or reporting

The influence of odour on the likelihood of occurrence of signs and symptoms was not explored as part of the current review. However, in light of reported physiological and/or psychological responses to odours, combined with the unpleasant smell of H_2S and its very low odour threshold, there is a distinct possibility that some individuals might respond at unusually low concentrations.

E. Eye

Despite repeated references in the published literature to the sensitivity of the eye to the irritant effects of H_2S (ACGIH, 1984; ATSDR, 1999; Alberta Health, 1988; Beauchamp *et al.*, 1984; NRCC, 1981) and the earlier claim that *"irreversible eye tissue damage can occur at 20 ppm H_2S …"* (Alberta Health, 1988), very little evidence of effects on the eye following short-term, "low-dose" exposure surfaced during the current review of the literature.

Clinical studies
Evidence of eye irritation rarely was reported in the controlled clinical studies that were reviewed, albeit a large number of the studies involved exposure to H_2S through a mouthpiece, thereby effectively precluding exposure of the eyes (Bhambhani *et al*., 1991, 1994, 1996a, 1997b, 1997).

Jappinen *et al*. (1990) found no evidence of eye irritation following controlled "whole body" exposure of asthmatic subjects to 2 ppm of H_2S for 30 minutes (Study ID ◆ 202), nor did Kangas and Savolainen (1987) report eye irritation among subjects exposed to H_2S at concentrations up to 30 ppm for 30 to 45 minutes (Study ID ● 207).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S for as little as 2 to 15 minutes complained of irritation of the eyes. The effects became more pronounced with continued exposure, with *"sharp pain in the*

eyes" reported after 1 to 4 hours (Study ID ● 444).

Non-clinical studies

With respect to the non-clinical studies, fewer than 10% of the investigations reported eye involvement in response to exposures to H_2S at concentrations between 0 and 100 ppm. The reported effects were typically non-descript. Specifically, Haider *et al.* (1980) reported *"itching and eye irritation"* following "whole-body" exposure of male guinea pigs to 20 ppm of H_2S for one hour per day for 11 days (Study ID ● 179). Kosmider *et al.* (1971) observed *"congestion of the conjunctiva"* among male and female rabbits exposed to 71 ppm of H_2S for one hour per day for 14 days (Study ID ● 595). Lopez *et al.* (1986) reported *"lacrimation"* among male rats exposed to 40 ppm of H_2S for 6 hours (Study ID ◆ 466). Mitchell and Yant (1925) reported *"irritation of the eyes"* progressing to *"pus in the eyes"* among rats, guinea pigs and dogs following continuous exposure to 35 to 100 ppm of H_2S for 8 to 48 hours. Lacrimation was also noted among the dogs (Study ID ● 444). In each case, the reported effects were not confirmed through *in situ* ophthalmoscopic examination or post-mortem histopathological evaluation of the eyes. It may be of some interest that most of the studies were assigned a "low" confidence index ranking, signaling serious weaknesses in experimental design, conduct and/or reporting. It is also of interest that Skrajny *et al*. (1996) reported that *"eye irritation was never observed in the rats"* as a result of exposure to H_2S at concentrations up to 100 ppm for three hours per day for 5 days (Study ID ◆ 291). No eye involvement was indicated in any of the remaining non-clinical studies.

Case-control studies

Case-control studies showed mixed results. Kilburn (1997) found that eye effects were not reported by subjects allegedly exposed to 1 to 50 ppm of H_2S for up to 24 hours at the time of exposure; however, upon evaluation two to six years later, eye involvement was reportedly indicated, as evidenced by abnormal colour vision and reduced visual fields as well as complaints of eye irritation (Study ID ● 216). Similarly, Kilburn (1999) reported eye effects among individuals allegedly exposed to H_2S at concentrations ranging from 1 to 40 ppm for up to one week as a result of emissions from a refinery explosion and fire. The effects presented as abnormal color vision and impaired visual fields based on testing performed three years after the event (Study ID ● 217). In addition, witnesses testifying as part of the Lodgepole blow-out inquiry recalled experiencing eye irritation at the time of the incident (Study ID ● 327). Similarly, a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole blow-out revealed a relatively high incidence of eye effects, including "burning eyes" and double vision (Study ID ● 424). As indicated earlier, the confidence index ranking assigned to each of these studies was "low" in light of serious deficiencies in experimental design, conduct and reporting.

It is clear from the above that little information is available suggesting eye involvement following short-term exposure to H_2S at concentrations in the range of 0 to 100 ppm. This finding is in apparent contradiction with the conclusions reached earlier (Alberta Health, 1988). Possible reasons for the discrepancy are outlined later (Chapter VI. Other Considerations).

Summary:

It would appear that very little reliable information exists in the literature concerning the effects of short-term exposure to H_2S at low concentrations on the eye and associated structures.

Clinical studies:

No evidence of eye involvement was indicated as part of the findings from clinical studies involving the controlled exposure of subjects to H_2S at concentrations up to 10 ppm for 30 minutes. However, for most of these studies, the H_2S was delivered via a mouthpiece, thereby effectively eliminating any exposure of the eyes.

Non-clinical studies:

Mixed results were obtained from non-clinical studies. Of the reliable studies, one investigation showed no signs of eye involvement at a concentration of 100 ppm after repeated subacute exposure, while another study reported irritation (as evidenced by

lacrimation) following a single exposure to 40 ppm of H_2S.

Case-control studies:
Human experience following incidents involving the accidental release of H_2S at low concentrations suggests eye irritation may occur, but the reliability of the information is questionable.

F. Respiratory System

The effects of short-term exposure to H_2S on the respiratory system presented as a spectrum of signs and symptoms as well as functional, structural, and biochemical changes that were consistent with the irritant properties of the gas. These changes are highlighted below.

Signs and Symptoms

Reports of respiratory discomfort or distress following short-term exposure to H_2S at concentrations up to 100 ppm were limited.

Clinical studies
Clinical signs of respiratory involvement were absent in most of the controlled clinical investigations.

Bhambhani *et al.* (1991, 1994, 1996a, 1996b, 1997) found no evidence of respiratory comfort or distress among healthy volunteer subjects following exposure to H_2S at concentrations up to 10 ppm for 30 minutes. The lack of signs and symptoms is especially noteworthy since the subjects inhaled the H_2S while performing moderate to vigorous exercise, which would act to augment the dose received.

Jappinen *et al.* (1990) reported that only initial transient dryness of the throat and nasal passages was experienced by asthmatic subjects during exposure to 2 ppm of H_2S for 30 minutes. Although two of the 10 subjects showed functional changes in airway resistance and airway conductance consistent with bronchial obstruction, clinical presentation was normal. Severe asthmatics were excluded from the study (Study ID ◆ 202).

Larsson *et al.* (1994) reported no airway symptoms among volunteer subjects during confinement in a swine containment building containing low levels of H_2S (< 0.05 ppm) for up to five hours (Study ID ● 662).

Mitchell and Yant (1925) observed coughing and respiratory discomfort among male subjects exposed to 100 to 150 ppm of H_2S for as little as 2 to 15 minutes. The work was described as *"preliminary"*,and the study was assigned a "low" confidence index ranking (Study ID ● 444).

Non-clinical studies
Specific references to signs and symptoms showing respiratory involvement were found in only three of the non-clinical studies reviewed. Specifically, Lopez *et al*. (1986) observed moderate respiratory distress among male rats exposed to 40 ppm of H_2S for 6 hours. The symptoms persisted for up to two hours post-exposure. Rats exposed to 300 ppm of H_2S in the same study showed severe signs of distress (Study ID ◆ 466). Kosmider *et al.* (1971) reported *"quicker breathing"* among rabbits of both sexes exposed to 71 ppm of H_2S for one hour per day for 14 days (Study ID ● 595). Finally, Mitchell and Yant (1925) observed signs of laboured breathing among rats and guinea pigs after 8 to 18 hours of continuous exposure to 100 to 140 ppm of H_2S (Study ID ● 444).

Case-control studies
Case-control studies provided little useful information. Kilburn (1997) reported no immediate signs of respiratory discomfort or distress among subjects exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational incidents. However, the same subjects reportedly complained of a number of respiratory symptoms when tested using a self-administered questionnaire two to six years following the events. The symptoms included shortness of breath, dry cough, cough with mucous, cough with blood, dryness of the mouth, and throat irritation. The study was assigned a "low" confidence index ranking owing to serious deficiencies in experimental design, conduct and reporting (Study ID ● 216). Witnesses testifying at the Lodgepole blow-out inquiry a year and a half after the event (1984) recalled experiencing a

number of upper and lower respiratory tract symptoms, including sore throat, nasal irritation, nosebleeds, pain on deep breathing and/or shortness of breath. Asthma and emphysema also were reportedly aggravated. Levels of H_2S measured in the vicinity of the well-site ranged from 0.1 to 15 ppm, with the lower concentrations (*i.e.*, < 1 ppm) predominating. The study also received a "low" confidence index ranking (Study ID ● 327). A retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole blow-out showed a high incidence of "respiratory problems", often accompanied by sore throat and cough. The highest frequency of complaints were registered in areas in which "peak" concentrations of H_2S ranged from 0.5 to 5 ppm, with mean daily average concentrations ranging from 0.02 to 0.4 ppm. The study received a "low-to-moderate" confidence index ranking (Study ID ● 424).

Functional

Clinical studies

Investigation of the effects of short-term exposure to H_2S on pulmonary function was generally confined to the clinical studies involving exposures under controlled conditions. Notable in this regard was the series of investigations completed by Bhambhani *et al*. (1991, 1994, 1996a, 1996b, 1997) involving the assessment of changes in physiological, metabolic and biochemical indices of aerobic and anaerobic metabolism in response to H_2S exposure. The studies typically involved brief exposures of young, healthy subjects to H_2S while exercising. Concentrations ranged from 0.5 to 10 ppm, and exposure times varied from 15 to 30 minutes. In all cases, the H_2S was delivered by breathing through a specially-fitted mouthpiece. Assessment of pulmonary function included measurement of ventilation rate, O_2 uptake, CO_2 output, forced vital capacity (FVC), forced expiratory volume (FEV_{1sec}), forced expiratory flow ($FEF_{25-75\%}$), and/or peak expiratory flow (PEFR), and calculation of respiratory exchange ratio (RER). Exposure-related changes in pulmonary function were generally absent. Although modest shifts in some parameters were reported in some instances, the differences from control exposures (0 ppm) typically were less than 10 percent, and only rarely achieved statistical significance. The most frequent change was a trend toward increased oxygen uptake, especially under high exercise intensity. The changes were not uniformly consistent across subjects, sexes, and studies, signaling that the effects due to exposure, if any, were marginal (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121, Study ID ▲ 122).

Pulmonary function also was assessed by Jappinen *et al*. (1990) among male and female asthmatic subjects exposed to 2 ppm of H_2S for 30 minutes under controlled conditions in a "whole-body" exposure chamber. No changes in FVC, FEV_{1sec}, or $FEF_{25-75\%}$ were recorded, nor were significant effects on airway resistance and airway conductance observed. However, airway resistance was reported to be increased on average by 26%, and airway conductance decreased on average by 8%, with considerable variability between subjects. For two of the 10 subjects, changes in airway resistance and conductance were both greater than 30%, signaling bronchial obstruction at the functional level. None of the subjects complained of any airway symptoms. The findings were not segregated according to the severity of the subjects' asthma, but severe asthmatics were excluded from the study. The authors concluded that *"exposure for a relatively short time to hydrogen sulphide concentrations appreciably higher than those existing in ambient air do not cause noticeable effects on respiratory function"* (Study ID ◆ 202).

Non-clinical studies

Pulmonary function was assessed in one of the non-clinical studies. Hulbert *et al.* (1989) observed changes in pulmonary function parameters among guinea pigs exposed to 100 ppm of H_2S for one hour. Exposure reportedly produced a transient 10-fold decrease in airway resistance and a corresponding increase in dynamic compliance, which, according to the study investigators, signaled *"an acute bronchodilatory effect"*. Modest changes in tidal volume and tidal frequency also were recorded. The nature of the overall response was unusual and unexpected since the more common response

following H_2S exposure is bronchial obstruction. The confidence index ranking assigned to the study was "low" in light of a number of serious weaknesses in experimental design, execution and reporting, including failure to even report the number of animals on test. The clinical relevance of the findings was difficult to assess since the experiment was designed *" to simulate a mouth-breathing condition"*, wherein exposure involved intra-tracheal treatment of the guinea pigs and use of mechanical ventilation to assist breathing (Study ID ● 460).

Case-control studies
Changes in pulmonary function were not specifically assessed as part of the case-control studies that were reviewed.

Structural

Clinical studies
Assessment of changes to the respiratory tract at the structural level did not form part of the clinical studies that were reviewed.

Non-clinical studies
Changes in the morphology and structural integrity of the respiratory tract at either the gross or microscopic level following short-term exposure to H_2S at concentrations in the range of 0 to 100 ppm were noted in several of the non-clinical studies.

At the macroscopic level, changes in the respiratory tract were mixed depending largely on the status of the animal when presented to necropsy. Weedon *et al*. (1940) reported deep red discoloration of the lungs accompanied by massive hemorrhage among mice that died on test following exposure to 63 ppm of H_2S for up to 16 hours. The lungs of a single rat that died on test under the same exposure conditions were less markedly affected, with only some evidence of hemorrhage. The lungs of animals exposed to higher concentrations of H_2S (> 250 ppm) were more severely affected, with massive hemorrhages covering all lobes. Macroscopic findings from the rats and mice that survived exposure (including animals exposed to 16 ppm of H_2S) were unremarkable (Study ID ◆ 316).

Lopez *et al*. (1986) observed no macroscopic lesions among male rats exposed to 40 ppm of H_2S for 6 hours when sacrificed at 0, 18 or 42 hours post-exposure. However, among rats that died following exposure to 300 ppm of H_2S, the lungs were reportedly congested and hemorrhagic, with froth also detected in the upper airways (Study ID ◆ 466). Curtis *et al*. (1975) found no macroscopic evidence of injury to the respiratory tract of pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416).

At the microscopic level, changes to the morphological architecture of the nasal passages and lungs were described by Lopez *et al*. (1986, 1987, 1988a, 1988b) in a series of studies involving "whole body" exposure of male rats to concentrations of H_2S ranging from 10 to 440 ppm for up to six hours. Examination extended to the ultra-structural level in some cases. At concentrations less than 100 ppm, changes detected by light microscopy were mild and generally transient, with involvement of the nasal passages being most pronounced. The changes included: i) the isolated occurrence of only sporadic "background" nasal lesions in rats exposed to 10 ppm of H_2S for four hours (Study ID ◆ 233); ii) mild to severe necrosis of the nasal epithelium, with or without evidence of mild pulmonary oedema among rats exposed to 40 ppm for six hours (Study ID ◆ 466); and, iii) evidence of mild perivascular oedema in rats exposed to 83 ppm for four hours (Study ID ◆ 232). In all cases, the appearance of oedema was transient, with signs clearing within several hours post-exposure. The damage to the nasal epithelium was largely localized to the lateral aspects of the intermediate sections of the passages. Evidence of repair was observed within a few days post-exposure. The changes were more progressive and severe at the higher exposure concentrations (> 200 ppm). At the ultra-structural level, the only change recorded at the lower exposure concentrations was evidence of mild transient distension of the perivascular space among the rats exposed to 83 ppm for four hours (Study ID ◆ 232). As part of a separate investigation, Hulbert *et al*. (1989) found no microscopic changes to the respiratory tract of

guinea pigs following a single exposure to 100 ppm of H_2S for one hour (Study ID ● 460).

Case-control studies
Assessment of changes to the respiratory tract at the structural level did not form part of the case-control studies that were reviewed.

Biochemical

Clinical studies
No cytological, enzymatic or biochemical markers within the respiratory tract were monitored as part of the clinical studies that were reviewed.

Non-clinical studies
A number of non-clinical studies were devoted to the examination of the effects of H_2S exposure on biochemical indices, primarily in an attempt to increase understanding of the mechanism of action. Since the respiratory tract represents the most significant portal of entry for H_2S, changes in these biochemical indices within the respiratory tissues was of interest to several investigators. Emphasis was most often directed at examining the effects of exposure on the activity of the respiratory chain enzymes (*e.g.*, cytochrome oxidase) at different levels within the respiratory tree.

Khan *et al*. (1990) reported that "whole body" exposure of male rats to H_2S at concentrations ranging from 10 to 700 ppm for four hours produced dose-related changes in the activities of several pulmonary mitochondrial enzyme complexes associated with oxidative metabolism and cellular energy production. Changes were confined largely to high exposure concentrations (> 200 ppm), both in terms of the number of enzyme complexes affected and the severity of the effects. No changes were observed at an exposure concentration of 10 ppm. At 50 ppm, effects were limited to a moderate reduction in cytochrome *c* oxidase activity only. At higher concentrations, marked inhibition of cytochrome *c* oxidase and succinate oxidase was reported. At the higher exposure concentrations (> 200 ppm), correlation between the inhibition of cytochrome *c* oxidase within the lung tissue and signs and symptoms of intoxication was evident. However, despite moderate to marked inhibition of enzyme activity at 50 to 200 ppm, no obvious clinical evidence of toxicity was presented (Study ID ◆ 210).

In a more recent investigation "nose-only" exposure of male rats to H_2S at concentrations ranging from 10 to 400 ppm on an "acute" (*i.e.*, 3 hours) or "subacute" (*i.e.*, 3 hours per day for 5 days) basis resulted in biochemical changes in the nasal passages and lungs, as evidenced by shifts in tissue sulphide content, cytochrome oxidase activity and the concentrations of sulphide metabolites (Dorman *et al*., 2002). Changes were reportedly most pronounced in the nasal olfactory epithelium and lung, with lesser effects noted in the nasal respiratory epithelium. In most instances, the nature, time-course and dose-responsiveness of the changes were consistent with the widely-accepted mechanism of action of H_2S, with the effects on cytochrome oxidase activity and tissue sulfide levels within the lung being inversely related. Inhibition of cytochrome oxidase was evident following exposure to H_2S at concentrations as low as 30 ppm. Changes were not evident at 10 ppm. Recovery from the changes was rapid, with certain of the biochemical indices returning to pre-exposure values within one hour after treatment. Regrettably, signs and symptoms were not monitored as part of the study to assist in the interpretation of the clinical relevance of the findings (Study ID ◆ 151).

Lopez *et al*. (1987) examined the early injury and inflammatory response within the respiratory tract of male rats exposed to H_2S through analysis of a series of cellular and enzymatic markers found in nasal and bronchoalveolar lavage fluids. "Whole body" exposures were performed for four hours at concentrations of 10, 200 or 400 ppm. The cellular and biochemical indices monitored included fluid composition, protein content, lactate dehydrogenase activity, alkaline phosphatase activity and gamma-glutamyl transpeptidase activity. Apart from a transient increase in total cell count within the nasal lavage fluid, no changes in the indices were reported at 10 ppm. The change in cell count had resolved within 20 hours post-exposure, demonstrating

"the remarkable reparative capacity of the respiratory epithelium". Notable changes to the biochemical and cytological markers were generally confined to the animals exposed to 400 ppm. The responses were consistent with the known irritant properties of H_2S, and presented as cytotoxic effects on the nasal passages and oedematogenic effects within the lung. No symptomatic correlates of the changes were evident as the animals showed no signs of respiratory discomfort or distress (Study ID ◆ 231).

Case-control studies
No cytological, enzymatic or biochemical markers within the respiratory tract were monitored as part of the case-control studies that were reviewed.

Other

A limited number of non-clinical studies were aimed at understanding the potential effects of H_2S exposure on the anti-bacterial defense system of the lung given reports of secondary pneumonia occurring subsequent to accidental poisoning from the gas (*e.g.*, Kemper, 1966; Donham, 1984).

Rogers and Ferin (1981) followed the inactivation of *Staphylococcus epidermidis* delivered via an aerosol challenge to male rats immediately following "whole body" exposure to 45 ppm of H_2S for up to six hours. Exposure for two hours resulted in no changes in the antibacterial defense system of the exposed rats, with the number of colony forming units (CFUs) not differing from control values. However, after 4 and 6 hours of exposure, the inactivation of *S. epidermidis* was significantly reduced (by up to 50-fold). The exact mechanism by which H_2S exposure effected the change was not determined, but the authors postulated that it could be related to altered macrophage function (Study ID ◆ 269).

In a more recent study, Khan *et al.* (1991) examined the viability of pulmonary alveolar macrophages harvested from male rats immediately following exposure to H_2S at concentrations ranging from 50 to 400 ppm for four hours. No significant changes in the viability of the cells were detected at the lower exposure concentrations (≤ 200 ppm). At 400 ppm, a significant decrease in viability was recorded (Study ID ◆ 211).

Summary:
The effects of short-term exposure to H_2S at concentrations up to 100 ppm on the respiratory system have been well studied. Effects have been examined at the symptomatic, functional, structural and biochemical level.

Clinical studies:
Evidence from clinical studies involving controlled exposures of humans suggests that clinical indications of respiratory discomfort are unlikely and that pulmonary function will remain normal among healthy individuals following brief exposures to concentrations up to 10 ppm. The evidence is particularly compelling since the subjects in these studies inhaled the gas directly through a mouthpiece while performing moderate to strenuous exercise. Asthmatics and individuals with other respiratory disorders may be more responsive, as suggested by the work of Jappinen et al. Specifically, although no signs of respiratory discomfort or distress and no impairment of pulmonary function on average were found among the 10 asthmatic subjects, two of the individuals showed functional changes consistent with bronchial obstruction. The latter finding is significant considering that exposure was relatively brief (i.e., 30 minutes), the concentration was low (i.e., 2 ppm), responses were highly variable between subjects, and severe asthmatics were excluded from the study.

Non-clinical studies:
At the structural level, the work of Lopez et al. was particularly revealing, and suggested that changes to the morphology of the respiratory tract following short-term exposure to low concentrations of H_2S are generally mild and reversible, but progress with increasing concentration. No tissue injury was found following exposure of rats for four hours to 10 ppm of H_2S, whereas damage to the nasal epithelium was evident at concentrations as low as 40 ppm, and mild pulmonary oedema was evident at 80 ppm. Recovery from the damage

was rapid, with evidence of repair observed within several hours post-exposure. Interpretation of the clinical relevance of these findings should respect the fact that the rat is an obligate nasal breather. Accordingly, the observed damage to the lining of the nasal passages may have been exaggerated compared to the expected human condition. It is of interest that the reported structural changes correlated well with the reported changes in biochemical and enzymatic markers. A similar pattern of response was evident, with few, if any, "biochemical" effects found following acute or subacute exposure of rats to 10 ppm of H_2S. Moderate inhibition of cytochrome oxidase activity was reported at concentrations as low as 30 ppm. Additional changes in biochemical and enzymatic indices were found as the concentration increased, possibly signaling early evidence of cytotoxic damage. Again, recovery was rapid, with a return to pre-exposure values within as little as one hour after treatment.

The work of Rogers and Ferin suggested that the pulmonary anti-bacterial defense system could be impaired by a relatively brief exposure to a low concentration of H_2S (i.e., 45 ppm). The clinical relevance of this finding is not fully understood; however, it might explain the frequent occurrence of secondary pneumonia reported to occur after accidental poisoning with H_2S in which pulmonary oedema is manifest.

Case-control studies:
Case-control studies provided little reliable information with respect to symptomatic, functional, structural or biochemical effects of short-term exposure of H_2S at concentrations up to 100 ppm on the respiratory system. Signs and symptoms consistent with respiratory involvement were reported (e.g., cough, sore throat, nasal irritation) but confidence in the study findings was low given the weaknesses in experimental design, conduct and reporting.

G. Skin

Very few studies reported changes to the skin and/or integumentary system as a result of short-term exposure to H_2S at concentrations up to 100 ppm.

Clinical studies
Assessment of changes to the skin did not form part of any of the clinical studies reviewed.

Non-clinical studies
Weedon *et al.* (1940) observed preening of the facial area among rats and mice exposed to H_2S for up to 16 hours under controlled conditions. The preening was suggestive of possible irritation of the skin and mucous membranes. However, the response was only observed at exposure concentrations of 250 ppm and higher. No preening or grooming of the skin was evident among the animals exposed to 16 or 63 ppm of H_2S (Study ID ◆ 316). Similarly, Mitchell and Yant (1925) observed *"washing of the face"* and/or *"rough hair"* among rats and guinea pigs following exposure to 35 to 100 ppm of H_2S, possibly suggestive of skin irritation (Study ID ● 444).

Case-control studies
Kilburn (1997) found that subjects reportedly exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational incidents, complained of skin irritation and fingernail changes when completing a self-administered questionnaire 2 to 6 years after the exposure events. The study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting. The deficiencies included use of a very limited sample size (*i.e.*, only 6 subjects were evaluated), possible subject bias in the reporting of complaints, unreliable exposure estimates, and excessive lag period between the reported exposures and the testing of the subjects (Study ID ● 216).

As part of a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole sour gas well blow-out, certain effects on the skin and integumentary system were identified. The effects were described as "skin allergies", loss of hair and ruptured septum. However, the reliability of the findings is questionable since the study

suffered from several weaknesses in design, conduct and reporting (Study ID ● 424).

Summary:
Clinical studies:
Assessment of changes to the skin did not form part of any of the clinical studies reviewed.

Non-clinical studies:
No reliable evidence was found to suggest that short-term exposure to H_2S at concentrations up to 100 ppm has an effect upon the skin or integumentary system.

Case-control studies:
Complaints of skin effects have been reported among subjects exposed to low levels of H_2S following accidental releases, but the evidence is largely circumstantial and the reliability of the findings is questionable.

H. Olfactory System

Very few reports showing effects of short-term exposure to H_2S on the olfactory system (*i.e.*, sense of smell) surfaced from the literature reviewed. Although it has been widely reported that high concentrations of H_2S (>150 ppm) can produce olfactory paralysis, no convincing evidence was presented that concentrations up to 100 ppm can elicit the same effect. However, evidence did suggest that olfactory fatigue can occur with concentrations up to 100 ppm.

Clinical studies
Jappinen *et al*. (1990) reported that male and female asthmatic subjects complained of an unpleasant odour immediately following "whole body" exposure to H_2S at a concentration of 2 ppm for 30 minutes. All subjects detected the odour, but rapidly became accustomed to it, presumably due to olfactory fatigue. The response was judged to be distinct from olfactory paralysis which has been documented to occur at significantly higher concentrations of H_2S (*i.e.*, >150 ppm). Several of the subjects also complained of headache following the exposure. (Study ID ◆ 202).

Mitchell and Yant (1925) reported that male subjects exposed to 100 to 150 ppm of H_2S complained of a loss of the sense of smell within as little as 2 to 15 minutes of commencement of exposure (Study ID ● 444).

Non-clinical studies
At the structural level, evidence suggests that the nasal olfactory epithelium may be especially responsive to H_2S, albeit effects were only reported following exposure of rats to concentrations greater than 100 ppm. Specifically, Lopez *et al*. (1988) reported necrosis and disruption of the cellular architecture of the olfactory nasal epithelium among rats exposed to 400 ppm of H_2S for four hours. Recovery from the damage was slow, with evidence of cellular degeneration still present at 4 days post-exposure. The effects on the olfactory epithelium were much more pronounced than those recorded for the respiratory nasal epithelium (Study ID ◆ 233).

At the cellular level, Dorman *et al*. (2002) also reported the olfactory epithelium to be highly responsive to H_2S. Significant inhibition of cytochrome oxidase within the olfactory nasal epithelium was found among rats exposed to H_2S at concentrations of 80 ppm and higher on either an acute or subacute basis. The response in the olfactory epithelium was greater than that found in the respiratory nasal epithelium. According to the authors, the finding was not unexpected "*as neurons are known to be exquisitely sensitive to chemical-induced hypoxic damage*" (Study ID ◆ 151).

Case-control studies
Kilburn (1997) reported that subjects exposed for "hours" to H_2S at concentrations between 1 and 50 ppm complained of a decreased sense of smell when completing a self-administered questionnaire 2 to 6 years after the exposure. As already indicated, confidence in the study findings was rated "low" in light of serious deficiencies in experimental design, conduct and reporting (Study ID ● 216).

Loss of the sense of smell was reported very infrequently as part of a retrospective analysis of the nature and frequency of health-related complaints associated with the Lodgepole well

blow-out. Of several hundred complaints registered following the incident, only one related to the loss, or the sense, of smell (Study ID ● 424).

Summary:
Consistent with earlier reports (Leonardos et al. 1969; Ruth et al., 1986; Amoore and Hautala, 1983), the present review revealed that the odour of H_2S can be detected at low concentrations. Assessment of the physiological and/or psychological responses to the odour of H_2S was not performed as part of the present review. Accordingly, it cannot be determined whether responses occurring secondary to the smell of the gas might present at concentrations up to 100 ppm.

Clinical studies:
In the only reliable study in which reference was made to the smell of the gas, it appeared that the response was transient, and the subjects quickly became accustomed to the odour (i.e., Jappinen et al.). However, the concentration tested was low (i.e. 2 ppm) and the exposure was relatively brief. The subjective response to higher concentrations could not be determined.

Non-clinical studies:
Evidence suggests that cytotoxic injury to the olfactory epithelium may occur at concentrations as low as 80 ppm, thereby introducing the possibility of olfactory paralysis at concentrations approaching 100 ppm. Caution should be exercised when interpreting the clinical relevance of the latter finding since the information relies only on testing with rats, which are obligate nasal breathers and possess a unique nasal architecture.

Case-control studies:
Case control studies revealed only very limited evidence of effects of short-term exposure to low levels of H_2S on the olfactory system. Some indication of impaired sense of smell following exposure was presented. However, confidence in the study findings was rated "low" in light of numerous weaknesses in experimental design, conduct and reporting.

I. Cardiovascular System

Given the high energy and oxygen demands of the heart muscle, together with the interference in tissue oxygen utilization and energy production known to be caused by H_2S, several studies were identified in the published literature in which the effects of short-term exposure to H_2S at concentrations between 0 and 100 ppm on cardiac function were examined. All study types were represented (*i.e.*, clinical, non-clinical and case-control). Unfortunately, much of the information was judged to be unreliable and/or of questionable relevance.

Clinical studies
In a series of clinical trials involving exposure of male and female subjects to H_2S at concentrations ranging from 0.5 to 10 ppm for up to 30 minutes while performing moderate to strenuous exercise, Bhambhani *et al*. (1991, 1994, 1997) reported no significant changes in heart rate, systolic blood pressure, diastolic blood pressure, or rate pressure product. The consistent lack of response in each parameter led the authors to conclude that *"men and women with cardiovascular disease who may be exposed to H_2S due to the nature of their occupations or otherwise, may not be at an increased risk in such an environment"*. Despite the conclusion, the evidence for the absence of cardiac effects is weak since heart rate and blood pressure are imprecise indicators of cardiac toxicity (Study ID ▲119, Study ID ▲120, Study ID ▲122).

Non-clinical studies
Reference to changes in cardiac function were found in two non-clinical studies, both of which involved exposure of male and female rabbits to H_2S on either an "acute" or "subacute" basis (Kosmider *et al*. 1967, 1971). In the earlier study, exposure of the rabbits to 71 ppm of H_2S on either a single occasion (*i.e.*, 1.5 hours) or repeatedly (*i.e.*, 30 minutes per day for 5 days) reportedly caused cardiac irregularities, as evidenced by changes in ECG tracings and by inhibition of myocardial enzymes. The changes in ECG readings signaled interference in ventricular repolarization as well as the appearance of arrhythmias in the form of ventricular extrasystoles. Recovery occurred within several

days post-exposure. Heart rate was alternately slowed or increased, with no consistent response between animals (Study ID ● 223). In the later study, rabbits were exposed to 71 ppm of H_2S for one hour per day for 14 days. The only reference to cardiac function was a reported increase in pulse rate (Study ID ● 595). Both studies were assigned a "low" confidence index ranking in light of deficiencies in experimental design, conduct and reporting. The deficiencies included inadequate description of the exposure chamber and gas delivery system, failure to analytically confirm the target exposure concentration, inadequate description of exposure conditions, use of a single exposure concentration only, and general lack of control data, especially in relation to the ECG tracings.

Case-control studies
As part of a case-control study to examine the effects of H_2S exposure on neurobehavioral function, Kilburn (1997) found that subjects exposed to concentrations of H_2S ranging from 1 to 50 ppm for "hours" complained of chest tightness and palpitations when completing a self-administered questionnaire 2 to 6 years following exposure. One of the 6 subjects evaluated also recalled experiencing pain in the chest and back at the time of the exposure. The complaints are suggestive of cardiac involvement. However, the study was assigned a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting (Study ID ● 216).

Several signs and symptoms suggestive of cardiac involvement were recorded as part of a retrospective analysis of the nature and frequency of complaints associated with the Lodgepole sour gas well blow-out. The symptoms included chest pain, suspected heart attack, and high blood pressure. Confidence in the study findings was rated low-to-moderate in light of a number of weaknesses in design, conduct and reporting (Study ID ● 424).

Summary:
Little reliable and/or relevant information exists on the effects of short-term exposure to low concentrations of H_2S on the cardiovascular system.

Clinical studies:
The work of Bhambhani et al. consistently showed no effects following brief exposures to H_2S at concentrations up to 10 ppm on heart rate and blood pressure; however, these measures are relatively imprecise and not overly reliable in assessing cardiac toxicity. Moreover, the subjects who participated in the studies were young, healthy and aerobically fit, and therefore, may not have been representative of the general population. On the other hand, the measurements were performed while the subjects inhaled the H_2S directly through a mouthpiece while exercising at moderate to high intensity, thereby adding considerably to the inhaled dose of the gas compared to that which might be received under normal resting conditions.

Non-clinical studies:
The non-clinical evidence suggested cardiac irregularities in response to short-term exposure to low concentrations of H_2S; however, the studies were weak in terms of experimental design, conduct and reporting, and reliance should not be placed on the findings.

Case-control studies:
Case-control studies revealed evidence suggestive of cardiac involvement following short-term exposure to low levels of H_2S (e.g., chest pain, chest tightness, palpitations); however, the symptoms were non-specific and weaknesses in experimental design, conduct and reporting undermined confidence in the study findings.

J. Liver

Very few studies aimed at examining the potential hepatic effects associated with short-term exposure to H_2S at concentrations up to 100 ppm were discovered in the published literature.

Clinical studies
Assessment of hepatic effects did not form part of any of the clinical studies reviewed.

Non-clinical studies
Kosmider *et al.* (1971) reported that exposure of male and female rabbits to 71 ppm of H_2S for one hour per day for 14 days caused liver injury, as

evidenced by changes in serum glutamic oxaloacetate transaminase activity (SGOT) and serum glutamic pyruvate activity (SGPT). Although the authors claimed that the activity of both enzymes increased in response to exposure (*i.e.*, indicating possible liver damage), careful review of the study findings revealed the SGPT activity actually declined. The lack of consistency in the response of these two well-recognized enzymatic markers of liver integrity hindered interpretation of the toxicological significance of the findings. Histochemical analyses of the liver tissue also revealed changes in the activities of several metallo-enzymes; however, the responses differed among the individual rabbits, and the differences from control values did not achieve statistical significance. The study was assigned a "low" confidence index ranking owing to a number of significant deficiencies in experimental design, conduct and reporting (Study ID ● 595).

Dorman *et al*. (2002) reported changes in cytochrome oxidase activity and sulphide concentrations in liver samples taken from male rats following a single 3-hour exposure to H_2S at concentrations between 10 and 400 ppm. The changes in cytochrome oxidase activity applied to all exposed groups; however, a dose-related pattern was not evident and enzyme activity was *increased* rather than decreased. The increased activity was unexpected, and the authors reported that the biological significance of the change was *"unclear"*. Tissue sulphide concentrations were increased following exposure; however, the changes again showed no obvious dose-related pattern, and differences from control values achieved statistical significance at the higher exposure concentrations only (*i.e.*, $\geq$ 200 ppm) (Study ID ▲151).

Weedon *et al.* (1940) reported discolouration and/or enlargement of the liver as part of the necropsy findings from rats and mice which died on test following a single exposure to 63 ppm of H_2S lasting up to 16 hours. The discolouration was accompanied by congestion in animals found dead after exposure to 250 or 1000 ppm (Study ID ◆ 316).

No reference to hepatic effects was found in the remaining non-clinical studies.

Case-control studies
Assessment of hepatic effects did not form part of any of the case-control studies reviewed.

Summary:
The potential effects of short-term exposure to low concentrations of H_2S on the liver have received little attention.

Clinical studies:
Assessment of hepatic effects did not form part of any of the clinical studies reviewed.

Non-clinical studies:
Possible injury at the cellular level, as evidenced by changes in liver enzyme activities, has been suggested, but the findings are inconclusive. Changes consistent with generalized systemic toxicity have been observed upon gross examination of animals found dead on test following exposure. In both cases, the findings are not especially revealing, nor informative.

Case-control studies:
Assessment of hepatic effects did not form part of any of the case-control studies that were reviewed.

K. Kidney

Even fewer studies aimed at examining the potential renal effects of short-term exposure to H_2S were located in the published literature.

Clinical studies
Assessment of renal effects did not form part of any of the clinical studies reviewed.

Non-clinical studies
Kosmider *et al*. (1971) reported that repeated exposure of rabbits to 71 ppm of H_2S for one hour per day for two weeks caused *"disturbed kidney metabolism"*, as evidenced by changes in the activities of several renal metallo-enzymes determined through histochemical analyses of kidney tissue samples. Interpretation of the biological significance of the observed changes

was hindered by the lack of consistent response between animals and the variable nature of the effects (*i.e.*, some enzymes were inhibited, others were stimulated). Comparison against the control animals was precluded by the lack of reporting of most data (Study ID ● 595).

Weedon *et al.* (1940) reported pale discolouration of the kidneys among rats and mice which died on test following exposure to 63 ppm of H_2S for up to 16 hours. The discolouration was accompanied by congestion among the animals which died after exposure to 250 or 1000 ppm (Study ID ◆ 316).

Case-control studies
Assessment of renal effects did not form part of any of the case-control studies reviewed.

Summary:
Very little information was discovered concerning the potential effects of short-term exposure to low concentrations of H_2S on the kidney.

Clinical studies:
Assessment of renal effects did not form part of any of the clinical studies reviewed.

Non-clinical studies:
Disturbance in kidney "metabolism" as evidenced by changes in the activity of certain renal enzymes was reported, but the data should be considered suspect owing to serious weaknesses in the design, conduct and reporting of the study. The change noted on gross examination of animals which died on test following exposure (i.e., congestion) is non-specific and indicative of generalized systemic toxicity only.

Case-control studies:
Assessment of renal effects did not form part of any of the case-control studies reviewed.

L. Gastrointestinal System

Very few reports of effects of short-term exposure to H_2S at concentrations up to 100 ppm on the gastrointestinal system were found in the literature.

Clinical studies
Gastrointestinal effects were not reported in any of the clinical studies reviewed.

Non-clinical studies
Weedon *et al*. (1940) reported that stomachs from rats and mice that died on test following exposure to 63 ppm of H_2S for up to 16 hours were distended and marked with small hemorrhagic infiltrates when examined at necropsy. The distension was more pronounced among animals that died following exposure to 250 or 1000 ppm (Study ID ◆ 316).

Case-control studies
Complaints of indigestion and swollen stomachs following exposure to H_2S were recorded by individuals as part of a self-reported questionnaire developed by Kilburn (1997). The individuals had reportedly been exposed to 1 to 50 ppm of H_2S for "hours", largely as a result of occupational mishaps, 2 to 6 years earlier (Study ID ● 216).

Witnesses at the Lodgepole blow-out inquiry (1984) testified that they had experienced signs of gastrointestinal upset, such as loss of appetite, nausea and diarrhea at the time of the incident, which they claimed were linked to exposure to H_2S. Concentrations of H_2S measured in the area at the time were routinely less than 1 ppm, but did reach 15 ppm on occasion (Study ID ● 327).

Retrospective analysis of the nature and frequency of health-related complaints registered following the Lodgepole blow-out revealed a number of symptoms consistent with gastrointestinal involvement, including diarrhea, nausea, vomiting, stomach cramps and abdominal pain. However, confidence in the study findings was undermined by several weaknesses in design, conduct and reporting, such as lack of reliable exposure estimates, possible subject bias, and reliance on self-reported symptoms (Study ID ● 424).

Summary:
Little reliable and/or relevant information respecting the effects of short-term exposure to low concentrations of H_2S on the gastrointestinal system was found.

Clinical studies:
No gastrointestinal effects were reported in any of the clinical studies reviewed.

Non-clinical studies:
The findings recorded from the necropsy of animals that died on test following exposure to H_2S are non-specific and cannot be assigned conclusively to treatment.

Case-control studies:
The complaints of gastrointestinal upset and dysfunction reported following accidental releases of H_2S are largely circumstantial.

M. Immunological System

No studies *specific* to the effects of short-term exposure to low concentrations of H_2S on the immune system were found. However, reference to immune function was included in some studies.

Clinical studies
Larsson *et al.* (1994) reported that two of 14 volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for up to five hours tested positive for IgE. However, the positive test results were based on a pre-exposure assessment completed two weeks earlier. No indication of any change in the immunological status of these subjects or the other volunteers due to exposure in the building was found (Study ID ● 662).

Non-clinical studies
Rogers and Ferin (1981) found impairment of the pulmonary anti-bacterial defense system among rats exposed to 45 ppm of H_2S for up to six hours, but the changes could not be specifically or conclusively related to disturbances in cell-mediated or humoral immune function (Study ID ◆ 269).

Although Khan *et al*. (1991) reported a reduction in the viability of pulmonary alveolar macrophages isolated from the lungs of male rats exposed to H_2S for four hours, the change was only expressed at a concentration of 400 ppm. Viability was unaffected by exposure to 50 or 200 ppm of H_2S (Study ID ◆ 211).

Case-control studies
Assessment of immunological effects did not form part of any of the case-control studies reviewed.

Summary:
No reliable and/or relevant information concerning the effects of short-term exposure to H_2S at low concentrations on the immunological system was discovered as part of the current literature review.

Clinical studies:
A single clinical study reported positive IgE test results among selected subjects exposed to very low levels of H_2S for up to five hours in a swine confinement building. However, positive tests were unrelated to H_2S exposure.

Non-clinical studies:
Impairment of the pulmonary anti-bacterial defense system among rats exposed to 45 ppm H_2S was reported; however, the mechanism by which this effect was elicited is unknown.

The reported reduction in the viability of pulmonary alveolar macrophages in response to exposure may be of some significance; however, the concentration at which the effect was observed (i.e., 400 ppm) was outside the range of interest.

Case-control studies:
Assessment of immunological effects did not form part of any of the case-control studies that were reviewed.

N. Hematopoietic System

No studies specific to the effects of short-term exposure to H_2S at concentrations up to 100 ppm on the hematopoietic system (*i.e.*, blood elements, bone marrow, spleen) were located. Some generalized findings are listed below.

Clinical studies
Larsson *et al*. (1994) did report a reduction in leucocyte counts among volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for up to five

hours, possibly related to an intense inflammatory reaction in the airways; however, the authors admitted that the responses were unlikely related to H_2S given the low concentration found. It was concluded that the changes were most likely related to the presence of endotoxins and grain dust (Study ID ● 662).

Non-clinical studies
Studies in which routine necropsy was performed on animals which either died on test or survived exposure to low concentrations of H_2S did not suggest any involvement of the spleen. For example, Curtis *et al.* (1975) found no evidence of gross alterations in the *"visceral organs"* of pigs exposed to H_2S either alone (8.5 ppm) or in combination with ammonia (2 ppm) for up to 19 days (Study ID ● 416). Similarly, Weedon *et al.* (1940) did not report any gross evidence of injury to the spleen among rats and mice that died on test during exposure to 63 ppm of H_2S for up to 16 hours. Even at higher concentrations (*i.e.*, up to 1000 ppm), the spleen was not involved (Study ID ◆ 316).

Case-control studies
Assessment of hematopoietic effects did not form part of any of the case-control studies reviewed.

Summary:
The potential effects of short-term exposure to low concentrations of H_2S on the hematopoietic system have received little attention.

Clinical studies:
A single clinical study reported a reduction in leucocyte counts in subjects exposed to very low levels of H_2S for up to five hours in a swine confinement building. However, the author attributed the response to the presence of contaminants other than H_2S.

Non-clinical studies:
No specific evidence was discovered to suggest that exposure is or is without effect on the blood elements, bone marrow, spleen or other components of the hematopoietic system.

Case-control studies:
Assessment of hematopoietic effects did not form part of any of the case-control studies that were reviewed.

O. Nervous System

The effects of short-term exposure to H_2S on both the developing and mature nervous system have received considerable attention. The effects present as a spectrum of symptomatic, functional, structural, behavioral, and biochemical changes. Changes specific to the mature system are highlighted below. Changes specific to the developing nervous system are described later (see Reproductive System). In many cases, the biological significance and clinical relevance of the effects could not be established since the studies showed weaknesses in experimental design, conduct and/or reporting that effectively precluded meaningful interpretation of the findings.

Signs and Symptoms

Clinical studies
Clinical responses suggestive of nervous system involvement were mixed, and depended on the nature of the study, the exposure concentration(s) used, and the duration of exposure. With respect to the clinical studies that were subjected to review, clinical signs of nervous system involvement were generally absent.

Bhambhani *et al.* (1991, 1994, 1996a, 1996b, 1997) reported no clinical symptoms among subjects exposed to H_2S via a mouthpiece at concentrations up to 10 ppm over a 15 to 30 minute period while exercising at moderate to strenuous intensity. None of the subjects complained of headache, fatigue, dizziness, disorientation or other indicators of nervous system involvement (Study ID ▲ 118, Study ID ▲ 119, Study ID ▲ 120, Study ID ▲ 121. Study ID ▲ 122).

Similarly, no evidence of clinical symptoms consistent with nervous system involvement was reported by Kangas and Savolainen (1987) in a study involving "whole body" exposure of

volunteer subjects to H_2S at concentrations up to 30 ppm for 30 to 45 minutes (Study ID ● 207).

No signs or symptoms signaling nervous system involvement were reported by Mitchell and Yant (1925) following exposure of male subjects to 100 to 150 ppm of H_2S for up to 8 hours (Study ID ● 444).

Jappinen *et al.* (1990) reported that three of ten asthmatic subjects exposed to 2 ppm of H_2S for 30 minutes complained of headache following exposure. The subjects also complained of an unpleasant odour at the time of exposure. (Study ID ◆ 202).

Larsson *et al.* (1994) reported that volunteer subjects placed in a swine confinement building containing very low levels of H_2S (*i.e.*, 0.05 ppm) for 2 to 5 hours complained of malaise and drowsiness, and less frequently of headache and nausea. The symptoms varied by subject. Other chemicals were also measured inside the building including ammonia, carbon dioxide, dust and endotoxins, each of which singly or in combination might have contributed to the symptoms (Study ID ● 662).

Non-clinical studies
With respect to non-clinical studies, short-term exposure to H_2S at concentrations up to 100 ppm range produced mixed responses, ranging from an absence of any signs and symptoms consistent with nervous system involvement (Khan *et al.*, 1990 – Study ID ◆ 210; Lopez *et al.*, 1987 – Study ID ◆ 231; Lopez *et al.*, 1988 – Study ID ◆ 233; O'Donoghue, 1961 – Study ID ● 255), to initial hyperactivity, often followed by lethargy and drowsiness (Haider *et al.*, 1980 – Study ID ● 179; Kosmider *et al*., 1971 – Study ID ● 595; Lopez *et al*., 1986 – Study ID ◆ 466; Mitchell and Yant, 1925 - Study ID ● 444; Savolainen *et al*., 1980 – Study ID ● 280; Weedon *et al.*, 1940 – Study ID ◆ 316), to unconsciousness (Kosmider *et al.*, 1967 – Study ID ● 223).

Case-control studies
In terms of case-control studies, a wide spectrum of signs and symptoms consistent with nervous system involvement have been reported; however, in all cases, the studies were assigned a "low" confidence index ranking owing to serious weaknesses in study design, conduct and reporting that hindered the interpretation of the findings. Kilburn (1997) reported that subjects exposed to 1 to 50 ppm of H_2S for "hours", primarily as a result of occupational incidents, complained of headache, excessive fatigue, syncope, and/or loss of consciousness at the time of the exposure. The same subjects subsequently complained of a variety of symptoms consistent with nervous system involvement (*e.g.*, headache, dizziness, loss of consciousness, extreme fatigue, loss of balance, nausea) as part of a self-administered questionnaire completed 2 to 6 years after the exposure events (Study ID ● 216).

Witnesses testifying at the Lodgepole blow-out inquiry (1984) recalled experiencing headache and nausea at the time of the incident two years earlier. However, in general, few complaints suggestive of nervous system involvement were registered. Levels of H_2S measured in the surrounding area at the time of the blow-out were typically less than 1 ppm (Study ID ● 327).

A retrospective analysis of the nature and frequency of health-related complaints registered following the Lodgepole blow-out did reveal a number of symptoms indicative of nervous system involvement. The symptoms included headache, nausea, loss of appetite, dizziness, insomnia, tiredness, numbness and tingling of the limbs. Exposures occurred over 2 two-week periods, during which time H_2S was released directly into the atmosphere. Concentrations of H_2S measured in areas from which complaints were heard ranged from "peak" values of 0.3 to 5.5 ppm to mean daily average values of 0.01 to 0.4 ppm (Study ID ● 424).

Functional

Clinical studies
Changes in nervous system function were not specifically assessed as part of the clinical studies reviewed.

Non-clinical studies
Only a single study aimed at examining functional changes in the nervous system

following short-term exposure to H_2S at low concentrations was located. Skrajny *et al*. (1996) reported that male rats exposed to H_2S at concentrations ranging from 25 to 100 ppm for three hours per day for 5 days showed changes in EEG activity signaling disturbance in voluntary motor function (*e.g*., walking, running, rearing). Autonomic motor function (*e.g*., shivering) was unaffected. Changes were confined to the hippocampal region of the brain. EEG readings in the neocortex remained normal. The biological significance of the changes was unclear, with the authors admitting that further study was needed to better differentiate the responses and to determine the underlying mechanism (Study ID ◆ 291).

Case-control studies
Changes in nervous system function were not specifically assessed as part of the case-control studies that were reviewed.

Structural

Clinical studies
Effects on the structural integrity of the nervous system were not specifically assessed as part of the clinical studies reviewed.

Non-clinical studies
No studies directed specifically at the examination of changes to the nervous system at the structural level following short-term exposure to H_2S at concentrations up to 100 ppm range were found. However, Weedon *et al.* (1940) noted *"congestion"* of the brains of rats and mice that died on test during exposure to 63 ppm of H_2S for up to 16 hours at the time of necropsy (Study ID ◆ 316). Discussion surrounding the effects of H_2S on nervous system development in neonatal rats can be found in Chapter V-Section P: Reproductive system.

Case-control studies
Changes in nervous system structure were not specifically assessed as part of the case-control studies reviewed.

Biochemical

Clinical studies
No cytological, enzymatic or biochemical markers within the nervous system were monitored as part of the clinical studies that were reviewed.

Non-clinical studies
A sizeable number of studies were identified in which a variety of different biochemical markers within brain tissue were measured in response to short-term exposure to H_2S at concentrations within the range of interest (*i.e*., 0 to 100 ppm). It appeared that in most instances, the studies were aimed primarily at furthering understanding of the mechanism of action of H_2S at the cellular level. The biochemical markers included enzymes, neurotransmitters, RNA, protein, lipids and other cell constituents. It is noteworthy that most of the studies received a "low" confidence index ranking in light of significant deficiencies in experimental design, conduct and/or reporting.

In an early study, Kosmider *et al.* (1967) determined that brain alkaline phosphatase activity was decreased among male and female rabbits following "whole body" exposure to 71 ppm of H_2S for 1.5 hours relative to control values. The finding supported the contention that H_2S has an affinity to bind to metallo-enzymes, disrupting activity. However, interpretation of the biological significance of the change was hindered by the use of only a single exposure concentration (*i.e*., dose-responsiveness could not be determined) and by the lack of any symptomatic correlates signaling nervous system dysfunction (*i.e*., no signs or symptoms were reported). A similar decrease in the activity of serum alkaline phosphatase also was observed (Study ID ● 694).

Elovaara *et al*. (1978) reported that "whole body" exposure of female mice to 100 ppm of H_2S for two hours produced alterations in protein metabolism in the brain, as evidenced by a reduction in the rate of leucine incorporation into cerebral protein and myelin, and inhibition of acid proteinase. However, the changes were not accompanied by any shift in the protein content of the brain or myelin. Although the animals were

reportedly *"agitated"* at the start of exposure, no other signs and symptoms consistent with nervous system involvement were recorded. Interpretation of the biological significance of the changes was hindered by the use of only one exposure concentration, and a lack of uniformity in the time-course of the response following exposure (Study ID ● 155).

Savolainen *et al*. (1980) discovered changes in a number of metabolic and enzymatic parameters in brain tissue obtained from female mice exposed on a 4-day interval to 100 ppm of H_2S for up to 16 days. The changes included progressive inhibition of cytochrome oxidase, reduction in RNA synthesis, increased superoxide dismutase activity, increased glutathione concentration, and increased activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase. Apart from some initial *'excitement"* at the initiation of treatment, the changes were not accompanied by any signs of intoxication. The biological significance of the changes was unclear. The majority of changes were relatively modest, and the time-course of the responses was highly variable. Dose-responsiveness could not be determined owing to the use of a single exposure concentration only (Study ID ● 280).

Haider *et al.* (1980) found that subacute exposure of male guinea pigs to 20 ppm of H_2S for 11 days caused regional-specific reductions in the total lipid and phospholipid content of brain tissue. The changes were apparent in the brain stem and cerebrum, but not in the cerebellum. Cholesterol levels were unaffected in all regions. Signs and symptoms consistent with nervous system involvement also were reported and included fatigue, somnolence and dizziness. The biological significance of the shifts in lipid content was not stated. Interpretation was hindered by the use of a single exposure concentration only, which precluded assessment of dose-responsiveness (Study ID ● 179).

In a more recent study, Nicholson *et al*. (1998) reported that "whole body" exposure of male rats to 100 ppm of H_2S for three hours per day for 5 days produced increased levels of L-glutamate in the hippocampal region of the brain. The authors postulated that, because of its role as a neurotransmitter, the changes in L-glutamate levels might contribute to the neurotoxic effects of H_2S. The findings should be considered preliminary in nature since even the authors offered that *"further study is warranted"*. Interpretation of the biological significance of the finding is difficult since only a single exposure concentration was used, and no symptomatic nor pathological correlates of the change could be identified (Study ID ◆ 398).

In a study aimed primarily at examining the neurobehavioral effects of short-term exposure to H_2S, Struve *et al*. (2001) found no changes in brain catecholamine levels among male rats following exposure to H_2S at concentrations up to 400 ppm for three hours per day for 5 days. The catecholamines measured included dopamine, serotonin, homovanillic acid, 5-hydroxyindole acetic acid, and 3, 4-dihydroxyphenylacetic acid (Study ID ◆ 296).

Case-control studies
No cytological, enzymatic or biochemical markers within the nervous system were monitored as part of the case-control studies that were reviewed.

Behavioral

Three studies were located in which the effect of short-term exposure to low concentrations of H_2S on neurobehavioral function was assessed. Two of the studies were "case-control" in nature and involved the evaluation of neurobehavioral function in subjects reportedly exposed to H_2S for several hours using a battery of neurophysiological and neuropsychological tests. The remaining study was a non-clinical investigation in which rats were subjected to a series of tests to assess learning and memory as well as spontaneous motor activity following "whole body" or "nose only" exposures to H_2S on a subacute basis.

Clinical studies
Assessment of behavioral changes was not specifically conducted as part of the clinical studies reviewed.

Non-clinical studies

Struve *et al*. (2001) reported that neurobehavioral function of male rats exposed to 10, 30, 80, 200 or 400 ppm of H_2S for three hours per day for 5 days was largely unaffected at the lower dose levels (*i.e*., < 200 ppm), as evidenced by normal performance in a water maze and no change in fixed interval operant behavior. The findings suggested that memory and learning were unaffected by treatment. The only change noted at 80 ppm was a reduction in spontaneous motor activity following "nose only" exposure. The change was judged to be of questionable significance since only 2 of 10 rats were affected, and the response was not seen following "whole body" exposure at the same concentration. No changes were observed among the animals exposed to 10 or 30 ppm of H_2S (Study ID ◆ 296).

Case-control studies

Kilburn (1997) reported that subjects exposed to H_2S at concentrations estimated to range between 1 and 50 ppm for "hours" showed deficits in neurobehavioral performance when evaluated 2 to 6 years later. Deficits were reportedly found for balance, choice reaction time, dexterity, coordination, colour discrimination, hearing, and grip strength. Mood also was affected. In addition, subjects complained of memory loss, loss of concentration, irritability, loss of libido and mood swings. The subjects evidently showed no deficits in most measures of cognitive function, including non-verbal non-arithmetical intelligence, integrative capacity, vocabulary and verbal recall. As already indicated, the study was assigned a "low" confidence index ranking in light of serious deficiencies in experimental design, conduct and reporting that essentially precluded meaningful interpretation of the biological significance or clinical relevance of the findings (Study ID ● 216).

In a subsequent study, Kilburn (1999) found that subjects exposed to H_2S at concentrations estimated to range between 1 and 40 ppm for up to several days as a result of emissions from a refinery explosion and fire showed neurobehavioral deficits upon evaluation 3 years later using essentially the same battery of tests. However, the measures affected were not in good agreement with the earlier findings, and inconsistencies in the results were even evident between the two sub-cohorts of subjects included in the study. For instance, unlike the earlier findings, dexterity and coordination appeared to be unaffected, but cognitive function was impaired based on measurement of vocabulary and verbal recall. In addition, conflicting findings were reported for the two exposed sub-cohorts for choice reaction time, balance (eyes closed), verbal recall, long-term memory, dexterity, and coordination. Confidence in the study findings was rated "low" owing to serious weaknesses in design, conduct and reporting. The weaknesses included possible selection bias in the choice of subjects, unreliable exposure estimates, excessive lag period between the time of exposure and subsequent evaluation of subjects, and failure to control for obvious confounding variables (*i.e*., exposure to other chemicals) (Study ID ● 217).

Summary:

A significant amount of information describing the effects of short-term exposure to H_2S at concentrations up to 100 ppm on the nervous system was discovered. Highlights are necessarily limited to the information obtained from studies which achieved a confidence index ranking of "moderate" or "high". No reliable evidence was found to suggest that short-term exposure to low concentrations of H_2S is associated with neurobehavioral impairment.

Clinical studies:

Clinical signs signaling nervous system involvement are unlikely to be seen following exposure to H_2S at concentrations up to 10 ppm. The work of Bhambhani et al. consistently showed a lack of any signs or symptoms among the test subjects following brief exposure to 0.5, 2, 5 or 10 ppm of H_2S, despite inhaling the gas directly through a mouthpiece while exercising at moderate to high intensity. Complaints of headache surfaced during the work of Jappinen et al.; however, only three of the 10 subjects complained.

Non-clinical studies:

At concentrations above 10 ppm, reliance can only be placed on the findings from non-clinical studies, with obvious limitations. Moreover, these

studies showed mixed results. However, the information from those studies judged to be most reliable revealed no signs or symptoms consistent with nervous system involvement among the test animals even at concentrations up to 100 ppm.

Information respecting the effects of short-term exposure to low concentrations of H_2S on the nervous system at the functional, structural and "biochemical" level proved difficult to interpret. Although changes in a variety of metabolic, enzymatic and biochemical indices have been reported following exposures to concentrations up to 100 ppm, the toxicological significance and clinical relevance of the findings remain elusive. Part of the difficulty relates to the fact that much of the information comes from studies with serious weaknesses in experimental design, conduct and reporting. Very little, if any, confidence could be placed in the results and conclusions from these studies. Accordingly, the reported changes in protein metabolism, lipid content, RNA synthesis, and enzyme activities in the nervous tissue cannot be assigned biological significance. In addition, even the findings from the studies which achieved a "moderate" confidence index ranking and suggested possible functional changes in the brain (i.e., altered EEG activity) or disturbances in neural transmission (i.e., shifts in L-glutamate levels) could not be assigned toxicological significance owing to a lack of understanding of the mechanisms involved and/or the clinical implications. Even the authors admitted that the results should be considered preliminary and that further study was warranted

Case-control studies:
No reliable evidence of effects of short-term exposures to low levels of H_2S on the nervous system was found. Any evidence that was discovered was largely circumstantial. In addition, the evidence was suspect owing to numerous weaknesses in the design, conduct and reporting of the studies.

P. Reproductive System

It was reported earlier (Alberta Health, 1988) that the reproductive effects of low concentrations of H_2S had received little attention, and that any effects observed needed *" to be corroborated by further studies"*. Since that time several non-clinical studies aimed at examining the reproductive and developmental toxicity of H_2S have been completed. In many instances, the duration of exposure extended over several weeks, with evaluation of reproductive and developmental parameters made during the pre-mating, mating, gestational and post-natal periods. Although the exposure duration was beyond "subacute", the studies were included in the present review in light of the importance of identifying and understanding any potential impacts of H_2S on pregnancy, reproductive outcome, and early development. The inclusion of the studies also respected the potential increased sensitivity of the developing fetus to the effects of H_2S. Various parameters were measured during the course of the studies, and were broadly classified as signs and symptoms, reproductive, structural, developmental and biochemical for review purposes. Confidence index rankings assigned to the studies varied from "high" to "low".

Signs and Symptoms

Clinical studies
No clinical reproductive studies were identified for review.

Non-clinical studies
Despite the widely accepted recommendation that signs and symptoms be routinely monitored and recorded during the course of reproductive and developmental toxicity studies, the results of routine observation of the test animals were rarely reported as part of the non-clinical studies. The exception was a well-designed study by Dorman *et al*. (2000) that followed a conventional study protocol and was performed in compliance with USEPA Good Laboratory Practice (GLP) regulations. No signs and symptoms of toxicity were observed among the parental animals following "whole body" exposure to H_2S at concentrations ranging from 10 to 80 ppm for six hours per day for at least 70 days (*i.e.*, throughout a two-week pre-mating period, a two-week mating period, a three-week gestation period, and a three-week post-natal period). However, both

the male and female parents showed reduced feed intake, accompanied by a slowing of body weight gain following initial exposure to 80 ppm of H_2S, possibly indicative of generalized and/or non-specific toxicity (Study ID ▲ 149).

In a series of studies completed by Roth and co-workers (Roth and Hannah, 1989; Hannah *et al*., 1990; 1989; Hayden *et al*., 1990a, 1990b; Hannah and Roth, 1991; Skrajny *et al*., 1992) exposures evidently were performed in a specially-constructed chamber that allowed for clear observation of the test animals. However, no signs and symptoms were reported. Information respecting the feed intake of the dams was provided in one of the studies of the series and showed reduction in consumption during the initial stage of gestation following exposure to 50 or 75 ppm of H_2S, again suggestive of generalized stress and/or non-specific toxicity (Study ID ● 630, Study ID ●180, Study ID ● 402, Study ID ◆ 564, Study ID ◆ 12, Study ID ● 11, Study ID ◆ 290).

Similarly, Saillenfait *et al*. (1989) failed to report signs and symptoms as part of a study to examine developmental defects following exposure of pregnant rats to H_2S at concentrations up to 150 ppm from Day 6 to Day 20 of gestation, either alone or in combination with carbon disulphide (Study ID ◆ 278).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters did not form part of the case-control studies reviewed.

Reproductive

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Dorman *et al*. (2000) reported no changes in reproductive parameters following exposure of male and female rats to 10, 30 or 80 ppm of H_2S for six hours per day throughout a two-week pre-mating, two-week mating, and three-week gestation period. Mating index, fertility index, post-implantation losses, number of late resorptions, length of gestation, delivery time, number of stillbirths, number of females with live pups, and number of implants per pregnant female were unaffected by exposure (Study ID ▲ 149).

Hayden *et al.* (1990b) found no effects on gestation length, litter size, pup viability and pup sex ratio following exposure of pregnant rats to H_2S at concentrations ranging from 20 to 75 ppm for seven hours per day from Day 6 to Day 21 of gestation. However, the exposed dams reportedly experienced dystocia (*i.e.*, prolonged and difficult delivery), in an apparent dose-related fashion. Careful examination of the data revealed that the dystocia was very much animal-dependent, with many of the dams having a normal length of delivery. The incidence of dystocia among the exposed dams also was within the range reported for the various sets of control dams used in the study. No evidence was provided that the dystocia adversely affected the health of the dams, and apart from a *"few"* fetuses that reportedly may have died from asphyxiation due to the prolonged delivery, the fetuses also were unaffected. The effect was judged to be of questionable significance (Study ID ◆12). No evidence of dystocia was reported by Dorman *et al*. (2000)

Saillenfait *et al.* (1989) exposed pregnant rats to 50, 100 or 150 ppm of H_2S for six hours per day from Day 6 to Day 20 of gestation, either alone or in combination with carbon disulphide. The authors reported that exposure to 50 ppm or 100 ppm of H_2S was without effect on reproductive parameters, including fertility index, number of implantation sites, number of resorptions, and number of live and dead fetuses. No clear adverse effects were even evident at 150 ppm. Some evidence of maternal toxicity at the highest exposure concentration was provided by the observation of reduced maternal body weight gain (Study ID ◆ 278).

Reproductive parameters were not reported in any of the remaining non-clinical studies.

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters was not specifically conducted as part of the case-control studies that were reviewed.

Structural

Regrettably, very few of the studies included reference to the health and appearance of the pups at the time of delivery. Again, this serves to emphasize the fact that many of the studies included in the present review failed to follow conventional testing protocols. As a result, much useful information was "lost", and, in this case, assessment of the potential teratogenic effects of H_2S was hindered.

Clinical studies
No clinical reproductive/developmental toxicity studies were identified for review.

Non-clinical studies
Dorman *et al.* (2000) reported isolated instances of external malformations among pups borne by dams exposed to 10, 30 or 80 ppm of H_2S throughout gestation, but the findings were assigned little significance since the differences from control values were not statistically significant and dose-responsiveness was not evident for any of the structural anomalies (Study ID ▲149).

Saillenfait *et al*. (1989) found no evidence of external, skeletal or soft tissue abnormalities related to treatment among pups borne by dams exposed to 50, 100 or 150 ppm of H_2S from Day 6 to Day 20 of gestation, even in the presence of maternal toxicity at the higher exposure concentrations (Study ID ◆ 278).

The findings suggest that H_2S is not teratogenic.

Case-control studies
Assessment of the effect of H_2S exposure on reproductive parameters was not specifically conducted as part of the case-control studies reviewed.

Developmental

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Developmental landmarks were monitored in two of the reproductive toxicity studies as indicators of the potential effects of H_2S exposure on the growth and development of the offspring. Hayden *et al*. (1990b) found no change in the elapsed time required for incisor eruption, eyelid opening and development of surface righting reflex among pups exposed *in utero* and postnatally to H_2S at concentrations ranging from 25 to 75 ppm compared to corresponding controls. However, the time required for pinnae detachment and hair development was reportedly altered by H_2S exposure. The biological significance of the latter findings was judged to be questionable since the changes were not uniform, not dose-responsive, and/or not statistically significant. No changes in pup body weights as a result of exposure were reported (Study ID ◆ 12).

Dorman *et al*. (2000) noted that developmental landmarks were unaffected by *in utero* and postnatal exposure of pups to H_2S at concentrations ranging from 10 to 80 ppm, as evidenced by no change in the elapsed time required for pinnae detachment, incisor eruption, negative geotaxis, eyelid opening and vaginal patency or preputial separation compared to controls. In addition, pup body weights were unaffected by exposure (Study ID ▲149).

Case-control studies
Assessment of the effect of H_2S exposure on developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Behavioral

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with

short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
The effects of H_2S exposure on neurobehavioral development were followed in a single study only. Dorman *et al*. (2000) reported that pups exposed *in utero* and postnatally to H_2S at concentrations ranging from 10 to 80 ppm showed no neurobehavioral deficits, as evidenced by normal scores when tested for spontaneous motor activity, acoustic startle response, and passive avoidance. Assessment through a functional observation battery (FOB) also revealed no behavioral effects due to exposure. The assessment included examination for signs of ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration, or abnormal pupil reflex, as well as evaluation of tail pinch response, approach response, visual placing response, hind and forelimb grip strength, hind-limb splay, urine and feces production, rearing behavior, neuromuscular dysfunction, and appearance. All parameters were reported to be unaffected by treatment (Study ID ▲149).

Case-control studies
Assessment of the effect of H_2S exposure on neonatal behavior and development was not specifically conducted as part of the case-control studies that were reviewed.

Pathology

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Gross and microscopic examination of the organs and tissues from parental animals and/or offspring formed part of the design of a number of the reproductive toxicity studies, with an emphasis on neuropathology.

Using a conventional study design, Dorman *et al*. (2000) found very few changes in the tissues of parental animals following "whole body" exposure to H_2S at concentrations ranging from 10 to 80 ppm for six hours per day for up to 70 days when assessed grossly and microscopically. Adrenal weights of selected parental males were decreased relative to controls, but the response was not dose-related and was considered unrelated to treatment. Selected dams showed reduced ovarian weights, but again, the response was not dose-related. All remaining organ weights of the parental animals were normal (*i.e*., liver, kidney, heart, lungs, spleen, brain, testes). Evidence of mild to marked changes in the olfactory mucosa was found upon microscopic examination, but details concerning dose-responsiveness were not available. The finding was not unexpected given the irritant properties of H_2S and the fact that the rat is an obligate nose breather. In addition, some evidence of seminiferous tubular generation was reported among the male rats exposed to 80 ppm of H_2S, but the biological significance of the finding was questionable since the cases were isolated and sperm production was normal. Pathological findings among the offspring were reported to be unremarkable, with no changes in the brain, spinal cord or peripheral nerves noted on microscopic examination. No changes in pup organ weights (*i.e*., adrenal glands, brain, heart, kidneys, liver, lungs, spleen, testes) due to exposure were found (Study ID ▲149).

As part of a series of studies, Roth and co-workers examined the morphological effects of exposure to low concentrations of H_2S on the developing nervous system of the rat, with particular emphasis on changes to the density and architecture of cerebellar Purkinje cells (Hannah and Roth, 1991; Roth and Hannah, 1989). Pups were exposed *in utero* for seven hours per day from Day 5 to Day 21 of gestation to H_2S at concentrations up to 75 ppm. Increased numbers of Purkinje cells in the cerebellum were discovered following exposure. The architecture of the cells also was reportedly altered, with structural changes evident in the "dendritic tree" (*e.g*., altered dendritic segment length, terminal path length, segment frequency). The authors offered that the changes were indicative of

abnormal development at the "growth front" of the cells, which could pre-dispose the animals to abnormal neurological function. However, it was admitted that interpretation of the findings was *"very difficult"* owing to the complexity of the developing nervous system. The biological significance of the findings was judged to be questionable in light of a lack of dose-responsiveness. Interpretation of the clinical relevance of the findings was hindered by the failure to record any signs and symptoms of toxicity which might have revealed symptomatic correlates of the changes in cellular density and architecture. The studies were assigned a "low" or "low to moderate" confidence index ranking owing to weaknesses in experimental design, conduct and reporting (Study ID ● 11 and Study ID ● 630).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Biochemical

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
Roth and co-workers examined a number of biochemical indices as part of a series of investigations aimed at determining the potential developmental effects of H_2S. The indices were assessed in dams and/or offspring following "whole body" exposure to H_2S at concentrations ranging between 20 and 75 ppm, typically for seven hours per day from Day 5 to Day 21 of gestation. The biochemical indices chosen for study allowed for assessment of the potential effects of H_2S on carbohydrate metabolism (*i.e.*, serum glucose), lipid metabolism (*i.e.*, serum and/or tissue triglycerides and cholesterol), protein metabolism (*i.e.*, tissue protein and RNA content), and neural transmission (*i.e.*, serum and/or tissue taurine, glycine, glutamate, aspartate, gamma-aminobutyric acid, serotonin, norepinephrine). Confidence index rankings for the studies were generally marginal (*i.e.*, "low" or "low-to-moderate") in light of weaknesses in experimental design, conduct and/or reporting. The studies did not follow a conventional design, and deviated significantly from the recommendations contained in testing guidelines for developmental toxicity studies.

In an early study in the series, Hannah *et al.* (1989) exposed pregnant rats to 75 ppm of H_2S during much of the gestational period, and reportedly found changes in the levels of selected amino acid neurotransmitters in the brains of the offspring. The authors postulated that the changes might result in long-term behavioral problems; however, it was offered that the consequences of the changes were *"as yet unknown"*, and that the interpretation of the biological significance of the findings *"was very difficult"*. Interpretation was hindered especially by a lack of uniformity in the time-course of the changes and the use of a single exposure concentration only, which precluded any assessment of dose-responsiveness. In addition, the exact role of the amino acids in neural transmission was stated to be unknown. No symptomatic correlates of the changes could be determined owing to a lack of reporting of any signs or symptoms (Study ID ● 180). The findings departed somewhat from those reported by Struve *et al.* (2001) in which no changes in brain catecholamine levels were found among male rats repeatedly exposed to H_2S at concentrations up to 400 ppm. The difference in findings may be age-related since Struve *et al.* used adult animals. In addition, each set of investigators monitored a different battery of amine neurotransmitters.

In a subsequent study, Hannah *et al*. (1990) reported that exposure to 50 ppm of H_2S during Day 6 to Day 21 of gestation resulted in elevated serum taurine levels among the dams both at the time of delivery and weaning of the pups. Although the authors claimed that the elevated maternal taurine levels could have the potential to cause neurological abnormalities in the offspring, the toxicological significance of the finding was unclear. The corresponding levels of taurine in the pups were not monitored, and no signs or

symptoms of toxicity were recorded. Dose-responsiveness could not be assessed owing to the use of a single exposure concentration only. The study was assigned a "low-to-moderate" confidence index ranking, signaling weaknesses in experimental design, conduct and reporting (Study ID ● 402).

As part of the same series of studies, Hayden *et al*. (1990a) monitored a number of enzymatic and metabolic indices among the dams and offspring following exposure to H_2S at concentrations ranging from 20 to 75 ppm for seven hours a day throughout gestation. Changes were limited to a dose-related increase in circulating glucose levels and a corresponding decrease in serum triglyceride levels in the dams. The same response was not seen in the pups, albeit serum triglyceride levels were modestly depressed and cholesterol levels were marginally increased. All other parameters were unaffected by exposure (*i.e*., serum protein level, serum alkaline phosphatase activity, SGOT activity, and SGPT activity). The biological significance of the changes in maternal serum glucose and trigyceride levels is unclear, with even the authors offering that *"further studies will be required"* to understand the basis of the responses (Study ID ◆564).

Hayden *et al*. (1990b) subsequently exposed pregnant rats to 20, 50 or 75 ppm of H_2S during much of gestation and found no changes in a number of biochemical indices among both the dams and offspring. Liver protein levels, liver DNA levels, and liver cholesterol levels were unaffected by exposure. Similarly, brain protein levels, brain DNA levels, and brain cholesterol levels remained unchanged following treatment (Study ID ◆ 12).

In the most recent study of the series, Skrajny *et al*. (1992) monitored monoamine neurotransmitter levels in the brains of pups borne by dams exposed to 20 or 75 ppm of H_2S for seven hours per day from Day 5 to Day 21 of gestation. Treatment reportedly caused shifts in the levels of serotonin and norepinephrine in the developing cerebellum and/or frontal cortex, which the authors postulated could result in permanent structural and neurochemical alterations in the central nervous system. However, interpretation of the findings was confounded by a lack of clear dose-responsiveness in the changes, as well as a highly variable time-course of response for each parameter. No symptomatic correlates of the changes could be determined since no signs or symptoms were reported.(Study ID ◆ 290). The findings contradict those reported by Struve *et al*. (2001).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Other

Clinical studies
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were found in the published literature.

Non-clinical studies
The potential mitogenic effects of H_2S in embryonic tissues were examined by Barilyak and Vasil'eva (1974). Samples of liver and kidney were harvested from pups borne by dams exposed to 7 ppm of H_2S in combination with carbon bisulphide throughout gestation. No significant changes in the mitotic index of the hepatic or renal embryonic tissues were found. The study was assigned a "low" confidence index ranking in light of serious weaknesses in experimental design, conduct and reporting (Study ID ● 497).

Case-control studies
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Summary:
Clinical studies:
No clinical studies of the potential reproductive and/or developmental effects associated with short-term exposures to low levels of H_2S were

found in the published literature.

Non-clinical studies:
It is regrettable that much of the work did not follow conventional testing protocols, and as a result, much useful information has been "lost" and interpretation of the biological significance of certain of the findings has been hindered. The work of Dorman et al. features prominently since it was performed in accordance with recognized testing guidelines and in compliance with the USEPA Good Laboratory Practice (GLP) regulations. The findings from this work suggest that H_2S is neither a reproductive toxicant, nor developmental toxicant. Routine measures of reproductive performance were unchanged among the parental animals following repeated daily exposure to H_2S at concentrations up to 80 ppm throughout pre-mating, mating and gestational periods. No structural malformations related to treatment were found among the fetuses borne by the exposed dams, suggesting that H_2S is non-teratogenic. The results in this respect were supported by the previous work of Saillenfait et al., albeit only a "low" level of confidence could be assigned to the earlier findings. Growth and development of the offspring appeared to be unaffected by treatment, with no changes in survival, body weights, and developmental landmarks resulting from in utero and postnatal exposure. No neurobehavioral deficits were noted among the offspring when evaluated through a battery of tests. Pathology of the parental animals and offspring was generally unremarkable.

The work of Roth et al. also features prominently; however, interpretation of the biological significance of many of the findings was hindered by the failure to follow conventional testing protocols as well as by weaknesses in study design, conduct and reporting. Apart from these practical difficulties in interpretation, the assessment of the significance of the findings also was hindered by the sheer complexity of the developing nervous system which served as the focus of much of the work. Even the authors admitted that interpretation of much of the work was "very difficult" and that the consequences of certain of the changes were "as yet unknown" and require "further study". Accordingly, although the reported changes in the density and architecture of Purkinje cells, the shifts in amino acid neurotransmitter levels, and the indications of possible disturbances in carbohydrate and lipid metabolism during development may be of some interest, the biological significance and clinical relevance of the findings are unknown.

Case-control studies:
Assessment of the effect of H_2S exposure on reproductive or developmental parameters was not specifically conducted as part of the case-control studies reviewed.

Q. Metabolic Systems

Study of the potential metabolic effects of short-term exposure to H_2S at concentrations up to 100 ppm has been aimed primarily at identifying and understanding the metabolic and physiologic implications surrounding the inhibition of cytochrome *c* oxidase and the subsequent impairment of oxygen utilization and disruption of energy production at the cellular level. Study has also been directed at examining the metabolic outcomes surrounding the inactivation of metallo-enzymes other than cytochrome oxidase following H_2S exposure.

Clinical studies
Reliance can be placed on a series of studies performed by Bhambhani and co-workers in which a number of physiological, hematological, metabolic and biochemical indices were monitored among male and/or female volunteer subjects during and following exposure to H_2S under strictly controlled conditions (Bhambhani and Singh, 1991; Bhambhani *et al*., 1994; Bhambhani *et al*., 1996a; Bhambhani *et al*., 1996b; Bhambhani *et al*., 1997). The intent was to explore the presumed shift that would occur from aerobic to anaerobic metabolism following exposure in view of the expected inhibition of cytochrome oxidase and the subsequent disruption of mitochondrial oxidative phosphorylation. Subjects were exposed to H_2S at concentrations ranging from 0.5 to 10 ppm under conditions of moderate to strenuous exercise for 15 to 30 minutes. In all cases, exposure to the gas was via a specially-fitted mouthpiece, thereby precluding any confounding of findings as a

result of possible physiological and/or psychological responses to the odour of H_2S. In most instances, exposures were performed in replicate, adding to the reproducibility of the findings. Subjects were young and generally fit. Confidence index ranking for the entire series of studies was "high" as the investigations were well-designed and well-executed (Study ID ▲118, Study ID ▲119, Study ID ▲ 120, Study ID ▲122).

In the initial study of the series (Bhambhani and Singh, 1991) male subjects were exposed to 0.5, 2 or 5 ppm of H_2S for approximately 15 minutes while performing exercise at three levels of intensity. Pulmonary and cardiovascular indices remained largely unchanged by exposure (*i.e.*, heart rate, oxygen uptake, CO_2 output, ventilation rate), except for a modest increase in oxygen uptake at the highest concentration while exercising at maximum intensity. In terms of metabolic parameters, blood lactate concentrations showed a tendency to increase during exposure, with the change reported to be significant at 5 ppm. The change was consistent with the response that might be expected; however, it was assigned little significance since, even at 5 ppm, the increase in lactate concentration (up to 65%) was well below that resulting from changes in exercise intensity alone (450%). The subjects experienced no discomfort (Study ID ▲ 119).

In a subsequent study aimed at investigating possible differences in physiologic and metabolic responses to H_2S exposure between males and females, subjects were exposed to 5 ppm of H_2S for 30 minutes while exercising at moderate intensity. Exposure was found to be without effect on metabolic, pulmonary, cardiovascular, and arterial blood parameters, irrespective of sex. A numerical, but not statistically significant, increase in blood lactate concentration (~10%) was observed in both sexes. None of the subjects complained of any clinical symptoms. The lack of response among the female subjects was unexpected given the lower blood volumes and hemoglobin content typically reported for the female sex (Study ID ▲ 120).

In a study aimed at investigating the effects of H_2S exposure on the metabolic profile of skeletal muscle (Bhambhani *et al*. 1996a), a series of enzymatic markers of aerobic and anaerobic metabolism was monitored in male and female subjects following exposure to 5 ppm of the gas for 30 minutes while performing moderate exercise. Other than a modest to moderate decrease in the activity of citrate synthase (~20%) among the exposed male subjects, pulmonary, metabolic and enzymatic parameters were reportedly unchanged by treatment. However, careful review of the data also revealed modest shifts in muscle lactate concentration, lactate dehydrogenase activity and cytochrome oxidase activity among the male subjects that were possibly consistent with a shift from aerobic to anaerobic metabolism. No such changes were evident for the female subjects. The clinical presentation of all subjects was unaffected by the exposure (Study ID ▲118).

In the most recent study of the series, the effects of exposure to 10 ppm of H_2S on pulmonary, cardiovascular, metabolic and biochemical parameters were assessed in male and female subjects exposed for 30 minutes while performing moderate exercise (Bhambhani *et al*., 1997). Results were mixed, with a modest (~ 10%), but unexpected decline in oxygen uptake observed for both sexes, and a modest (~ 11%), but unexpected *increase* in cytochrome oxidase activity noted for the female subjects. In addition, both sexes showed a modest (~ 7%), but unexpected reduction in muscle lactate dehydrogenase activity. These findings argued against any shift from aerobic to anaerobic metabolism as a result of exposure; however, other changes were consistent with such a shift (*i.e.*, increased muscle lactate concentration, reduced citrate synthase activity in both genders). Clinical signs were absent among all subjects. Although the authors concluded that the findings provided cause to question the validity of the current occupational exposure limit (OEL) for H_2S (*i.e.*, 10 ppm), the conclusion was judged to be tenuous given the lack of consistency or responses(Study ID ▲ 122).

Non-clinical studies

Earlier metabolic work performed by Kosmider *et al.* (1971) using rabbits exposed to 71 ppm of H_2S for one hour per day for 14 days showed disturbances in the acid/base balance and mineral

status of the blood as well as shifts in the activities of various serum and tissue enzymes that reportedly indicated damage to the liver and impaired kidney function. Interpretation of the significance of the findings was hindered by serious weaknesses in experimental design, conduct and reporting, including inadequate description of the exposure chamber and gas delivery system, use of a single exposure concentration only, failure to include conventional measures of toxicity as part of the overall study design, and failure to properly confirm the target exposure concentration. The study was assigned a "low" confidence index ranking (Study ID ● 595).

Case-control studies

The effects of H_2S exposure on metabolic parameters were not specifically examined as part of the case-control studies reviewed.

Summary:

The results suggest that brief exposures to H_2S at concentrations up to 10 ppm are well tolerated at the "metabolic" level, with some evidence of a possible shift from aerobic metabolism to anaerobic metabolism owing to inhibition of cytochrome oxidase.

Clinical studies:

The "metabolic" work performed by Bhambhani et al. has contributed to a better understanding of the clinical, physiological, and cellular responses to short-term exposures to low concentrations of H_2S. The work was well-designed and well-executed, thereby adding to the significance of the findings. However, at the exposure concentrations tested, the metabolic shift was faint, and the response varied by individual and by sex. Any changes at the metabolic level were not accompanied by clinical expressions of toxicity, as none of the subjects complained of any symptoms. The findings are especially revealing since the subjects inhaled the H_2S directly through a mouthpiece while performing moderate to strenuous exercise, thereby adding considerably to the inhaled dose of the gas compared to that which might be received under normal resting conditions. Interpretation of the clinical relevance of the findings should respect the fact that the apparent shift from aerobic metabolism to anaerobic metabolism is a routine and integral part of the body's response to increased oxygen and energy demands (i.e., physical exercise) as well as to dwindling carbohydrate reserves (i.e., fasting). It does not represent a unique response to H_2S exposure. Interpretation should also consider the fact that the subjects used in the studies were young, healthy, and generally "fit", and therefore, may not represent the general population.

Non-clinical studies:

Disturbances in acid/base balance and the mineral status of the blood, as well as shifts in the activities of various serum and tissue enzymes were reported in rabbits repeatedly exposed to 71ppm H_2S. However, confidence in the study findings and conclusions was judged to be low.

Case-control studies:

The effects of H_2S exposure on metabolic parameters were not specifically examined as part of the case-control studies reviewed

VI. OTHER CONSIDERATIONS

Several items of interest relating to the health effects associated with short-term exposures to H_2S surfaced during the course of the present review as well as during the meetings of the Expert Panel, and these items require extra consideration. The items are of scientific interest, and present rather unique challenges in terms of interpretation. They are perhaps best expressed in the form of questions. Specifically:

- What level of significance should be assigned to the deaths recorded by Weedon *et al*. (1940) and Mitchell and Yant (1925) among laboratory animals exposed to H_2S at relatively low concentrations for up to several hours?
- Why does the current review differ from the earlier review (Alberta Health, 1988) concerning the effects of short-term exposure to H_2S on the eye?
- What information can be gathered from the present review either to support or refute the increased sensitivity of certain individuals to H_2S?

Discussion around each of these items follows.

A. Mortality

Weedon *et al*. (1940) reported that 4 of 4 mice and 1 of 8 rats died following exposure to 63 ppm of H_2S for up to 16 hours. Deaths occurred within as little as 60 minutes among the exposed mice, and all mice were dead within 48 hours from start of exposure. The single rat reportedly died on test during the 16-hour exposure period. Signs and symptoms were generally mild, but consistent with discomfort and respiratory distress. Necropsy of the animals revealed signs of generalized systemic toxicity consistent with poisoning, with the lungs appearing to be most severely affected. Dose-response was evident among both species, with no deaths reported following exposure to 16 ppm of H_2S, and higher frequencies and generally shorter times to death at concentrations of 250 ppm and greater. The study received a "moderate" confidence index ranking (Study ID ◆ 316).

Similarly in a second older study, Mitchell and Yant (1925) reported deaths among rats exposed *"continuously"* to 100 to 140 ppm of H_2S. The deaths occurred within 18 to 48 hours following the onset of exposure. Deaths were also recorded for guinea pigs and dogs exposed to 103 ppm of H_2S, with the animals succumbing within 8 to 48 hours after the start of exposure. Canary birds were found to be especially sensitive to H_2S, with deaths recorded following exposure to 35 to 100 ppm within 4 to 18 hours after the onset of exposure. Higher concentrations resulted in a higher incidence of mortality and shorter times to death in all species tested. Signs and symptoms were consistent with irritation of the eyes, skin and/or mucous membranes, progressing to evidence of systemic toxicity in a dose-related manner. Details respecting pathology were limited. The study received a "low" confidence index ranking owing to serious weaknesses in experimental design, conduct and reporting (Study ID ● 444).

The issue surrounding the recorded deaths concerns the considerable discrepancy between the above findings and those of the remaining studies. The mortality findings from the various studies are summarized in Appendix 7. Highlights are listed below.

- For the majority of studies, information respecting whether or not deaths occurred was missing. Although it might be reasonable to presume that had exposures caused deaths, it would have been reported, the actual results remain unknown.

- Although the focus of the present review was on exposure concentrations in the 0 to 100 ppm range, mortality data for concentrations beyond this range were included for completeness and to provide for more meaningful interpretation of the weight-of-evidence. Similarly, additional studies were included that did not satisfy the eligibility criteria *per se*, but did report mortality

findings that should bear on the interpretation of the overall data set. Both positive and negative findings emerged from this additional set of studies. The studies were excluded from detailed review since they were not peer-reviewed, did not appear as full-length publications or involved exposures that extended beyond acute or sub-acute duration. The additional set of studies should not be considered all-inclusive, but rather the studies were chosen since they used exposure concentrations within the range of interest, they were readily available, and they are often cited in the published literature.

- With only one exception, the studies received a "low" or "moderate" confidence index ranking, signaling weaknesses in experimental design, conduct or reporting. Thus, caution must be exercised in the interpretation of the findings.

- Experimental designs varied considerably. Some studies involved single exposures only, while others used repeated exposures, either intermittent or daily, over several days or weeks. Since exposure duration will directly affect the "toxic load" of H_2S received, the interpretation of the mortality data must necessarily extend beyond simple comparison of the findings across studies. No attempt was made in the present review to reach this level of interpretation.

- Apart from the findings of Hays (1972), Mitchell and Yant (1925) and Weedon *et al*. (1940), which showed deaths among various laboratory animal species following exposures to H_2S within the concentration range of interest (*i.e*., 0 to 100 ppm), no evidence of mortality was discovered among the remaining 32 studies for the same concentration range. Confidence in the findings from the former three studies is weakened by the fact that the studies not only are "dated", but also showed weaknesses in experimental design, conduct and reporting.

- Although some exceptions occurred, the deaths observed among test animals exposed to H_2S at concentrations up to 100 ppm, typically followed continuous exposure for periods of 8 to 16 hours and beyond. In only one instance was a death reported to occur within one hour of exposure to concentrations less than 100 ppm (*i.e*., Weedon *et al.*, 1940).

Based on the mortality data contained in Appendix 7, it would appear that the likelihood of death occurring as a result of short-term exposure to H_2S at concentrations up to 100 ppm is very remote.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

B. Eye Effects

The present review revealed very little evidence of eye irritation following short-term exposures to H_2S at concentrations up to 100 ppm. This is in sharp contrast to the earlier report (Alberta Health, 1988), wherein it was concluded that the eye *"is very susceptible to the irritant action of H_2S"*, and *"irreversible eye tissue damage can occur at 20 ppm H_2S …"*. The discrepancy may be explained, in part, by the reliance placed on review articles in the earlier assessment. It would appear that the earlier conclusions relied heavily on statements made by Milby (1962) and Gosselin *et al*. (1976) attesting to *"irreversible eye tissue damage at concentrations $\geq$ 20 ppm for several hours' exposure"*. Neither statement represents original research. Instead, the following statement by Milby cites Yant (1930), *"exposure to concentrations above 50 ppm for a period exceeding 1 hour may produce irritation of the conjunctival and corneal tissues"*. The statement does not suggest "irreversible" eye damage, nor does it make reference to effects at 20 ppm. Moreover, the report by Yant also does not represent original research. Alberta Health (1988) also reported *"blurred vision at 0.08 ppm (Kleinfeld et al., 1964)*"; however, this exposure concentration was not stated in the cited document. Thus, it appears that unsubstantiated opinions have been propagated through several review articles, dating as far back as 1930.

As already indicated, the current review relied exclusively on original scientific findings. No reliable evidence was discovered showing eye involvement following short-term exposures to H_2S at concentrations up to 100 ppm, especially evidence of irreversible damage. Circumstantial evidence exists, but the level of confidence that can be assigned to such evidence is low. However, this is not to say that the eye is not responsive to H_2S. Rather, it simply indicates that information showing effects on the eye at concentrations up to 100 ppm is lacking. In all likelihood, given the sensitivity of the eye to irritants in general, it will respond to H_2S. The dose-responsiveness and time-course of the effect(s) at low concentrations is yet to be determined.

C. Hypersusceptibility

Although the present review was not specifically concerned with identifying, examining and/or understanding the health effects of H_2S on hypersusceptible individuals[7], information did emerge during the course of the work that could bear on this issue.

Concerns surrounding the increased susceptibility of certain individuals to the health effects of H_2S were discussed at some length in the earlier report (Alberta Health, 1988), and surfaced again during the deliberations of the Provincial Advisory Committee on Public Safety and Sour Gas (2000). Interest in this area extends back to work completed by the Illinois Institute for Environmental Quality (IIEQ, 1974), which suggested that: *"Knowledge of the toxicology of H_2S permits the identification of individuals who, because of some disease process, metabolic disorders or psychological disturbance, cannot tolerate H_2S or its by-products and therefore possess enhanced sensitivity to this gas".* Among the categories of individuals who might be affected are:

- Individuals with eye or respiratory tract problems. The suggestion is that exposure to H_2S could aggravate pre-existing conditions such as conjunctivitis, eye infections, tuberculosis, asthma, emphysema or chronic bronchitis.
- Individuals with anemia – the suggestion being that the detoxification of H_2S through oxidation via hemoglobin in the blood could be impaired in these individuals.
- Individuals with lowered resistance to bacterial or viral infections.
- Individuals with heart disease – the suggestion being that since H_2S can interfere with oxygen utilization and energy production at the cellular level, the heart muscle may be particular susceptible given its high oxygen demand and need for uninterrupted energy supply.

Findings that emerged during the present review pertaining to the issue of hypersusceptibility are discussed below:

First, the work of Jappinen *et al*. (1990) relates to the first category of individuals since testing was performed with asthmatics. Unfortunately, the results were mixed. Although the authors found that when asthmatic subjects were exposed under controlled conditions to 2 ppm of H_2S for 30 minutes *"no significant changes in respiratory function occurred"*, the changes varied considerably between individuals, with two of the ten subjects showing evidence of bronchial obstruction. The authors reported that these same subjects showed no clinical signs of respiratory discomfort or distress. Interpretation of the findings from the study should necessarily respect the fact that exposure was relatively brief (*i.e.*, 30 minutes), the exposure concentration was low (*i.e.*, 2 ppm), and that severe asthmatics were excluded from testing. On balance, the findings appear to suggest that asthmatics may be at increased risk from H_2S exposure. The degree of susceptibility will depend on the individual's

[7] The term "hypersusceptible" was used in the context of the description provided by the Ad Hoc Committee on H_2S Toxicity (Alberta health, 1988) and referred to individuals who might be at increased risk due to pre-existing medical conditions that could be aggravated by exposure to H_2S. The term was not used to describe individuals who may be intrinsically more sensitive to chemicals in general and might respond to lower concentrations of H_2S than the general population.

condition and the exposure circumstances, but even at low concentrations of H_2S, responses might be expected. The finding is not unexpected given the etiology of the disorder and the irritant nature of the gas.

Second, as outlined earlier, no firm evidence was found to confirm or refute the suggestion that the eyes will respond to short-term exposures to H_2S at concentrations in the range of interest (*i.e.*, 0 to 100 ppm). Accordingly, it is impossible to state with certainty that individuals with pre-existing eye disorders will be extra-sensitive to H_2S within this concentration range. However, first principles and "common sense" suggest that susceptibility would be heightened.

With respect to the second category of individuals (*i.e.*, anemics), no findings emerged from the present review to support the increased susceptibility of this group. The work of Bhambhani *et al.* (1994, 1996, 1997) suggested that female subjects were *less* responsive to the "metabolic" effects of short-term exposure to H_2S compared to their male counterparts despite a lower blood volume, lower hemoglobin content, and lower oxygen carrying capacity. The authors admitted that the findings were unexpected, and suggested that the inhaled dose of H_2S received by the women may have been lower than that received by the men due to a smaller inspiration volume, thereby possibly explaining the unpredicted results. In addition, the women were young, healthy and "fit", with their aerobic capacity heightened by involvement in systematic endurance training programs. As a result, it is difficult to extrapolate the findings to the general population, especially with respect to assessing whether or not the results support or refute the suggestion that anemics may be more susceptible to H_2S. Perhaps it should be pointed out that the suggestion of increased susceptibility of individuals with anemia is based strictly on theoretical grounds. In the earlier review (Alberta Health, 1988), it was concluded that the heightened susceptibility would likely only apply in cases of severe anemia.

In terms of the third category of individuals (*i.e.*, people with increased susceptibility to bacterial and viral infections), the work of Rogers and Ferin (1981) is of interest since it showed that short-term exposure of rats to a low concentration of H_2S (*i.e.*, 45 ppm) caused impairment of the pulmonary anti-bacterial defense system. These findings were supported, in turn, by the work Khan *et al*. (1991) who found that short-term exposure of rats to H_2S could reduce the viability of the pulmonary alveolar macrophages, but the response was only witnessed at a concentration of 400 ppm. No change in cell viability was evident at 50 or 200 ppm of H_2S. Although the information is limited, on balance there is some evidence to support the suggestion of heightened susceptibility to H_2S among individuals with lowered resistance to bacterial and viral infections. However, further study is needed to elucidate the mechanisms that may be involved as well as the dose-response characteristics of the changes.

With regard to the fourth category of potentially susceptible individuals (*i.e.*, people with heart disease), no reliable evidence emerged from the present review to suggest an increased risk of cardiac irregularities from short-term exposure to H_2S at low concentrations. However, as with the former categories, information is limited, and no firm evidence to contest the suggestion of increased susceptibility is available. Bhambhani *et al.* (1991,1994, 1997) did report that heart rate, systolic blood pressure and diastolic blood pressure were unaffected among volunteer subjects by brief exposure to H_2S at concentrations up to 10 ppm, prompting the authors to suggest that "*men and women with cardiovascular disease who may be exposed to H_2S due to the nature of their occupations or otherwise, may not be at an increased risk in such an environment*". However, when interpreting the significance of these findings, it must understood that the subjects were young, healthy, and "aerobically fit", with no indication of pre-existing cardiac abnormalities. By the same token, it must be pointed out that the subjects inhaled the H_2S directly through a mouthpiece while performing moderate to strenuous exercise, thereby significantly heightening the amount of the gas received. Finally, as indicated earlier (Alberta Health, 1988) heart rate and blood pressure are imprecise indicators of cardiac toxicity.

VII. CONCLUSIONS

The conclusions that emerged from the present review are listed below. Consistent with the recommendations of the Expert Panel, the conclusions are segregated into those based on evidence from human studies and those which rely on the results from animal testing. The general basis of each concluding statement is summarized in the attached 'conclusions and commentary' summary tables (see p. 63 to 71). The tables identify the studies that featured most prominently in arriving at the conclusions. Comments bearing on the interpretation of the individual study findings are included in the tables to permit better understanding of the strengths and limitations of the conclusions. The reader is strongly encouraged to consult the full text and the detailed reviews of the individual studies (see Appendix 6) to fully appreciate the basis of the conclusions.

A. Evidence from Human Studies

Young normal healthy adults can tolerate up to 10 ppm H_2S without significant adverse effects. At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings.

Based on observations from clinical studies involving the controlled exposure of individuals with mild to moderate asthma, short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance (*i.e.*, specific airway resistance and airway conductance). Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations remains unclear.

No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified.

B. Evidence from Animal Studies

Based on observations from non-clinical studies involving the exposure of test animals under controlled conditions, it would appear that short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant adverse effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting, and the toxicological significance and clinical relevance of the responses remain largely unknown.

Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations from the non-clinical studies continued to support an absence of significant adverse effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects are unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms which did appear, were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed. In addition, some equivocal evidence of irritation of the eyes and mucous membranes was reported among rats and guinea pigs following exposure for several hours.

Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations from the non-clinical studies were conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one older study of moderate quality (Weedon *et al*., 1940). In a second older study conducted by Mitchell and

Yant in 1925, deaths were recorded among rats, guinea pigs and dogs following prolonged exposure to concentrations ranging from 100 to 140 ppm; however, the reliability of the information was highly suspect. Serious adverse effects, including death, were absent from all of the remaining studies. The weight-of–evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely.

Information also suggests that species variation may exist in sensitivity to H_2S, based on mortality rates, but the evidence is inconclusive.

No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen in the animal species tested. Comparable studies using other species could not be found

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
"Young normal healthy adults can tolerate up to 10 ppm H_2S without significant adverse effects."	Bhambhani *et al.* (1996) Study ID ▲ 118 Bhambhani *et al.* (1991) Study ID ▲ 119 Bhambhani *et al.* (1994) Study ID ▲ 120 Bhambhani *et al.* (1996) Study ID ▲ 121 Bhambhani *et al.* (1997) Study ID ▲ 122	• The exposure time was limited and participants were typically young, healthy and "aerobically fit", and therefore, unlikely to be representative of the general population. • The inhaled dose was higher than that which might be received under resting conditions since the gas was administered directly via a mouthpiece while the subjects routinely engaged in moderate to strenuous exercise. • Changes were limited to alterations in pulmonary and biochemical parameters consistent with a shift from aerobic to anaerobic metabolism. Although the effects are consistent with the accepted mechanism of action of H_2S, they are not unique, but rather occur to varying degrees during normal daily activity and as part of normal diurnal rhythms. • Changes were generally mild in nature and varied by subject. Females were less responsive than males. • None of the subjects experienced any unusual signs or symptoms (*e.g.*, headache, nausea, sore throat, etc), nor any reduction in their exercise capacity.
"At concentrations above 10 ppm, the clinical studies reviewed were not of sufficient technical quality to permit meaningful assessment of the significance of the findings."	Kangas and Savolainen (1986) Study ID ● 207	• No signs and symptoms were monitored. Health effects *per se* were not examined.
	Kilburn (1997) Study ID ● 216	• Decrements in neurobehavioral performance and neurophysiological function were reported among six subjects exposed to H_2S, for up to 24 hours, from a variety of point sources. The exposure concentrations to which subjects were exposed were estimated. It is likely that the exposure concentrations for some subjects were misrepresented as being in the 1 to 50 ppm range, considering that one subject was rendered unconscious and two subjects experienced olfactory paralysis at the time of exposure. • Twelve of the sixteen subjects were plaintiffs in lawsuits. Thus, subject bias my have impacted results since certain parameters were assessed based on a self-administered questionnaire. • An excessive lag period occurred between the exposure events and the testing of the subjects (*i.e.*, up to 6 years).
	Kilburn (1999) Study ID ● 217	• Neurobehavioral deficits were reported to occur in subjects exposed during the course of one week to H_2S released from a refinery explosion and fire, based on "scores" recorded through a battery of neuropsychological and neurophysiological tests. However, test scores were inconsistent for a number of the parameters monitored. The scores for most of these inconsistent responses were classified as "abnormal" by the author, with no apparent basis. • Exposure levels were estimated and inconsistently reported within the study. • An incident of this type would release numerous chemicals, apart from H_2S. These additional substances were not quantified nor was their potential impact assessed. • An excessive lag period occurred between the exposure incident and the testing of subjects (*i.e.*, more than 3 years). Consequently, other events and/or exposures occurring subsequent to the refinery explosion and fire may have contributed to the reported neurological changes. The long lag period also undermines confidence in the results, especially since the study relied, in part, on a self-administered questionnaire.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Lodgepole Blowout Inquiry Panel (1984) Study ID ● 327	• The Lodgepole well reportedly remained unignited for 26 days. During this time, the concentration of H_2S measured in the local area exceeded 10 ppm for 31 hours and 5 ppm for a period of 61hours. Concerns were raised during the inquiry over the adequacy of the monitoring equipment and the number of monitoring units employed. In fact, the Panel concluded, *"there is insufficient data to derive meaningful average hourly concentrations."* • Thirty residents within a 50km radius of the well-site complained of headache, eye irritation and upper/lower respiratory tract symptoms coincidental with the blowout. Symptoms were not verified by medical practitioners. As stated by the panel,, there was a lack *"of scientific data available for analysis"*. • It is not known to what degree the witnesses were representative of the general population within the communities surrounding the well-site. Subject bias was possible. • The role of other chemical agents or other possible causative factors in inducing the observed symptoms was not explored.
	Alberta Social Services and Community Health (1983) Study ID ● 424	• During the Lodgepole blowout, the well was unlit for a total of 28 days, during which time, local monitoring stations assessed peaks levels to be $\leq$ 5.5 ppm. However, the actual concentration to which people were exposed is unknown, since the precise location of monitoring equipment with respect to the peoples' homes was not stated. • Complaints filed with Alberta Environment during this period included general malaise, headache, nausea, diarrhea, respiratory problems, burning eyes, sore throat, etc. All symptoms were self-reported and not verified by medical practitioners. Symptom frequency in an unexposed population was not included in the analysis. • The percent of residents adversely affected by the release was not reported. Thus, no conclusions with respect to the universality of the signs and symptoms can be stated. • As a highly publicized event, subject bias likely skewed results of the study.
	Mitchell and Yant (1925) Study ID ● 444	• Male subjects exposed to 100-150 ppm of H_2S reportedly experienced coughing, irritation of the eyes and loss of sense of smell within 2 to 15 minutes. Symptoms progressed in severity with continued exposure, with salivation and mucous discharge and a sharp pain in the eyes reportedly after 1 to 4 hours of exposure. • Study was described by the authors as being "preliminary". • Male subjects only were used. • Details concerning exposure conditions, exposure chamber, and gas delivery system were generally lacking. • Details concerning subjects (*e.g.*, age, health status) were completely lacking.
"…short-term exposure to H_2S at a concentration of 2 ppm may be capable of inducing bronchial obstruction, based strictly on measures of pulmonary compliance…Since airway symptoms were absent, and the responses varied considerably between subjects, the overall clinical significance of the observations remains unclear.	Jappinen *et al* (1990) Study ID ◆ 202	• The reported changes in airway resistance (*i.e.*, 26.3% increase on average) and airway conductance (*i.e.*, 8.4% decrease on average) were not statistically significant. • Some subjects were non-responsive or showed decreased airway resistance and/or increased airway conductance. • The response appears to be very much subject-dependent and possibly influenced by the severity of the asthmatic condition. In 2 of the 10 exposed asthmatics marked changes in airway conductance and resistance indicative of bronchial obstruction were reported, however, no signs or symptoms consistent with respiratory discomfort or distress were observed. • Subjects had a history of mild to moderate asthma. Individuals with severe asthma were excluded from the study. • The authors noted that the results were preliminary and required further study. They concluded that the exposure caused *"no specific changes in respiratory function"*, and "*although the*

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – HUMAN STUDIES[1]		
CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
		airway resistance increased somewhat after exposure, it was not reflected in the subjects' clinical condition". • The exposure duration was brief and the exposure concentration relatively low. The authors concluded that: *"it is not possible to predict what kind of symptoms asthmatic subjects could have at higher concentrations".*
"No reliable studies of the effects of short-term exposures to H_2S on human reproductive performance were identified."	No studies complied with the eligibility criteria.	• Not applicable.

[1] The reader is strongly encouraged to consult the individual study reviews found in Appendix 6 to obtain a full understanding of the various factors that should be considered in the interpretation of the findings and conclusions from the cited studies.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
"short-term exposures to H_2S at lower concentrations (*i.e.*, up to 35 ppm) are well tolerated, with no clear evidence of significant adverse effects. In the majority of cases, the effects observed were minor in nature and occurred only at the cellular level. In many instances, the effects were shown to be reversible. The scientific evidence supporting these minor effects often was conflicting, and the toxicological significance and clinical relevance of the responses remains largely unknown."	Dorman, *et al* (2002) Study ID ◆ 151	• The only change observed following nose-only exposure of male rats to 30 ppm H_2S was inhibition of cytochrome oxidase in the lung, nasal olfactory epithelium and nasal respiratory epithelium. In the liver, an opposite response was observed, the activity of cytochrome oxidase increased in response to exposure to concentrations at low as10 ppm. In the absence of conventional measures of toxicity, the clinical significance of the observed changes is unknown.
	Haider *et al* (1980) Study ID ● 179	• Whole-body exposure of male guinea pigs to 20 ppm H_2S for 1h/d for 11 days reportedly caused regional-specific reductions in total lipids and phospholipids in brain tissue and increased lipid peroxidation. The changes were not consistent across all regions of the brain, and varied according to lipid type, rendering the interpretation of the significance of the findings difficult. • Evidence of eye irritation was reported, but was not confirmed by *in situ* ophthalmoscopic examination.
	Lopez *et al* (1987) Study ID ◆ 231	• A transient increase in the absolute number of epithelial cells was detected in the nasal lavage fluid of male rats exposed to 10 ppm H_2S in a whole-body chamber. The authors cautioned that the toxicological significance of the response should not be overestimated *"since dramatic change can rapidly be restored due to remarkable reparative capacity of respiratory epithelium."*
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 25 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.
	Weedon *et al.*(1940) Study ID ◆ 316	• Mild dose-dependent signs of intoxication (*e.g.*, transient restlessness) were observed in rats exposed to 16 ppm H_2S. Necropsy findings at this concentration were uniformly non-remarkable. The level of confidence that can be assigned to the study findings is undermined by the use of relatively "crude" instrumentation and the associated uncertainty surrounding the actual exposure concentrations that were tested.
"Within the intermediate range of the concentrations of interest (*i.e.*, 35 to 60 ppm), the observations…continued to support an absence of significant adverse effects. Most effects remained minor in nature and confined to the cellular level. The toxicological significance and clinical relevance of these effects are unclear. Some mild signs and symptoms suggestive of systemic toxicity emerged in a few studies, but the overall evidence was mixed, with reports of no symptoms being equally common. Any signs and symptoms which did appear were generally transient in nature and resolved quickly. Some evidence of mild reversible inflammation of the lining of the nasal passages among rats was observed."	Khan *et al* (1990) Study ID ◆ 210	• Effects of whole body exposure of male rats to 50 ppm H_2S for 4 hours were limited to a reduction in cytochrome *c* oxidase activity. However, the level of inhibition detected post-exposure (*i.e.*, 15%) was comparable to the variability observed among control rats (*i.e.*, 18%). Despite, the enzyme inhibition, no signs and symptoms of toxicity were reported.
	Rogers and Ferin (1981) Study ID ◆ 269	• Exposure to 45 ppm H_2S over a period of 2 hours resulted in no significant changes in the pulmonary antibacterial defense capacity of exposed male rats; however, following 4 and 6 hours of exposure bacteria inactivation was significantly reduced. Time-responsiveness of the effect was demonstrated through the use of graded exposure times, but due to the use of a single exposure concentration the dose-responsiveness of the change could not be determined. Further, the manner by which control rats were handled was not entirely clear, thereby rendering interpretation of the findings difficult.
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 50 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Lopez *et al.* (1986) Study ID ◆ 466	• Exposure of male rats to 40 ppm H_2S for 6-hours resulted in initial weight loss and clinical signs of intoxication (*e.g.*, agitation, lacrimation, and moderate respiratory distress). Pathological examination post-exposure detected focal necrosis of the nasal epithelium and mild pulmonary oedema. However, responses were transient and generally resolved within hours post-exposure (*e.g.*, repair of the focal necrosis of the nasal epithelium was obvious at 42 hours post-exposure). In addition, the observed effects of H_2S on the nasal epithelium might be expected to be more severe in the rat compared to humans since rats are obligate nasal breathers.
	Mitchell and Yant (1925) Study ID ● 444	• Death among canary birds following exposure to 35 –65ppm for 8 to 18 hours. Canaries were described by authors as being extremely susceptible to poisonous gases. • Signs and symptoms among rats and guinea pigs exposed to 35-65ppm for as little as 1 to 4 hours. Signs and symptoms consisted of eye irritation and rough hair coat, which progressed in severity with continued exposure up to 100 hours. No deaths. • Information respected exposure conditions, exposure chamber and gas delivery system were generally lacking. • Study is very "dated".
"Within the upper range of the concentrations of interest (*i.e.*, 60 to 100 ppm), the observations…were very conflicting. Although the responses continued to be dominated by minor effects at the cellular level, serious outcomes did emerge, notably death among rats and mice in one study. Serious adverse effects, including death, were absent from all of the remaining studies. The reason(s) for the discrepancy in findings is not known. However, the weight-of-evidence suggests that death or other serious adverse outcomes following short-term exposure in the concentration range of interest is unlikely."	Dorman *et al.* (2002) Study ID ◆ 151	• Transient shifts in tissue sulfide content, cytochrome oxidase activity and/or the concentrations of sulfide metabolites were observed in male rats exposed to 80 ppm H_2S via a nose-only inhalation apparatus. In the absence of conventional measures of toxicity, the clinical significance of the observed changes is unclear.
	Elovaara *et al.* (1978) Study ID ● 155	• Whole body exposure of female mice to 100 ppm H_2S for 2 hours reportedly resulted in alterations in protein metabolism in the brain, although the protein content of both the brain and myelin was unaffected. The effect was inconsistent, varying with the duration of exposure. The increase in leucine incorporation detected during the first 24 hours of exposure was followed by a decrease relative to controls. This shift in response renders interpretation of the findings difficult. • Apart from transient agitation during the first 20 minutes of exposure, the mice remained clinically unaffected. Thus, the changes in protein metabolism were not accompanied by observable signs or symptoms of intoxication. No gross or histopathological examination of the brain or myelin tissues was performed to determine if the changes had macroscopic or microscopic correlates.
	Kosmider *et al.* (1967) Study ID ● 223	• Acute and subacute exposure of rabbits to 71 ppm H_2S reportedly caused electrocardiographic and histochemical changes in the heart muscle. Interpretation of the significance of the studies findings was limited by reporting deficiencies (*e.g.*, lack of control data). • Animals exposed for a single 1.5 hour exposure period lost consciousness, whereas those exposed repeatedly for 30 min/day for 5 days exhibited no signs or symptoms. In studies conducted by Kosmider in 1967 (Study ID ● 694) and 1971 (Study ID ● 595), rabbits exposed for 1.5 hours and 1 hour, respectively, were not reported to be rendered unconscious during exposure.
	Lopez *et al.* (1988) Study ID ◆ 232	• Changes to the morphological architecture of the lungs of male rats exposed to 83 ppm H_2S for four hours were relatively minor (*e.g.*, perivascular edema of pulmonary vessels and distention of perivascular space) and resolved quickly following cessation of exposure. These changes were not accompanied by observable signs or symptoms of intoxication.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Savolainen *et al.* (1980) Study ID ● 280	• Mice exposed to 100 ppm of H_2S for 2 hours at four-day intervals for up to 16 days reportedly showed cumulative changes in a number of metabolic and enzymatic cerebral parameters (*e.g.*, cytochrome oxidase activity, RNA synthesis, superoxide dismutase activity, glutathione levels, etc.). However, the changes rarely achieved statistical significance and generally varied less than 10% from control values. In addition, the nature of the change (*i.e.*, increase vs. decrease) for some parameters was not consistent across the entire treatment schedule. Thus, a treatment-related effect was not obvious. • Apart from some initial "excitement" at the initiation of each exposure period, metabolic and enzymatic changes were not accompanied by any observable signs or symptoms of intoxication.
	Skrajny *et al.* (1996) Study ID ◆ 291	• Male rats exposed to 75 ppm and 100 ppm H_2S for 3h/d for 5 consecutive days reportedly exhibited a cumulative, concentration-dependent increase in hippocampal activity, as evidenced by irregularities in EEG readings. Since conventional measures of toxicity were not included as part of the overall study design, clinical and/or pathological correlates of the changes could not be determined, and the toxicological significance and clinical relevance of the response are unknown.
	Struve *et al.* (2001) Study ID ◆ 296	• The motor activity of male rats receiving whole-body exposure to 80 ppm H_2S for 3h/d for five consecutive days was unchanged, whereas animals that received nose-only exposures showed reduced activity. However, the change was significant at only 2 of 10 testing intervals. Given the inconsistency of the response and the lack of progression of the change over the course of treatment, combined with the lack of response for all other measures of neurobehavioral function, the change in spontaneous motor activity was judged to be equivocal.
	Weedon *et al.* (1940) Study ID ◆ 316	• Rats and mice exposed to 63 ppm H_2S for up to 16 hours exhibited frank evidence of intoxication, especially among test mice, with deaths recorded as early as within one hour of exposure. All of the mice (4 of 4) and one of eight rats were dead within 40 hours. Considerable discrepancy exists between this finding and those reported by more recent studies. Specifically, whereas Weldon *et al.* reported deaths within 60 minutes, both Elovaara *et al*, 1978 (Study ID ● 155) and Savolainen *et al*, 1980 (Study ID ● 280) reported no deaths among mice following exposure to 100 ppm H_2S for 2 hours. Thus, it would appear from the available information that the high incidence of deaths reported by Weedon *et al.* is unusual. The results may reflect the age of the study since the exposure equipment and gas monitoring systems were necessarily "crude" by present-day standards. Some uncertainty exists surrounding the actual exposure concentrations to which the animals on test were exposed.
	Nicholson *et al.* (1998) Study ID ◆ 398	• Whole body exposure of male rats to 100 ppm H_2S for 3h/d for 5 days reportedly increased levels of L-glutamate in the hippocampus. The dose-responsiveness of this change could not be assessed since only a single exposure concentration was employed. The authors' claim that elevated L-glutamate levels may, due to their role as a neurotransmitter, contribute to cerebral neurotoxicity was not substantiated since no symptomatic or pathological correlates of the observed changes were reported owing to limitations in study design.
	Mitchell and Yant (1925) Study ID ● 444	• Signs and symptoms consistent with systemic toxicity (e.g., dizziness, laboured respiration, and unconsciousness) among canary birds following exposure to 100ppm for 1 to 8 hours. Deaths within 4 to 8 hours. • Deaths among guinea pigs (1 of 2), dogs (1 of 2) and rats (?) following exposure to 100-140ppm for at least 8 hours.

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Hulbert *et al.* (1989) Study ID ● 460	• One-hour exposure to 100 ppm H_2S reportedly produced a transient decrease in airway resistance and an increase in dynamic compliance in 50% of the guinea pigs under test. The response is contrary to that which might be expected given the irritant properties of H_2S and the commonly reported broncho-obstruction following exposure. • The mode of administration of H_2S involved intra-tracheal treatment of the animal under restraint, aided by mechanical ventilation. Consequently, the gas by-passed the "scrubbing" action provided by the mucosal epithelium lining the upper respiratory passages and therefore, the findings likely over-state the changes that might occur in the pulmonary tissue of a exposed human. • Microscopic examination of pulmonary tissue exposed to 100 ppm H_2S revealed no apparent damage.
	Kosmider *et al.* (1971) Study ID ● 595	• Rabbits exposed for 1h/d for 14 days reportedly exhibited changes in serum and/or tissue protein, mineral and enzyme levels. Clinical relevance was difficult to establish since the study did not outline the range of shifts typical of normal homeostatic mechanisms. According to the authors, some changes were indicative of liver and kidney damage; however, no histopathological evaluations were conducted to verify this conclusion. • Conventional measures of toxicity were largely lacking, but rapid respiration, elevated heart rate and initial excitement were observed at the time of exposure. • Eyes reportedly developed "congestion" of the conjunctiva, but the nature of the congestion was poorly described and was not investigated by *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination.
	Kosmider *et al.* (1967) Study ID ● 694	• Exposure of rabbits to 71 ppm H_2S for 1.5 hours reportedly resulted in a decrease in both serum and brain alkaline phosphatase activity. The clinical and toxicological significance of these changes are unknown. • No signs or symptoms were reported.
"No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen. Comparable studies using other animal species could not be found."	Hannah and Roth (1991) Study ID ● 11	• Offspring of pregnant female rats exposed to 20 and 50 ppm H_2S from day 5 post-coitus until day 21 post-natal were reported to exhibit abnormal development at the "growth front" of the cerebellar Purkinje cells. Considering specie specific gestation periods, the equivalent exposure time for humans would extend over several months. This coupled with the lack of dose-responsiveness and the minimal degree of change observed renders interpretation of the significance of the findings difficult. • The absence of clinical data precludes any consideration as to whether the reported changes may result in behavioral abnormalities or if the architectural alterations represent direct H_2S toxicity or secondary responses to maternal stress.
	Kosmider *et al.* (1971) Study ID ● 595	• Rabbits exposed for 1h/d for 14 days reportedly exhibited changes in serum and/or tissue protein, mineral and enzyme levels. Clinical relevance was difficult to establish since the study did not outline the range of shifts typical of normal homeostatic mechanisms. According to the authors, some changes were indicative of liver and kidney damage; however, no histopathological evaluations were conducted to verify this conclusion. • Conventional measures of toxicity were largely lacking, but rapid respiration, elevated heart rate and initial excitement were observed at the time of exposure. • Eyes reportedly developed "congestion" of the conjunctiva, but the nature of the congestion was poorly described and was not investigated by *in situ* ophthalmoscopic examination or post-sacrifice histopathological examination.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Kosmider *et al.* (1967) Study ID ● 694	• Exposure of rabbits to 71 ppm H_2S for 1.5 hours reportedly resulted in a decrease in both serum and brain alkaline phosphatase activity. The clinical and toxicological significance of these changes are unknown. • No signs or symptoms were reported.
"No indications of significant adverse effects on reproductive performance, pregnancy, fetal development, or growth and development of the offspring were observed among rats exposed throughout gestation to H_2S at concentrations up to 80 ppm. These findings suggest that H_2S is neither a reproductive toxin nor teratogen. Comparable studies using other animal species could not be found."	Hannah and Roth (1991) Study ID ● 11	• Offspring of pregnant female rats exposed to 20 and 50 ppm H_2S from day 5 post-coitus until day 21 post-natal were reported to exhibit abnormal development at the "growth front" of the cerebellar Purkinje cells. Considering specie specific gestation periods, the equivalent exposure time for humans would extend over several months. This coupled with the lack of dose-responsiveness and the minimal degree of change observed renders interpretation of the significance of the findings difficult. • The absence of clinical data precludes any consideration as to whether the reported changes may result in behavioral abnormalities or if the architectural alterations represent direct H_2S toxicity or secondary responses to maternal stress.
	Hayden *et al.* (1990) Study ID ◆ 12	• Exposure of gravid rat dams to 20, 50, or 75 ppm H_2S from day 6 of gestation to day 21 post-partum reportedly caused dystocia. However, the reported dystocia was based on the observation of a limited number of animals (*i.e.*, about ¼ of the number recommended by guidelines for developmental toxicity studies). Moreover, the actual incidence of the dystocia was not reported. Large standard deviations of mean parturition times indicate a high degree of inter-animal variability. Parturition times also varied across trials, as evidenced by differences in the times reported for each of the control groups. Consequently, it is very difficult to assign significance to the findings. • A transient depression in feed intake was noted in treated dams during the first few days following initiation of treatment; however, normal feed intake was restored within one week and the body weights of treated dams were unaffected. • A 6% increase in maternal liver cholesterol was noted following exposure to 75 ppm. However, this did not appear to be a dose-responsive change as exposure to 20 ppm and 50 ppm H_2S decreased maternal cholesterol relative to controls. • The reported changes in offspring development (*e.g.*, altered pinna detachment and hair development) were not dose-responsive and did not uniformly achieve statistical significance. • All remaining maternal parameters and pup parameters monitored were unaffected (*e.g.*, organ weights, liver/brain protein and DNA content, brain cholesterol levels, etc.). Similarly, routine reproductive parameters were normal.
	Dorman *et al.* (2000) Study ID ▲ 149 CIIT (1999) Study ID ▲ 594	• The response of male and female rats exposed to 0, 10, 30, or 80 ppm H_2S for 6h/d during the course of a two-week pre-breeding period, a two-week mating period, the gestation period and a three-week postnatal period was limited to a transient reduction in body weight and feed consumption among the high-dose F_0 males, a typical response to a chemical stressor in laboratory rodents. • Any differences in reproductive, developmental or pathological parameters were generally isolated, minor in nature, non-dose-related and/or of unknown biological significance. Although, malformations were only observed in newborn pups from dams exposed to H_2S, careful review of the incidence of the malformations revealed no evidence that the structural defects were related to treatment. The majority of the pups were from a single litter and the most common structural defects were described as "skin and dermis detachment", which was likely of genetic origin. • The authors concluded that the findings suggest that H_2S is neither a reproductive toxin nor teratogen.

Confidence Index Ranking: High or high-to-moderate ▲ Moderate-to-high, moderate or moderate-to-low ◆ Low or low-to-moderate ●

CONCLUSIONS AND COMMENTARY- SUMMARY TABLE – ANIMAL STUDIES[1]

CONCLUSION:	SUPPORTING STUDIES:	CONSIDERATIONS:
	Hannah *et al.* (1989) Study ID ● 180	• Exposure of pregnant rats to 75 ppm H_2S for 7h/day from day 5 post-coitus until day 21 resulted in alterations in levels of selected amino acids in regions of the brain in the offspring. However, variations in regional and temporal responses, and the absence of symptomatic data make the interpretation of the significance of the changes difficult. Further, the equivalent exposure time in humans would extend over several months, an exposure period which would be unlikely to occur at this exposure concentration.
	Saillenfait *et al.* (1989) Study ID ◆ 278	• Exposure of pregnant rats to 50, 100, and 150 ppm H_2S reportedly resulted in a dose-related change in mean implantation sites per litter and changes in both maternal and fetal body weights. However, the changes were confined to the higher exposure groups and replicate experiments yielded different degrees of change in certain parameters.
	Skrajny *et al* (1992) Study ID ◆ 290	• *In utero* and post-natal exposure of rats to 20 ppm and 75 ppm H_2S for 7h/d from day 5 post-coitus to day 21 post-partum reportedly resulted in changes in the levels of serotonin and norepinephrine in the developing cerebellum and/or frontal cortex. However, a lack of dose-responsiveness, inconsistency in the nature of responses at different sampling times and the absence of observational, histopathological and histochemical data renders interpretation of the findings very difficult. The changes were of unknown biological significance and clinical relevance.
	Hannah *et al.* (1990) Study ID ● 402	• Exposure of pregnant rats to 50 ppm H_2S for 7h/d from day 6 post-coitus until day 21 postnatal was associated with an average 30% increase in maternal blood taurine levels relative to corresponding control values. Interpretation of the significance of the findings with respect to animal health is hampered by the absence of observational data. The clinical relevance of the finding is unclear.
	Roth and Hannah (1989) Study ID ● 630	• *In utero* and post-natal exposure of rat pups to 75 ppm H_2S for 7h/d from day post-coitus to day 21 post-partum reportedly produced morphological changes in the cerebellum (*i.e.*, increased number of Purkinje cells). However, the lack of routine measures of toxicity (*e.g.*, observational data) prevents conclusions pertaining to the overall impact of the observed increase in Purkinje cell density on the functional integrity of the CNS. Further, the equivalent exposure time in humans would extend over several months, an exposure period which would be unlikely to occur at this exposure concentration.

[1] The reader is strongly encouraged to consult the individual study reviews found in Appendix 6 to obtain a full understanding of the various factors that should be considered in the interpretation of the findings and conclusions from the cited studies.

VIII. SUMMARY REMARKS

The Terms of Reference for the present work called for a review of the currently available health effects information surrounding short-term exposures to low concentrations of hydrogen sulphide. The review was prompted, in part, by the recommendations of the Provincial Advisory Committee on Public Safety and Sour Gas, which specifically encouraged development of a *"revised comprehensive health effects table"* for H_2S to replace the version first prepared in 1988 on the basis of the conclusions reached by the Ad Hoc Committee on H_2S Toxicity following a review of the scientific literature available at that time (Alberta Health, 1988). The earlier review assessed the scientific evidence for acute, sub-chronic and chronic effects from exposure to H_2S at concentrations up to 20 ppm. It concluded, in part, that:

1. *H_2S by itself is a broad-spectrum toxicant that can elicit numerous psychological and biological responses in the 0 to 20 ppm range with which this review was concerned. As with any chemical, all organ systems respond variably to different levels of H_2S with no given level affecting all systems equally at the same time or rate.*

2. *In general, single or repeated exposures of "normal, healthy adults" to H_2S in the 0 to 5 ppm range have not resulted in clinically-detectable irreversible biological or psychological effects. Whether this is true for hypersusceptible individuals is not currently known.*

3. *Although reversible adverse effects may result in temporary discomfort or changes in organ function, they have not been found to have a lasting impact to date.*

These conclusions helped to shape the 1988 version of the "health effects table".

In arriving at the conclusions from the current review of the health effects information on H_2S (see Chapter VII: Conclusions), several items surfaced that had a direct bearing on the meaning and level of significance that could be assigned to the collective findings from the various studies that were reviewed. An understanding of these items is critical since they speak directly to the nature of the conclusions that were developed and the limitations thereof. They also account for differences in the conclusions reached in the present review from those reached in 1988. The items are captured in the following questions:

- What major uncertainties exist in the health effects information surrounding short-term exposures to H_2S, and how do these uncertainties affect the conclusions concerning potential effects on humans?
- What limitations surround the current health effects database on short-term exposures to H_2S, and how does these limitations affect the strength of the conclusions?
- How does the present review differ from that completed in 1988, and how do these differences affect the current set of conclusions?

Discussion around each of these items follows.

A. Uncertainties in Information

Uncertainty always surrounds scientific research and the conclusions that can be drawn. The uncertainty takes many forms, ranging from that resulting from data gaps or missing information to that associated with potential errors in sampling or measurement. Such uncertainty is not unique to the health effects information on short-term exposures to H_2S. Rather, it applies to virtually all forms of scientific investigation. In the case of toxicological research aimed at understanding health effects, an additional layer of uncertainty exists in the form of intra-species (*i.e*., within species) and inter-species (*i.e*. between species) variability that can affect the response(s) to a chemical and any associated health outcomes. This variability essentially precludes direct extrapolation of findings from animal testing to the human condition without careful consideration of species differences. In

addition, it can preclude extrapolation of results from clinical or case-control studies to the general population in light of individual differences in response.

The uncertainty associated with inter-species variability can be addressed through different means, ranging from the systematic investigation of differences at the physiological, functional, structural and/or metabolic levels between species to better understand how the findings might apply to the human condition, to the largely arbitrary use of "safety factors" which assumes that humans are more sensitive than test animals. Similar approaches can also be applied to account for intra-species differences.

Notwithstanding the above, no attempt was made in the present review to extrapolate the findings from the animal studies to the human condition. Rather, the information was simply segregated according to study type, with the clinical and non-clinical data clearly differentiated. No attempt was made to adjust the findings to account for differences between species in factors such as pulmonary dynamics and pharmacokinetics that would act to determine the "toxic load" of H_2S received in each of the studies reviewed. Similarly, no attempt was made to extrapolate the findings from the clinical and case-control studies to the general population. Such exercises were considered to be beyond the scope of the current literature review. This position was accepted by the Expert Panel, with the proviso that the clinical and non-clinical information be clearly distinguished.

In light of the above, the conclusions arising from the present review have been separated into those based on evidence from human studies and those based on results from animal testing. Caution must be exercised in the interpretation and use of the latter conclusions since they should not be directly extrapolated to the human condition without further analysis.

B. Limitations in Information

The present review revealed some rather serious limitations in the scope and quality of information respecting the health effects of short-term exposures to H_2S. These limitations affected the strength of the conclusions that were drawn. In some respects, the limitations remained the same as those identified by the Ad Hoc Committee on H_2S Toxicity in 1988. For example, the earlier review made frequent reference to the need for *"further research"* owing to the *"lack of good scientific data"* and the *"little attention"* afforded to the study of the effects of H_2S on certain organ systems. Although a significant body of new information has emerged since 1988, particularly with respect to reproductive and developmental toxicity, data on many systems remain lacking. Moreover, a considerable amount of the earlier and newer work is of limited usefulness because of weaknesses in experimental design, conduct and/or reporting, which preclude critical interpretation of the biological significance and clinical relevance of the findings. The level of confidence that could be assigned to the findings and conclusions from this work was judged to be very limited. Examples of the limitations were provided earlier, but they are repeated here since they are especially telling and must be considered in the overall interpretation and use of the health effects information.

- Only *one* of the studies reviewed was performed under Good Laboratory Practice (GLP) conditions. No reference to GLP was discovered in any of the remaining studies.
- Close to 50 percent of the studies received a "low" confidence index ranking, indicating serious weaknesses in study design, execution and/or reporting when measured against recommended testing guidelines developed by leading scientific and regulatory authorities. Only 15 percent of the studies achieved a "high" confidence index ranking.
- Significant departures from even basic protocol requirements were common. Close

to 50 percent of the controlled studies used only a single exposure concentration, thereby precluding assessment of the dose-responsiveness of any observed changes and hindering any conclusions as to whether or not responses were truly treatment-related. Forty percent of the studies involved use of a single sex, thereby limiting the extrapolation of the results to the general population.

- Close to 80 percent of the non-clinical studies followed unconventional designs, hindering both the interpretation of the results and the comparison of findings across studies as a means to assess the reproducibility of any observed outcomes.
- The bulk of information respecting the health effects of short-term exposures to H_2S was derived from animal testing. Human data were very limited, further highlighting the uncertainty surrounding the extrapolation of the health effects information to the general human population.
- For many organ systems, reliable information on effects following short-term exposure to H_2S is almost completely lacking. Very little information was found with respect to effects on the skin, liver, kidney, gastrointestinal tract, hematopoietic and immunological systems. However, effects on the nervous system (*i.e.*, the primary systemic target) and respiratory system (*i.e.*, the portal of entry for inhaled H_2S) were well documented.

Based on the above shortcomings, the need for further research on the health effects of H_2S still remains. The requirement that future research follow recommended testing guidelines should be strongly encouraged. Such work will likely allow for the conclusions respecting the health effects of short-term exposure to H_2S at concentrations up to 100 ppm to be strengthened and broadened. In the meantime, the conclusions reached in the present review are judged to accurately reflect the currently available scientific information. Although circumstantial evidence exists that possibly contests certain of the conclusions, the information should be viewed as questionable and should not be relied upon as part of a scientific review.

C. Comparison with 1988 Review

The present review bears some resemblance to the earlier review completed by the Ad Hoc Committee on H_2S Toxicity, which ultimately led to the development of the 1988 "health effects table". Both the current review and the earlier work focused on the potential health effects associated with low-level exposures to H_2S, and in each case, "low level" was defined as concentrations up to 100 ppm. In addition, both reviews involved an extensive review of the scientific literature on the health effects of H_2S. Third, both reviews "weighed" the quality of the scientific information, albeit to different degrees. Further, both reviews relied on technical expertise as well as professional judgement in assessing the information. Finally, both reviews were performed under the auspices of an expert committee or panel, with members drawn from various stakeholder groups.

Despite these similarities, important differences in the approach used in each review become evident on closer examination. Specifically, the present review:

- Captured health effects information found in the scientific literature published up to 2002. (The earlier review was necessarily limited to publications appearing before 1988).
- Focused only on the assessment of health effects following acute or sub-acute exposures, extending over a few hours or a few days. (The former review included sub-chronic and chronic exposures, lasting several months or several years).
- Relied only on information found in "primary" papers, in which the original research work was described. (The earlier review placed more reliance on abstracts and review articles).
- Used a pre-defined set of objective "quality" criteria to gauge the technical strengths and

weaknesses of each scientific paper, and to assess the level of confidence that could be assigned to the study findings and conclusions. (The former review simply used professional judgement to "weigh" the quality of the information according to its source).

- Focused on the responses that might follow short-term exposure to H_2S at low concentrations on "healthy" individuals, with no specific prediction of the potential effects of H_2S on "hypersusceptible" people. Nevertheless, any specific information on "hypersusceptible" people should have been captured by this review. (The earlier review sought to clarify the effects of low levels of H_2S on sensitive individuals).
- Clearly distinguished effects on the basis of study type, with the evidence from human studies deliberately segregated from the results of animal testing. (In the former review, the information from clinical and non-clinical studies was merged and conclusions were common to both study types).

These differences in approach are important since they have a direct bearing on the interpretation of the health effects information presented in each review, as well as on the conclusions drawn. Accordingly, side-by-side comparison of the two sets of conclusions is not easily performed. Any comparison must necessarily carefully weigh the differences in approach used as well as the source and basis of the statements.

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