SARA GROUP

Sudbury Area Risk Assessment
Volume II

Appendix A1:

Detailed Toxicological Profile of Arsenic

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A1: DETAILED TOXICOLOGICAL PROFILE OF ARSENIC

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APPENDIX A1 DETAILED TOXICOLOGICAL PROFILE OF ARSENIC

SUMMARY

The purpose of arsenic toxicological profile is to: i) outline the most relevant toxicological and epidemiological information on this substance; ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the arsenic exposure limits selected for use in the human health risk assessment of the Sudbury area. The toxicological review of arsenic is based primarily on secondary sources, such as ATSDR and EPA toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for arsenic. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Arsenic

Several arsenical compounds are typically found in environmental and biological systems. These include inorganic forms: trivalent arsenic (As[III] or As^{III}), the valency found in arsenite (AsO_3^{3-} or AsO_2^{1-}) and arsenic trioxide; pentavalent arsenic (As[V] or As^{V}), the form found in arsenate (AsO_4^{3-}, $HAsO_4^{2-}$, or $H_2AsO_4^{1-}$); as well as organic arsenic compounds: monomethylarsonic acid (MMA^{V}; $CH_3AsO[OH]_2$) and dimethylarsinic acid (DMA^{V}; $[CH_3]_2AsO[OH]$), the predominant metabolites of inorganic arsenic in humans; as well as arsenobetaine ($[CH_3]_3As^{+}CH_2COOH$) and arsenocholine, organic arsenicals found predominantly in fish and other seafood.

Absorption and Toxicokinetics of Arsenic

Water solubility of arsenic compounds is well known to have the greatest impact on oral bioavailability (Hrudey *et al.,* 1996). Greater than 95% of water-soluble inorganic trivalent arsenic may be absorbed from the gastrointestinal tract of humans (Coulson *et al.,* 1935; Ray-Bettley and O'Shea, 1975; Pomroy *et al.,* 1980). Similarly, the results of animal studies indicate that soluble arsenic compounds are also readily absorbed following oral exposure. Reduced oral bioavailability is observed with the less soluble forms of arsenic.

The oral bioavailability of inorganic arsenic has been reported to be considerably reduced when administered in a soil or dust matrix, such that soil-bound arsenic is unlikely to be accumulated by exposed human receptors to a high level (Hrudey *et al.,* 1996). A number of studies demonstrate that the absorption of arsenic in ingested dust or soil is likely to be considerably less than absorption of arsenic from ingested salts (Davis *et al.,* 1992, 1996; U.S. EPA 1997; Freeman *et al.,* 1993, 1995; Pascoe *et al.,* 1994; Rodriguez *et al.,* 1999). However, it has also been shown that arsenic bioavailability in a soil matrix is highly variable across sites and is greatly dependent on site-specific soil properties, and the predominant arsenic species present.

When inhaled, it is the water soluble forms of arsenic that are most rapidly and efficiently absorbed. The absorption of particulate-bound arsenic following inhalation is greatly dependent on the dynamics of deposition, retention and clearance of airborne particles by the respiratory system.

Dermal absorption of arsenic is believed to be low and occurs slowly. The available weight-of-evidence suggests that generally less than 5% of soluble arsenic compounds would be absorbed dermally and negligible amounts of particulate bound (dust or soil) arsenic is likely to cross the skin and enter the systemic circulation.

Once absorbed *via* any route, arsenic is widely distributed to various tissues and organs within the body, in both humans and experimental animals.

Most mammals metabolize inorganic arsenic *via* methylation to methylarsonic acid (MMA) and dimethylarsinic acid (DMA). While much scientific progress has been made in recent years, there is much about the metabolism of inorganic arsenic that is not fully understood (Vahter, 2002). In general, once absorbed, some dissolved As (V) is reduced to As (III), while some As (III) is oxidized to As (V) (Vahter, 2002; Malachowski, 1990). Both species are metabolized in many tissues, but primarily in the liver *via* methylases, resulting in formation of methylarsonic acid, dimethylarsinic acid, and trimethylarsenic compounds. In many species, the two main steps in arsenic metabolism are: (1) reduction of pentavalent to trivalent arsenic, and, (2) oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form mono-, di- and trimethylated products using S-adenosyl methionine (SAM) as the methyl donor and GSH as an essential co-factor.

An unusual feature of arsenic metabolism is that there are extreme qualitative and quantitative interspecies differences in methylation to the extent that some species do not appear to methylate arsenic at all (Styblo *et al.,* 1995; Vahter, 1999). There is also evidence of substantial inter-individual variability

in the degree of methylation in arsenic-exposed humans, and patterns of methylation products between a number of studies have not correlated well with arsenic exposure (WHO-IPCS, 2001). It is postulated that this may reflect genetic polymorphisms or differing methylation capacity by gender and age class.

While methylation reactions in biometabolism are generally considered a detoxification process (as methylated compounds tend to be rapidly excreted), recent studies indicate that reduced trivalent intermediates, monomethylarsenous acid (MMAIII), and dimethylarsinous acid (DMAIII), are of greater toxic potency than either the inorganic parent compounds or other methylated arsenic species (Styblo and Thomas, 1997; Petrick *et al.,* 2000; Styblo *et al.,* 2000; 2002; NRC, 2001). However, at this time, the extent to which MMAIII and DMAIII contribute to the observed toxicity following exposure to inorganic arsenic is not fully understood (Vahter, 2002).

The primary pathway of elimination of all arsenic species from the body is excretion *via* the urine. A very small fraction of excreted arsenic compounds occur in the feces, bile, sweat and breast milk (Malachowski, 1990). Urinary arsenic is routinely used as a bioindicator of arsenic exposure.

Toxicology of Arsenic

Evaluating the toxic effects of arsenic is complicated by the fact that arsenic can exist in several different valence states and can occur as multiple inorganic and organic compounds. The majority of cases and situations involving human toxicity from exposure to arsenic have been associated with exposure to inorganic arsenic compounds. The most common inorganic arsenical in air is arsenic trioxide (As_2O_3), while a variety of inorganic arsenates $(AsO_4)^{-3}$ or arsenites $(AsO_2)^{-1}$ occur in water, soil, and foods. A number of studies have noted differences in the relative toxicity of these compounds, with trivalent arsenites tending to be somewhat more toxic than pentavalent arsenates (ATSDR, 2005). The relative toxicity of arsenic compounds in both animals and humans is generally known to decrease as follows: arsine (III) > organo-arsine derivatives > arsenites (III) > arsenoxides (III) > arsenates (V) > pentavalent organic compounds (V) > arsonium metals (I) > metallic arsenic (0), where the Roman numeral indicates the oxidation state (HSDB, 2003). However, it should be recognized that in many studies: i) the differences in the relative potency are small, and often within the bounds of uncertainty around a NOAEL or LOAEL; ii) different forms of arsenic may be rapidly interconverted, both in the environment and the body; and, iii) in many studies of human exposure to arsenic, the chemical speciation is not known (ATSDR, 2005).

Organoarsenicals are generally considered to be less toxic than the inorganic forms, although several methyl and phenyl arsenical pesticides may pose a possible human health concern. The major organoarsenicals that have been studied are monomethyl arsonic acid (MMA) and its salts, (monosodium methane arsonate [MSMA] and disodium methane arsonate [DSMA]), dimethyl arsinic acid (DMA, also known as cacodylic acid) and its sodium salt (sodium dimethyl arsinite, or sodium cacodylate), and roxarsone (3-nitro-4-hydroxyphenylarsonic acid). In addition, a number of organic arsenic species are known to occur in fish and shellfish (*e.g.*, arsenobetaine, arsenocholine, various arsenosugars). Available studies on these forms of arsenic have revealed that they are readily excreted from the body in a virtually unchanged form and are essentially non-toxic (ATSDR, 2005).

Another type of arsenical that has been studied is arsine (AsH_3) and its methyl derivatives. While this form of arsenic is known to have a greater toxic potency than any other known form of arsenic, it is not associated with smelter emissions and would not be expected to occur in the ambient environment surrounding a smelter to any significant extent. While trace amounts of arsine and methylarsines can be formed from inorganic arsenic species through natural biological transformation processes, arsine is a gas at room temperature, which limits its ability to accumulate in soils or water bodies, and it appears to rapidly undergo a variety of reactions which rapidly transform or degrade these compounds to other forms of arsenic (HSDB, 2003). Thus, the emphasis in this arsenic profile is on inorganic arsenic compounds, although relevant studies on organoarsenicals are described and discussed where data are available.

An important aspect of arsenic toxicology to note is that most laboratory animals appear to be substantially less susceptible to arsenic-induced adverse health effects than humans. Thus, certain animals may not be good quantitative models for studying arsenic toxicity in humans.

The toxicology review of arsenic describes and discusses a large number of studies that investigated the toxicity (acute, subchronic and chronic) of various arsenic compounds (including organoarsenicals) in humans and experimental animal species, *via* the oral, inhalation and dermal routes of exposure. In addition, the reproductive and developmental toxicology, carcinogenicity, and genotoxicity/mutagenicity of arsenic compounds following oral, inhalation or dermal exposure in humans and animals are reviewed in detail.

The toxicological database on arsenic clearly indicates that this substance is a systemic toxicant in humans and animals that acts on a number of organs and tissues within the body. Well characterized target tissues and organs for arsenic toxicity are the skin, cardiovascular system, peripheral nervous system (sensory and motor nerves), liver, and blood.

The available weight-of-evidence from animal studies suggests that arsenic compounds are not teratogenic and have not been found to cause significant reproductive or developmental toxicity at environmentally relevant exposure levels or doses, administered *via* oral, inhalation or dermal routes. In humans, the reproductive and developmental toxicity database for humans is much more limited and equivocal, and many studies show conflicting results. Further confounding the interpretation of human reproductive or developmental toxicity is the fact that all available human epidemiological studies suffer from various limitations in study design, and failure to account for, or report key variables in the studies.

Genotoxicity and mutagenicity studies of arsenic compounds in a variety of test systems (including bacterial, mammalian and human cell lines, *in vitro* and *in vivo* studies), and under varying exposure conditions, have generally produced mixed results. Thus, the available evidence for genotoxicity and mutagenicity of arsenic compounds is equivocal. A number of recent studies indicate that certain arsenic compounds can induce apoptosis in a variety of human and other mammalian cell lines.

Evidence from human epidemiological studies clearly shows that exposure to inorganic arsenic increases the risk of cancer. Evidence from studies of inhalation occupational exposure has shown an increased risk of lung cancer, primarily in copper smelter workers exposed mainly to arsenic trioxide, but also in chemical plant workers exposed to arsenate, and in residents near copper smelters or chemical plants using arsenic compounds. However, there are also studies of exposed communities where no link could be established between arsenic exposure and lung cancer rates. Oral exposure (primarily drinking water exposures) has been positively associated with skin cancers. Epidemiology studies have also proposed an association between liver, kidney, lung, prostate, colon and bladder cancers with oral exposure to arsenic (Tchounwou *et al.,* 2003).

While evidence is suggestive of an association between inhalation and oral arsenic exposure and cancer, a number of studies have been critical of such evidence, citing numerous uncertainties, confounding variables, and limitations of the studies that reported positive associations.

In animals, the database for the carcinogenicity of arsenic compounds is limited, and has generally been considered inadequate, with no consistent demonstration of carcinogenic activity. Although, some recent studies are suggestive of a role for certain arsenic compounds in the development of cancer in experimental animals.

There are presently three modes of action for arsenic carcinogenesis that have some degree of positive evidence in experimental systems (animal and human cells), and in human tissues. These are

chromosomal abnormality, oxidative stress, and altered growth factors (Tchounwou *et al.,* 2003). Other modes of action that have been examined include altered DNA repair, altered DNA methylation patterns, enhanced cell proliferation, promotion/progression, suppression of P53, gene amplification, and interactions with other chemicals. The genotoxicity database is equivocal with respect to demonstrating a clear mechanism of action for arsenic-induced carcinogenesis. While various hypotheses to explain the carcinogenic activity of arsenic have been proposed, details of molecular mechanisms by which inorganic arsenical compounds induce cancer remain poorly understood (Tchounwou *et al.,* 2003). The mode-of-action studies have suggested a role for arsenic as a cocarcinogen, a promoter, or a progressor of carcinogenesis. Also, the determination of the specific arsenic form(s) responsible for carcinogenic activity remains a matter of some dispute, partially related to the fact that the mechanism of carcinogenic action of arsenic has not been fully determined (WHO-IPCS, 2001; Tchounwou *et al.,* 2003). Some recent studies have suggested that trivalent MMAIII may be a potential contributor to arsenic-induced carcinogenicity (Bernstam and Nriagu, 2000; Yu *et al.,* 2000; Kitchin, 2001), as humans produce more of this metabolite than animals, and some epidemiology studies have associated higher levels of MMAIII with increased odds ratios for developing skin cancer. Trivalent DMAIII has also been suggested as a potential key arsenic metabolite associated with carcinogenesis (Kitchin, 2001). Overall, there is unlikely to be a single mode of action for arsenic carcinogenicity.

Exposure Limits

The following paragraphs relate to inorganic arsenic species only, as all regulatory TRVs that exist for arsenic have been developed from data on inorganic arsenic exposure. Furthermore, human exposure to arsenic in the Greater Sudbury Area is expected to be dominated by inorganic arsenic species.

The following organizations were consulted to select exposure limits for arsenic; the U.S. EPA; MOE; ATSDR; Health Canada; RIVM; NRC; WHO; and, OEHHA. Exposure limits derived by the U.S. EPA were selected for use in this assessment, with the exception of the inhalation RfC, for which the U.S. EPA has not derived a value. Thus, the chronic REL developed by OEHHA was used as a threshold inhalation exposure limit.

The U.S. EPA derives exposure limits for both threshold and non-threshold effects when data is available. The Reference Dose (RfD) and Reference Concentration (RfC) are based on the assumption that a threshold exists for certain toxic non-carcinogenic effects. In general, the RfD (or RfC) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population

(including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The U.S. EPA (1998) has classified arsenic as a Group A carcinogen (human carcinogen). Based on the weight-of-evidence of carcinogenicity in humans by more than one route of exposure, inorganic arsenic compounds are classified by Health Canada (1992) as "carcinogenic to humans" (Group 1). Inorganic arsenic compounds are classified by IARC in Group 1 (carcinogenic to humans) on the basis of sufficient evidence for carcinogenicity in humans and limited evidence for carcinogenicity in animals (IARC, 1987).

Oral Exposure Limits

Non-Carcinogenic (Threshold) Effects

The U.S. EPA (1993) calculated an oral RfD of 0.3 µg As/kg body weight/day based on the epidemiological studies of chronic exposure to arsenic through drinking water (Tseng *et al.,* 1968; Tseng, 1977). Critical effects were hyperpigmentation, keratosis, and possible vascular complications at a lowest-observable-adverse-effects-level of 14 µg As/kg body weight/day. The RfD was based on a NOAEL of 0.8 µg As/kg body weight/day, with the application of an uncertainty factor of three to account for both lack of data on reproductive toxicity in humans, and for differences in individual sensitivity. The U.S. EPA (1993) noted some limitations of the studies, in that the exposure levels were not well-characterized (particularly from foods) and other contaminants were present. Also, there was not a clear consensus among U.S. EPA scientists on the oral RfD, and arguments were made for alternate values that are within a factor of two or three of the currently recommended RfD value (*i.e.*, 0.1 to 0.8 µg/kg/day)(U.S. EPA, 1993). New data that could possibly impact on the recommended RfD for arsenic will be evaluated by the U.S. EPA Work Group as it becomes available. Confidence in the chosen principal study and the resulting oral RfD is considered medium. MOE (1996) adopted 0.3 µg As/kg body weight/day, based on information provided on IRIS in 1993, as the chronic RfD as part of the *Guideline for Use at Contaminated Sites in Ontario* (MOE, 1996). For the purposes of the current assessment, the more conservative exposure limit from U.S. EPA (1998) of 0.3 µg As/kg body weight/day, was selected as the oral exposure limit for non-carcinogenic effects.

Carcinogenic (Non-threshold) Effects

Arsenic exposure *via* the oral route was considered by the U.S. EPA to be carcinogenic to humans, based on the incidence of skin cancers in epidemiological studies examining human exposure through drinking water (Tseng *et al.,* 1968; Tseng, 1977). Based on the application of a linear-quadratic mathematical model to the data from these studies, the U.S. EPA (1998) calculated an oral slope factor of 0.0015 (μg As/kg body weight/day)$^{-1}$. The slope factor (SF) is based on the assumption that carcinogenic effects do not have a threshold (*i.e.,* dose-response relationship is linear to zero exposure). It was assumed that the Taiwanese individuals had a constant exposure from birth, and that males consumed 3.5 L drinking water per day, and females consumed 2.0 L per day. Doses were converted to equivalent doses for U.S. males and females based on differences in body weights and differences in water consumption and it was assumed that background skin cancer risk in the U.S. population would be similar to the Taiwanese population. The multistage model with time was used to predict dose-specific and age-specific skin cancer prevalence rates associated with ingestion of inorganic arsenic; both linear and quadratic model fitting of the data were conducted. The SF of 0.0015 (μg As/kg body weight/day)$^{-1}$ was adopted as the oral exposure limit for carcinogenic effects of arsenic for this assessment.

Recently, there has been concern on the part of regulators regarding the applicability of the arsenic cancer potency estimates for cancers at other sites (specifically bladder cancer) in setting exposure limits for arsenic. The National Research Council (NRC) (1999; 2001) recently re-evaluated drinking water criteria for the United States, based on bladder cancer incidence data in the Taiwanese population as presented in Wu *et al.,* 1989; Chen *et al.,* 1992; Smith *et al.,* 1992. NRC, 1999; 2001 emphasized that the evaluation of cancer potency factors for bladder cancer has been limited by the amount and the quality of data available for use in the linear model. While the bladder cancer value would yield a greater cancer potency than that based on skin cancer, these data are still limited by many of the same problems as the potency factor for skin cancer, including large uncertainty of total daily exposure to inorganic arsenic (*i.e.,* the poor linkage between water concentrations of arsenic and individual exposure, and lack of data on arsenic intake from food), concomitant exposures to other chemicals and carcinogens (which would be especially important if arsenic is a cancer promoter), and differences in nutritional and health status between Taiwanese and North American populations. As the intended use of the cancer potency factor is in the estimation of risk to a particular population in comparison to a "background" or "typical" population, and risks for both will be assessed with the same methodologies and the same exposure limit, the use of the skin cancer potency factor is considered acceptable and conservative.

MOE (1996) selected 0.00175 (µg As/kg body weight/day)$^{-1}$ as the oral cancer potency factor as part of the *Guideline for Use at Contaminated Sites in Ontario*, (MOE, 1996) based on information provided on IRIS in 1993. This number was considered outdated and was not used in the current study.

The SF of 0.0015 (µg As/kg body weight/day)$^{-1}$, corresponding to an RsD of 0.00067 µg As/kg body weight/day for an acceptable risk level of one-in-one million, was adopted as the oral exposure limit for carcinogenic effects of arsenic for this assessment.

Inhalation Exposure Limits

Non-cancer (Threshold) Effects

The U.S. EPA has not established an inhalation RfC or dose for arsenic. Thus, the chronic REL developed by OEHHA (2000) was used.

The OEHHA (2000) used the study by Nagymajtenyi *et al.* (1985) as the basis for deriving the chronic REL. This was accomplished by using the average experimental exposure for the LOAEL group (determined to be 33 µg As/m^3) and applying a cumulative uncertainty factor of 1,000 (10-fold each for use of a LOAEL, interspecies extrapolation and intraspecies differences in sensitivity) to yield a chronic REL of 0.03 µg As/m^3. According to the OEHHA (2000), route-to-route conversion of the LOAEL in the key study indicates that this chronic REL should also be protective of non-cancer adverse effects that have been observed in studies with oral exposures, either in food or drinking water. Also, OEHHA considers that had available human data been used instead of animal data in the REL derivation, a similar value would have been obtained. Thus, the chronic REL from animal data is believed to be protective of potential adverse health effects in humans.

Cancer (Non-threshold) Effects

The U.S. EPA (1998) considers arsenic to be a non-threshold carcinogen. Based on this assumption, the U.S. EPA (1998) calculated an inhalation unit risk value of 0.0043 (µg As/m^3)$^{-1}$, based on studies by Brown and Chu (1983a,b,c), Lee-Feldstein (1983), Higgins *et al.* (1982), and Enterline and Marsh (1982) which indicated increased lung cancer mortality of exposed populations. A geometric mean was obtained for data sets obtained with distinct exposed populations (Anaconda smelter and ASARCO smelter), and then the final estimate was the geometric mean of those two values. It was assumed that the increase in age-specific mortality rate of lung cancer was a function only of cumulative exposures. The unit risk was

converted to a slope factor (SF) of 0.015 (µg As/kg body weight/day)$^{-1}$ assuming a 70 kg adult breathes 20 m^3/day. It should be noted that all of the studies used to derive the U.S. EPA unit risk value had a number of confounding factors and uncertainties. These included: confounding by concurrent exposure to airborne dusts, sulphur dioxide and other chemicals; lack of measured air concentrations in some studies; failure to consider latent periods for lung cancer development; and, confounding by smoking.

Dermal Exposure Limit

There are currently no dermal arsenic exposure limits that have been developed by regulatory agencies. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in a human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF_{dermal}). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary:

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).
AF*dermal* = the fraction of the applied chemical absorbed through the skin.
AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

Criteria Summary

The following table summarizes the arsenic exposure limit selected for the current study.

Summary Table of Exposure Limits for Arsenic Selected for the HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Cancer (Non-threshold) Effects					
Oral	0.0015 $(\mu g/kg/day)^{-1}$	SFo	skin cancer	Tseng *et al.*, 1968; Tseng, 1977	U.S. EPA (1998)
Inhalation	0.0043 $(\mu g/m^3)^{-1}$	IUR	lung cancer	Brown and Chu (1983a,b,c); Lee-Feldstein (1983); Higgins *et al.* (1982); Enterline and Marsh (1982)	U.S. EPA (1998)
	0.015 $(\mu g/kg/day)^{-1}$	SFi			
Dermal	NA		NA	NA	NA
Non-cancer (Threshold) Effects					
Oral	0.3 µg/kg/day	RfD	hyperpigmentation, keratosis, and possible vascular complications	Tseng *et al.*, 1968; Tseng, 1977	U.S. EPA (1993)
Inhalation	0.03 $\mu g/m^3$	Chronic REL	increased fetal malformations	Nagymajtenyi *et al.* (1985)	OEHHA (2000)
Dermal	NA		NA	NA	NA

NOTES:
NA = not available; SFo = oral slope factor; IUR = inhalation unit risk; SFi = inhalation slope factor; RfD = reference dose; REL = reference exposure level.

References

For a complete list of references, see Section A1-5.0 of the detailed toxicological profile on the accompanying CD.

A1-1.0 CHEMICAL CHARACTERIZATION AND GENERAL OVERVIEW

Several arsenical compounds are discussed in the following sections, including inorganic forms: trivalent arsenic (As[III] or As^{III}), the valency found in arsenite (AsO_3^{3-} or AsO_2^{1-}) and arsenic trioxide; pentavalent arsenic (As[V] or As^{V}), the form found in arsenate (AsO_4^{3-}, $HAsO_4^{2-}$, or $H_2AsO_4^{1-}$); as well as organic arsenic compounds: monomethylarsonic acid (MMA^{V}; $CH_3AsO[OH]_2$) and dimethylarsinic acid (DMA^{V}; $[CH_3]_2AsO[OH]$), the predominant metabolites of inorganic arsenic in humans; as well as arsenobetaine ($[CH_3]_3As^{+}CH_2COOH$) and arsenocholine, organic arsenicals found predominantly in fish and other seafood. For the purposes of this discussion, the term seafood is meant to indicate fish and shellfish (crustaceans and bivalves) derived from freshwater or marine environments for human consumption, and does not include seaweed- or kelp-based products. Structures of these arsenic compounds are shown in Figure A1-1.

In general, there is much less information available on the pharmacokinetics of organoarsenicals than there is on inorganic arsenic compounds. Nonetheless, available evidence indicates that organoarsenicals are metabolized to a much lesser extent than inorganic arsenic and are more rapidly eliminated in both laboratory animals and humans (ATSDR, 2005; WHO-IPCS, 2001).

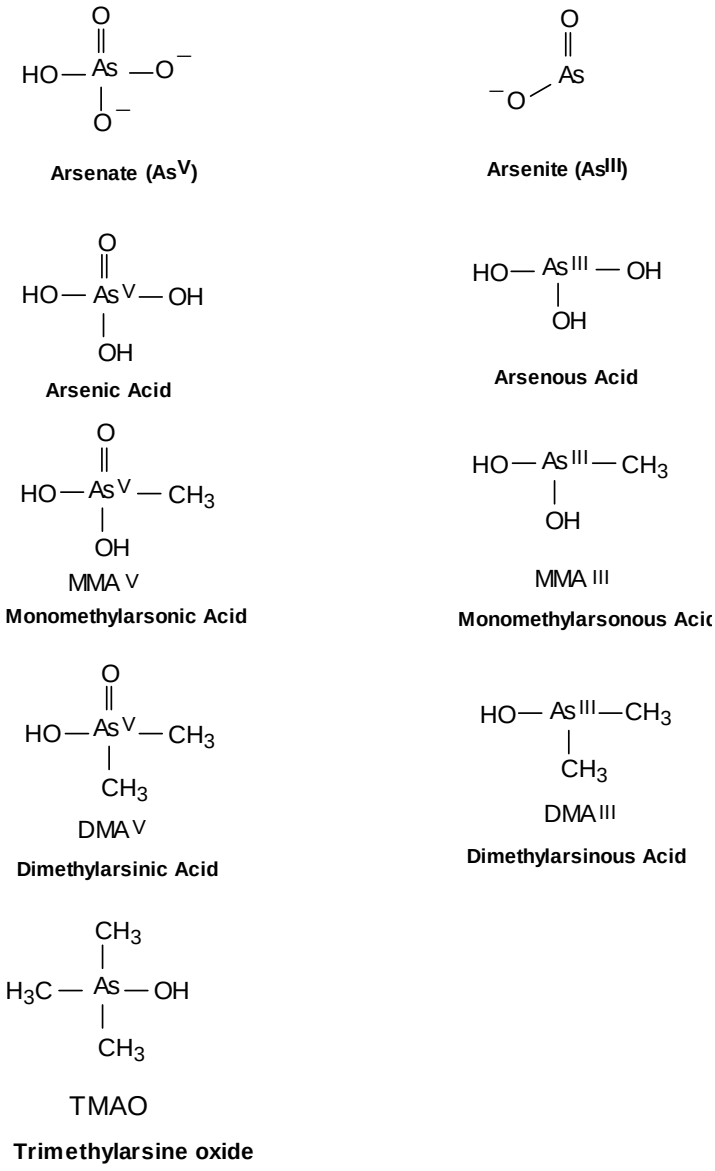


Figure A1-1 Structures Of Some Toxicologically Relevant Arsenic Compounds. Adapted From Hughes, 2002

A1-2.0 ABSORPTION AND TOXICOKINETICS OF ARSENIC

A1-2.1 Oral Absorption

Factors influencing the absorption of arsenic from the gastrointestinal tract include:

- The speciation of the arsenic compound under consideration;
- The physical-chemical properties of the arsenic species, especially its solubility;
- The magnitude of the dose;
- The matrix in which the exposure is received; and,
- The animal species, especially the physiological characteristics of the digestive system.

The impacts of these factors on the oral bioavailability of arsenic compounds are discussed in the following paragraphs.

With regard to matrix effects, it is generally true that the greater the solubility of arsenic in the matrix in which it is present, the greater the absorption (NAS, 1977). For oral ingestion, water solubility of the arsenic compounds is well known to have the greatest impact on oral bioavailability (Hrudey *et al.,* 1996). Absorption in the gastrointestinal tract appears to occur by simple passive diffusion in humans and mice, although there is some evidence for a saturable carrier-mediated transport process for arsenate in rats (Gonzalez-Zulueta *et al.,* 1995).

Greater than 95% of water-soluble inorganic trivalent arsenic may be absorbed from the gastrointestinal tract of humans (Coulson *et al.,* 1935; Ray-Bettley and O'Shea, 1975; Pomroy *et al.,* 1980). Similarly, the results of animal studies indicate that soluble arsenic compounds are readily absorbed following oral exposure, based on proportions of the administered dose of arsenic recovered in the faeces. For example, the percentage absorption ranges from 68 to 98% (mice and monkeys), 60 to 90% (hamsters) and 51 to 67% (rabbits and rats) (Charbonneau *et al.,* 1978a,b; Vahter and Norin, 1980; Odanaka *et al.,* 1980; Yamauchi and Yamamura, 1985; Marafante *et al.,* 1987; Marafante and Vahter, 1987; Freeman *et al.,* 1993; 1995). Reduced oral bioavailability is observed with the less soluble forms of arsenic (*e.g.*, arsenic trisulfide, lead arsenate, arsenic triselenide) in both humans (Mappes, 1977) and laboratory animals (Marafante and Vahter, 1987).

A1-2.1.1 Oral Bioavailability in Soil

The oral bioavailability of inorganic arsenic has been reported to be considerably reduced when administered in a soil or dust matrix, such that soil-bound arsenic is unlikely to be accumulated by exposed human receptors to a high level (Hrudey *et al.,* 1996).

There are a number of studies which demonstrate that the absorption of arsenic in ingested dust or soil is likely to be considerably less than absorption of arsenic from ingested salts (Davis *et al.,* 1992, 1996; U.S. EPA 1997; Freeman *et al.,* 1993, 1995; Pascoe *et al.,* 1994; Rodriguez *et al.,* 1999). Freeman *et al.* (1993) found that the oral bioavailability of arsenic associated with a soil matrix was 24% in rabbits, when normalized for concentrations of arsenic in urine following intravenous (*i.e.,*) administration of the same compound. Freeman *et al.* (1995) studied the bioavailability of arsenic in soil and house dust from the vicinity of a mine and smelter facility following administration to female Cynomolgus monkeys *via* gel capsules. The mean absolute percentage oral bioavailability values were reported to be 19 and 14% (based on normalization for urinary concentrations following i.v. administration) or 10 and 11% (based on normalization for blood concentrations following i.v. administration) for arsenic in house dust and soil matrices, respectively. These values are in agreement with that of Groen *et al.* (1994), who reported the oral bioavailability of inorganic arsenic (arsenic pentoxide) in a bog ore-containing soil matrix was 8.3% in dogs, based on normalization of urinary excretion following oral administration of a single dose of 0.6 mg As/kg body weight.

However, it has also been shown that arsenic bioavailability in a soil matrix is highly variable across sites and is greatly dependent on site-specific soil properties, and the predominant arsenic species. The U.S. EPA (1997) studied the bioavailability of arsenic in soil in juvenile swine that received daily oral doses of soil or sodium arsenate (in food or by gavage) for 15 days. The soils were from various mining and smelting sites and contained arsenic at concentrations ranging from 100 to 300 µg/g, and lead at concentrations ranging from 3,000 to 14,000 µg/g. The daily arsenic doses ranged from 1 to 65.4 µg/kg/day. The mean relative bioavailability of soil-borne arsenic ranged from 0 to 98% in soils from seven different sites (mean ± SD = 45% ± 32). Estimates for relative bioavailability of arsenic in samples of smelter slag and mine tailings ranged from 7 to 51% (mean ± SD, 35%±27). In a similar study with juvenile swine, Rodriguez *et al.* (1999) estimated the relative bioavailability of arsenic in mine and smelter wastes (soils and solid materials). The wastes included iron slag deposits and calcine deposits and had arsenic concentrations that ranged from 330 to 17,500 µg/g. Relative bioavailability was found to range from 3 to 43% for 13 samples (mean = 21%) and was higher in iron slag wastes (mean = 25%)

than in calcine wastes (mean = 13%). Ng and Moore (1996) investigated the comparative bioavailabilities of arsenic in soil from a site contaminated with copper chrome arsenate (CCA) and soil contaminated by arsenic solutions used in cattle tick control, using a rat model. Soil from the cattle dip site had a bioavailability of 8.1 ± 4%, 14.4 ± 7% and 60 ± 3.4% when compared with orally administered sodium arsenite, calcium arsenite and sodium arsenate respectively. For CCA-contaminated soil the bioavailabilities were 13.0 ± 4.5%, 32.2 ± 11.2% and 38.0 ± 13.2%. In a subsequent study, Ng *et al.* (1998) used a rat model to determine the the absolute bioavailability of arsenic in soils containing 32 to 1597 µg As/kg (0.32 to 56% arsenite) from a combination of arsenical pesticides and natural geological formations in a residential area. The absolute bioavailability was found to range from 1.02 to 9.87% relative to arsenite, and from 0.26 to 2.98% relative to arsenate. This study also attempted to develop a suitable leachate test as an index of bioavailability. However, the results indicated that there was no significant correlation between the bioavailability and leachate test data, using either neutral pH water or 1M hydrochloric acid.

The basis for the reduction of bioavailability of arsenic compounds in soils and house dusts in the vicinity of mining and smelter operations is believed to be related to the fact that arsenic from these sources tend to be in less soluble forms (*e.g.*, metal-arsenic oxides and phosphates) and, as a result of containment within a solid matrix, to be less accessible for dissolution or uptake (Davis *et al.,* 1996). This has been supported by studies conducted with *in vitro* simulations of the gastric and/or intestinal fluids (*e.g.*, Hamel *et al.,* 1998; Rodriguez *et al.,* 1999; Ruby *et al.,* 1996, 1999; Williams *et al.,* 1998). These studies have found that when soils containing arsenic are incubated in simulated gastrointestinal fluids, only a fraction of the arsenic becomes soluble. Estimates of the soluble, (*i.e.,* bioaccessible) arsenic fraction have ranged from 3 to 50% for various soils and mining and smelter wastes (Rodriguez *et al.,* 1999; Ruby *et al.,* 1996).

More recently, Turpeinen *et al.* (2003) reported a significant positive correlation between the concentration of total water-soluble As and its bioailability in contaminated soils. However, the bioavailability varied widely between sampling sites (*i.e.,* bioavailable arsenic ranged from 3 to 77% of total water-soluble As in soil). The authors also noted that aging and sequestration of As occurs in contaminated soils which can make arsenic compounds progressively less bioavailable with time.

The results of these studies clearly demonstrate the importance of obtaining site-specific information on arsenic speciation and bioavailability, as the use of default absorption factors for assessing human health risk may substantially overestimate actual absorption from soils (Casteel *et al.,* 2001).

A1-2.1.2 Food

There were no data identified in the published literature for the bioavailability of inorganic arsenic compounds in food. The OMOE (1994) cited an assumed value of 90% absorption of arsenic compounds in various food types; however, this value appears high given the information presented above on studies of various inorganic arsenic species. There is some evidence that diets high in fibre may result in lower rates of inorganic arsenic absorption than diets containing little fibre. For example, Kenyon *et al.* (1997) found that feeding a diet lower in fibre or "bulk" to female B6C3F1 mice increased absorption of sodium arsenate by roughly 10% compared to a standard rodent chow diet, after a single oral dose of 5 mg As/kg.

Organic arsenic compounds, including arsenocholine, MMA and DMA, are readily absorbed following oral administration in humans and laboratory animals, with absorption reported to be greater than 75% (Stevens *et al.,* 1977; Buchet *et al.,* 1981a; Yamauchi and Yamamura, 1984; Marafante *et al.,* 1987; Yamauchi *et al.,* 1988). Tam and Lacroix (1980) reported greater than 99% absorption of organic arsenic in fish in two human volunteers. However, this form of arsenic is not metabolized and is excreted in urine in an unaltered form.

A1-2.2 Inhalation Absorption

The published literature indicates that the key parameters upon which pulmonary absorption of arsenic depends includes particle size, and resultant degree of deposition of particulate-borne arsenic in the lungs, as well as the specific arsenic compound and its solubility. Buchet *et al.* (1997) states that absorption of arsenic in the lungs depends on a combination of the following factors: chemical species, particle size, and dust load in the lung.

The role of solubility of the arsenic species on absorption in the lungs has been the subject of many studies in which arsenic was administered *via* intratracheal instillation. In general, water soluble forms of arsenic are more rapidly absorbed than less soluble forms (Hrudey *et al.,* 1996). It has been reported that 95 to 99% of doses of soluble arsenic compounds (as sodium arsenite, sodium arsenate, arsenic trioxide) were absorbed from the lung within 1 day (and 99.6 to 99.8% are absorbed within 3 days) following intratracheal instillation in rats and hamsters (Inamasu *et al.,* 1982; Pershagen *et al.,* 1982; Rhoads and Sanders, 1985; Marafante and Vahter, 1987). Insoluble arsenic compound (*e.g*., arsenic trisulfide, lead arsenate and calcium arsenate) were absorbed from the lung more slowly (98.7, 55 and 50%, respectively, after 3 days; Marafante and Vahter, 1987). About 64 to 74% of particulate-bound arsenic (bound to fly ash and copper smelter dust) was absorbed from the lungs of hamsters 48 hours after intratracheal

administration, whereas arsenate and arsenite were completely absorbed from the lungs in the same time period, when administered with an inert dust material (Buchet *et al.,* 1995).

The importance of the chemical form of arsenic is demonstrated by Yager and Wiencke (1997). These authors noted that the predicted urinary arsenic out-put for workers exposed to 10 $\mu g/m^3$ arsenic was more than one-third lower for boiler maintenance workers in a coal-fired power plant than it was for copper-smelter workers. This finding was attributed to the fact that the arsenic in coal fly ash in their study was predominantly in the form of calcium arsenate, whereas in the copper smelter work environment the arsenic was in the form of As_2O_3.

As mentioned, absorption of particulate-bound arsenic following inhalation is greatly dependent on the dynamics of deposition, retention and clearance of airborne particles by the respiratory system. Based on the airborne particle dynamics model, the smaller airborne particles (<2 um) are respirable and would enter, and be retained in the respiratory system, and arsenic associated with these particles would be subject to absorption within the lungs. Larger airborne particles would be cleared from the respiratory system by mucociliary action and swallowed; arsenic adsorbed to these particles would be subject to gastrointestinal absorption (U.S. EPA, 1984b).

Pinto *et al.* (1976) and Smith *et al.* (1977) studied the relationship between concentrations of arsenic in the urine of copper smelter workers and in workplace air, based on particle size distributions. It was noted that there was a stronger correlation between urinary concentrations and concentrations in the particulate fraction composed of larger particles (> 5 um), indicating that in exposures to particulate-borne arsenic, clearance and swallowing of particulates, with subsequent absorption in the gut, may be a more important route of exposure than inhalation.

Holland *et al.* (1959) studied the deposition and absorption of arsenic in the respiratory system of hospitalized lung cancer patients. Radiolablelled arsenite aerosols were administered *via* cigarette usage. Approximately 40% of the arsenic inhaled was actually deposited in the lungs, and of this, 75 to 85% was absorbed, resulting in an overall absorption of 30 to 34% (*i.e.*, 75 to 85% of 40% equals 30 to 34%). While the health status of the subjects were not controlled in this study, these results are in general agreement with the range of values for arsenic absorption from the lung as discussed above, and what is known about general airborne particle dynamics. Given that for typical airborne exposures, about 13% of airborne particles are respirable (Brain and Mosier, 1980), and that soluble arsenic compounds are almost completely absorbed in the lungs (about 99%), the absorption of particle-borne soluble arsenic would approximate 13%, a value lower than the range indicated by Holland *et al.* (1959). The U.S. EPA (1984a)

used the lower value of 30% from Holland *et al.* (1959) to represent the fraction of inhaled arsenic that is absorbed by humans.

There are little data available regarding the absorption of organic arsenic *via* inhalation. Stevens *et al.* (1977) reported that 92% of a dose of DMA was absorbed following intratracheal instillation in the rat, indicating that, like inorganic forms of arsenic, once in the lower regions of the lung, absorption of organic arsenic is almost complete.

In summary, the bioavailability of inorganic arsenic to which subjects are exposed *via* inhalation appears to be primarily determined by airborne particle dynamics, and would be in the range of 30 to 34%..

A1-2.3 Dermal Absorption

There are few data available on the absorption of arsenic compounds through the skin, although the available studies indicate that the dermal bioavailability of arsenic is low. Dutkiewicz (1977) conducted a study of the absorption of sodium arsenate through the skin of rats whose tails were immersed in an aqueous solution for 1 hour. Arsenic concentrations in blood, liver, and spleen increased over 5 days following exposure. Based on this observation, ATSDR (2000) suggested that arsenic may initially bind to the skin and then be slowly absorbed into the blood stream even if exposure is discontinued.

Measurements of *in vitro* percutaneous absorption of As^{III} and As^{V} using artificial human skin showed evidence of strong affinity for human keritinocytes (Bernstam *et al.,* 2002). Data from permeation experiments resulted in etention of approximately 30% of the As^{V} passing through artificial skin preparations. By comparison, over 90% of As^{III} applied in this system was retained in artificial skin and not passed through. It may be inferred from these results that a higher percentage of As^{V} applied to the skin surface of a human would get into the systemic circulation, and thus result in exposure to internal organs. On the other hand, direct application of As^{III} to skin would be expected to result in a greater retention of arsenic in the epidermal layer due to the higher affinity of As^{III} for keratinocyte proteins (Bernstam *et al.,* 2002).

Basing the rate of absorption of arsenic species through artificial skin, Bernstam *et al.* (2002) determined a permeability constant (K_p) for arsenic according to the equation:

$$\mathbf{K_p\ (cm/h) = Q/(A \times C \times t)}$$

Where *Q* is the total absorbed dose in micrograms, *A* is the surface area exposed, *t* is the timein hours, and *C* is the concentration gradient across the skin membrane. The percutaneous absorption constants were used to roughly estimate the dermalabsorption of arsenic from bathing water. Using the average surface areas for an adult (20,000 cm^2) and an eight year old child (10,000 cm^2) and the average body weights of 70 kg for an adult and 28.2 kg for a child as recommended in the EPA Exposure Factors Handbook (U.S. EPA 1999), the exposure from showering for 15 minutes per day with water containing different levels of arsenic could be calculated (Bernstam *et al.,* 2002). These calculations show that an adult showering with water containing 100 µg/L As^{III} can account for about 20% of the recommended RfD for arsenic (0.3 µg/kg day^{-1}). At a concentration of 500 µg/L of either As^{III} or As^{V} in shower water, 50% of the recommended RfD for Arsenic could be achieved from solely from dermal absorption in adults or a child (Bernstam *et al.,* 2002).

In a study that employed mature pig skin, Trukall *et al.* (2003) examined the rate of penetration of As^{V} (H_3AsO_4) alone or when in the presence of a mixture of organic compounds commonly found at hazardous waste sites. The binding capacity of arsenic (^{73}As) alone amounted to 44.2% of the dose. Approximately 60% of the dose could be washed from the surface with soap when arsenic was applied alone. The effect of the application of As in a mixture with organic chemicals (chloroform, toluene, phenanthrene) was to enhance binding and to reduce the capability for soap wash to remove bound arsenic from skin preparations.

Wester *et al.* (1993) reported that dermal absorption of arsenic in monkeys from soil ranged from 3.2 to 4.5% (high and low dose respectively), while comparable rates of absorption As in water ranged from 2 to 6.4%. Only 0.8 to 1.9% of inorganic arsenic, in soil and water respectively, was found to penetrate preparations of human skin *in vitro* (Wester *et al.,* 1993).

Wester *et al.* (2004) have examined the permeability of arsenic (As^{V} as sodium arsenate heptahydrate in water) when applied to the skin of the Rhesus monkey. The Rhesus monkey is a relevant animal model for *in vivo* human percutaneous absorption (Wester *et al.,* 2004). Urinary arsenic data following dermal application of As^{V} produced variable results in the monkeys tested. For the three animals examined, total arsenic mass excreted over a period of 96 hours ranged from 7.3 to 39.7 to 51.3%. The percent absorption over the same period resulted in 0.6, 3.4 and 4.4% (2.8% ± 1.9 [standard deviation]) of applied dermal dose of soluble arsenic. These recent results in monkeys generally confirm the earlier results of Wester *et al.* (1993).

In contrast to results of exposure to soluble inorganic arsenic, Wester *et al.* (2004) found that arsenic bound to finely ground particulate prepared from chromated copper arsenate (CCA) wood structures was not absorbed. In similar tests with monkeys, only 0.04 % (± 0.07) of arsenic was bioavailable for percutaneous absorption. Thus the form of As in the environment is extremely important for the assessment of dermal exposure. A detailed analysis of the bioavailablity of arsenic from CCA treated wood structures has been reported by Hemond and Solo-Gabriele (2004). These authors used the conservative assumption of 100% bioavailability *via* the skin that in light of Wester *et al.* (2004) may greatly overestimate actual exposure.

There are limited data available on the *in vivo* and *in vitro* dermal absorption of arsenical herbicides in laboratory animals. Rahman and Hughes (1994) found that a constant fraction of the dose (12.4%) in water vehicle was absorbed during a 24 h period over the entire applied dose range (10 to 500 µg), for monosodium and disodium salts of monomethylarsonate applied to clipped dorsal skin of B6C3F1 mice. This observation was unaffected by vehicle volume. Using the same experimental system with DMA, Hughes *et al.* (1995) found no significant dose-dependency in absorption over a 24 h period. However, in this study, vehicle volume did have a significant effect on absorption, which ranged from 7 to 40% and decreased with increasing volume of water. In both these studies percutaneous absorption of the arsenical herbicides from soil was estimated to be very low (< 1%). Shah *et al.* (1987) studied the *in vivo* percutaneous absorption of MMA (monosodium salt) and DMA (disodium salt) in young (33 day old) and adult (82 day old) Fischer 344 rats. Three dose levels of each compound were applied in an aqueous vehicle, and absorption over 72 h was determined. Both compounds exhibited similar absorption values in both young and adult animals over the dose range studied, but the young animals absorbed significantly less of each. The total percutaneous absorption, expressed as a mean of all doses for both compounds, was 15.1 and 3.01% of the recovered dose in old and young rats, respectively.

A1-2.4 Distribution

Distribution of arsenic within the body is affected by the route through which exposure occurs. Once absorbed into the body's bloodstream, As binds to the globin portion of hemoglobin and is rapidly distributed to various tissues and organs in red blood cells (Malachowski, 1990).

Arsenic is widely distributed within the body and arsenic concentrations in any given tissue have been found to be highly variable in the available phamacokinetic studies (WHO-IPCS, 2001).

The available studies indicate that oral exposure resulted in higher initial distribution to the liver, in comparison to intravenous and subcutaneous exposures (Charbonneau *et al.,* 1979; Vahter, 1981; Marafante *et al.,* 1985). In contrast, the distribution of arsenic in the body following inhalation and intravenous exposure resulted initially in accumulation in the liver, lungs, muscle and gastrointestinal epithelium in mice, marmoset monkeys and rabbits (Lindgren *et al.,* 1982; Vahter *et al.,* 1982; Vahter and Marafante, 1983).

However, given sufficient time for equilibration within the body, arsenic generally tends to be evenly distributed amongst various tissues within the body, with slight elevations in the concentrations of arsenic in nails and hair; this has been observed in humans following oral exposure (Liebscher and Smith, 1968; Kurttio *et al.,* 1998) and in mice, monkeys, rabbits and hamsters following oral, intravenous and intratracheal administration (Stevens *et al.,* 1977; Vahter and Norin, 1980; Lindgren *et al.,* 1982; Vahter *et al.,* 1982; Vahter and Marafante, 1983, 1988; Rhoads and Sanders, 1985; Yamauchi and Yamamura, 1985; Hood *et al.,* 1987, 1988; Marafante and Vahter, 1987). Tissue distribution studies have demonstrated that arsenic readily transfers across the placenta in mice (Hood *et al.,* 1987, 1988; Lindgren *et al.,* 1984), and that arsenic can penetrate the blood-brain barrier (Ghafgazi *et al.,* 1980; Valkonen *et al.,* 1983).

However, the levels in brain tissue are low relative to other tissues, which indicates that arsenic (when administered in the form of sodium salts in acute dosing studies) does not readily cross the blood–brain barrier or accumulate in brain tissue (WHO-IPCS, 2001). There is also evidence that arsenic readily crosses the placenta in humans. Concha *et al.* (1998b) reported similar arsenic concentrations in cord blood and maternal blood (roughly 9 µg/L) of maternal–infant pairs exposed to drinking-water containing high levels of arsenic (around 200 µg/L). Tissue analysis of organs taken from an individual following death from ingestion of 8 g of arsenic trioxide (roughly 3 g of arsenic) showed higher concentrations of arsenic in liver (147 µg/g) than in kidney (27 µg/g) or muscle, heart, spleen, pancreas, lungs, or cerebellum (11 to 12 µg/g) (Benramdane *et al.,* 1999). Smaller amounts were found in other parts of the brain (8 µg/g), skin (3 µg/g), and hemolyzed blood (0.4 µg/g).

The distribution of specific arsenic compounds within the body is dependent on interactions with certain tissue types, as well as physiological characteristics of different animal species. Vahter and Marafante (1983) observed that more arsenic is retained in the tissues of mice and rabbits when injected as arsenite than when injected as arsenate. This difference in distribution was believed to be due to the stronger affinity of trivalent arsenic (As[III]) for sulfhydryl groups based on the observed concentrations in

sulfhydryl-rich tissues such as keratin, found in hair and nails. This is supported by numerous studies that found that skin, hair, and other tissues rich in squamous epithelium (*e.g.,* mucosa of the oral cavity, oesophagus, stomach and small intestine) have a strong tendency to accumulate and maintain higher levels of arsenic (*e.g.,* Lindgren *et al.,* 1982; Yamauchi and Yamamura, 1985). As^{III} as MMA^{III} binds to proteins crich in sulfhydryl groups such as keratin in these tissues (Lindgren *et al.,* 1982; Hughes *et al.,* 2003). Regardless of the form of arsenic administered, trivalent arsenic tends to be the primary form interacting and binding to tissues in most species (Vahter and Marafante, 1983; Vahter, 1994).

Pentavalent arsenic (As[V]) may also interact with tissues. Based on its similarity in chemical properties to phosphate, it may substitute for phosphate in enzyme-catalyzed reactions and become incorporated into bone (Mitchell *et al.,* 1971; Vahter, 1981; Vahter and Marafante, 1988). However, the metabolic fate of As^{V}, discussed below, tends to limit this form of tissue interaction. As^{V} also tends to be rapidly cleared from the blood stream into the kidney, where it may be excreted or, based on its similarity to phosphate, be actively reabsorbed (Vahter and Marafante, 1988). These differences in distribution between trivalent and pentavalent arsenic, dependent on mechanisms of metabolism and excretion, result in different profiles of tissue interaction and retention, and thus possibly toxic potency, as discussed below.

There are limited data regarding the distribution of organic forms of arsenic following absorption; however, the few available laboratory studies indicate that DMA and MMA are rapidly cleared from the blood and distributed amongst all tissues of the body following oral and intratracheal exposure (Stevens *et al.,* 1977; Yamauchi and Yamamura, 1985; Yamauchi *et al.,* 1988; Vahter *et al.,* 1984). Stevens *et al.* (1977) also reported that DMA is capable of crossing the placenta of rats. One study investigating the tissue distribution of organoarsenicals in human volunteers ingesting ^{74}As-labelled arsenobetaine reported that arsenobetaine is rapidly and widely distributed in soft tissues with no major concentration in any region or organ, and that greater than 99% of radioactivity was eliminated from the body within 24 days (Brown *et al.,* 1990).

While the distribution of arsenic species within the body is generally similar for most species studied, including humans, a significant deviation from the distribution profile described above is seen in the rat. In the rat, a large proportion of arsenic has been observed to bind to hemoglobin in the erythrocytes, in the form of DMA^{V} (*i.e.,* following metabolism, discussed below) (Vahter, 1994). Arsenic distribution in rats is strongly associated with high-molecular-weight cellular components in liver and kidney, whereas in rabbits it is associated with low-molecular-weight, more readily diffusible cellular components (Marafante *et al.,* 1982). Also, in the marmoset monkey, arsenite (As^{III}) or arsenate (As^{V}) show a unique

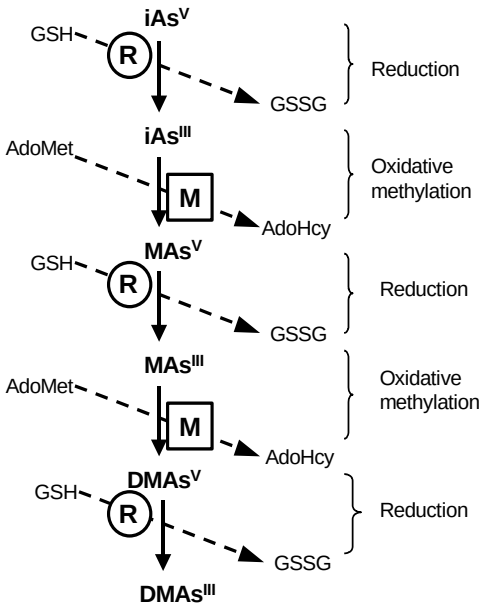
tendency to bind strongly with the rough endoplasmic reticulum in the liver. This binding has not been readily observed in other laboratory animals (Vahter *et al.,* 1982; Vahter and Marafante, 1985).

Clearance of arsenic from the blood of experimental animals is di- or triphasic with estimates of clearance half-lives from blood ranging from approximately 24 to 200 hours (Vahter 1983; Tam *et al.,* 1979; DeKimpe *et al.,* 1996; Marafante *et al.,* 1985). It has been reported that clearance of As from the blood occurs faster following oral exposure than it does after inhalation exposure (Magos, 1991). Zhang *et al.* (1998) report that inorganic arsenic also binds to other serum proteins, with transferrin the main carrier protein. However, these authors studied individuals with renal disease (patients with this condition tend to accumulate arsenic in serum), thus; these results may not be typical of the general population. Distribution of As to liver, kidney, spleen, lung and GI tract often occurs within the first day of exposure with arsenic incorporation into hair, nails, skin and bone occurring within 2 to 4 weeks (U.S. EPA, 1985; Vahter, 1983; Malachowski, 1990). Bone, muscle and skin tissue are the major depots of the arsenic body burden (Hrudey *et al.,* 1996).

A1-2.5 Metabolism

Most mammals metabolize inorganic arsenic *via* methylation to methylarsonic acid (MMA) and dimethylarsinic acid (DMA). The methylation occurs *via* alternating reduction of pentavalent arsenic (As^{V}) to trivalent As^{III}) and addition of a methyl group. The postulated sequence of events includes binding of As^{III} to a dithiol, a carrier protein, before the methyl groups are attached (Vahter, 2002). *S*-Adenosylmethionine (SAM) is the main methyl donor in arsenic methylation. Chemical inhibition of the SAM dependent methylation by periodate-oxidized adenosine in mice and rabbits has been shown to result in a marked decrease in the methylation of arsenic (Marafante *et al.,* 1985). Reduced dietary intake of foods rich in methionine (a source of methyl groups) resulted in lower arsenic methylation (Vahter and Marafante, 1987). *In vitro* studies of rat liver preparations incubated with As^{III} confirmed a requirement of SAM for the formation of MMA and DMA (Buchet and Lauwerys, 1988; Styblo *et al.,* 1995).

The nature of the interaction of arsenic with various tissues is dependent on the chemical form of arsenic. Thus, there are a large number of animal studies that have examined the toxicological effect of As administered in its trivalent or pentavalent form, as well as an inorganic or organic compound.



Note: DM is Dimethylated Arsenic. GSH is Glutathione; GSSG is Glutathionedisulphide. AdoMet is *S*-adenosylmethionine; AdoHcy is *S*-adenosylhomocysteine; R is As^{V} Reductase; M is As^{III} Methyltransferase. Adapted from Styblo *et al.,* 2002

Figure A1-2 Scheme Showing The Metabolic Conversions of Inorganic Arsenic (iAs) in Humans. MAs^{V} is Methylated As(V). MAs^{III} is Methylated As(III).

While much scientific progress has been made in recent years, there is much about the metabolism of inorganic arsenic that is not fully understood (Vater, 2002). In general, once absorbed, some dissolved As^{V} is reduced to As^{III}, while some As^{III} is oxidized to As^{V} (Vahter, 2002; Malachowski, 1990). Both species are metabolized in many tissues, but primarily in the the liver (especially after ingestion) *via*methylases, resulting in formation of methylarsonic acid, dimethylarsinic acid, and trimethylarsenic compounds. In many species, the two main steps in arsenic metabolism are: (1) reduction of pentavalent to trivalent arsenic, and (2) oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form mono-, di- and trimethylated products using S-adenosyl methionine (SAM) as the methyl donor and GSH as an essential co-factor; see Figure A1-2.

An unusual feature of arsenic metabolism is that there are extreme qualitative and quantitative interspecies differences in methylation to the extent that some species do not appear to methylate arsenic at all (Styblo *et al.,* 1995; Vahter, 1999). For example, methylated metabolites are low to undetectable in the urine of marmoset monkeys administered either arsenite (As^{III}) or arsenate (As^{V}) (Vahter *et al.,* 1982;

Vahter and Marafante, 1985) and chimpanzees administered arsenate (Vahter *et al.,* 1995). Studies of liver cytosol from marmoset and tamarin monkeys (Zakharyan *et al.,* 1996) and guinea pigs (Healy *et al.,* 1997) also indicate that these species are deficient in methyltransferase activity when compared to species such as the rabbit, which readily methylate inorganic arsenic compounds.

Methylation reactions in biometabolism are generally considered a detoxification process (as methylated compounds tend to be rapidly excreted). Recent studies indicate that reduced trivalent intermediates, monomethylarsenous acid (MMA^{III}), and dimethylarsinous acid (DMA^{III}), but particularly MMA^{III} are of greater toxic potency than either the inorganic parent compounds or other methylated arsenic species (Styblo and Thomas, 1997; Petrick *et al.,* 2000; Styblo *et al.,* 2000; 2002; NRC, 2001). MMA^{III} and DMA^{III} have been detected in bile and urine of humans exposed to inorganic arsenic (Aposhian *et al.,* 2000; Gregus *et al.,* 2000; Vahter, 2002). Due to the greater toxicity of these trivalent methylated forms as compared with pentavalent methylated forms and to As^{III}, it is an oversimplification to assume that methylation leads specifically to detoxification. The formation and distribution of trivalent methylated forms of arsenic might be associated with increased toxicity (NRC, 2001). At this time, the extent to which MMA^{III} and DMA^{III} contribute to the observed toxicity following exposure to inorganic arsenic is not fully understood (Vahter, 2002).

As shown in Figure A1-2, the basic metabolic reactions affecting the fate of inorganic arsenic include reduction-oxidation reactions and methylation. After absorption of inorganic arsenic into the bloodstream, and during its subsequent distribution and excretion, a proportion of As^{V} (methylated) is reduced to As^{III} (methylated). The urinary profile of arsenic compounds in most species, including humans, indicates a rapid reduction of As^{V} to As^{III} followed by methylation (Yamauchi and Yamamura, 1979; Lerman and Clarkson, 1983; Vahter and Marafante, 1988).

The methylation process of As^{III} (Figure A1-2) is mediated by enzymes known as methyltransferases, with glutathione (GSH) and *S*-adenosylmethionine (SAM) acting as essential cofactors in the reaction (Buchet and Lauwerys, 1985; 1987; 1988; 1994; Styblo *et al.,* 1996, 2002). The methyltransferase enzymes are believed to contain a thiol group, through which a dithiol-arsenic intermediate functions as a methyl receptor (Thompson, 1993; Mushak and Crocetti, 1995). In laboratory studies, species lacking methyltransferase activity, such as marmoset monkeys, did not metabolize inorganic arsenic into DMA^{V}, but rather accumulated it or excreted it as inorganic arsenic (Vahter, 1994; Aposhian, 1997). Based on the results of inhibition and substrate-substitution studies, Buchet and Lauwerys (1985; 1994) postulated that while different enzyme systems were responsible for the production of MMA^{V} and DMA^{V} in

humans, the same methyl donors were used in both processes. The time-course of appearance of MMA^V and DMA^V and inorganic arsenic in the urine of humans also led several other authors (Foà *et al.,* 1984; Apostoli *et al.,* 1997) to the conclusion that there are two successive methylating processes responsible for production of MMA^V and DMA^V. Under normal conditions, availability of *S*-adenosylmethionine, acting as the methyl group donor, is not the rate-limiting factor in the methylation of arsenic (Buchet *et al.,* 1981a; Buchet and Lauwerys, 1987). While the exact role of glutathione in the methylation reaction is not known, decreased concentrations of glutathione in the liver have been associated with decreased rates of arsenic methylation (Buchet and Lauwerys, 1987) and the inhibition of DMA^V production (Hirata *et al.,* 1989). Glutathione may play a role in the metabolic fate of arsenic at other stages of metabolism, as it has been observed that availability of glutathione is also important for reduction of As^V to As^{III} and for the uptake of As^{III} into hepatocytes (Lovell and Farmer, 1985; Buchet and Lauwerys, 1987; 1994).

Following absorption or its production in the bloodstream, As^{III} undergoes oxidative methylation to form MMA^V and DMA^V. This methylation pathway has been observed in rats, mice, rabbits, hamsters, dogs, cattle and humans (Lakso and Peoples, 1975; Crecelius, 1977; Buchet and Lauwerys, 1987, 1994; Vahter and Marafante, 1988). Irrespective of the type and extent of exposure to arsenic, the average relative distribution of arsenic metabolites in urine of various population groups seems to be fairly constant (Vater, 2002). These are 10 to 30% inorganic arsenic, 10 to 20% MMA and 60 to 70% DMA in urine.

Based on urinary excretion patterns, the predominant metabolite of inorganic arsenic is DMA^V in humans, rats, mice, rabbits, hamsters and dogs (Charbonneau *et al.,* 1979; Yamauchi and Yamamura, 1979; Buchet *et al.,* 1981a,b; Maiorino and Aposhian, 1985; Farmer and Johnson, 1990; Offergelt *et al.,* 1992; Hopenhayn-Rich *et al.,* 1993; Hopenhayn-Rich *et al.,* 1996a,b; Yoshida *et al.,* 1997; 1998). The available data indicate that while MMA^V is also formed in these species, only in humans and the Flemish Giant rabbit is MMA^V considered a metabolite of any significance (Tam *et al.,* 1979; Buchet *et al.,* 1981a,b; Vahter, 1981; Vahter and Marafante, 1983; 1988; Marafante and Vahter, 1987; DeKimpe *et al.,* 1999). In the rabbit, the ratio of arsenic methylated metabolites is similar to that of human adults (Maiorino and Aposhian 1985), suggesting that rabbits may be the most appropriate animal model for toxicokinetics in humans. In contrast, the guinea pig and the marmoset and tamarin monkeys do not methylate inorganic arsenic (Healy *et al.,* 1998; Vahter and Marafante 1985; Vahter *et al.,* 1982; Zakharyan *et al.,* 1996), and thus, may be poor models for humans.

Recently, Csanaky and Gregus (2002) reported that while the rat is not a good model for arsenic disposition in humans, it may be useful in metabolism studies, particularly with respect to studies on As^{III}

methyltransferase and the *in vivo* formation of MMA^V. MMA^{III} was observed in the bile of rats (the most efficient producers of this metabolite) that received arsenate (As^V) or arsenite (As^{III}). A comparative investigation of five animal species (rats, mice, hamsters, rabbits, and guinea pigs) injected with As^V or As^{III}, and an examination of the excretion of As^V, As^{III} and their metabolites showed that all species (except guinea pigs), formed MMA^{III} and excreted it into bile.

Apostoli *et al.* (1997) reported that arsenobetaine was observed in the urine of humans to whom inorganic arsenic (as arsine, AsH_3) had been administered. No external source of the arsenobetaine could be identified, and the authors concluded that its detection may have been an analytical artifact. Yoshida *et al.* (1997; 1998) reported that after exposure of rats to MMA^V and DMA^V, relatively small but measurable amounts of trimethylarsine oxide (TMAO) and tetramethylarsonium ($TMAs^+$) were detected in the urine. In general, however, the organic forms of arsenic (DMA^V, MMA^V, and arsenocholine (AsC)) tend to be excreted with minimal or no metabolic change (Buchet *et al.,* 1981a; Marafante *et al.,* 1984). Inorganic arsenic was not observed as a product formed during metabolism in these studies.

The primary site of arsenic methylation is believed to be the liver (Vahter, 2002), although some methylating ability has been observed in the kidney as well (Lerman and Clarkson, 1983; Buchet and Lauwerys, 1987; Vahter and Marafante, 1988). Arsenic methylating activity has been detected in several different tissues of male mice including the testes, kidney, liver and lung (Healy *et al.,* 1998). Studies of tissue methylation of arsenic showed little significant methylating capacity in red blood cells, lung, brain, intestine, or kidney tissues of the rat (Buchet and Lauwerys, 1985), or in alveolar cells of rabbits (Marafante *et al.,* 1987). *In vitro* studies indicate that while arsenite is readily methylated in the cells of the liver, methylation of arsenate in the hepatocytes is minimal; in contrast, in kidney cells, much more DMA was produced following exposure to arsenate, as opposed to arsenite (Lerman and Clarkson, 1983; Lerman *et al.,* 1985). *In vivo* studies do indicate, however, that regardless of whether arsenate or arsenite was administered, the first and most predominant site of DMA formation is the liver (Marafante *et al.,* 1985; Charbonneau *et al.,* 1979; Vahter, 1981).

Vahter and Marafante (1988) proposed that in addition to the liver, the tissues of the kidney could be responsible for the methylation of that proportion of arsenate (As^V) that was reabsorbed in the tubules of the kidney. These findings suggest that although the liver may have the greatest capacity to methylate arsenite (As^{III}) (on the basis of tissue mass), extrahepatic metabolism may also be significant, particularly for routes of exposure such as inhalation, where there is opportunity for first-pass metabolism in the lung (WHO-IPCS, 2001).

While blood and tissue concentrations would be the most accurate measure of the degree of methylation of arsenic in the body, the proportions of inorganic arsenic and methylated metabolites in the urine also provides a measure of arsenic methylation Urinary excretion is the main pathway of elimination of arsenic, although the urinary profile does not provide information on the fraction of arsenic (generally as As^{III}) retained in the body (see section 1.4 above). The route of exposure, chemical species administered, and time following exposure can all affect the proportions of inorganic arsenic forms and their methylated metabolites. In humans, urinary concentrations of the proportions of major forms of arsenic range from 10 to 30% for inorganic arsenic, 10 to 25% for MMA and from 40 to 80% for DMA (Crecelius, 1977; Smith *et al.,* 1977; Tam *et al.,* 1979; Buchet *et al.,* 1981a,b; Vahter, 1986; Farmer and Johnson, 1990; Johnson and Farmer, 1991; Hopenhayn-Rich *et al.,* 1993; Hopenhayn-Rich *et al.,* 1996a,b; Yoshida *et al.,* 1998). Based on product concentration profiles in the urine (except for MMA), arsenic has a similar metabolic fate in the hamster, mouse, rabbit and humans (Maiorino and Aposhian, 1985; Vahter and Marafante, 1988; Carmignani *et al.,* 1985; Yoshida *et al.,* 1998). The mouse appears to have the greatest capacity for methylation, followed by rats, rabbits, humans, and hamsters (Vahter and Marafante, 1988). In experimental animals, inorganic arsenite (As^{III}) was generally more efficiently methylated than arsenate (As^{V}), based on the proportions of methylated and unmethylated species recovered in urine (Vahter and Marafante, 1983).

A1-2.5.1 Differences in Individual Methylation Capacity

A1-2.5.1.1 Individual Differences Independent of Exposure Levels

A large number of controlled ingestion studies indicate that both arsenate and arsenite are extensively methylated in humans, although to a lesser degree in humans than in some laboratory animals (WHO-IPCS, 2001). There is evidence of substantial inter-individual variability in the degree of methylation in arsenic-exposed humans, and patterns of methylation products between a number of studies have not correlated well with arsenic exposure (WHO-IPCS, 2001). Individual variation in methylating capacity within human populations has been associated with smoking, gender, pregnancy, years of exposure, and ethnic background (Hopenhayn-Rich *et al.,* 1996b; Vahter, 2000), although these factors only explain roughly 20% of the observed variability (WHO-IPCS, 2001). Liver disease is also known to affect inorganic arsenic metabolism (Buchet *et al.,* 1984). The large inter-individual variation in arsenic methylation might indicate that a genetic influence is more important than environmental factors (Vahter, 2002).

The considerable variation in human rates of arsenic methylation and recent studies that revealed evidence of MMA^{III} in human urine and bile (Gregus *et al.,* 2000; Aposhian *et al.,* 2000) suggest there is a genetic polymorphism for methyltransferases among humans (Vahter, 2000; Vahter and Concha, 2001; Concha *et al.,* 1998a; Mushak and Crocetti, 1995). Concha *et al.* (1998a) reported a significant variation in methylation capacity in a South American population. The indigenous peoples of the Andes (mainly Atacameños) have been chronically exposed for thousands of years to high levels of arsenic in drinking water. The urine of children from this population had much higher proportions of inorganic arsenic (50%) than did adult women (32%) or children from other populations experiencing much lower exposures (18%). A review by ATSDR (2000) suggested that these findings may also indicate that the metabolism of arsenic in children is less efficient than in adults. Kurttio *et al.* (1998) and Vahter *et al.* (2000) have reported a greater capacity for methylation in adults relative to children; however, Kalman *et al.* (1990) did not support this observation.

Chung *et al.* (2002) have provided additional support for a genetic basis to inter-individual variation in methylation capacity. Although based on a small sample size, Chung *et al.* (2002) observed that methylation patterns aggregate in families and are significantly correlated in siblings, but not between parents. Others have also observed that differences in the activities of methyltransferases can be explained by the existence of genetic polymorphisms (Weinshilboum *et al.,* 1999). At this time, the specific methyltransferases involved in arsenic biomethylation have not been well characterized (Vahter, 2000).

Gender differences in arsenic metabolism have been investigated in humans by Hopenhayn-Rich *et al.* (1996a) and Hsu *et al.* (1997) who reported that relatively more DMA^{V} was excreted by women than men. Similarly, Concha *et al.* (1998b) reported significant increases in the percentage of DMA^{V} excreted in urine in Argentinian women during pregnancy. Others have also observed gender differences in arsenic methylation. Vahter *et al.* (2000) reported that among children of native Andean origin, an exposure-related increase in the percent of DMA^{V} detected in urine likely resulted from an induced level of enzyme. This was in contrast to previous studies on adult populations in Chile and Mexico that showed increasing levels of arsenic exposure resulted in a decrease in the relative amount of DMA^{V} in urine and a corresponding increase in inorganic arsenic and MMA^{V}. In the Argentinean population groups, the authors reported a marked increase in the relative amount of DMA^{V} in urine at the end of pregnancy. The pattern of methylation was similar in newborn infants, indicating induction of arsenic methylation during pregnancy. Thus, children and pregnant adults of specific populations have

demonstrated an ability to respond to increased organic arsenic exposure through increased methylase activity.

A study of adults and children in Bangladesh, Chowdhury *et al.* (2003) showed that in that population the first methylation step for arsenic species was more active in adults than children. A significant increase in the ratio of DMA^V/MMA^V in children compared with adults indicated that the second methylation step (dimethylarsinic acid) could be more active in children than in adults, and also that the value of this ratio decreased with the age of the children in the comparison group. The authors suggested that children may retain less arsenic compared to adults (DMA^V is more readily excreted), which may also explain why children do not typically show the skin lesions observed in adults exposed to the same arsenic concentrations in drinking water.

A1-2.5.1.2 Threshold of Methylation Theory

The threshold of methylation theory has been based on a limited number of human studies that have reported that methylated arsenic compounds in the urine decreased in proportion to the total arsenic exposure. It has been theorized that total arsenic exposures slightly in excess of 1 mg As/day represents a saturation threshold for the rate of methylation of arsenic in the body (Buchet *et al.,* 1981b; Marcus and Rispin, 1988; Petito and Beck, 1990; Stohrer, 1991). For arsenic ingestion, it has been suggested that the methylation process reaches saturation at an oral intake rate of around 200 to 250 µg per day in adults (North, 1992; U.S. EPA, 1990). Valberg *et al.* (1994) used the Michaelis-Menton equation that describes enzyme kinetics to calculate a saturation half-point for arsenic methylation of 0.7 µg As/day. Prediction of a threshold means that above certain exposure levels, the rate of methylation, and therefore the proportion of methylated arsenic products, would decrease relative to the total arsenic exposure. At this time, the precise dose rate at which methylation capacity becomes saturated cannot be defined (ATSDR, 2005).

Hopenhayn-Rich *et al.* (1993) found that levels of non-methylated arsenic in human urine were fairly constant (around 20%), regardless of the received dose. A comparison of methylated metabolites in the urine with doses of inorganic arsenic, showed no significant increase in the proportions of inorganic arsenic, or a decrease in the amount of MMA^V or DMA^V. No difference was observed between humans exposed to arsenic through the workplace, through drinking water or *via*exposure to background concentrations in the environment. This relationship held for a wide range of exposures that ranged from 4.4 to 245 µg As/L in drinking water, as indicated by total speciated arsenic concentrations recorded in the study population.

Some investigators have suggested that the approach to a threshold of saturation for arsenic methylation is best indicated by the rate of increase in the concentration ratio of MMA^V to DMA^V (Beck *et al.,* 1995; Slayton *et al.,* 1996). Several study populations have shown a decreased capacity for dimethylation (*i.e.,* a decreased proportion of DMA) after chronic exposure to high concentrations of arsenic in drinking water (Yamauchi *et al.,* 1989a; Hsueh *et al.,* 1995; Del Razo *et al.,* 1997). Others do not agree that epidemiological data support a significant increase in the MMA^V: DMA^V ratio (Smith *et al.,* 1995; Mushak and Crocetti, 1996). Hopenhayn-Rich *et al.* (1996a) conducted a study of the metabolism of arsenic in a Chilean population, and observed a slight increase in the proportion of inorganic arsenic, as well as a slight decrease in the MMA^V: DMA^V ratio. After an intervention that reduced the drinking water concentrations of arsenic for this population, there was a slight decrease in both the proportion of inorganic arsenic and the MMA^V: DMA^V ratio (Hopenhayn-Rich *et al.,* 1996b). In an analogous study, Warner *et al.* (1994) studied the metabolism of arsenic in a population in Nevada, comparing a group exposed to drinking water concentrations greater than 500 µg As/L to a group exposed to an average of 16 µg As/L. No significant differences in the proportions of MMA^V to DMA^V were noted in urine samples. Both of these studies reported large individual variability, which statistical analysis attributed to ethnicity, gender, and smoking.

An alternative theory to explain a reduction in the rate of DMA compared to MMA proposed the depletion of methyl donors was the cause as a consequence of inadequate nutrition. The methylation of absorbed arsenic is dependent on the supply of methyl donors which are related to nutritional state (*e.g.*, proteins rich in the amino acids cysteine and methionine) (Beck *et al.,* 1995). Vahter and Marafante (1987) studied the impact of a methionine/cysteine reduced diet on the methylating activity of rabbits, and reported a significant decrease in methylation of inorganic arsenic following a 25% reduction in dietary intake of the amino acids.

The importance of nutrition in maintaining adequate levels of methyl donors has also been proposed as a reason for increased cancer risks in subsets of the Taiwanese population. The content of the typical Taiwanese diet was compared to that of the typical North American population. In an epidemiological study of cancer incidence in a Taiwanese village, Hsueh *et al.* (1995) found that malnutrition, indexed by a high consumption of dried sweet potato as a staple food, was a risk factor for skin cancer. However, Smith *et al.* (1995) reviewed the Taiwanese intake of protein, and found it adequate by current standards. Beck *et al.* (1995), in rebuttal to the review by Smith *et al.* (1995), pointed out that current standards dictate intakes required for normal bodily processes, and may be deficient and inadequate to methylate an excessive and sustained intake of arsenic.

Several authors have described mechanisms for inhibition of dimethylation (*i.e.*, conversion of MMA^V to DMA^V) in humans. Prolonged exposure to arsenic may result in the development of tolerance. Such tolerance could be observed to result from increased excretion and enhanced methylation or to an increase in some other excretory mechanism. Studies in mice have indicated that exposure to arsenic does not induce arsenic methylation activity (Healy *et al.,* 1998). These authors found that mice receiving up to 0.87 mg As/kg/day as sodium arsenate (As^V) in drinking water for 91 days had the same arsenic methylating activity as unexposed controls. Hughes and Thompson (1996) reported similar findings in a study of subchronic (28 day) exposure of mice to 25 or 2,500 µg As/L as arsenate in drinking-water . There was no increased urinary excretion of methylated metabolites noted. However, studies in the older literature (Bencko and Symon, 1969; Bencko *et al.,* 1973) of mice exposed to arsenite suggest that there is enhanced tissue clearance upon continuous exposure. Albores *et al.* (1992) reported that metallothionein is inducible *in vivo* in rats injected with arsenite (As^{III}), but not with arsenate (As^V). Kreppel *et al.* (1990) also reported that arsenite induced metallothionein in mice more efficiently *in vivo* than *in vitro*.

The significance of these findings is unclear. It may be that there are differences in the ability of arsenate *versus* arsenite to induce certain metabolic processes. Also, the reason for arsenite-induction of metallothionein is not known as arsenite does not bind metallothionein (Albores *et al.,* 1992).

A1-2.5.1.3 Toxicological Significance of the Threshold of Methylation Theory

Saturation of the capacity to methylate arsenic would result in excretion of increased amounts of inorganic arsenic (As[III] or As[V]) and decreased amounts of MMA^V and DMA^V. The toxicological consequences of an increased urinary MMA^V:DMA^V ratio and the resultant changes in species proportions in the body is not completely clear. The methylation saturation theory predicts that the body tissue concentrations of inorganic arsenic and/or MMA^V would increase, while relative concentrations of DMA^V would decrease. Accumulation of either inorganic arsenic, or MMA^{III} would increase the concentration of the most toxic arsenic species.

In general, methylation reactions are viewed as a detoxification process (methylated As compounds tend to be rapidly excreted). Recent studies have found that reduced trivalent intermediates, monomethylarsenous acid (MMA^{III}), and dimethylarsinic acid (DMA^{III}), particularly MMA^{III} are of greater toxic potency than either the inorganic parent compounds or other methylated species (Styblo and Thomas, 1997; Petrick *et al.,* 2000; Styblo *et al.,* 2000; Styblo *et al.,* 2002; NRC, 2001). These species have also been detected in bile and urine of humans exposed to inorganic arsenic (Aposhian *et al.,* 2000;

Gregus *et al.,* 2000; Vahter, 2002). At this time, the extent to which MMA^{III} and DMA^{III} contribute to the observed human toxicity following exposure to inorganic arsenic is not fully understood (Vahter, 2002).

A1-2.5.2 Metabolism of Organoarsenicals

Yamauchi *et al.* (1988) demonstrated that *in vivo*, MMA undergoes methylation to dimethylated and trimethylated products, but that methylation is not extensive, and that most of the absorbed MMA is excreted unchanged in urine, regardless of the dose. There was no evidence that MMA was demethylated in these studies. Similar findings were reported by Hughes and Kenyon (1998) for female $B6C3F_1$ mice administered MMA intravenously. These authors also noted a decrease in DMA excretion with increasing dose that was observed in both hamsters and mice. It was suggested that this could have been due to either dose-dependent saturation or inhibition of MMA methylation (Hughes and Kenyon, 1998).

A number of studies have demonstrated that DMA is methylated to trimethylarsenic compounds to a limited extent in mice, rats and hamsters (Yamauchi and Yamamura, 1984; Marafante *et al.,* 1987; Yoshida *et al.,* 1997; 1998).

Given that radiolabelled inorganic arsenic is not detected in the urine of mice, rats, hamsters and humans after administration of ^{74}As-DMA suggests that demethylation is insignificant in these species (Vahter *et al.,* 1984; Marafante *et al.,* 1987; Yoshida *et al.,* 1998). Based on limited studies in hamsters, it appears that trimethylarsenic compounds do not undergo further methylation or demethylation reactions, but are either oxidized, reduced or excreted (Yamauchi *et al.,* 1989b).

Several important organoarsenical compounds occur in marine species (Figure A1-3). Human exposure is primarily *via*ingestion of shellfish and seafood. Arsenobetaine was discovered as early as 1977, and is commonly found in marine biota. It has been speculated that it is formed *via* degradation of arsenosugars (algal dimethylarsinoyl-ribosides (Ritchie *et al.,* 2004). Studies in mice, rats, rabbits and hamsters that were administered arsenobetaine either intravenously or orally found that this compound was not biotransformed or demethylated (Vahter *et al.,* 1983; Yamauchi *et al.,* 1989a). Arsenobetaine is metabolically stable and is rapidly excreted through the kidney and therefore presents no toxic hazard (Ritchie *et al.,* 2004). Arsenocholine is also a product found in marine organisms that is produced from arsenosugars *via* reduction and methylation of dimethylarsinoylethanol. It is not known to be produced in mammals (WHO-IPCS, 2001; Milstein *et al.,* 2003). Arsenocholine in mammalian tissues is also not demethylated, but it is metabolized extensively to arsenobetaine and excreted (Marafante *et al.,* 1984).

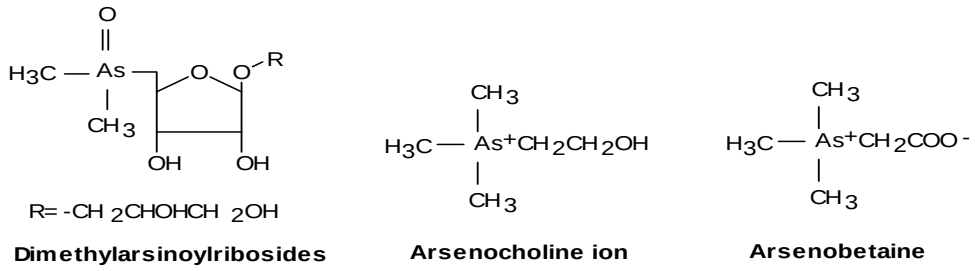


Figure A1-3 Structures of Organoarsenicals Derived Primarily from Marine Species

A1-2.6 Excretion

The primary pathway of elimination of all arsenic species from the body is excretion *via* the urine. A very small fraction of excreted arsenic compounds occur in the feces, bile, sweat and breast milk (Malachowski, 1990). Investigations of the magnitude of urinary excretion following exposure to inorganic arsenic indicate that, for oral, inhalation, and dermal administration, 45 to 85%, 30 to 65%, and 50% respectively of the administered dose is excreted in the urine within 1 to 3 days (Holland *et al.,* 1959; Pinto *et al.,* 1976; Crecelius, 1977; Mappes, 1977; Tam *et al.,* 1979; Pomroy *et al.,* 1980; Buchet *et al.,* 1981a,b; Vahter *et al.,* 1986; U.S. EPA, 1988; Buchet *et al.,* 1995; Apostoli *et al.,* 1997; Kurttio *et al.,* 1998). In repeat-dose studies, it has been determined that when equilibrium between intake and output was reached, urinary excretion of arsenic following oral administration to human volunteers accounted for 40 to 60% of the daily dose (Farmer and Johnson, 1990; Johnson and Farmer, 1991). Studies in mice, hamsters and rabbits indicate that urinary excretion accounts for 50 to 87% of the doses administered by intravenous injection (Odanaka *et al.,* 1980; Vahter and Marafante, 1983; Maiorino and Aposhian, 1985). About 15 to 48% of arsenic administered intratracheally to hamsters as fly ash or copper smelter dust was eliminated in the urine within 48 hours (Buchet *et al.,* 1995). Experimental results in laboratory animals for excretion *via* the urine following other routes of exposure are generally supportive of similar excretion rates observed in humans (Charbonneau *et al.,* 1979; Odanaka *et al.,* 1980; Vahter and Norin, 1980; Rhoads and Sanders, 1985; Marafante and Vahter, 1987). There are interspecies variations in overall retention times, as discussed below.

Hopenhayn-Rich *et al.* (1993) reported that roughly 80 to 90% of all excreted arsenic is in a methylated form following exposure to inorganic arsenic. Organic arsenic compounds, such as arsenobetaine, are directly excreted in urine without further metabolism in both animals and humans (ATSDR, 2005; Le *et*

al., 1993). Limited data from studies where multiple dose levels were used (Yamauchi *et al.,* 1988; Hughes and Kenyon, 1998) suggest that urinary elimination of arsenic is dose-independent – where the percentage of the dose eliminated in urine does not change significantly with increasing or decreasing dose levels.

Volatile metabolites of some organoarsenicals are eliminated in expired air after oral administration. After a high oral dose of DMA^V (1,500 mg/kg), mice eliminate dimethylarsine, but not tetramethylarsonium ions, in expired air (Yamanaka and Okada, 1994). On the other hand, for mice orally administered 14,400 mg/kg TMAO, tetramethylarsonium ions were detected in expired air (Kaise *et al.,* 1989). Hamsters also eliminate tetramethylarsonium ions in expired air after administration of either TMAO or tetramethylarsonium salts (Yamauchi *et al.,* 1989b).

Faecal elimination of arsenic compounds is indicative of biliary excretion, and in the case of oral administration, of the proportion of arsenic not absorbed during passage through the gastrointestinal tract. The literature indicates that this is not a significant route of elimination of arsenic for most species (Ray-Bettley and O'Shea, 1975). Following oral administration of radiolabelled arsinic acid to humans, Pomroy *et al.* (1980) observed a total faecal elimination of about 6% over 7 days. Fecal elimination in laboratory animals ranged from 33 to 49% of an orally administered dose, although following intravenous administration, only 0.8 to 1.4% was found in the faeces (Odanaka *et al.,* 1980). Dutkiewicz (1977) observed that in the rat, excretion of arsenic following dermal administration was equivalent *via* urine and feces. Vahter (1994) postulated that faeces elimination may be more important in the rat due to a greater biliary excretion which would result from glutathione being the primary thiol in rat bile.

The time course of elimination of arsenic is of relatively short duration in both humans and experimental animals. It is well established that the urinary excretion of absorbed arsenic occurs primarily within the first 24 to 48 hours of exposure and declines rapidly in the following days if there is no further exposure (Hrudey *et al.,* 1996; ATSDR, 2005).

The whole body clearance of arsenic in humans following ingestion was reported to have half-times of 40 to 60 hours (Mappes, 1977; Buchet *et al.,* 1981b), while Crecelius (1977) estimated biological half-lives of inorganic arsenic and its methylated metabolites to be 10 and 30 hours, respectively. Pomroy *et al.* (1980) observed a triphasic elimination of arsenic in humans following oral administration; about 66% of the dose had a half-life of 2.1 days, 30% had a half-life of 9.5 days, and 4% had a half-life of 38 days. Data from laboratory animals generally indicate that retention of arsenic is dependent on methylating capabilities as well as species-specific tissue interactions. Thus, high-methylating species such as dogs,

rabbits and mice have shorter retention times, while the non-methylating marmoset monkey has a longer retention time than humans (Vahter, 1981; Vahter *et al.,* 1982; Vahter and Marafante, 1983; 1988; Vahter, 1994). The rat is an exception to this trend, as it readily methylates inorganic arsenic, but due to the interaction of DMA with hemoglobin in the red blood cells of this species, overall retention times for the rat are much longer in comparison to that observed in humans (Vahter, 1981; Vahter and Marafante, 1988).

Studies of inhalation exposure in humans have indicated similar results to those cited above. Occupational studies indicate that urinary arsenic levels closely mirror the time-course of daily exposures (Vahter *et al.,* 1986). Apostoli *et al.* (1997) observed a triphasic elimination profile following inhalation exposure of humans; approximately 75% of the dose was cleared with a half-life of 4 days, while the remainder had a half-life of 10 days. Studies of arsenate and arsenite retention in rats and hamsters

following intratracheal instillation indicate half-lives of 1 day or less (Rhoads and Sanders, 1985; Marafante and Vahter, 1987; Buchet *et al.,* 1995). Although dependent on chemical species, small amounts of arsenic may be retained in the lung with a half-time of several months (Rhoads and Sanders, 1985).

In general, the retention of arsenic in the body is a function of the chemical species. Vahter and Marafante (1983) compared the fate of arsenate (As^{V}) and arsenite (As^{III}) in mice and rabbits. It was observed that in the rabbit, the retention time was greater following exposure to As^{III} than after exposure to As^{V}, based on urinary excretion profiles. Despite the greater methylation of As^{III}, the retention of this form through tissue interaction resulted in a longer overall retention in the body. The tissue interaction of As^{III} may be partially compensated by methylation, in species with greater capacity, such as the mouse, in which there was no significant difference in the whole body retention of As^{V} and As^{III}. Apostoli *et al.* (1997) also observed differences in retention of arsenic species. The half-lives of different chemical species ranged from 27 to 86 hours following inhalation, with the shortest half-life exhibited by As^{V}, followed, in order of increasing half-life, by MMA^{V}, $As^{III,}$ DMA^{V}, and arsenobetaine^{V} (AsB) (Apostoli *et al.,* 1997).

Pomroy *et al.* (1980) studied the whole-body retention of ^{74}As (6.4 μCi, 0.06 ng As) administered once orally as pentavalent arsenic acid in healthy male volunteers (ages 28 to 60 years) using whole-body counting for periods of < 103 days. The averaged whole-body clearance data for the six subjects in the study were best described by a triexponential model, but inter-individual variation was noted to be quite

high. It was reported that 65.9% of the administered dose was cleared with a half-life of 2.09 days, 30.4% with a half-life of 9.5 days and 3.7% with a half-life of 38.4 days.

Retention of arsenic in the body is related to interactions of arsenic compounds with cellular and biochemical processes. It is well established that pentavalent inorganic arsenic (arsenate) can act as a phosphate analogue. At the molecular level, arsenate can compete with phosphate for active transport processes. This is evidenced by studies showing that the addition of phosphate can decrease intestinal uptake of arsenate (Gonzalez-Zulueta *et al.,* 1995). Arsenate can also substitute for phosphate in the hydroxyapatite crystal of bone, which accounts for the higher concentrations of arsenic-derived radioactivity in bone after administration of arsenate compared to arsenite (Lindgren *et al.,* 1982). At the biochemical level, arsenate can uncouple oxidative phosphorylation in mitochondria by substituting for inorganic phosphate in the synthesis of ATP (Gresser, 1981), and can also inhibit glycolysis by competing with phosphate to form 1-arseno-3-phosphoglycerate (which has no biochemical function), rather than 1:3-diphosphoglycerate (Mayes, 1983). Trivalent arsenic (arsenite) reacts readily with the sulfhydryl groups of a variety of enzymes and proteins. It is this affinity of arsenite for sulfhydryl groups that accounts for its accumulation in keratin-rich tissues such skin, hair and nails. Arsenite also interacts with the sulfhydryl-containing cellular tripeptide glutathione (GSH) at many different levels in the methylation process (Styblo *et al.,* 1996; 2002) (Figure A1-2).

A1-2.7 Urinary Excretion as a Bioindicator of Daily Exposure to Inorganic Arsenic

A1-2.7.1 Arsenic Exposure and Urinary Excretion

Urinary excretion is the primary route of elimination of arsenic. Concentrations of arsenic compounds in the urine are considered to be a reliable index of recent exposure to arsenic (Hindmarsh and McCurdy, 1986; Johnson and Farmer, 1989; Buchet *et al.,* 1996a; Gebel *et al.,* 1998). The objective of developing and validating a model relating urinary concentrations to daily exposures to arsenic would be to provide a non-intrusive technique for evaluating not only arsenic exposure, but to also provide an indicator of the health status of populations exposed to arsenic.

Total urinary arsenic concentration reflects intake of all forms of arsenic. In addition to sources of inorganic arsenic, intake of organic arsenicals such as MMA, DMA, arsenobetaine, arsenocholine and arsenosugars also affects the total urinary concentration of arsenic detected as As (Kalman *et al.,* 1990; Goessler *et al.,* 1997) (see Figure A1-1). Exposure to organoarsenicals has been associated primarily with intake of arsenic from dietary sources. Particularly high concentrations of organic arsenic compounds

have been found in seafood products (Buchet *et al.,* 1996b; Goessler *et al.,* 1997; Walker and Griffin, 1998). Organoarsenicals can constitute the majority of the total urinary arsenic measurements in people who have recently consumed seafood (Kalman *et al.,* 1990, Goessler *et al.,* 1997). These organoarsenicals (such as arsenobetaine) are thought to be relatively non-toxic and are believed to leave the body metabolically unchanged (Vahter *et al.,* 1983; Johnson and Farmer, 1989; Kalman *et al.,* 1990; Le *et al.,* 1993; Gebel *et al.,* 1998). A number of studies have found that the ingestion of organoarsenicals has not been associated with increased concentrations of inorganic arsenic in the body (Buchet *et al.,* 1981a; Luten *et al.,* 1982; Buratti *et al.,* 1984; Marafante *et al.,* 1984; Johnson and Farmer, 1991; Le *et al.,* 1993; Goessler *et al.,* 1997). Thus, the inclusion of organoarsenicals in the urinary arsenic-exposure model is not relevant in terms of relating exposures to potential adverse health effects (Johnson and Farmer, 1991). Because tissue interaction, and thus toxic potency, has been attributed to primarily inorganic As^{III} (Vahter and Marafante, 1988), a urinary concentration-exposure model should be limited to quantifying exposures to arsenic species which are of greatest toxicological concern (*i.e.,* inorganic As[III] and As[V]).

Chen *et al.* (2002) found that arsenic species in freshly collected urine (pH 5.5 to 7) remained stable up to 6 months when stored at temperatures of – 20^{o}C or less. For aqueous arsenic standards (stored at 4^{o}C), MMA and DMA remained stable for at least 4.5 months, whereas As^{V} reduced to As^{III} within four weeks of preparation. Chen *et al.* (2002) also found that arsenic species in urine showed greater stability than aqueous standards and that they last longer than the aqueous standards. The added stability may be because of the complex matrix and pH of urine, as well as the sample storage temperature. Arsenic trapped in protein-urine matrix is termed insoluble, but could represent a significant fraction of total detectable urinary arsenic.

The interconversion of As^{V} and As^{III} in aqueous solution is a recognized phenomenon. Depending on the pH, temperature, oxygen content, light, and the presence of other substances more or less of the As^{III} and As^{V} may be detected in a given sample. Transformation between As^{III} and As^{V} in human urine (freshly collected urine samples), was not frequently observed Chen *et al.,* 2002). Only low concentrations of As^{III} and As^{V} were present in the most freshly collected urine samples. It is possible that the matrix of urine stabilizes the distribution of arsenic species. On the other hand, in the blood, most As^{V} is reduced to As^{III}. Some of the reduced form of As can then be methylated into the less toxic forms (MMA^{V} and DMA^{V}). Finally, the remaining As^{III} binds to sulfhdryl proteins, and is retained in the keratin of skin, or hair, or in the gastrointestinal tract (Chen *et al.,* 2002).

As discussed in previous sections, the predominant forms of arsenic and its metabolites in the human body following exposure to inorganic arsenic are: As^{III}, As^{V}, MMA^{V} and DMA^{V} In order to capture only those arsenicals in urine that are ultimately derived from exposure to inorganic arsenic, routine urine analysis for indication of arsenic exposure should provide information on "speciated arsenic", consisting of inorganic arsenic, MMA and DMA (Buchet *et al.,* 1981a; Kalman *et al.,* 1990; Farmer and Johnson, 1990; Offergelt *et al.,* 1992; Walker and Griffin, 1998). Some additional background sources contribute to arsenic exposure, and these should be accounted separte from environmental inhalation exposure sources. Extraneous, but not insignificant sources of As include smoking, drinking water, and consumption As naturally present in the typical Canadian diet (MOEE, 1994; Environment Canada, 1993). All contributions to intake of must be considered in the application of the urinary arsenic-exposure model. A clear distinction should be drawn between arsenic intake from sources rich in organic arsenical compounds such as seafood, since these may have little or no impact on concentrations of inorganic arsenic in the body, and thus have limited to no relevance for potential adverse health effects.

Consumption of organoarsenicals in seafood has been found to result in significant increases in total urinary arsenic levels, but does not result in a significant increase in urinary concentrations of inorganic forms of arsenic (*i.e*., As^{III}, As^{V}) and their related methylated metabolites (*i.e.,* MMA and DMA) (Buchet *et al.,* 1981a; Buratti *et al.,* 1984; Foà *et al.,* 1984; Marafante *et al.,* 1984; Le *et al.,* 1993; 1994). Nonetheless, several recent studies have suggested that consumption of certain types of seafood can influence speciated arsenic concentrations. Gebel *et al.* (1998) found an association between seafood consumption and concentrations DMA in urine. Goessler *et al.* (1997) found that urinary concentrations of As^{III}, As^{V} and MMA in a single human volunteer were not influenced by either the consumption of codfish or exposure to gaseous trimethylarsine. However, significant increases (2.8 to 4.3 µg As per gram creatinine and 4.9 to 26.5 µg As per gram creatinine) were observed for the level of DMA after the first and second instance of codfish consumption, respectively (Gebel *et al.,* 1998). Arbouine and Wilson (1992) found that mean total urinary speciated arsenic levels (*i.e.,* the total of As^{III}, As^{V}, DMA and MMA concentrations) increased between 1.8 and 6.9 times after the consumption of various seafood products, relative to levels measured prior to consumption. This increase was attributed to the presence of DMA in seafood. Buchet *et al.* (1996b) also observed an increase in urinary concentrations of DMA following seafood consumption and postulated that DMA may be a breakdown product of arsenobetaine formed during cooking. Arsenobetaine was generally found to be excreted unchanged in urine (Ritchie *et al.,* 2004). In another study, the consumption of seaweed and kelp was reported to cause increased urinary concentrations of arsenobetaine, but also the concentration of inorganic arsenic and DMA (Le *et al.,*

1994). Le and Ma (1998) also observed substantial increases in DMA concentrations in urine of four volunteers after the ingestion of seaweed. The increase of urinary DMA concentration was attributed to the metabolism of arsenosugars (see Figure A1-3) that are present in the seaweed. These authors suggest that the commonly used urinary biomarkers of exposure linked to inorganic arsenic are not reliable when arsenosugars are ingested from the diet. Thus, the attempt to link urinary speciated arsenic concentrations to specific environmental exposure (*e.g.,* a point source) may be hampered by normal consumption of fish and seafood. It is therefore important that dietary intake of seafood be considered in any urinary arsenic-environmental exposure model (Goessler *et al.,* 1997; Gebel *et al.,* 1998, Le and Ma, 1998).

A1-2.7.2 The Urinary Speciated Arsenic - Daily Exposure Model

Several attempts have been made to develop a model that describes the relationship between arsenic concentrations detected in urine and total daily exposure. Validation of the predicted concentrations generated by such a model have compared measured urinary concentration with monitored or calculated daily exposure (based on environmental media of concern [*i.e.*, soil, drinking water or indoor air] and receptor characteristics such as water consumption, *etc.*). The model estimates urinary concentrations based on calculated daily exposures, or alternately, utilizes actual urinary concentrations to predict estimated daily exposure. The model calculations have been widely employed in occupational settings to predict exposure.

Vahter *et al.* (1986) attempted to correlate urinary concentrations of speciated arsenic with inhalation exposures experienced by smelter workers. The best empirical fit established a linear relation for occupational inhalation exposure: As_{URINE} (µg As/L) = 2.90 x As_{AIR} (µg As/m^3) + 15.5. The model did not take into account differences in environmental arsenic speciation and bioavailability of arsenic species.

Johnson and Farmer (1989) used a simple model to relate urinary concentrations to daily exposure to inorganic arsenic. These authors examined published literature values and assumed that 40 to 60% of the daily intake of arsenic is excreted in the urine. Using typical daily creatinine outputs (1.5 and 0.75 g for adults and children, respectively), urine concentrations (as µg As per g of creatinine) were converted into estimates of daily intake. These were generally in agreement with modelled daily intakes that were based on concentratons of As in local soil and drinking water. It should be noted that the range of proportional excretion into the urine indicates that these authors are considering a soluble form of arsenic administered *via* oral or inhalation routes of exposure (*i.e.*, approaching complete absorption). Indeed, Farmer and Johnson (1990) note that much of the available data on the absorption of arsenic is restricted to the more

soluble forms, which may not be representative of the forms of arsenic typically encountered in environmental media.

Walker and Griffin (1998) published the results of the application and validation of the urinary arsenic model endorsed by the U.S. EPA. In this model, urinary arsenic concentrations were linked to calculated daily exposures for children living near a smelting facility in Anaconda, Montana. The overall study has been published in several parts: the urinary arsenic analyses and exposure assessment were conducted by Calabrese *et al.* (1993) and Hwang *et al.* (1997); while the model validation was published by Walker and Griffin (1998). In order to conduct a comparison of estimated daily exposure (mg As/day), as an absorbed dose, and urinary arsenic levels (µg As/L), Walker and Griffin (1998) used the following relationship:

$$EXC = (ABS \times CF_{abs}) / (RATE \times CF_{exc})$$

Where:

EXC	=	Urinary arsenic excreted (µg As/L)
ABS	=	Estimated absorbed intake of arsenic per day for each person (mg As/day)
CF_{abs}	=	Conversion factor (1,000 µg/mg)
RATE	=	Estimated Urinary output per day (mL/day)
CF_{exc}	=	Conversion factor (0.001 L/mL)

In the course of this study, detailed site-specific exposure assessments were conducted for the children based on air, soil, indoor dust, drinking water and food (Calabrese *et al.,* 1993; Hwang *et al.,* 1997). The resultant calculated daily exposure was then applied to the equation above, in order to predict urinary arsenic concentrations (using measured urinary outputs for RATE), which were then compared to actual measured urinary arsenic concentrations from 366 children (Hwang *et al.,* 1997). The results of this comparison indicated that the predicted and actual urinary arsenic concentrations were in reasonably good agreement, especially when based on urinary speciated arsenic concentrations.

Walker and Griffin (1998) noted several factors which were considered to affect variability in the actual or predicted urinary concentrations, including:

- Accuracy of assumptions regarding the soil ingestion rate;

- The exposure scenario (*i.e.,* at home *versus* away from home), and the impact different scenarios would have on exposure;

- The importance of exposure to soil *versus* indoor dust: although a small fraction of time is spent out of doors, as much as 45% of the total daily soil/dust intake is derived from outdoor soils;

- Seasonality of urinary arsenic concentrations; urinary concentrations of arsenic have been reported to be highest in late spring and summer, intermediate in fall and early spring, and lowest in winter (Hwang *et al.,* 1997);

- Assumptions regarding urinary output volumes,

- Dietary intake of arsenic, especially with regard to impact on total arsenic concentrations following seafood ingestion; and,

- Soil and dust collection methods, in that smaller particles have a higher proportion of phases to which arsenic may bind (Davis *et al.,* 1996).

There were some limitations to the model employed in this study that should be recognized. The model inherently assumes that 100 % of the estimated daily absorbed intake is completely excreted *via* the urine on a daily basis. However, as noted above, several studies have shown that, at equilibrium, only 40 to 60% of the oral dose of inorganic arsenic is excreted on a daily basis (Buchet *et al.,* 1981b, Farmer and Johnson, 1990). In addition, although creatinine concentrations were determined (Hwang *et al.,* 1997), the authors did not normalize the urinary arsenic concentrations, and thus did not consider the impact of urinary density.

A1-2.7.2.1 Recommendations for Exposure Assessment and Model Selection

Based on the information presented above, several elements are recommended for inclusion in the development and use of a model describing the relationship between urinary speciated arsenic concentrations and total daily exposure to inorganic arsenic. These elements are summarized below:

Exposure estimates are usually expressed as either a daily intake rate (µg As day^{-1}) or as an uptake rate per kilogram body weight (µg As/kg body weight day^{-1}). Intake is based on all relevant pathways of exposure. Exposures should be attributed to different pathways (*e.g.*, oral, inhalation, dermal), and total daily exposures should be expressed in terms of the proportion of arsenic absorbed into the body.

Calculation of absorbed arsenic must consider bioavailability of arsenic for each pathway of exposure, and should consider differences in chemical species, as well as the matrix in which they are found.

Excretion of inorganic arsenic and its metabolites, MMA and DMA, has been estimated to account for 40 to 60% of the total absorbed dose (Holland *et al.,* 1959; Buchet *et al.,* 1981b; Farmer and Johnson, 1990). These studies have reported that urinary excretion of soluble arsenicals (*i.e.*, for which absorption in the gut or lung would be almost complete) approximates an average of 50%.

Urinary arsenic concentration is a surrogate for daily urinary excretion at equilibrium (*i.e.*, when, based on a relatively constant chronic daily intake, the total daily arsenic excretion is relatively constant). As such, it could be expressed as the amount of arsenic excreted in the urine per day. This requires monitoring 24-hour urinary output. A more practical approach would be to take a limited number of urine samples for arsenic analysis (to determine µg As per L of urine), and to then relate these back to total daily excretion. This can be accomplished by accounting for varying densities of the urine during the day (*e.g.*, the first void of the morning is generally more concentrated, and therefore of greater specific gravity). Standardization of chemicals in urine is based on concentrations of creatinine, a by-product of protein metabolism. The daily rate of creatinine elimination is relatively constant (estimated based on typical daily urine volumes and daily creatinine excretion rates). Normalized urinary arsenic concentrations are expressed as "µg As per gram creatinine". Actual urine concentrations for each individual should be normalized using actual creatinine concentrations from the same individual. In the model, predicted urine concentrations would be normalized using the typical population-based record of creatinine concentration.

Speciation of arsenic is important for (1) validation of the urinary arsenic-exposure model, and (2) application of the model to estimate daily exposures to inorganic arsenic. Inorganic arsenic species are of greatest relevance to risk assessment.

A1-2.8 Physiologically-Based Pharmacokinetic (PBPK) Models for Arsenic

There are three PBPK models available for arsenic: The Mann model (Mann *et al.,* 1996a,b); the Yu model (Yu, 1998a, 1998b); and the Menzel model (Menzel *et al.,* 1994). The Mann model simulates the absorption, distribution, metabolism, elimination, and excretion (ADME) of As^{III}, As^{V}, MMA, and DMA after oral and inhalation exposure in hamsters, rabbits, and humans. The Yu model simulates the ADME of As^{III}, As^{V}, MMA, and DMA after oral exposure to inorganic arsenic in mice and rats. The Menzel model is a more preliminary model that predicts internal organ burdens of arsenic during specific oral

exposures, simulating the metabolism and distribution to organs and binding to organs in mice, rats, and humans. The Mann and Yu models are considered "well-derived" and consist of multiple compartments and metabolic processes, and have been validated using experimental data (ATSDR, 2005). The Menzel model has not been validated. None of these models are currently being widely used in the risk assessment of arsenic. These models are described in detail in ATSDR (2000) as well as in the original studies.

Recently, Gentry *et al.* (2004) applied the PBPK models described by Mann *et al.* (1996a,b) to predict the pharmacokinetics of arsenic in the mouse. The model evaluation indicated that pharmacokinetic factors do not explain the difference in outcomes across the various mouse bioassays. The authors suggest that other possible explanations for these differences may relate to strain-specific differences, or to the different durations of dosing in the various mouse studies.

A1-3.0 TOXICOLOGY

Evaluating the toxic effects of arsenic is complicated by the fact that arsenic can exist in several different valence states and occurs in nature and industry as multiple inorganic and organic compounds. The majority of cases and situations involving human toxicity from exposure to arsenic have been associated with exposure to inorganic arsenic compounds. The most common inorganic arsenical in air is arsenic trioxide (As_2O_3), while a variety of inorganic arsenates $(AsO_4)^{-3}$ or arsenites $(AsO_2)^{-1}$ occur in water, soil, and foods. A number of studies have noted differences in the relative toxicity of these compounds, with trivalent arsenites tending to be somewhat more toxic than pentavalent arsenates (ATSDR, 2005). The relative toxicity of arsenic compounds in both animals and humans is generally known to decrease as follows: arsine (III) > organo-arsine derivatives > arsenites (III) > arsenoxides (III) > arsenates (V) > pentavalent organic compounds (V) > arsonium metals (I) > metallic arsenic (0), where the Roman numeral indicates the oxidation state (HSDB, 2003). However, it should be recognized that in many studies: i) the differences in the relative potency are small, and often within the bounds of uncertainty around a NOAEL or LOAEL; ii) different forms of arsenic may be rapidly interconverted, both in the environment and the body; iii) in many studies of human exposure to arsenic, the chemical speciation is not known (ATSDR, 2005).

Organoarsenicals are generally considered to be less toxic than the inorganic forms, although several methyl and phenyl arsenical pesticides may pose a possible human health concern. The major organoarsenicals that have been studied are monomethyl arsonic acid (MMA) and its salts, (monosodium methane arsonate [MSMA] and disodium methane arsonate [DSMA]), dimethyl arsinic acid (DMA, also known as cacodylic acid) and its sodium salt (sodium dimethyl arsinite, or sodium cacodylate), and roxarsone (3-nitro-4-hydroxyphenylarsonic acid). In addition, a number of organic arsenic species are known to occur in fish and shellfish (*e.g.*, arsenobetaine, arsenocholine, various arsenosugars). Available studies on these forms of arsenic have revealed that they are readily excreted from the body in a virtually unchanged form and are essentially non-toxic (ATSDR, 2005). Another type of organoarsenical that has been studied is arsine (AsH_3) and its methyl derivatives. While this form of arsenic is known to have a greater toxic potency than any other known form of arsenic, it is not associated with smelter emissions and would not be expected to occur in the ambient environment surrounding a smelter to any significant extent. While trace amounts of arsine and methylarsines can be formed from inorganic arsenic species through natural biological transformation processes, these forms of arsenic are volatile, which limits their ability to accumulate in soils or water bodies, and they appear to rapidly undergo a variety of reactions which rapidly transform or degrade these compounds to other forms of arsenic (HSDB, 2003). Thus, the

emphasis in the following sections is on inorganic arsenic compounds, although relevant studies on organoarsenicals are provided where data are available.

In a recent review of the toxicokinetics and toxicology of methylated arsenicals, Cohen *et al*. (2006) note that the animal carcinogenicity data for MMAV and DMAV are equivocal. These authors also state that the metabolism and disposition of MMAV and DMAV , when formed endogenously during the metabolism of inorganic arsenic, differs from the metabolism and disposition of these methylated species when exposure is exogenous. Furthermore, the trivalent arsenicals that known to be cytotoxic and indirectly genotoxic in vitro are formed in negligible amounts in organisms exposed exogenously (ingestion) to MMAV or DMAV due to low cellular uptake and limited metabolism of these compounds. Cohen *et al*. (2006) conclude that at anticipated environmental exposures to MMAV and DMAV, carcinogenic risk to humans is unlikely. In a science issue paper produced by the U.S. EPA Office of Pesticide Programs on a mode of carcinogenic action for DMAV (cacodylic acid) (U.S. EPA OPP, 2005), it is also noted that there are differences in methylation efficiency and cellular uptake between direct exposure to DMAV and exposure to inorganic arsenic, with subsequent metabolism to DMAV and other methylated species. The U.S. EPA paper also notes that direct exposure to DMAV results in the production of fewer arsenical metabolites relative to metabolism that occurs following direct exposure to inorganic arsenic. Thus, exposure to inorganic arsenic results in a more complex mixture of metabolites and transformation products. The U.S. EPA paper also states that there is presently insufficient evidence to establish pentavalent MMA and DMA species as the ultimate carcinogenic forms of inorganic arsenic. Rather, it is likely that several inorganic and organic arsenical species may be involved in various modes of action in different target tissues. For DMAV, U.S. EPA OPP (2005) suggests that this substance is a threshold carcinogen with a carcinogenic mode of action that is non-linear. As such, a reference dose has been proposed using benchmark dose modelling.

An important aspect of arsenic toxicology to note is that most laboratory animals appear to be substantially less susceptible to arsenic-induced adverse health effects than humans. Thus, certain animals may not be good quantitative models for studying arsenic toxicity in humans.

A1-3.1 Systemic Toxicity

A1-3.1.1 Animal Studies

There is some evidence which suggests that inorganic arsenic may be an essential micronutrient in goats, chicks, mini pigs and rats (NRC, 1989). However, confirmation of an essential biological role has not been demonstrated to date (NRC, 1999; 2001).

A detailed description of animal studies identified with systemic effects of arsenic exposure have been reviewed elsewhere (WHO-IPCS, 2001; ATSDR, 2005; NRC, 2001).

A1-3.1.1.1 Inhalation Exposure to Inorganic Arsenic

Relatively few inhalation exposure animal studies were identified in the scientific literature. An abbreviated summary of results for some of the inhalation studies in rodents are presented in Table A1.2. In other studies, damage to alveolar macrophages, in the absence of direct cytotoxicity, was reported in in rats exposed to arsenite and arsenate *via* intratracheal instillation (Lantz *et al.,* 1994, 1995). Evidence of an immunological effect of inhaled arsenic in animals has been associated with decrease a humoral response to antigens and in several complement proteins in mice given an intratracheal dose of 5.7 mg As/kg as sodium arsenite (Sikorski *et al.,* 1989). A single intratracheal instillation of 17 mg arsenic trioxide in rats induced an acute fibrogenic response in the rat lung. Exposures to arsenic trioxide resulted in multifocal interstitial pneumonia and focal proliferative bronchiolitis and alveolitis observed at necropsy 14 days post-exposure (Webb *et al.,* 1986).

A1-3.1.1.2 Oral Exposure to Inorganic Arsenic

Oral LD_{50} values (single doses that result in death of 50% of the animals) for arsenite, arsenate and arsenic trioxide in rodents have been reported to range between 4 and >400 mg As/kg bw (Dieke and Richter, 1946; Harrisson *et al.,* 1958; Gaines, 1960; IARC, 1980; Kaise *et al.,* 1989; Brown and Kitchin, 1996; WHO-IPCS, 2001; Schulz *et al.,* 2002). Effects in acute repeat-dose studies included gastrointestinal irritation (with symptoms of vomiting and diarrhea) and mild histological changes of the liver (enlargement of bile ducts) (Heywood and Sortwell, 1979).

Summary results for some oral arsenic studies are presented in Table A1.2.

In other studies, male ddY mice that received oral doses of 0, 3, or 10 mg arsenic trioxide per kg body weight/day 14 days had increased locomotor activity in the low-dose group, which decreased in the high-dose group (Itoh *et al.,* 1990). In addition, several monoamine levels were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum. Chronic exposure to arsenic has also been associated with persistent changes in the concentrations of neurotransmitter substances in the brains of developing and adult Wistar rats (Nagaraja and Desiraju, 1993).

Male and female Wistar rats treated from age 2 days to 60 days by oral gavage with daily administration of 5 mg As/kg body weight (as sodium arsenate) showed decreases in body weight, brain weight, and food consumption by 160 days post-exposure (Nagaraja and Desiraju, 1993; Nagaraja and Desiraju, 1994). As well, acetylcholinesterase (AChE) and glutamic acid decarboxylase (GAD) activity and gamma-aminobutyric acid (GABA) levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period, all of which returned to normal post-exposure with the exception of AChE. Changes in operant conditioning were also observed among the exposed animals.

Santra *et al.* (2000) exposed male BALB/c mice to either to drinking water contaminated with arsenic (3.2 mg/L) or to arsenic-free water (<0.01 mg/L, control) *ad libitum* for up to 15 months. At the end of the study, liver weights increased significantly as did serum levels of aspartate aminotransferase and alanine aminotransferase. At 6 months of treatment, hepatic glutathione and the enzymes glucose-6-phosphate dehydrogenase and glutathione peroxidase were significantly lower than those of the control group. Hepatic catalase activity was significantly reduced at 9 months in the arsenic-fed group. Glutathione-S-transferase and glutathione reductase activities were significantly reduced at 12 and 15 months exposure. Plasma membrane Na^+/K^+ ATPase activity was reduced after 6 months while lipid peroxidation increased significantly after 6 months of arsenic feeding. Liver histology remained normal for the first 9 months, but fatty infiltration was noted after 12 months. At fifteen months, there histologic evidence of liver fibrosis was found in exposed mice.

Schulz *et al.* (2002) exposed male Wistar rats for 4, 8, and 12 weeks with 3.33, 6.66, 13.3, or 26.6 mg/kg of inorganic arsenic (as sodium arsenite) by gavage. Changes in behavioural and electrophysiological parameters were determined. Treated rats exhibited hypoactivity of horizontal ambulation in open field tests and also showed reduced rates of grooming. There were no significant electrophysiological changes throughout the study duration. The weight of organs responsible for immune response (thymus, spleen, adrenals), was found to be significantly reduced, as were delayed-type hypersensitivity (DTH) reactions

and mean cell volume (MCV) of red blood cells. The authors suggest that subchronic low-level exposure to arsenic may affect immune responses and/or spontaneous behaviour of rats.

Garcia-Vargas *et al.* (1995) reported that subchronic treatment of mice with sodium arsenite (20 mg/L) or sodium arsenate (50 mg/L) in drinking water produced a time-dependent porphyric response. Urinary porphyrins were increased by 3 weeks following initial exposure to arsenic, which corresponded with increased activities of some hepatic and renal porphyrin-metabolizing enzymes, and decreased activity of others. Arsenite was more potent in inducing this response than arsenate.

A1-3.1.1.3 Dermal Exposure to Inorganic Arsenic

Data on the systemic toxicological effects in animals in response to dermal exposure to arsenic in animals are extremely limited. Direct irritation of the skin in mice was noted following dermal exposure to 4 mg As/kg-bw/day as potassium arsenite for 30 weeks (Boutwell, 1963). Dermal exposure to 2.5 mg As/kg bw for 18 weeks, 11 times a week, was reported to result in local hyperplasia and skin irritation in mice (Boutwell, 1963). In contrast, no significant dermal irritation was noted in guinea pigs exposed to aqueous solutions containing 4,000 mg As/L as arsenate or 580 mg As/L as arsenite (Wahlberg and Boman 1986). These studies indicate that direct skin contact may be of concern at high exposure levels, but lower levels are unlikely to cause significant acute skin irritation. Other data regarding the acute dermal toxicity indicates that arsenic is not likely to cause lethality or systemic toxicity. No effects were observed in rats following dermal doses up to 1000 mg As/kg-bw as As^{III} or As^{V} (Gaines, 1960).

A1-3.1.1.4 Animal Exposures to Organoarsenicals

Systemic toxicity resulting from acute and subchronic exposures to organoarsenical compounds has been comprehensively reviewed (WHO-IPCS, 2001; ATSDR, 2005; NRC, 2001). Some of the more recent subchronic studies are discussed below.

Few animal inhalation exposure studies with organic arsenic compounds were identified in the literature. A 2-hour LC_{50} of 2117 mg As/m^3 was reported for DMA in female rats (Stevens *et al.,* 1979). Rats and mice exposed to DSMA (the disodium salt of MMA) at concentrations up to 2485 mg As/m^3 in rats and 2811 mg As/m^3in mice did not suffer lethality. Respiratory distress, diarrhea, rhinorrhoea, porphyrin-like encrustation of the eyes, irritation and reduced body weight was evident at these concentrations. Although there was no consistent pattern of gross pathological lesions, female rats showed impacted caeca, blood in the intestine, bright red lungs, and dark spots in the lung.

Acutely lethal oral LD_{50} values ranged from about 102 to >10,000 mg As/kg (Jaghabir *et al.,* 1988; Kerr *et al.,* 1963; NTP 1989; Rogers *et al.,* 1981; Kaise *et al.,* 1985; 1989; Gaines and Linder, 1986). The highest LD_{50} value was for arsenobetaine. The lowest value came from a study by Jaghabir *et al.* (1988) on MMA in rabbits. General weakness and decreased activity, appetite and urine volume, discolored urine and diarrhea were observed. Ahmad *et al.* (1999) reported that mice exposed to one or two oral doses of 720 mg DMA/kg had decreased liver glutathione, cytochrome P450 content, and serum ornithine decarboxylase activity.

Studies involving acute, intermediate and chronic oral exposures to DMA in rats found decreases in body weight gain (Murai *et al.,* 1993; NTP 1989; Rogers *et al.,* 1981; Siewicki 1981). The lowest dose to produce a decrease in growth (LOAEL) was identified as being approximately 4 mg As/kg/day (NTP, 1989). There is also limited animal evidence that organoarsenicals may cause adverse neurological effects (Edmonds and Baker 1986; Rice *et al.,* 1985; Kennedy *et al.,* 1986; NTP 1989).

A1-3.1.2 Human Studies

Arsenic has not been demonstrated to be essential in humans (WHO-IPCS, 2001). Some animal evidence exists to suggest that arsenic might be essential to humans; however, the exact mechanism has not been established and deficiencies in human populations have not been reported.

The majority of data on human health effects from As exposure come from studies of workers and communities exposed *via* inhalation to smelter emissions, and orally to naturally-elevated arsenic concentrations in drinking water supplies. In particular, there have been extensive studies conducted on populations in Taiwan, China, Chile, Argentina and West Bengal-Bangladesh that are chronically exposed to elevated drinking water concentrations. Studies of these populations have contributed much of what is known about arsenic toxicology in humans.

The toxicological database on arsenic clearly indicates that this substance is a systemic toxicant in humans that acts on a number of organs and tissues within the body. Summary results for some human arsenic studies are presented in Table A1.3.

A1-3.1.2.1 Inhalation exposure to inorganic Arsenic

Although there are many studies of the effects of arsenic inhalation in humans, there were no cases of lethality or severe impacts from short-term exposure in the scientific literature. This was interpreted by ATSDR (2000) as indicating that mortality is not likely to be of concern, even at the very high exposure

levels (1 to 100 mg As/m^3) that were once associated with workplace exposures. Acute inhalation exposure may result in severe irritation of the mucous membranes of the upper and lower respiratory tract; symptoms include cough, dyspnea, and chest pain (Friberg *et al.,* 1986). Garlicky breath and gastrointestinal symptoms such as vomiting and diarrhea may also occur (HSDB, 2003). Acute arsenic intoxication may also manifest itself as dermatitis, nasal mucosal irritation, laryngitis, mild bronchitis, and conjunctivitis (Friberg *et al.,* 1986). The mode of action for acute toxicity of arsenic is believed to be due to inflammation of the mucous membranes and increased permeability of the capillaries (HSDB, 2003).

NIOSH lists a revised *Immediately Dangerous to Life and Health* (IDLH) level of 5 mg/m^3 on the NIOSH web site (http://www.cdc.gov/niosh), which is derived from an oral lethality study of calcium arsenate in dogs.

Health effects of subchronic and chronic occupational exposures to arsenic dusts *via* inhalation tend to be related to the irritation of mucous membranes (sequelae include generally mild laryngitis, bronchitis, or rhinitis, and, at high concentrations, perforation of the nasal septum) (Dunlap, 1921; Perry *et al.,* 1948; Pinto and McGill, 1953; Morton and Caron, 1989), and, at high concentrations, the gastrointestinal tract (resulting in nausea, vomiting, and diarrhea) (Beckett *et al.,* 1986; Bolla-Wilson and Bleecker, 1987; Morton and Caron, 1989). These effects typically disappear when exposure ceases. A NOAEL air concentration for a subchronic (2 months) inhalation exposure was reported to be 0.11 mg As/m^3 of inorganic arsenic; the only reported effects at this concentration were nausea and anorexia in one worker (Ide and Bullough, 1988).

Feldman *et al.* (1979) reported an increased prevalence of clinically diagnosed peripheral neuropathy in arsenic-exposed smelter workers (43%), relative to controls (12%). However, the difference in mean nerve conduction velocities (NCV) was not statistically significant.

In a case-control study, copper smelter workers (n = 47) exposed to arsenic for 8 to 40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom *et al.,* 1985). The workers displayed a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in two or more peripheral nerves was reported as "more common" among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to range from 0.05-0.5 mg

As/m^3 (average of 0.36 mg As/m^3), with arsenic trioxide being the predominant chemical form. Daily arsenic uptake was estimated at less than 300 μg/day and was confirmed with workers urinary excretion data. The majority of workers did not receive any significant exposure to other heavy metals.

Lagerkvist *et al.* (1986) further examined these smelter workers for prevalence of Raynaud's phenomenon and for vasospastic tendency (as determined by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%). The FSP% was found to co-vary with the duration of exposure to arsenic, and the prevalence of Raynaud's phenomenon was significantly increased among exposed workers. A second follow-up study (Lagerkvist *et al.* (1988) later found that vasospasticity measurements in exposed workers had improved concurrent with a reduction in arsenic exposure levels, although peripheral vascular effects were still common in the workers (Lagerkvist *et al.*, 1988). Further followup on this group of workers (5 years later) found that the prevalence of abnormally low nerve conduction velocity remained significantly increased in the exposed workers, but that the decrease in mean NCV was now also statistically significant in the tibial (motor) and sural (sensory) nerves (Lagerkvist and Zetterlund, 1994). Blood lead was monitored in this study as a potential confounder, but levels were low and were considered unlikely to have influenced the findings of this study by the authors.

A cross-sectional study of 46 workers in Denmark with varying and unquantified occupational exposure to arsenic in different occupations found that systolic blood pressure was significantly increased in the arsenic-exposed workers, relative to controls (Jensen and Hansen, 1998). Diastolic pressure was also increased in exposed workers, although the difference from controls was not statistically significant.

Various cohort mortality studies of arsenic-exposed workers at smelters (Enterline *et al.*, 1997; Lee-Feldstein 1983; Welch *et al.*, 1982; Wall 1980), orchards (Tollestrup *et al.*, 1995), and tin mines in China (Qiao *et al.*, 1997; Xuan *et al.*, 1993) have all reported an increased risk of mortality from cardiovascular disease, specifically ischemic heart disease and cerebrovascular disease. However, none of these studies provided conclusive evidence that the observed increase in risk was due to arsenic exposure alone, and clear dose-response relationships could not be established.

The same cohort of smelter workers (2802 men) evaluated in Enterline *et al.* (1997) was evaluated in a followup study by Hertz-Picciotto *et al.* (2000). The authors included a 20 year lag time and attempted to account for the healthy worker effect. They found no significant increased incidence of cerebrovascular disease in the workers, but the previoulsy noted association between arsenic exposrue and ischaemic heart disease was strenghthened. Relative risks for cerebrovascular disease ranged from 0.9 to 1.7 at inorganic

arsenic air concentrations ranging from <0.75 to >20 mg/m^3; however, there was no correlation between exposure level and higher relative risk. In fact, the highest exposure level studied (>20 mg/m^3) had a lower relative risk than the 8 to 19.999 mg/m^3 exposure level.

No significant increase in mortality rate due to arteriosclerosis and ischaemic heart disease or cerebrovascular disease was observed among the members of a Montana smelter cohort (Lubin *et al.,* 2000). The findings were similar when the analysis was repeated with an attempt to adjust for the healthy worker effec (Lubin and Fraumeni, 2000). A number of other studies have also found no association between arsenic-exposed workers and cardiovascular diseases. In a Japanese smelter cohort, Tokudome and Kuratsune (1976) found a deficit in mortality from heart diseases (7 observed and 14.9 expected cases). Mortality from circulatory diseases in Australian gold-miners (Armstrong *et al.,* 1979), French gold miners (Simonato *et al.,* 1994), and U.S.arsenical pesticide workers were all significantly lower than expected mortality rates.

Dermatitis has frequently been observed in industrial workers exposed to airborne arsenic, with the highest rates occurring in the workers with the greatest arsenic exposure (Dunlap 1921; Holmqvist 1951; Lagerkvist *et al.,* 1986; Pinto and McGill 1953; Vallee *et al.,* 1960). However, there is limited information available as to the exposure levels that produce dermatitis. A cross-sectional study of workers at a factory where sodium arsenite was prepared found that workers with the highest arsenic exposure (mean air levels ranging from 0.384 to 1.034 mg As/m^3) displayed pigmented skin with hyperkeratinization and multiple warts (Perry *et al.,* 1948). Workers with lower arsenic exposure (estimated to average 0.078 mg As/m^3) were much less affected, but still showed an increased incidence of keratosis relative to controls. Eye irritation has also been frequently reported in conjunction with dermatitis in occupational studies although it is possible that such irritation effects are more related to the physical irritant effects of dust exposure than they are to arsenic.

Mohamed (1998) reported that dermatitis (as characterized by hyperpigmentation, folliculitis, and superficial ulcerations) was observed in 11 employees in one department of a Malaysian tin smelter who were exposed to mean arsenic trioxide concentrations ranging from 0.005 to 0.014 mg/m^3.

Complaints of keratosis were roughly two-fold higher (relative to unexposed controls) in female packaging workers exposed to arsanilic acid at an average concentration of 0.05 mg As/m^3 and in male manufacturing workers exposed to an average concentration of 0.13 mg As/m^3 in a chemical factory (Watrous and McCaughey 1945). However, the reliability of these observations are questionable due to

limitations in study methodology (*e.g.*, alternate causes of keratosis were not investigated, workers tend to not report minor complaints to company officials).

Oral Exposure Studies

Acute high dose oral exposures to inorganic arsenic result in gastrointestinal irritation, including nausea, vomiting, diarrhea, and abdominal pain. These types of symptoms may also occur in longer term, lower dose oral exposures (ATSDR, 2005). Severe gastrointestinal effects, such as hematemesis, hemoperitoneum, gastrointestinal hemorrhage, and necrosis have been reported in cases where acute oral exposure exceeded 8 mg As/kg (Civantos *et al.,* 1995; Fincher and Koerker 1987; Levin-Scherz *et al.,* 1987; Quatrehomme *et al.,* 1992), and also in some cases involving long term human ingestion of 0.03 to 0.05 mg As/kg/day as a medicinal preparation (Lander *et al.,* 1975; Morris *et al.,* 1974). Minimum lethal oral doses for humans have been reported to be in the range of 1 to 3 mg As/kg (Vallee *et al.,* 1960; Armstrong *et al.,* 1984; Levin-Scherz *et al.,* 1987). Other effects from acute and sub-acute doses include haematological effects (anemia and leukopenia), hepatic effects (hepatitis and elevated serum transaminase levels), renal effects (proteinuria and elevated serum creatine), respiratory effects (haemorrhagic bronchitis), neurotoxicity (encephalopathy [with symptoms ranging from confusion, lethargy, headache, seizures to coma], peripheral neuropathy, acute demyelinating polyneuropathy) (Armstrong *et al.,* 1984; Fincher and Koerker, 1987; Greenberg, 1996). An acute oral LOAEL in humans of 1 mg As/kg body weight/day was reported for these types of effects (Armstrong *et al.,* 1984).

In a suicide attempt, severe gastrointestinal irritation (vomiting, diarrhea, abdominal pain) but no adverse cardiovascular effects were noted after acute human ingestion of 793 mg/kg arsenic of an organoarsenical (monosodium methanearsenate) (Shum *et al.,* 1995). Gastrointestinal irritation and sinus tachycardia were noted in a case of human acute ingestion of 77 mg/kg arsenic (as dimethyl arsenic acid and dimethyl arsenate) (Lee *et al.,* 1995).

Subchronic exposures *via* oral administration have been reported to result in cardiovascular effects (abnormal electrocardiogram), gastrointestinal irritation, haematological effects (anaemia and leukopoenia), hepatic effects (mild hepatomegaly), and dermal effects (conjunctivitis, edema of eyelids, and hyperkeratosis) (Holland, 1904; Mizuta *et al.,* 1956; Wagner *et al.,* 1979; Franzblau and Lilis, 1989). Based on these effects, a subchronic LOAEL of 0.05 mg As/kg body weight/day was reported for humans fed arsenic for 2 to 3 weeks (Mizuta *et al.,* 1956).

While respiratory effects are not generally associated with repeated oral ingestion of low arsenic doses, a few studies have reported minor respiratory symptoms, such as cough, sputum, rhinorrhea, and sore throat, in people with repeated oral exposure to 0.03 to 0.05 mg As/kg/day (Ahmad *et al.,* 1999; Mizuta *et al.,* 1956). In acute poisoning incidents, respiratory symptoms are more commonly reported but are likely secondary effects of cardiovascular injury (ATSDR, 2005).

One of the most characteristic effects of inorganic arsenic ingestion is a pattern of skin changes that include generalized hyperkeratosis and formation of hyperkeratotic warts or corns on the palms and soles, along with areas of hyperpigmentation interspersed with small areas of hypopigmentation on the face, neck, and back. These and other dermal effects have been noted in a large majority of human studies involving chronic oral exposure to inorganic arsenic in drinking water and the diet, and appear to be a sensitive indicator of arsenic exposure and toxicity (ATSDR, 2005). The weight of available evidence suggests that dermal effects may be expected when oral ingestion of inorganic arsenic exceeds 0.01 mg As/kg/day; the available literature indicates that such effects are not observed when the rate of arsenic ingestion is <0.01 mg As/kg/day (ATSDR, 2005). Interestingly, the hallmark dermal lesions associated with human oral exposure to arsenic have not been found to occur in experimental animals. In addition, organoarsenicals do not appear to cause these types of skin effects in either humans or animals.

A large number of epidemiological studies and case reports also demonstrate that ingestion of inorganic arsenic may result in neurological effects in both sensory and motor nerves. Acute, high-dose exposures (>2 mg As/kg/day) have been associated with encephalopathy, headaches, lethargy, mental confusion, hallucination, seizures, peripheral neuropathy and coma, while longer term exposures to lower levels (0.03 to 0.1 mg As/kg/day) have been associated with such effects as muscular weakness, paresthesia (of the limbs and extremities, ranging from numbness to prickling sensation), electromyographic abnormalities, functional denervation, decreased sensitivity to stimuli, peripheral neuropathy, and neurobehavioural abnormalities (ATSDR, 2005). Generally, such neurological effects are not observed in populations chronically exposed to doses of <0.006 mg As/kg/day (Harrington *et al.,* 1958; Hindmarsh *et al.,* 1977; Southwick *et al.,* 1981). Hindmarsh *et al.* (1977) assessed the effect of drinking water with high arsenic concentrations on electromyographic abnormalities. Out of 110 persons exposed to elevated arsenic concentrations in drinking water, 32 were studied using electromyography (EMG), and compared to 12 non-exposed referents. There was a positive relationship observed between EMG abnormalities and water and hair arsenic concentrations. Among those drinking water containing >1 mg As/L, the frequency of EMG abnormalities was 50%. Fatigue, headache, dizziness, insomnia, nightmare, and numbness of the extremities were among the symptoms reported at 0.005 mg As/kg/day in a study of

31 141 inhabitants of 77 villages in Xinjiang, China (Lianfang and Jianzhong, 1994). Recovery from neurological effects of arsenic in humans has been described as slow and generally incomplete following cessation of exposure (Le Quesene and McLeod, 1977; Murphy *et al.,* 1981; Fincher and Koerker, 1987; Kiburn, 1997). Similar to skin effects, animals appear less susceptible to the neurological effects of inorganic arsenic than humans. Among animals, neurological effects have been observed only in monkeys and rabbits (Heywood and Sortwell, 1979; Nemec *et al.,* 1998).

In addition to skin and neurological effects, a large number of studies in humans have demonstrated that arsenic ingestion may lead to adverse effects on the cardiovascular system. Cardiovascular effects attributed to chronic arsenic exposure included Blackfoot disease, arterial thickening, myocardial depolarization, arrythmias, Raynaud's disease, ischemic heart disease and thrombosis, cerebrovascular disease, hypertension, hypotension; and in several epidemiological studies, increased mortality due to cardiovascular disease was reported (ATSDR, 2005; WHO-IPCS, 2001).

Various other effects on other organs and tissues have also been reported following arsenic exposure. These include hematological effects such as anemia, leukopenia, suppression of erythropoiesis, which have been observed in a number of (but not all) acute, intermediate and chronic exposure studies in humans. Various hepatic effects have also been reported following oral exposure to arsenic compounds including: edema, altered enzyme activity, hepatomegaly, portal fibrosis and hypertension, bleeding from esophageal varices; central and vascular fibrosis; cirrhosis, ascites, and fatty liver (ATSDR, 2005). The kidney does not appear to be target organ of inorganic arsenic toxicity based on the weight of available evidence from both human and animal studies; any renal effects that do occur are considered secondary effects of injury or changes to other tissues and organs (ATSDR, 2005). However, limited data from animal studies suggests that oral exposure to organoarsenicals can result in kidney effects (Abdo *et al.,* 1989; Kerr *et al.,* 1963; NTP 1989; Jaghabir *et al.,* 1989).

A large number of studies have been conducted on populations in villages in Taiwan that obtain their drinking water from artesian wells containing elevated inorganic arsenic concentrations. A number of these studies are described and discussed in the following paragraphs.

Tseng *et al.* (1968) reported increased incidences of hyperpigmentation and keratosis with age and dose in a population exposed to arsenic in drinking water. The overall prevalences of hyperpigmentation and keratosis in the exposed groups are 184 and 71 per 1,000, respectively.

Tseng (1977) found an increased incidence of Blackfoot disease that increased with age and dose. The prevalences (males and females combined) at the low dose were 4.6 per 1,000 for the 20 to 39 year age group, 10.5 per 1,000 for the 40 to 59 year group, and 20.3 per 1,000 for the >60 year group. However, a later study suggests that the prevalence rates of Blackfoot disease may not be entirely due to arsenic exposure (Lu, 1990).

Southwick *et al.* (1983) demonstrated a marginally increased incidence of a variety of skin lesions (palmar and plantar keratosis, diffuse palmar or plantar hyperkeratosis, diffuse pigmentation, and arterial insufficiency) in the Taiwanese individuals exposed to arsenic. The incidences were 2.9% (3/105) in the control group and 6.3% (9/144) in the exposed group, which were not significantly different. There was also a slight, although statistically insignificant, increase in the percent of exposed individuals with abnormal nerve conduction. However, the investigators excluded all individuals older than 47 years from the nerve conduction portion of the study. This limits interpretation of the findings as these individuals would be most likely to have the longest exposure to arsenic. Furthermore, the number of subjects in this study was insufficient to establish statistical significance. Although neither the increased incidence of skin lesions nor the increase in abnormal nerve conduction was statistically significant, it was considered by the U.S. EPA (1998) that these effects may be biologically significant as the same abnormalities occur at higher doses in other studies.

Wu *et al.* (1989) found significantly increased mortality rates of peripheral vascular diseases and cardiovascular diseases that were associated with concentrations of arsenic in well water in Taiwan.

Lai *et al.* (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus in 891 adult residents of villages in southern Taiwan where arseniasis is endemic. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes medical treatment. Cumulative arsenic exposure (CAE) was determined from detailed history of drinking artesian well water. A dose-response relationship was found between CAE and prevalence of diabetes mellitus, which remained significant after adjustment for age, sex, body mass index, and activity level at work. Multiple logistic regression analysis gave multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1 to 15 mg/L-year and >15.0 mg/L-year, relative to unexposed controls.

Chen *et al.* (1995) investigated the association between long-term exposure to inorganicarsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in Taiwan villages where arseniasis was endemic. Arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and measured concentrations in well water.

Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension, relative to residents in areas where arseniasis is not endemic. A number of variables including duration of well water consumption, average As water concentration, and cumulative As exposure were all found to be significantly associated with hypertension.

Tseng *et al.* (1996) investigated the relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in Blackfoot disease-endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle and brachial arteries and estimation of their long-term arsenic exposure. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A positive dose-response relationship was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77, and 4.28 for those who had cumulative As exposures of 0.1 to 19.9 and ≥20 (mg/L-years), respectively. A follow up study (Tseng *et al.,* 1997) demonstrated that PVD incidence was correlated with ingested As rather than abnormal lipid profiles, which are often associated with arterial diseases such as atherosclerosis. The possible influence of platelet aggregation and coagulation profiles were not accounted for in this study.

Chen *et al.* (1996) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure in Taiwan. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were evaluated with respect to an association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 recorded ISHD deaths, the cumulative ISHD mortalities from birth to age 79 were reported to be 3.4, 3.5, 4.7, and 6.6% in association with median As concentrations of < 0.1, 0.1 to 0.34, 0.35 to 0.59, and >0.6 mg/L, respectively. Multivariate-adjusted relative risks associated with CAE from well water were 2.46, 3.97, and 6.47 for 0.1 to 9.9 mg/L, 10.0 to 19.9 mg/L, and >20 (mg/L-years), respectively.

Chiou *et al.* (1997) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3,901 households were studied. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response relationship was most prominent for

cerebral infarction with multivariate-adjusted odds ratios of 1.0, 3.4, 4.5, and 6.9, for households consuming well water with As concentration of 0, 0.1 to 50.0, 50.1 to 299.9, and >300 μg/L, respectively. Odds ratios for cerebrovascular disease and cerebral infarction were also significantly greater in the As-exposed groups *versus* controls.

In a recent ecological study in Taiwan, Tsai *et al.* (1999) studied age and sex-specific mortality rates from different causes from 1971 to 1994, and compared these rates to local rates in both the Chiayi-Tainan county and the whole of Taiwan. The total number of deaths and person-years for the study group was 20,067 and 2,913,382. An excess mortality from ischaemic heart diseases was found with a standardized mortality ratio (SMR) of 175, along with a small but significant excess in the mortality from cardiovascular disease (SMR of 114). Tsai *et al.* (1999) also examined mortality from diabetes mellitus in 4 townships in southwestern Taiwan. The mean concentration of arsenic in well-water consumed in these townships from the 1900's to the 1970's was 780 :g/L. Standard mortality ratios for males were 1.35 and 1.14 using a local and national reference group respectively. For females, the corresponding ratios were 1.55 and 1.23.

Tseng *et al.* (2000) studied diabetes mellitus in 3 villages in southwestern Taiwan with similar exposure conditions. The CAE was adjusted for age, sex and body-mass index. The relative risk for developing type II diabetes mellitus among the study population was 2.1 for those with a CAE >17 mg/L-years compared to those with CAE <17 mg/L-years. When CAE is considered as a continuous variable, the adjusted relative risk is 1.03 for every 1 mg/L-year of exposure.

Studies of the effects of oral arsenic exposure have been conducted in a number of other countries as well. Rahman and Axelson (1995) investigated the association between diabetes mellitus and arsenic in drinking water using 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (based on death certificates) were compared with 31 controls. The controls had no incidence of cancer, cardiovascular or cerebrovascular disease. There was a weakly significant increase in odds ratios for diabetes mellitus with increasing arsenic exposure. It must be recognized however, that the small number of subjects in this study limits any meaningful conclusions on arsenic exposure and diabetes. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30

U.S. counties from 1968 to 1984. The mean As levels in drinking water ranged from 5.4 to 91.5 μg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries for counties exceeding 20 μg/L in drinking water were 1.9 for females and 1.6 for males. Elevated incidence of

congenital abnormalities of the heart and circulatory system was also noted in the >20 µg/L group. A study of arsenic exposure from drinking water and mortality outcome in Millard County, Utah, was conducted by Lewis *et al.* (1999). A statistically significant association with mortality from hypertensive heart disease was reported. Median drinking water concentration of arsenic ranged from 14 to 166 µg/L for the 946 subjects evaluated in the study. The standard mortality ratios (SMR) without considering specific exposure levels were 2.20 for males and 1.73 for females. However, when cumulative arsenic exposure was considered, there was no apparent dose response relationship between arsenic exposure in drinking water and hypertensive disorders. The study also found a deficit in the mortality from cardiovascular and ischaemic heart diseases. The low smoking rates among the church members may have explained the low SMRs for those vascular causes of death related to cigarette smoking (Villanueva and Kogevinas, 1999).

In recent years, there have been a large number of studies conducted regarding the adverse effects of ingestion of arsenic in drinking water in the Gangetic plain area of India and Bangladesh, where over 30 million people have potentially been exposed to elevated arsenic concentrations in well-water (NRC, 2001). This is the largest population known to be incurring chronic exposure to elevated arsenic concentrations in drinking water. A number of these studies are summarized in the following paragraphs.

Skin Lesions

Mazumder *et al.* (1998) studied the relationship between skin keratosis and hyperpigmentation and consumption of arsenic-contaminated drinking water in a cross-sectional study of a population in West Bengal, India. Each of 7,683 participants from areas of both high and low arsenic-exposure were questioned about drinking water sources, water intake, diet, medical symptoms, height, weight, and other variables. Participants were also medically examined, with an emphasis on skin lesions. Water samples were collected from each tube-well used by the study participants. The age-adjusted prevalence of keratosis rose from zero out of 100 in the lowest exposure level (< 50 µg As/L) to 8.3 per 100 for females drinking water containing > 800 µg As/L. For males, the age-adjusted prevalence of keratosis increased from 0.2 per 100 in the lowest exposure category to 10.7 per 100 for males in the > 800 µg As/L category. For females, the age-adjusted prevalence of hyperpigmentation rose from 0.3 per 100 in the lowest exposure category to 11.5 per 100 in the highest exposure category; for males the age-adjusted prevalence rose from 0.4 per 100 in the lowest exposure category to 22.7 per 100 in the highest exposure category. Comparisons of dose on a body weight basis found that men had roughly 2 to 3 times the prevalence of both keratosis and hyperpigmentation compared to women apparently ingesting the same dose of arsenic

from drinking water. Subjects below 80% of their body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility to this skin condition. No such difference was observed for hyperpigmentation though.

OEHHA (2000) reports that a dose-response analysis was conducted on the data sets from Mazumder *et al.* (1998) using the benchmark dose (BD) software provided by U.S. EPA (BMDS, version 1.2, 1999). Since the response for keratosis ranged only to 11 percent, a response criterion of one percent was used instead of five percent. A linear dose-response model was fit to the data for male skin keratoses. An effective dose (ED_{01}) of 58.5 µg As/L and a benchmark dose (BD_{01}) of 49.7 µg As/L were obtained. The fit of the model to the data was considered statistically acceptable. Assuming an average body weight of 60 kg and daily water consumption of three L/d, OEHHA estimated the BD_{01} would be equivalent to 2.5 µg As/kg/day, and could be considered a chronic oral NOAEL for skin effects. A ten-fold uncertainty factor for intraspecies variability was applied to this value to obtain a health-protective, oral exposure criterion of 0.25 µg/kg/day. A similar estimate based on the male hyperpigmentation data yielded a lower value (0.09 µg/kg/day), which was rejected due to poor model fit. Although derived using a different approach, the BD_{01} value of 0.25 µg/kg/day is very similar to the U.S. EPA oral RfD of 0.3 µg/kg/day that is based on skin effects observed in an earlier study by Tseng (1977) (IRIS, 1998). Tondel *et al.* (1999) examined skin lesions in 1481 subjects, 30 years of age in four villages in Bangladesh. All subjects had a history of arsenic exposure through arsenic-contaminated tube wells. Arsenic concentrations in the drinking water ranged from 10 to 2,040 µg As/L. There were 430 individuals with skin lesions (pigmentation changes or keratosis). The age-adjusted prevalence rate of skin lesions increased from 18.6 per 100 in the lowest exposure category (<150 µg As/L) to 37.0 per 100 in the highest exposure category (>1,000 µg As/L) for males, and from 17.9 per 100 in the lowest exposure category to 24.9 per 100 in the highest exposure category for females. The trends were statistically significant for both males and females. A similar relationship was also observed when the exposure was considered on a dose basis.

In a cross-sectional survey of melanosis in three contiguous rural villages in Bangladesh, Ahsan *et al.* (2000) found a relatively high prevalence of skin lesions in subjects whose drinking-water samples had very low arsenic concentrations. It was found that 13.9% of all subjects with skin lesions were currently drinking water with an arsenic concentration <10 :g/L.

Hypertension

Rahman *et al.* (1999) performed a cross-sectional evaluation of blood pressure in 1,595 adults (>30 years of age) living in 4 villages in rural Bangladesh. Most of the well water used by the study population for drinking had arsenic concentrations in excess of 50:g/L. Using Mantel-Haenzel-adjusted prevalence rations for hypertension, the study found that both average and cumulative exposure to arsenic in drinking water are associated with hypertension. The ratios were 1.2, 2.2 and 2.5 for exposure groups at <500, 500 to 1,000 and >1,000 :g of arsenic/L. CAE was calculated by multiplying the arsenic concentration in the drinking water well by the number of years using that well. The ratios for cumulative exposure were 0.8, 1.5, 2.2 and 3.0 for exposure groups at <1.0, 1.0≤5.0, 5.0≤10.0 and >10 mg/L-years.

Neurological Effects

Mukerjee *et al.* (2002) reported on neurological effects observed in patients with arsenicosis from West Bengal. A total of 451 patients from three districts (Murshidabad, Nadia, and Burdwan) were studied (267 males; 184 females) ranging in age from 11 to 79 years. All patients had skin lesions, positive biomarkers for arsenicosis and were clearly exposed to arsenic-contaminated drinking water. Peripheral neuropathy was the predominant neurological complication in these patients, and was characterized by paresthesias, pain in the distal parts of extremities, distal limb weakness/atrophy. Sensory effects were more common than motor impairment in patients with neuropathy. Nerve conduction and electromyographic studies performed in 88 cases revealed dysfunction of sensory nerves in 45 and 27%, and of motor nerves in 20 and 16.7% of patients with moderate and mild clinical neuropathies, respectively. Evoked potential studies performed in a subset of 20 patients were largely normal except for two instances each of abnormal visual evoked potential and brainstem auditory evoked potential findings.

Dermal Exposure Studies

Data regarding the toxicity of arsenic in humans following dermal exposure are extremely limited. In most cases of human arsenic exposure, dermal exposure has co-occurred with oral and/or inhalation exposures, making it difficult to separate out any effects that may be specific to the dermal route.

Bourrain *et al.* (1998) reported a case of sensitization to DMA in a 26-year old woman who was occupationally exposed to DMA and experienced eczema on her face. Patch testing confirmed an allergic reaction to DMA, and subsequent avoidance of DMA resulted in disappearance of the symptoms.

Occupational exposures to arsenic dusts causes contact dermatitis (typified by erythema and swelling, while papules and vesicles occurring in more severe cases) (Holmqvist, 1951; Pinto and McGill, 1953). These studies, in combination with the animal studies, suggest that the induction of dermatitis is likely limited to relatively high concentrations (ATSDR, 2005). Studies of dermal sensitization *via* patch tests (Holmqvist, 1951; Wahlberg and Boman, 1986) have yielded mixed results. ATSDR (2000) concluded that the relevance of the positive patch test results to typical environmental exposures was doubtful.

A1-3.2 Reproductive and Developmental Toxicity

A1-3.2.1 Animal Studies on the Reproductive and Developmental Effects of Arsenic Exposure

Summary results for some animal reproductive and developmental studies are presented in Table A1.4.

Reproductive toxicity is the occurrence of adverse effects on the reproductive system, while developmental toxicity is the occurrence of adverse effects on the developing organism resulting from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation.

An expert panel convened by TERA on June 14, 1999 (TERA, 1999) reviewed a series of studies sponsored by Elf Atochem North America, Inc. on the potential developmental effects of inorganic arsenic. The reviewed studies included DeSesso *et al.* (1998) and a series of peer-reviewed papers describing new experimental data (Nemec *et al.,* 1998; Holson *et al.,* 1999; 2000; Stump *et al.,* 1999). In addition, as inorganic arsenic has been commonly considered to be a teratogenic agent, causing neural tube defects in particular, in hamsters, mice, and rats exposed primarily *via* intravenous or intraperitoneal injections, the Panel sponsor conducted an examination of this "older" literature database, and concluded that it was not appropriate for determining whether inorganic arsenic poses a risk of developmental effects in humans. This conclusion is in agreement with that of DeSesso *et al.* (1998), and is based on the following points:

The older studies were not designed for use in risk assessment, but were designed to study the formation of neural tube defects; thus, single, high doses of an agent known to cause these defects were administered, typically by intravenous or intraperitoneal injection (which are not relevant environmental routes of exposure), at a known critical period in neural tube development;

Dose-response relationships were not determined;

Small numbers of animals were used; and

Study methods and results (including maternal effects) were not clearly or completely described.

In light of the inadequate nature of the existing laboratory animal and epidemiology literature, Elf Atochem North America, Inc. sponsored and reported a series of new experimental developmental toxicity studies in rats, mice, and rabbits. All studies were conducted in compliance with U.S. regulatory guidelines and Good Laboratory Practices. All studies employed a dose-response design, with a concurrent control group and three or four dose groups that were selected based on preliminary range-finding studies. The methods and results of these studies are summarized in the following paragraphs.

At the onset of these studies, single high dose intraperitoneal injections of sodium arsenate (the compound most frequently used by earlier investigators), and arsenic trioxide on gestational day 9 of the rat were used to replicate the findings of the older literature and to validate the rat as an animal model. The *i.p.* exposures resulted in such effects as maternal toxicity and lethality, increased post-implantation loss, decreased fetal weights, and increased malformation rates (especially neural tube and eye defects) (TERA, 1999). These findings were consistent with those of the older studies on neural tube defects.

Even at oral doses of arsenic trioxide that caused maternal toxicity and/or lethality, no increase in the rate of fetal malformations was observed (Stump *et al.,* 1999). The findings of this study are consistent with previous studies in which single high doses of orally administered inorganic arsenic failed to produce malformations in laboratory animals. Repeated oral administration of inorganic arsenic to rats, mice, and rabbits did not increase the incidence of malformations, even at doses that caused maternal toxicity and lethality (TERA, 1999). Administered doses were as high as 10 mg/kg/day, which resulted in reduced fetal weights but post-implantation loss and the incidence of malformations were not affected at this dose level. Another study exposed mice and rabbits by gavage to arsinic acid daily throughout the period of organogenesis, using doses up to 48 mg/kg/d (mice) and 3 mg/kg/d (rabbits) (Nemec *et al.,* 1998). Maternal toxicity and lethality occurred in mice at the middle and high doses and in rabbits at the high dose only. Post-implantation loss increased and fetal weights decreased at the high dose in mice. These effects were not observed in rabbits. Overall, the incidence of malformations and variations were not increased by repeated oral exposures to inorganic arsenic in mice or in rabbits (TERA, 1999).

Holson *et al.* (1999; 2000) conducted a study in which rats received repeated inhalation exposures to arsenic trioxide, for a period that extended from before mating and throughout gestation. The exposure concentrations tested were as high as 10 mg/m^3 in air. No prenatal developmental toxicity as indicated by

a change in malformation rate, incidence of post-implantation loss, and mean fetal weight was observed in association with these inhalation exposures. Maternal toxicity was observed at the high dose of 10 mg/m^3.

The TERA (1999) Panel Report summarized the results of the new research as showing an association between exposure of pregnant animals to inorganic arsenic and neural tube defects in offspring is a consequence of high systemic doses. Such doses may only be obtainable by injection, a non-relevant route of exposure for animals and humans in the ambient environment (TERA, 1999). No developmental or teratogenic effects occurred following inhalation exposure.

As pointed out above, there is a long history of reproductive and developmental studies conducted to assess the effects of arsenic on laboratory animals and their offspring. The majority of these studies have been conducted *via* parenteral administration. These studies found that intravenous and intraperitoneal administration of inorganic arsenic was associated with fetal resorption, delayed growth and developmental malformations (primarily of the central nervous system, but also of the eye, skeleton, kidney and/or gonads) in hamsters, mice and rats (*e.g.,* Ferm and Carpenter, 1968; Ferm and Kilham, 1977; Hood *et al.,* 1977, 1978; Hood and Harrison, 1982; Willhite and Ferm, 1984; Ferm and Hanlon, 1986; Carpenter, 1987; Mason *et al.,* 1989; Domingo *et al.,* 1992). Statistical analyses have indicated a NOAEL of 2.5 mg As/kg, based on hamsters dosed on gestational days 8, 11 or 12 (Hood and Harrison, 1982) and a LOAEL of 5 mg As/kg, based on several studies. The effects reported to result from parenteral administration are supported by the results of *in vitro* studies of the developmental impacts of arsenic. Growth retardation, developmental abnormalities and fetotoxicity were reported to be treatment-related effects following incubation of post-implanted mouse embryos with sodium arsenite and arsenate (Chaineau *et al.,* 1990). Tabacova *et al.* (1996) conducted an *in vitro* study of the effects of arsenate and arsenite in whole mouse embryo culture. Based on ED_{50} values for growth, development and malformation (consisting of neural abnormalities such as cranial neural tube non-closure), arsenite (As[V]) was about three-fold more potent than arsenate (As[III]). Tabacova *et al.* (1996) observed a decreasing susceptibility to neural tube defects with increasing gestational age of embryos. Data on the rates of neural tube defects in cultured whole embryos were interpreted by Tabacova *et al.* (1997) as indicating a possible oxidative damage mechanism of action for this effect.

The severity of developmental toxicity of arsenic in animals exposed *via* oral and inhalation routes differs significantly from that following parenteral administration. While oral and inhalation studies have indicated increased fetal resorptions and/or decreased offspring survival in rabbits, hamsters and mice,

these effects were accompanied by maternal toxicity, and were not accompanied by significant increases in the occurrence of fetal malformations (Baxley *et al.,* 1981; Willhite, 1981; Kamkin, 1982; Hood and Harrison, 1982; WIL Research Laboratories, 1988a,b; Hood *et al.,* 1998). In rats, exposure to arsenic *via* oral or inhalation exposure did not result in adverse embryonic effects, nor were resorptions or teratogenicity observed in several studies (Kojima, 1974; Hood *et al.,* 1977; Stump *et al.,* 1998a, b). A few studies indicated increased rate of malformations in mice (Hood *et al.,* 1978; Nagymajtényi *et al.,* 1985) and delayed neurological development in pups (Earnest and Hood, 1981) following oral or inhalation exposure, but no data was available regarding maternal toxicity in these studies, and therefore the role of maternal toxicity in the occurrence of these effects cannot be ruled out. Colomina *et al.* (1996) observed that concomitant maternal exposure to arsenic and maternal stress (induced by restraint) resulted in an increase in fetal malformations, but neither treatment alone caused malformations.

A number of relevant reproductive or developmental toxicity studies in experimental animals using either the oral or inhalation route of exposure are described in the following paragraphs.

Nagymajtenyi *et al.* (1985) exposed pregnant mice to 0.26, 2.9, or 28.5 mg/m³ arsenic trioxide for four hours per day on days 9 to12 of gestation. A dose-related decrease in fetal weight was observed in the offspring of exposed dams. However, significantly increased fetal malformations were observed only in the highest dose group (primarily delayed ossification), with an apparent positive dose-related trend in the number of fetuses with malformations (3, 7, and 31, respectively in the three dose groups). The proportion of dead fetuses per dose group also increased in a dose-related manner. Maternal toxicity was not reported in this study; thus, the possibility that adverse maternal effects accounted for some of the observed fetal effects cannot be discounted.

Despite the fact that maternal toxicity was not accounted for, the OEHHA (2000) derived an acute 4 hour Reference Exposure Level (REL) of 0.19 μg As/m^3 based on the study by Nagymajtenyi *et al.* (1985). A cumulative uncertainty factor of 1000-fold (10-fold each for use of a LOAEL, interspecies extrapolation and intraspecies differences in sensitivity) was applied to the study LOAEL (which was adjusted to reflect only the arsenic component of arsenic trioxide). The OEHHA (2000) also used this same study as the basis for their chronic REL. This was accomplished by using the average experimental exposure for the LOAEL group (determined to be 33 μg As/m^3) and applying a cumulative uncertainty factor of 1,000 (10-fold each for use of a LOAEL, interspecies extrapolation and intraspecies differences in sensitivity) to yield a chronic REL of 0.03 μg As/m^3. According to the OEHHA (2000), route-to-route conversion of the LOAEL in the key study indicates that this chronic REL should also be protective of adverse effects

that have been observed in studies with oral exposures, either in food or drinking water. Also, OEHHA considers that had available human data been used instead of animal data in the REL derivation, a similar value would have been obtained. Thus, the chronic REL from animal data is believed to be protective of potential adverse health effects in humans.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to arsenic trioxide at a concentration of 40 mg/m³ for 48 hours (Kamil'dzhanov, 1982). Intravenous injection studies of radioactive arsenate (V) or arsenite (III) in several rodent species, was found to result in accumulation of arsenic in the lumen of the epididymal duct, suggesting that long term exposure of sperm may occur *in vivo* following acute exposure to As (Danielsson, 1984).

Omura *et al.* (1996a,b) administered arsenic trioxide intratracheally in hamsters and rats. Animals were dosed twice a week for 6 to 8 weeks at a level of 1.3 mg/kg. There were no effects on hamster or rat body weight or weights of the testes or epididymis.

Holson *et al.* (1999; 2000) administered arsenic trioxide by whole body inhalation to groups of 25 Crl:CD (SD)BR female rats for six hours per day, beginning fourteen days prior to mating and continuing throughout mating. The mean exposure levels were 0.24, 2.6, and 8.3 mg As/m^3. Maternal toxicity, as evidenced by rales, laboured breathing, gastrointestinal lesions, a decrease in net body weight gain, and decreased food intake during pre-mating and gestation, was observed only at the highest concentration tested. Necropsy revealed no evidence of lung lesions. The NOAEL for maternal toxicity was determined to be 2.6 mg As/m^3. No treatment-related fetal malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was therefore 8.3 mg As/m^3. The median mass aerodynamic diameter of particle sizes generated in the exposure chambers ranged from 1.9 to 2.2 μm for the three exposure groups, indicating that the dust particles were in the respirable range. No blood or urine arsenic analyses were conducted to measure delivered doses in target tissues (OEHHA, 2000).

Minimal developmental effects have been reported in offspring of animals exposed chronically to arsenic *via* oral administration. Schroeder and Mitchener (1971) observed no significant effects in mice exposed to 1 mg As/kg body weight/day, as sodium arsenite, *via* drinking water for 3 generations. Similarly, no effects were reported for the offspring of rats treated chronically *via* gastric intubation with an arsenic solution at a dose level of 25 μg/kg/day for a period of 7 months, including pregnancy (Nadeenko *et al.*, 1978). There were no significant embryotoxic effects and only infrequent slight expansion of ventricles

of the cerebrum, renal pelvic area and urinary bladder. At much higher doses, severe maternal and fetal toxicity (indicated by decreased body weights and reduced survival) was observed in mice administered arsenic acid in the diet for 2 generations (Hazleton Laboratories, 1990). Maternal and fetal NOAELs were reported to be 100 and 20 mg As/kg body weight/day, respectively, in this study. Hood *et al.* (1977) reported that very high single oral doses of arsenate solutions (up to 120 mg/kg) to pregnant mice were necessary to cause prenatal fetal toxicity, while multiple doses of 60 mg/kg for 3 days had little effect.

Male and female mice dosed with MMA (55 mg As/kg/day) prior to mating and during pregnancy, were found to produce fewer litters than normal, which was attributed to decreased fertility of the males (Prukop and Savage, 1986).

Rogers *et al.* (1981) administered DMA orally to pregnant mice (200 to 600 mg/kg per day) and rats (7.5 to 60 mg/kg per day) on days 7 to 16 of gestation. Significant fetal mortality in mice was observed at 600 mg/kg per day and in rats at 50 to 60 mg/kg per day. A significant decrease in fetal weight gain was observed in mice at 400 to 600 mg/kg and rats at 40 to 60 mg/kg. Cleft palate in mice was observed at the two highest doses. There was also a significantly decreased incidence of supernumerary ribs. In the 400 mg/kg group, four mouse fetuses had irregular palatine rugae. In rats, the average number of sternal and caudal ossifications was decreased at the two highest doses and the percentage of irregular palatine rugae increased significantly with dose. There was also a dose-related decrease in maternal weight gain and an increase in lethality at the highest dose for the mice (59%) and the two highest doses for the rat (14 to 67%

Hood (1998) investigated the toxicity of organic arsenic (DMA), and based on the extreme doses required for fetal toxicity, concluded that any prenatal toxicity observed following exposure to inorganic arsenicals is due to either direct or maternally-mediated effects of the parent compound, rather than to effects of the methylated metabolites.

Nemec *et al.* (1998) evaluated the developmental toxicity of inorganic arsenic in CD-1 mice and New Zealand White rabbits. Mice were exposed by gavage to aqueous arsenic acid at doses of 0, 7.5, 24, or 48 mg/kg/day on gestation days six through 15, and rabbits were exposed to 0, 0.19, 0.75, or 3.0 mg/kg/day on gestational days six through 18. In mice, treatment-related maternal toxicity, which included gastrointestinal lesions, was observed only at the two highest doses tested. Mortality occurred in 2 animals at the highest dose. Effects on mouse maternal weight gain were noted only on gestational days 6 to 9 and 15 to 18. While overall maternal weight gains were significantly reduced only at the highest dose, there was an apparent negative trend in decreased body weights with increasing dose. The authors

identified a NOAEL for maternal toxicity of 7.5 mg/kg/day; however, OEHHA (2000) suggests that the apparent effect on maternal weight gain during gestation indicates that 7.5 mg/kg/day may be better described as a LOAEL. Statistically significant adverse effects on offspring growth or survival in mice were seen only at the highest dose. There was an apparent negative trend in the number of live fetuses per litter with increasing dose. An increased incidence of fetal resorptions per litter was also seen in the high dose group. Mean fetal weight also showed an apparent negative trend with increasing dose. Overall, there were no statistically significant dose-related increases in the total incidences of fetal malformations, although the mean per cent of malformations per litter was about three-fold higher in the 48 mg/kg/day dose group than in the lower doses and controls. The NOAEL for developmental toxicity in mice appears to be 7.5 mg/kg/day. In rabbits, maternal toxicity (as evidenced by mortality, slight body weight loss, decreased urination and defecation, occasional prostration and ataxia) only occurred at the highest dose tested (3.0 mg/kg/day). On gestational day 29, maternal body weight appeared to be reduced in the high dose group. A significant loss in mean maternal gravid body weight occurred during the first six days of high-dose treatment during gestational days 6 to 12. This effect persisted and remained significantly different from controls for the entire treatment interval. There were no statistically significant increases in the incidences of any developmental parameters, including fetal malformations. Fetal survival, mean fetal weight, and sex ratios were not affected by the treatment. The number of live fetuses per litter was reduced and resorptions per litter increased in the high-dose group. The authors identified a NOAEL of 0.75 mg/kg/day for both maternal toxicity and developmental toxicity.

Stump *et al.* (1999) administered arsenic trioxide by gavage on gestational day 9 to 25 Crl:CD (SD) BR rats. The doses of arsenic trioxide were: 0, 5, 10, 20, and 30 mg/kg (0, 3.8, 7.6, 15.2, and 22.7 mg As/kg). In the highest dose group, 7 of 25 animals died. Clinical signs of toxicity in the two highest dose groups included changes in fecal consistency and decreased defecation. Food consumption on gestational days 9 and 10 was decreased in a dose-dependent manner across all treatment groups. A LOAEL of 3.8 mg As/kg was identified. Oral administration of arsenic trioxide caused no treatment-related fetal malformations. The study identified a NOAEL of 15.2 mg As/kg.

Rodriguez *et al.* (2001) exposed Sprague-Dawley rats to arsenite (36.70 mg As/L) in drinking water) from gestation day 15 or postnatal day 1, until rats were approximately 4 months old. Once pups were weaned, they continued receiving the same solution as their drinking water. Animals exposed from gestational day 15 showed increased spontaneous locomotor activity and both groups showed increased numbers of errors in a delayed alternation task, relative to controls. The concentration of total arsenic in brain tissue was

similar for both exposed groups. These results suggest rats exposed to arsenic during development show deficits in spontaneous locomotor activity and alterations in a spatial learning task.

A1-3.2.2 Human Studies on the Reproductive and Developmental Effects of Arsenic Exposure

The reproductive and developmental toxicity database for humans is limited and inconclusive (Goldman and Dacre, 1991; DeSesso *et al.,* 1998). Case-control epidemiological studies have indicated increased rates of spontaneous abortion, reduced birth weights, toxemia, and congenital malformations in the children of women working in or residing near smelter operations (Nordstrom *et al.,* 1978a,b, 1979a,b; Aschengrau *et al.*, 1989; Börzsönyi *et al.,* 1992; Tabacova *et al.,* 1994). DeSesso *et al.* (1998) cited the limitations of these studies as including: marginal increase in odds ratio, and inclusion of unity in the 95th percentile confidence interval indicating questionable causality of association between the proposed effects and arsenic exposure. DeSesso *et al.* (1998) also noted limitations in the human reproductive studies, including: uncertainty regarding exposure levels and lack of control of confounding factors.

The TERA (1999) Expert Panel identified twelve human epidemiological studies that address reproductive or developmental effects due to some form of arsenic. All twelve studies were ecologic in design and investigated areas around smelters, industrial facilities, and community drinking water supplies. Unfortunately, inorganic arsenic was not the focus of most studies, and no measurements of arsenic exposure were reported specifically during pregnancy for any of these studies. The studies also poorly controlled, or failed to control for key confounding variables, including maternal exposures to other agents, smoking, alcohol use, age, health, nutritional status, socioeconomic status, and prenatal care. Furthermore, only a limited subset of these studies looked at any particular reproductive or developmental endpoint. Thus, the Panel concluded that the existing epidemiologic studies are inadequate for determining whether inorganic arsenic exposure causes, or is even associated with adverse developmental effects in humans (TERA, 1999).

Ihrig *et al.* (1998) conducted a case-control study of moderate- and high-exposure groups of women dwelling near a facility producing arsenic-based pesticides. Air dispersion modelling was used to predict potential arsenic exposures. There was a statistically significant increase in the risk of stillbirth in the highest exposure category (>100 ng As/m^3). However, differences in incidences of stillbirth between various ethnic groups led Ihrig *et al.* (1998) to conclude that differential susceptibilities may be related to a genetic polymorphism affecting the metabolism of folate. DeSesso *et al.* (1998) reviewed this study, and concluded that various confounding factors, such as prenatal care, may have affected the correlation,

in that lower socioeconomic classes were associated with higher exposures. ATSDR (2000) also noted that interpretation of this study is limited by small numbers of cases and controls in the high exposure group, lack of data on smoking, potential confounding exposures to other chemicals from the factory, and failure to take into account previous years of deposition in the exposure estimates.

Zierler *et al.* (1988) reported that while there was no overall causal association between exposure in drinking water and congenital heart defects; a possible association between exposure and incidence of coarctation of the aorta (a localized malformation caused by constriction of the vessel lumen; stenosis) was noted. However, given uncertainties in determination of exposure levels, it was concluded that this correlation was not indicative of arsenic being a causative agent of teratogenicity.

An ecological study of infant mortality rates in three Chilean cities over a 46 year period (1950 to 1996) was conducted by Hopenhayn-Rich *et al.* (1997; 2000). The city of Antofagasta, in northern Chile, experienced very high arsenic levels in drinking water for a period of 12 years. In 1958 a new water source, containing arsenic concentrations around 800 µg/L was introduced. In 1970, an arsenic removal plant was installed (due to reports of arsenicism in the population), and levels in water decreased initially to around 100 µg/L, and then eventually to around 40 µg/L. The changes in infant mortality rates over time in Antofagasta were compared to those in Valparaiso, a city with similar demographic characteristics but low arsenic drinking water concentrations, and the capital, Santiago, with similarly low concentrations of arsenic in drinking water. The authors reported a close temporal relationship between the high arsenic exposure period and an increase in mortality rates in Antofagasta, whereas the other two cities showed a steady decline in infant mortality. Data on other contaminants or potential confounding factors related to infant mortality were not presented in this study, thus while arsenic appears to be associated with in infant mortality, causation has not been established.

In contrast to the studies cited above where limited possible associated effects were noted, Concha *et al.* (1998b,c) reported that there were no adverse reproductive or developmental effects associated with high chronic arsenic exposure in a native Andean population. A common limitation to all these epidemiological studies is the lack of sufficient information on the possible exposure to other chemicals, and uncertainties regarding determination of maternal exposures during critical periods of development (DeSesso *et al.,* 1998; Golub *et al.,* 1998).

DeSesso *et al.* (1998) conducted a comprehensive review of the embryotoxicity and teratogenicity of arsenic compounds in both humans and experimental animals, and concluded that the impact of arsenic on the developing fetus is greatly dependent on the route of exposure. These authors concluded that only

under intravenous or intraperitoneal administration would the "extreme" concentrations required for teratogenic effects to be achievable. They further concluded that because oral and inhalation studies have consistently shown a lack of dose-related teratogenicity, especially in studies compliant with Good Laboratory Practices, arsenic is unlikely to result in teratogenic effects in humans under typical environmental exposure situations. In an earlier review of the developmental toxicity databse for arsenic, Golub (1994) noted that maternal and developmental toxicity occurred in the same dose range. This review was re-visited in Golub *et al.* (1998), who attributed the differences in oral/inhalation studies and intravenous studies to differences in concentrations reaching the fetus. These authors characterized the main manifestation of the reproductive toxicity of arsenic as being detrimental impacts on development, resulting in a characteristic profile of malformations, growth retardation, and prenatal death; it was further concluded by these authors that environmental exposures could pose a risk to the fetus.

A1-3.2.2.1 Conclusion for Reproductive and Teratogenic Effects of Arsenic

The TERA Panel's consensus conclusions for human and animal studies were as follows (TERA, 1999):

The existing human epidemiological data are not adequate to demonstrate that inorganic arsenic exposure causes or is associated with structural malformations. However, because adequate studies have not been conducted to date, a definitive statement regarding association or causation could not be made.

Several forms of inorganic arsenic (sodium arsenate, sodium arsenite, arsenic acid, and arsenic trioxide) have been tested in several species of experimental animals (*i.e.*, rat, mouse, rabbit, and hamster) under single, repeated, and two-generation oral dosing protocols. These compounds did not induce malformations even at doses that cause maternal toxicity and lethality.

Rats exposed daily by inhalation to arsenic trioxide, from two weeks prior to mating through the end of gestation, did not show any induced malformations or other developmental effects even at doses that caused maternal toxicity.

The current scientific evidence did not support a conclusion that sustained exposure to ingested or inhaled inorganic arsenic at environmental levels causes prenatal structural malformations in humans.

The Panel considered the lack of malformations induced by inorganic arsenic as being most likely due to the lack of bioavailability to the target tissue.

Further research on issues of pharmacokinetics would be of interest in elucidating the route-specificity observed in the data.

A1-3.2.3 Effects of Arsenic on the Nervous System

In humans, arsenic interacts with the nervous system at several levels. In the peripheral nervous system, chronic arsenic exposure leads to peripheral neuropathy, while encephalopathy and impairments of superior neurological functions have been reported in patients with acute and occupational exposure to arsenic compounds and in children exposed through environmental pollution. Behavioural studies in rodents have shown that arsenic exposure affects locomotor activity and learning tasks. The demonstrated behavioural effects are dose-dependent, and have been linked to both the duration of exposure and developmental stage of the animals studied (Rodríguez *et al.,* 2003).

The neurotoxic effects of arsenic have been recently reviewed (Rodríguez *et al.,* 2003). Case reports of arsenic poisoning show that it can produce neuropathy following inhalation or ingestion. This may be characterized by symmetrical neuropatyies of sensory and motor nerves resulting in numbness in distal extremities. Among occupationally exposed smelter workersm chronic arsenic exposure can produce decreased nerve conduction velocity. Other exposures *via* drinking water resulted in forgetfulness, confusion, unusual visual sensations and other central disturbances. Arsenic exposure *via* combustion of high-arsenic coal produced auditory hearing loss in neighbouring children. Populations chronically exposed to low levels of arsenic rarely demonstrate those effects characteristic of large, acute exposure to arsenic. Neurobehavioural studies, on the other hand, may prove useful to detect effects of exposure to low doses of arsenic in the nervous system (Rodríguez *et al.,* 2003).

Among children living near a copper smelter, neurophysical examination and comparison with As in the urine it was found that arsenic in urine was inversely correlated with verbal intelligence quotient (IQ) scores that encompass higher brain functions (Calderon *et al.,* 2001). The studies on the adverse effects of arsenic on the nervous systems of otherwise healthy children have been summarized in Rodríguez *et al.* (2003).

Specific targets of arsenic neurotoxicity in the CNS include systems involved in neuromodulation. There have been a significant number of studies that have examined the effect of arsenic exposure on neurotransmitters including acetylcholine esterase (AChE) activity (cholinergic system), glytamic acid decarboxylase (GABAergic system), and monoaminergic systems involving dopamine and norepinephrine (Rodríguez *et al.,* 2003).

Arsenic either directly or indirectly affects genes that are responsible for the normal neural tube development. *In vivo* studies have shown neurobehavioural abnormalities in mice and rats exposed to arsenic during gestation or throughout postnatal development (see reproductive effects section).

A1-3.3 Genotoxicity of Inorganic Arsenic Compounds

A1-3.3.1 *In vitro* - Bacterial Systems

There are a limited number of studies of the mutagenicity of arsenic in bacterial systems. Negative results were reported for arsenate and arsenite (Lofroth and Ames, 1978) in *Salmonella typhimurium* assays, without metabolic activation. Studies using *Escherichia coli* have yielded both positive (Nishioka, 1975) and negative (Rossman *et al.,* 1980) results for arsenite, although Rossman *et al.* (1980) identified several limitations in the earlier study, which render the significance of the positive result questionable. Yamanaka *et al.* (1989b) reported positive results in *E. coli* in the presence of oxygen. Ulitzer and Barrak (1988) treated *Photobacterium fischeri* with As^{III} and As^{V}, and reported negative and positive results, respectively, for gene mutation.

Yamanaka *et al.* (1989b) found that DMA is mutagenic in *E. coli* B tester strains after >3 h incubation, which suggested to the authors that a metabolite was involved in this effect. Both dimethylarsine and trimethylarsine were detected in the gas phase of the cell suspensions containing DMA. However, only dimethylarsine was mutagenic when the two organoarsines were incubated with the bacteria. The mutagenic effect of dimethylarsine required the presence of oxygen, which suggests that a reactive species formed from the oxidation of dimethylarsine, perhaps a peroxyl radical, is the active mutagenic species.

A1-3.3.2 *In vitro* - Mammalian Systems

A number of short-term *in vitro* genotoxicity assays in mammalian systems have indicated mixed results. Inorganic arsenic does not appear to be a point mutagen in standard assays. Arsenite did induce large deletion mutations (multilocus mutations) in hamster-human hybrid cell lines (Hughes, 2002). Arsenic compounds were observed to induce sister chromatid exchange and chromosomal aberrations in hamster embryo cells (Larramendy *et al.,* 1981), mouse cells (Andersen, 1983; Moore *et al.,* 1997a), Chinese hamster ovary cells (Ohno *et al.,* 1982; Wan *et al.,* 1982; Kochhar *et al.,* 1996) hamster-human hybrid cell lines (Hei *et al.,* 1998) and human lymphocytes, lymphoblasts and fibroblasts (Petres and Hundeiker, 1968; Paton and Allison, 1972; Larramendy *et al.,* 1981; Nakamuro and Sayato, 1981; Nordenson *et al.,*

1981; Wan *et al.,* 1982; Andersen, 1983; Crossen, 1983; Jha *et al.,* 1992; Oya-Ohta *et al.,* 1996; Iwami *et al.,* 1997; Rasmussen and Menzel, 1997). Arsenic produced morphological transformations and DNA amplification in Syrian hamster embryo cells (Lee *et al.,* 1985, 1988; Barrett *et al.,* 1988). Arsenic-induced chromosomal aberrations and SCEs were dependent on active DNA replication, (Nordenson *et al.,* 1981; Crossen, 1983).

Negative results were reported for mutations, and specifically point mutations, in both mouse (Amacher and Paillet, 1980) and Chinese or Syrian hamster ovary (Rossman *et al.,* 1980, Lee *et al.,* 1985; Li and Rossman, 1991) test systems, and for sister chromatid exchange in mouse P388 cells (Andersen, 1983). In a time- and dose-related manner, inorganic arsenic was reported to both stimulate (at lower doses) and inhibit (at higher doses) DNA synthesis in human lymphocytes exposed *in vitro* (Meng, 1994). Iwami *et al.* (1997) observed that the organic metabolites of arsenic (DMA, MMA) were capable of inducing mitotic arrest and aneuploidy in cultured human lymphocytes. DMA was of greater potency with regard to aneuploidy, and was suggested to cause this effect through spindle disruption, as demonstrated by Ramirez *et al.* (1997).

A dose-related inhibition of lymphocyte proliferation was observed *in vitro* with human lymphocytes exposed to sodium arsenate or sodium arsenite (Gonsebatt *et al.,* 1992). A decrease in lymphocyte proliferation was observed with increased As exposure durations in human lymphocytes in the presence and absence of the lymphocyte proliferation stimulator phytohaemagglutinin (PHA)

Sodium arsenite induced DNA strand breaks in cultured human fibroblasts at 3 mM (Dong and Luo, 1993). Arsenite also inhibits the DNA repair process by inhibiting both excision and ligation processes (Jha *et al.,* 1992; Lee-Chen *et al.,* 1993). The inhibitory effect of arsenite on strand-break rejoining during DNA repair was reduced with excess glutathione (Huang *et al.,* 1993). The cytotoxic effects of sodium arsenite in Chinese hamster ovary cells correlate with the intracellular glutathione levels (Lee *et al.,* 1989).

V79 cells treated with MMA, DMA and TMAO undergo mitotic (Endo *et al.,* 1992; Eguchi *et al.,* 1997). Arsenocholine and arsenobetaine were not genotoxic in V79 cells (Eguchi *et al.,* 1997). DMA was found to inhibit human lymphocyte phytohaemagglutinin-M stimulated mitogenesis (Endo *et al.,* 1992). In mouse lymphoma cells (L5178Y/TK$^{+/-}$), incubation with organic arsenicals for 4 h induced both cytotoxicity and clastogenicity (Moore *et al.,* 1997a). MMA was more potent than DMA, but both organic forms were less potent than inorganic arsenicals (Moore *et al.,* 1997a). A number of studies (Tezuka *et al.,* 1993; Yamanaka *et al.,* 1993, 1995, 1997; Rin *et al.,* 1995) have reported DNA single-

strand scissions and DNA–protein cross-links in human alveolar type II (L-132) cells after incubation with DMA. The genotoxic effects of DMA are due to the peroxyl radical, formed from the reaction of dimethylarsine with oxygen (Yamanaka *et al.,* 1989b, 1990). Tezuka *et al.* (1993) proposed that DMA modifies DNA by forming a DMA–DNA adduct. The proposed adduct then renders DNA susceptible to single-strand scissions induced by agents such as UV light (Tezuka *et al.,* 1993) or superoxide radicals (Rin *et al.,* 1995). Apurinic or apyriminidic sites may also form (Yamanaka *et al.,* 1995), and damaged DNA may also undergo beta-elimination to form DNA single-strand scissions or a Schiff-base reaction to form DNA–protein cross-links.

Guillamet *et al.* (2004) have investigated the DNA damage induced by seven organic and inorganic arsenical (AsV) compounds in the TK6 human lymphoblastoid cell line using the alkaline Comet assay. They reported that both inorganic and organic forms of arsenic induce varying levels of single strand breaks as measured by this assay. Taking into account the high concentrations necessary to produce positive results, Guillamet *et al.* (2004) concluded that AsV compounds (including the methylated forms commonly found in human urine) were not genotoxins. Sodium arsenite (AsIII) was a positive clastogen in this assay.

Mass *et al.* (2001) have shown that trivalent methylated arsenicals (MMA^{III} and DMA^{III}) are directly genotoxic, while arsenite, arsenate, MMA^{V}, nor DMA^{V} were unable to nick supercoiled phage ΦX 174 DNA.

Hei *et al.* (1998) observed a significant reduction in mutagenic activity of arsenite in a hamster-human hybrid cell assay with an oxygen radical scavenger. The authors concluded that the mutagenicity of arsenic was dependent on a reactive oxygen species. In a number of human cell lines, Bau *et al.* (2002) reported oxidized guanine adducts in all cell types treated with arsenite, and that adducts and crosslinks were the major DNA lesions caused by arsenite.

Several studies reported different arsenic species exhibit varying mutagenic potencies in *in vitro* mammalian systems. Jacobson-Kram and Montalbano (1985) reviewed the mutagenicity of inorganic arsenic and concluded it is clastogenic, but a very weak mutatgen *in vitro.* Trivalent arsenic was an order of magnitude more potent than pentavalent arsenic. Kochhar *et al.* (1996) observed a similar difference in pthe potency of arsenite (As[III]) and arsenate (As[V]) for the induction of chromosomal aberrations and sister chromatid exchanges in mammalian cells. Similarly, Moore *et al.* (1997a) reported that arsenite had the lowest effective doses in the L5178/$TK^{+/-}$ assay, followed by arsenate, MMA and DMA. Oya-Ohta *et al.* (1996) compared the mutagenic and clastogenic activity of several arsenic compounds, showing

arsenite (As[III]) as the most potent followed by arsenate (As[V]), DMA, MMA, and TMA respectively. The depletion of cellular glutathione (GSH) increased the incidence of chromosome aberrations induced by arsenite, arsenate, and MMA, while markedly suppressing the clastogenic effects of DMA (Oya-Ohta *et al.,* 1996). Arsenobetaine, arsenocholine, an arsenosugar, and tetramethylarsonium iodide demonstrated relatively minor clastogenic potency.

The role of arsenical compounds in the carcinogenic process suggested by its genotoxic activity as described above may be in the role of progression rather than as a classical initiator (Moore *et al.,* 1994). DNA repair activity is inhibited by arsenic, but the inhibition is not a direct action of arsenic on repair enzymes. It appears that some type of effect on the signal to repair the DNA is generated in the presence of arsenic (Hughes, 2002).

A1-3.3.3 *In vivo* - Mammalian Systems

Studies of the mutagenicity of arsenic in *in vivo* mammalian systems have yielded mixed results. While Datta *et al.* (1996) reported increased chromosomal aberrations in rat bone marrow cells following oral exposure to As^{V}. Weakly positive (Nagymajtényi *et al.,* 1985) or negative (Poma *et al.,* 1987) results have been reported for various cell types of the mouse following oral or inhalation exposure to As^{III}. Intraperitoneal administration of arsenic to mice has yielded negative results for chromosomal aberrations in bone marrow cells, spermatogonia, dominant lethal mutation, and sperm morphology (Deknudt *et al.,* 1986; Poma *et al.,* 1987), while positive results were reported for micronuclei in bone marrow cells (Poma *et al.,* 1987; Tinwell *et al.,* 1991). Yamanaka *et al.* (1989a,b; 1991; 1995; 1996) reported that oral administration of DMA to mice at high doses resulted in DNA strand breaks or DNA-protein cross-links in the lungs. Similarly, Brown *et al.* (1997b) reported the induction of DNA damage in the lungs of rats following oral administration of DMA.

Simeonova *et al.* (2000) found a persistent increase in DNA binding of the nuclear transcription factor, AP-1, in bladder epethelium of mice exposed to 20 and 100 mg/L sodium arsenite in drinking water. They demonstrated that arsenite induces AP-1-mediated transcriptional activation in the urothelium *in vivo* and that arsenite alters the expression of several genes associated with cell growth. The authors concluded that these alterations likely contribute to arsenite's ability to cause cancer (NRC, 2001).

Tice *et al.* (1997) reported that the hepatic methyl donor status can affect arsenic-induced DNA damage in mice. Increases in micronucleated erythrocytes and a decrease in DNA migration were observed in choline-deficient mice (the methyl donor status in mice liver fed a choline-deficient diet is lower than in

mice fed a choline-sufficient diet), after multiple exposures to arsenite. Increased DNA damage in the skin of the choline-deficient mice was also observed.

Using the comet assay, Banu *et al.* (2001) orally administered 0, 0.13, 0.27, 0.54, 1.08, 2.15, 4.3 and 6.45 mg/kg body weight of arsenic trioxide to mice dissolved in distilled water. Blood samples from treated mice were collected at various times post treatment (4, 24, 48 and 72 h, 1 week and 2 weeks after exposure) and nucleated cells from whole blood (leukocytes) examined in a an alkaline Comet assay for evidence of DNA breaks. All doses induced a significant increase in comet tail length at 24 h post-treatment, with a clear dose-dependent increase from 0.13 to 2.15 mg/kg. Interestingly, there was a dose-dependent decrease at the higher doses (4.3 to 6.45 mg/kg) at 24 h post-treatment. By 48 h post-treatment, all tested doses showed a significant increase in comet tail-length when compared to 24 h post-treatment. A gradual decrease in the comet tail length was observed for all doses from 72 h post-treatment onwards, indicating a gradual repair in DNA damage. The results of this study indicate a non-linear dose and time response between DNA damage and varying doses of arsenic trioxide.

Similarly mixed results have been reported in humans after inhalation or oral exposure. Burgdorf *et al.* (1977), and Vig *et al.* (1984) failed to detect chromosomal aberrations in peripheral lymphocytes following oral exposure to arsenic, while others (Nordenson *et al.,* 1978, 1979; Gonsebatt *et al.,* 1997) did observe increases in chromosomal aberrations in lymphocytes following either oral or inhalation exposure. Positive (Burgdorf *et al.,* 1977; Lerda, 1994) and negative results (Vig *et al.,* 1984) have been reported for the induction of sister chromatid exchanges in peripheral lymphocytes of humans with oral exposure to arsenic. The relevance of the mutagenicity of arsenic species to carcinogenicity based on cell types that are not targets for cancer has been questioned (Rudel *et al.,* 1996).

Of greater relevance to human cancer incidence is the prevalence of micronuclei in the exfoliated bladder cells to induction of bladder cancer among a population exposed to arsenic *via* drinking water. It was observed that the incidence of micronuclei increased with urinary speciated arsenic concentrations in a dose-dependent manner (Warner *et al.,* 1994; Gonsebatt *et al.,* 1997; Moore *et al.,* 1997b). Background or baseline urinary speciated arsenic concentrations in these studies were 34 and 59 µg As/L (Gonsebatt *et al.,* 1997; Moore *et al.,* 1997b, respectively), while the concentrations in the urine of exposed populations were 740 and 54 to 729 µg As/L, respectively. An exception to the dose-response was observed by Moore *et al.* (1997b), who reported that the group with maximum urinary levels (729 to 1,894 µg As/L) had an incidence of micronuclei similar to the baseline, presumably as a result of toxic action of arsenic on the cells. Based on these results, the authors concluded that arsenic is clastogenic, and possibly

weakly aneuploidogenic (results in production of cells with either an excess or insufficient number of chromosomes).

A number of genotoxicity studies have been conducted in individuals from the Blackfoot disease (BFD) endemic area of Taiwan. Liou *et al.* (1996) examined the rates of SCEs and mitomycin C-induced SCEs in patients with cancer only (17 skin, 5 other types), BFD only, with both BFD and cancer (6 with skin cancer), and healthy controls from the BFD area. Healthy non-arsenic-exposed workers were used as external controls. Baseline SCEs did not differ among the 5 groups studied. Smokers consistently had higher SCEs than non-smokers across all groups, but no differences were observed between groups when stratified by smoking status. A nested case–control study (Liou *et al.,* 1999) was conducted on a cohort of 686 residents from the BFD endemic area. Blood samples obtained at the beginning of the cohort study for 22 cases with existing cancer. Controls (no cancer) were selected from the same cohort, and were matched by sex, age, village of residence and smoking status. No differences were found between the cases and controls with respect to overall frequency of SCEs, although the frequency of total chromosomal aberrations was significantly higher among the cancer cases. This study was limited by the apparent uniformity of arsenic exposure among both cases and controls (WHO-IPCS, 2001), which precludes a dose response analysis necessary to establish a definitive link between frequency of chromosomal aberrations and arsenic exposure. A number of studies of subjects in the BFD endemic areas have also investigated whether arsenic exposure resulted in increased mutations in the p53 gene which is closely associated with cancer etiology in humans (Shibata *et al.,* 1994; Hsieh *et al.,* 1994; Hsu *et al.,* 1999). The results have been equivocal (WHO-IPCS, 2001).

In a human biomonitoring study of a West Bengalese subpopulation (Chattopadhyday *et al.,* 2002) reported elevated mean values of the percentage of aberrant cells (8.08%) and SCEs per cell (7.26%) were also observed in the exposed individuals, relative to controls. The mean arsenic concentration in well water used by the group in the study was 211.7 µg/L.

A1-3.3.4 Apoptosis

Apoptosis is a morphologic pattern of cell death affecting single cells. It is marked by shrinkage of the cell condensation of chromatin, formation of cytoplasmic blebs, and fragmentation of the cell into membrane-bound bodies that are eliminated by phagocytosis. Aopotosis may be induced by chemical or other alterations of DNA that result in DNA damage and/or mutation. Cells bearing a P53 mutation or P53 null fail to initiate the process of apoptosis may survive through mutation. Loss of P53 function in humans has frequently been linked to tumour progression and expression.

Recent studies have found that inorganic arsenic induces apoptosis (programmed cell death) in CHO cells (Wang *et al.,* 1996), immature rat thymocytes (Bustamante *et al.,* 1997) and human HL-60 cells (Ochi *et al.,* 1996). A characteristic marker of apoptosis is internucleosomal DNA cleavage, which was observed in all cell types after exposure to inorganic arsenic. Arsenite has produced a greater response than arsenate in these studies. Apoptosis was increased in cells that were GSH-deficient. The mechanism of apoptosis in these cell lines is not fully understood. In CHO cells however, Wang *et al.* (1996) observed that arsenite induces a cascade of events that involves the generation of reactive oxygen species, production of hydroxyl radicals *via* a metal-catalysed Fenton reaction, protein synthesis and activation of protein kinase. Li and Broome (1999) have proposed that trivalent arsenic induces apoptosis, at least in leukaemia cells, by binding to tubulin. This results in inhibition of tubulin polymerization and eventual formation of microtubules.

Ochi *et al.* (1996) reported that DMA, but not MMA or TMAO, induces apoptosis in human renal carcinoma HL–60 cells. The apoptotic response (> 10%) induced by DMA is greater than that observed with sodium arsenite, but greater doses of DMA are required to elicit this effect. Depletion of GSH in the cells diminished the DMA-induced apoptotic response. Interestlingly, this is in contrast to the increased apoptotic response to inorganic arsenic in GSH-depleted cells.

Many other studies have demonstrated an apoptotic effect of inorganic and organic arsenicals in human fetal brain explants, neonatal rat brain cells, human mononuclear cells, and a wide range of cancer cell types (*e.g.*, Chattopadhyay *et al.,* 2002; de la Fuente *et al.,* 1998; Lin *et al.,* 2000; Li *et al.,* 2003; Cai *et al.,* 2003; Zhang *et al.,* 2003; Zhu *et al.,* 2003; Hayashi *et al.,* 2002; Wang *et al.,* 2003). The apoptotic effect on cancer cells has led to a large number of studies investigating the potential use of arsenicals in cancer therapy.

A1-3.4 Carcinogenicity

Currently three modes of action for arsenic carcinogenesis (modes of action are discussed in the U.S. EPA Guidelines for Carcinogen Risk Assessment published in 2001) have some degree of positive evidence in experimental systems (animal and human cells) and in human tissues. These are chromosomal abnormality, oxidative stress and altered growth factors (Tchounwou *et al.,* 2003). Other modes of action that have been examined include altered DNA repair, altered DNA methylation patterns, enhanced cell proliferation, promotion/progression, suppression of *P53* and gene amplification. While various hypotheses to explain the carcinogenic activity of arsenic have been proposed, details of molecular mechanisms by which inorganic arsenical compounds induce cancer remain poorly understood

(Tchounwou *et al.,* 2003). The mode-of- action studies have suggested a role for arsenic as a cocarcinogen, a promoter, or a progressor of carcinogenesis.

Evidence from epidemiological studies clearly shows that exposure to inorganic arsenic increases the risk of cancer. Evidence from occupational exposure by inhalation has shown an increased risk of lung acncer, while oral exposure has primarily been associated with skin cancer. Epidemiology studies have proposed an association between liver, kidney, lung, colon and bladder cancers with oral exposure to arsenic (Tchounwou *et al.,* 2003).

A1-3.4.1 Animal Studies

Summary results for some animal carcinogenicity studies are presented in Table A1.5.

The experimental animal database for the carcinogenicity of arsenic compounds is limited, and has generally been considered inadequate. There has been no consistent demonstration of carcinogenic activity by arsenic (IARC, 1987; U.S. EPA, 1998). In a carcinogenicity study conducted by Kanisawa and Schroeder (1967), no increase in tumour incidence was observed in rats or mice administered sodium arsenite [As^{III}] or sodium arsenate [As^{V}] in drinking water over their lifespan. Similarly, there was no evidence of carcinogenicity observed in a group of mice exposed by inhalation to an aqueous aerosol of sodium arsenite (Berteau *et al.,* 1978). Both of these studies employed a single exposure concentration, precluding their use in determining the carcinogenic potency of arsenic, and thereore were deemed inadequate. Several other studies also reported a lack of carcinogenicity following oral exposure in experimental animals (Byron *et al.,* 1967; Schroeder *et al.,* 1968; Kroes *et al.,* 1974).

Furst (1983) reviewed animal carcinogenicity studies for nine inorganic arsenic compounds (all oxidation states) in more than nine strains of mice, in five strains of rats, in dogs, in rabbits, in swine and in chickens. Chemical exposure was *via* the oral, dermal, inhalation, and parenteral routes. Furst (1983) concluded that no study demonstrated the carcinogenicity of inorganic arsenic in animals. Dimethylarsonic acid (DMA^{V}) was tested for carcinogenicity (a screening test) in two strains of mice and produced a negative result (Innes *et al.,* 1969). The implication of largely negative results for carcinogenicity with inorganic arsenic is uncertain; rodents and other common laboratory animals may not be a good model for arsenic carcinogenicity in humans (U.S. EPA, 1998).

Based on the lack of observed carcinogenicity in animal studies, arsenic has sometimes been called a "paradoxical" human carcinogen (Jager and Ostrosky-Wegman 1997). The basis for the lack of

tumourigenicity in animals is not known, but it may be related to species-specific differences in arsenic distribution, and induction of cell proliferation (Byrd *et al.,* 1996). However, Chan and Huff (1997) point out that a carefully controlled long-term carcinogenesis bioassay (*i.e.,* using the National Toxicology Program protocol) is yet to be conducted for either arsenic trioxide by inhalation exposure or for sodium arsenite by drinking water; thus these authors caution that labelling arsenic a non-carcinogen may be premature. Waalkes *et al.* (2004) have recently reported that male mice thet received an exposure to sodium arsenate during gestation, but no exposure post partum developed hepatocellular carcinomas and adrenal tumours in a dose-dependent manner corresponding to their maternal exposure. While liver tumours were not observed in female mice, they did develop lung tumours as well as uterine and oviduct preneoplasia.

Selected studies relevant to the carcinogenic potential of arsenic compounds in experimental animals are briefly described in the following paragraphs. Overall, the evidence for animal carcinogenicity is mixed, but some recent studies are suggestive of a role for certain arsenic compounds in the development of cancer.

A number of intratracheal instillation studies in hamsters have provided evidence that exposure to both arsenite and arsenate increased the incidence of lung adenomas and/or carcinomas (Ishinishi *et al.,* 1983; Pershagen and Bjorklund 1985; Pershagen *et al.,* 1984; Yamamoto *et al.,* 1987).

In a long-term carcinogenicity study in cynomolgus monkeys (*Macaca fascicularis*) showed no evidence of excess malignant tumours after being dosed orally for 5 days/week for fifteen years with sodium arsenate (0.1 mg/kg) (Thorgeirsson *et al.,* 1994). It should be noted that this strain of monkey exhibits a 1.5% incidence of spontaneous malignant tumours. Other findings included renal cortical adenomas in 2 monkeys, micronodular cirrhosis in one monkey, endometriosis in 3 monkeys and 3 monkeys displayed changes in the islets of Langerhans located in the pancreas. One of the monkeys with pancreatic changes developed diabetes.

A preliminary study by Ng *et al.* (1998; 1999) examined the effects of sodium arsenate in drinking water (500 µg As/L) on 90 female C57BL/6J mice, 140 female metallothionein knock-out transgenic mice, and 60 control females. The exposure period lasted up to 26 months, with the average arsenic intake of 2 to 2.5 µg As/day resulting in a daily arsenic dose rate for a 30 g mouse of 0.07 to 0.08 mg As/kg body weight. Eighty-one percent of the C57BL/6J and 74% of the transgenic mice survived, compared to 98% of the control group. One or more tumours were detected in 41% of the C57BL/6J mice and 26% of the transgenic mice. Tumours were found in the gastrointestinal tract, lung, liver, spleen, skin and

reproductive system of these two strains. No tumours were observed in controls. A small percentage of tumours were also observed in the skin and eyes of the C57BL/6J mice. The authors concluded that the capability to produce metallothionein had no protective effect in mice for developing tumours after arsenic exposure. This was the first experimental carcinogenicity study in rodents using a relevant route of exposure and relevant exposure level that clearly demonstrated a treatment-related increase in tumours (WHO-IPCS, 2001). This finding was in contrast to the many previous studies that have employed higher levels of exposure or strains with greater sensitivity due to a relatively elevated spontaneous tumour background.

Morikawa *et al.* (2000) described results that suggest thaty arsenic plays a role in tumour promotion. They found that skin tumour development in a skin-tumour sensitive strain of mice was significantly accelerated by topical application of 3.6 mg dimethylarsinic acid (DMA^V) twice a week for 18 weeks. A number of other studies also suggest that DMA^V acts as a promoter of urinary bladder, kidney, liver, skin and thyroid gland carcinogenesis in rats and mice (Yamamoto *et al.,* 1995, 1987; Wanibuchi *et al.,* 1996; Yamanaka *et al.,* 2001b). While limited, these studies in combination suggest that organic arsenicals might possess weak carcinogenic potential (ATSDR, 2005).

Several studies (Hayashi *et al.,* 1998; Chen *et al.,* 1999; Wei *et al.,* 1999; Yamanaka *et al.,* 2000) have demonstrated direct carcinogenic responses from high concentrations of DMA^V in drinking water in rats. However, it is noted that the limited experimental details provided in these studies and the known pharmacokinetic differences between rats and other species (including humans) limit the applicability to human populations (WHO-IPCS, 2001). While the evidence for DMA^V suggests that organoarsenicals may exhibit some weak carcinogenic activity (ATSDR, 2005), the studies are limited. A major drawback to interpretation of these studies is a reliance on high dose levels of DMA^V necessary (range: 10 to 100 mg/kg (WHO-IPCS, 2001). DMA^V is the primary metabolite of inorganic arsenic in most mammals; however, it is unlikely that exposure to the levels used in these studies could occur *in vivo* after exposure to inorganic arsenic.

Germolec *et al.* (1998) administered 48 mg As/kg/day as sodium arsenite (As^{III}) in the drinking water of transgenic TG:AC mice (that carry the activated *v-Ha-ras* oncogene) for 4 weeks. This was followed by dermal application of 12-O-tetradecanoylphorbol-13-acetate (TPA) to shaved back skin twice a day for 2 weeks. The incidence of skin papillomas in the TG:AC mice increased with TPA treatment when compared to other transgenic mice that received either TPA treatment or arsenic alone. Wild-type mice that received both TPA and arsenic showed no evidence of induction of skin papillomas.

Recently, Waalkes *et al.* (2003) conducted a transplacental carcinogenicity study in mice using sodium arsenite. Groups of 10 pregnant C3H mice were given drinking water containing sodium arsenite (As^{III}) at 0 (control), 42.5, and 85 mg/L, *ad libitum* from gestation days 8 through 18. The doses were well tolerated by the dams and body weights of the dams during gestation and of the offspring were not reduced. Offspring were weaned at 4 weeks and put into separate gender-based groups according to maternal exposure level. The offspring received no additional arsenic treatment. The study duration was 74 weeks in males and 90 weeks in females. At necropsy, a marked increase in hepatocellular carcinoma incidence was noted among male offspring. This tumourigenic response was dose-dependent (control - 12%; 42.5 mg/L - 38%; 85 mg/L - 61%). The number of tumours per liver was also increased relative to controls (5.6-fold greater in the high exposure group). Males also displayed a dose-related increase in adrenal tumour incidence and multiplicity. In female offspring, there were dose-dependent increases in ovarian tumour incidence (control - 8%; 42.5 mg/L - 26%; 85 mg/L - 38%) and lung carcinoma incidence (control - 0%; 42.5 mg/L - 4%; 85 mg/L - 21%). There was also an increased incidence of proliferative lesions of the uterus and oviduct. This is the first study to demonstrate that oral inorganic arsenic exposure can induce tumour formation in rodents *via* the transplacental route.

In another recent study, Wei *et al.* (2002) investigated the carcinogenicity of DMA^V in male F344 rats in a 2-year bioassay, and also included assessment of the genetic alteration patterns in the induced bladder tumours. An objective of these authors was to test the hypothesis that reactive oxygen species (ROS) play a role in DMA carcinogenesis, by testing for the presence of 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation in the rat bladder. In the initial experiment, 144 10-week old male F344 rats were randomly divided into four groups that received DMA^V *via* drinking water at concentrations of 0, 12.5, 50 and 200 mg/L, respectively, for 104 weeks. At weeks 97 to 104, bladder tumours were observed in 8 of 31 and 12 of 31 rats in the 50 and 200 mg/L groups respectively. DMA^V treatment did not induce tumours in other organs and no bladder tumours or preneoplastic lesions were evident in the 0 and 12.5 mg/L groups. DMA^V and trimethylarsine oxide ($TMAO^V$) were the major compounds detected in the urine, with small amounts of monomethylarsonic acid (MMA^{III}) and tetramethylarsonium (As^V) also detected. Genetic assessment of the tumours showed diverse changes including a significantly increased 5-bromo-2'-deoxyuridine (BrdU) labeling index in the groups treated with 50 and 200 mg/L, a 10% rate of H-*ras* mutations, cyclin D1 overexpression, decreased p27(kip1) expression, and increased COX-2 expression. In the second experiment, 8-OHdG formation in bladder cells was significantly increased in the 200 mg/L group after 2 weeks of exposure. The authors concluded that these two studies demonstrated that DMA^V is a carcinogen in rat bladder and suggested that DMA^V exposure may be

relevant to the carcinogenic risk of inorganic arsenic in humans. The diversity of genetic alterations observed in the DMA^V-induced bladder tumours suggests that multiple genes are involved in various stages of DMA^V-induced tumour development. The presence of 8-OHdG after 2 weeks exposure suggests that reactive oxygen species play an important role in the early stages of DMA^V carcinogenesis.

Shen *et al.* (2003) investigatd the carcinogenicity of monomethylarsonic acid (MMA^V) in male Fischer 344 rats. Rats were exposed *via* drinking water to 0, 50, and 200 ppm MMA^V, ad libitum, for 104 weeks. No significant differences were found between the control and the treated groups with respect to clinical signs, mortality, hematological effects, and serum biochemistry. Quantitative analysis of glutathione *S*-transferase placental form (GST-P) positive foci in liver revealed a significant increase of numbers and areas of foci in the 200 ppm group. In the urinary bladder, hyperplasia and significantly elevated levels of proliferating cell nuclear antigen in the urothelium were noted. A variety of tumours developed in rats of all groups, including the controls; however, all tumours were histologically similar to those known to occur spontaneously in F344 rats, and there were no significant differences observed between groups. It was concluded that while MMA^V induced lesions in the liver and urinary bladder, it did not cause tumour development in male F344 rats after 104 weeks of exposure.

A number of studies in mice have indicated that arsenic ingestion may actually decrease the incidence of some tumour types. For example, co-exposure to arsenic decreased the incidence of urethane-induced pulmonary tumours (Blakely 1980), spontaneous mammary tumours (Schrauzer and Ishmael 1974; Schrauzer *et al.,* 1976), and tumours resulting from injection of mouse sarcoma cells (Kerkvliet *et al.,* 1980). On the other hand, arsenic has also shown evidence of co-carcinogenicity (Hughes, 2002). Arsenic exposure effectively increased the growth rate of the tumours that did occur, resulting in a net decrease in survival time in the affected animals (Kerkvliet *et al.,* 1980; Schrauzer and Ishmael 1974). These observations suggest that arsenic *should not be* considered to possess anti-tumour properties in animals as it seems that different types of neoplastic cells respond differently (Schrauzer and Ishmael 1974; Shirachi *et al.,* 1983).

A1-3.4.2 Use of Arsenicals in Cancer Chemotherapy

Based on information obtained from the wealth of genotoxicity data for arsenic compounds and recent evidence that indicates oxidative stress and apoptosis can be induced in cancer cell lines by arsenic compounds, a large number of recent studies have investigated the use of arsenic-containing therapeutants in the chemotherapy of various cancers in *in vivo* and *in vitro* studies (Miller *et al.,* 2002; Duzkale *et al.,* 2003; Gortzi *et al.,* 2003; Ora *et al.,* 2000; Mathews *et al.,* 2002; Li *et al.,* 2002a,b; Dombret *et al.,* 2002;

Lu *et al.,* 2002; Pu *et al.,* 2002; Waxman and Anderson, 2001; Antman, 2001). Some arsenic compounds (*e.g.*, arsenic trioxide) are currently being tested in human clinical trials as novel chemotherapy agents (*e.g.*, Vuky *et al.,* 2002). Other forms of arsenic being tested in these types of studies include DMA, arsenoliposomes, and tetra-arsenic tetrasulfide.

A1-3.4.3 Human Studies - Inhalation & Lung Cancer

Summary results for some human carcinogenicity studies are presented in Table A1.6.

The epidemiological literature has indicated a positive association between inhalation exposure to arsenic and lung cancer rates, primarily in copper smelter workers exposed mainly to arsenic trioxide (As^V) (Pinto *et al.,* 1977, 1978; Axelson *et al.,* 1978; Wall, 1980; Enterline and Marsh, 1982; Welch *et al.,* 1982; Lee-Feldstein, 1983; Enterline *et al.,* 1987a; Järup *et al.,* 1989), but also in chemical plant workers exposed to arsenate (As^V) (Ott *et al.,* 1974; Mabuchi *et al.,* 1979; Sobel *et al.,* 1988), and in residents near copper smelters or chemical plants using arsenic compounds (Matanoski *et al.,* 1981; Cordier *et al.,* 1983; Brown *et al.,* 1984; Pershagen, 1985). However, there are studies of exposed communities where no link could be established between arsenic exposure and lung cancer. For example, Marsh *et al.* (1997; 1998) used decedent resident data for 1979 to 1990 to conduct a study of lung cancer mortality in six Arizona copper smelter towns. They identified 185 lung cancer cases with two matched controls per case. Information on lifetime residential, occupational, and smoking history and exposures was obtained. No statistically significant associations were observed between lung cancer risk and residential exposure to smelter emissions (these included arsenic as well as other chemicals), after adjustment for potential confounding factors (gender, Hispanic ethnicity, and smoking) were made.

The strongest evidence that arsenic causes lung cancer comes from quantitative dose-response data relating specific arsenic exposure levels to lung cancer risk in copper smelter workers (Enterline and Marsh 1982; Enterline *et al.,* 1987a, 1995; Mazumdar *et al.,* 1998; Lee-Feldstein 1986; Welch *et al.,* 1982; Enterline *et al.,* 1987b; Jarup and Pershagen 1991; Jarup *et al.,* 1989). These studies are described in the following paragraphs.

Enterline and Marsh (1982) reported a significant increase in respiratory cancer mortality in a cohort of 2,802 males (104 observed respiratory cancer deaths) employed for more than 1 year during the period between 1940 and 1964 at the ASARCO smelter in Tacoma, Washington (standard mortality ratio [SMR] = 189.4). Only 54.9 deaths were expected over the period 1941 to 1976. This cohort was further separated into low and high arsenic exposure groups, with mean estimated time-weighted average arsenic

exposures of 0.054 and 0.157 mg As/m^3, respectively (based on work history, urinary arsenic measurements, and an experimentally derived relationship between urinary and inhaled arsenic developed by Pinto *et al.* (1976)). Respiratory cancer mortality was found to be significantly increased in both groups in a concentration-dependent manner (SMR = 227.7 and 291.4 in the low and high groups, respectively).

Enterline *et al.* (1987a) conducted a reanalysis of the Tacoma, Washington data that included a review of the basis for the Pinto *et al.* (1976) equation. This re-analysis revealed several problems leading to under-estimation of exposure, and thus over-estimation of the unit risk. Analysis of the data using improved exposure estimates demonstrated a stronger relationship between cancer and arsenic exposure. Although no data on the smoking habits of these workers were available, the large excesses in respiratory cancer observed (*i.e.,* up to more than 4-fold) were unlikely to be attributable to smoking alone (Siemiatycki *et al.,* 1988; Blair *et al.,* 1985). An alternative analysis of these data by Mazumder *et al.* (1989) produced similar results. Enterline *et al.* (1997) extended the mortality follow-up from 1976 to 1986, and reported findings similar to the earlier study. Enterline (1997) noted that these studies all suffered from the inherent difficulty in estimation of air concentrations based on urinary arsenic concentration, as highly exposed workers typically wear protective gear such as respirators, which makes direct correlation difficult. Overall, the studies of the Tacoma smelters workers (Enterline and Marsh 1982; Enterline *et al.,* 1987a, 1995) indicate a supralinear exposure-response relationship (*i.e.,* the curve is steeper at lower doses) between arsenic exposure and lung cancer mortality. Hertz-Picciotto and Smith (1993) extended this observation to several other occupationally exposed cohorts with quantitative exposure information. These authors suggest that neither toxicokinetic mechanisms nor confounding from age, smoking, or other workplace carcinogens that differ by exposure level offer plausible explanations for this type of exposure-response relationship. The authors suggest the following as potential explanations: 1) synergistic effect of smoking; 2) long-term survivorship at higher exposures among the healthier, less susceptible individuals (healthy worker effect), and 3) exposure estimate errors that were more prominent at higher-exposure levels as a result of past inaccuracies in industrial hygiene sampling or worker protection practices.

Lee-Feldstein (1986) reported respiratory cancer mortality was significantly increased (SMR = 285) based on 302 observed respiratory deaths between 1938 and 1977 in a cohort of 8045 white male workers employed for at least 1 year between 1938 and 1956 at the Anaconda smelter in Montana. When workers were categorized according to cumulative arsenic exposure (CAE) and date of hire, lung cancer mortality was significantly increased in all groups hired between 1925 and 1947. An alternative analysis of a

subset of the Anaconda cohort (n = 1,800, including all 277 employees with heavy arsenic exposure) that included information on smoking and other occupational exposures was conducted by Welch *et al.* (1982). This analysis showed that lung cancer mortality increased with increasing time-weighted average arsenic exposure. The cohort members were more likely to be smokers than typical U.S. white males, but smoking rates did not differ among the arsenic exposure groups. Overall, the exposure-response analysis of smokers was similar to the analysis based on the full subcohort, while analysis of nonsmokers (which was limited by small group sizes) also showed a similar pattern, but with lower SMRs. Higgins *et al.* (1982) investigated the mortality as of 1981 of the Anaconda cohort. Based on actual measurements and extrapolation from existing data to fill gaps, three indices of exposure were estimated (time-weighted average exposure, 30 day ceiling exposure and cumulative exposure). There was an exposure-response relationship for excess mortality due to respiratory cancer and all three measures of arsenic exposure. The latest cohort update (Lubin *et al.,* 2000) evaluated 8014 white males, who were employed for at least 12 months before 1957. Industrial hygiene data (702 measurements), collected between 1943 and 1958, were used to categorize each work site by exposure category on a scale of 1 to 10, and work areas were then further grouped as representing "light", "medium" or "heavy" arsenic exposure. Based on estimates of workers' daily exposure time, time-weighted average (TWA) exposures for each category were estimated, and were considered to be 0.29, 0.58 and 11.3 mg/m^3 arsenic for the "light", "medium", and "heavy" exposure category. For each worker, the cumulative exposure was estimated from the time of working in different work areas. The authors note however that industrial hygiene measurements were not available for more than half of the working areas; no data were collected before 1943, and the measurements were often performed when an industrial hygiene control measure was instituted or after a process change occurred, and most often in areas where arsenic was thought to be a hazard. Thus, the sampling locations were not randomly selected. There were 446 deaths from respiratory cancer (SMR = 155) observed in this cohort, and a trend of increasing risk with increasing estimated exposure was observed.

Enterline *et al.* (1987b) studied lung cancer mortality rates from 1949 to 1980 in a cohort of 6,078 white males who had worked for 3 years or more between 1946 and 1976 at one of eight U.S. copper smelters in Arizona, Utah, Tennessee, and Nevada. Lung cancer mortality was reported to be significantly increased only in the Utah smelter (SMR = 226.7), which had the highest average arsenic exposure concentration (0.069 mg/m^3 *versus* 0.007 to 0.013 mg/m^3 at the other smelters) and also had the largest cohort size. A nested case-control study of these workers showed that arsenic exposure and cigarette smoking were both significant risk factors for lung cancer. Smoking rates were lower in the Utah smelter workers than in the

other smelter workers, but still higher than in the referent Utah population. The authors suggest that the risk attributable to arsenic in this study population was therefore somewhat lower than indicated by the SMR.

Jarup *et al.* (1989) reported significantly increased lung cancer mortality (SMR = 372), based on 106 lung cancer deaths in a cohort of 3916 male workers employed for >3 months between 1928 and 1967 at the Ronnskar smelter in Sweden. Workers were followed for mortality through to 1981. When the workers were separated into low, medium, and high arsenic exposure groups with mean time-weighted average exposure estimates of 0.05, 0.2, and 0.4 mg/m^3, respectively, lung cancer mortality was found to be significantly increased in all three exposure groups in a concentration-related manner. A nested case-control analysis of 102 lung cancer cases and 190 controls from this cohort showed that lung cancer risk increased with increasing arsenic exposure in nonsmokers, light smokers, and heavy smokers (Jarup and Pershagen, 1991). These results suggest that arsenic is a key risk factor for lung cancer in the smelter workers, but that a greater-than-additive interaction may exist between smoking and occupational arsenic exposure. In this case control analysis, in contrast to the cohort study, lung cancer risk due to arsenic was increased only in the higher arsenic-exposure groups. Potential explanations for this difference include a higher proportion of smokers in the smelter workers than in the regional referent population used in the cohort study, and limited statistical power to detect increased risk in the case-control study due to the smaller group sizes that were evaluated.

In a cancer incidence study (Sandström *et al.,* 1989) that partially overlapped with the Ronnskar Smelter mortality study, cancer risk in smelter workers was observed to decrease steadily between 1976 and 1979 and from 1980 to 1984. Further follow-up by Sandström and Wall (1993) showed a decreasing trend in lung cancer incidence and mortality, but the elevated lung cancer incidence among the workers remained when compared with Swedish men from the general population.

Based on the weight of epidemiological evidence from the smelter worker studies, the U.S. EPA has concluded that arsenic is a human carcinogen (U.S. EPA, 1984a,b; 1998). The U.S. EPA derived a unit risk estimate (the excess risk of lung cancer associated with lifetime exposure to 1 :g/m^3) of 4.3 x 10^{-3} (:g/m^3)$^{-1}$ based on the dose-response relationships between arsenic exposure and excess lung cancer mortality in smelter workers reported in the studies described above. The U.S. EPA relied on four reports relating lung cancer mortality to arsenic exposure to derive four separate unit risk estimates.

Data from Higgins *et al.* (1986) and Welch *et al.* (1982) was used by the U.S. EPA to generate a unit risk for mortality due to respiratory cancer of 4.30 $x10^{-3}$ $(\mu g\ As/m^3)^{-1}$ (U.S. EPA, 1984a) (absolute linear risk model).

The risk specific dose (RsD) for lifetime risk one-in-one million cancer risk for the average adult (body weight of 70 kg, and inhalation rate of 23 m^3/day) was 0.000067 µg As/kg body weight/day.

Using the multistage model equation supplied by Brown and Chu (1983a,b,c), the U.S. EPA calculated a unit risk of 0.00125 $(\mu g\ As/m^3)^{-1}$ (U.S. EPA, 1984a). This was converted to an RsD of 0.00026 µg As/kg body weight/day.

Using data from the Lee-Feldstein (1983) study, and supplemental data reported in Morris *et al.* (1974) who estimated arsenic airborne concentrations at this smelter, based on 702 samples collected from 1943 to 1958, the unit risk was reported to be 0.0028 $(\mu g\ As/m^3)^{-1}$ (U.S. EPA, 1984a). This equates to an RsD of 0.00012 µg As/kg body weight/day.

Viren and Silvers (1994) re-evaluated the unit risk estimate using the same methods as the U.S. EPA, but incorporated updated results from Enterline *et al.* (1987a), from Mazumdar *et al.* (1998) as well as findings from Jarup *et al.* (1989). This analysis yielded a revised unit risk of 1.28 x 10^{-3} $(:g/m^3)^{-1}$. When pooled with the earlier unit risk estimate, Viren and Silvers (1994) calculated a composite unit risk of 1.43 x 10^{-3} $(:g/m^3)^{-1}$. This unit risk estimate is a factor of three smaller than the U.S. EPA current unit risk estimate of 4.3 x 10^{-3} $(:g/m^3)^{-1}$.

All of the studies used to derive the U.S. EPA unit risk value had a number of confounding factors and uncertainties. These included: confounding by concurrent exposure to airborne dusts, sulphur dioxide and other chemicals; lack of measured air concentrations in some studies; failure to consider latent periods for lung cancer development; and, confounding by smoking.

A number of studies that examined the histopathology of arsenic-associated lung cancer in smelter workers have noted a variety of lung cancer types, including epidermoid carcinoma, small cell carcinoma, and adenocarcinoma (*e.g.*, Axelson *et al.,* 1978; Newman *et al.,* 1976; Pershagen *et al.,* 1987; Qiao *et al.,* 1997; Wicks *et al.,* 1981). The incidence of the various cell types varied from population to population, indicating that arsenic does not specifically increase the incidence of one particular type of lung cancer (ATSDR, 2005). Guo *et al.* (2004) used the National Cancer Registry Program data base to compare the proportionof each major lung cancer cell type between an endemic area of arsenic intoxication with

exposures through drinking water. Lung cancer patients from the endemic area had higher proportions of squamous cell and small cell carcinomas, but a lower proportion of adenocarcinomas. They suggested that the carcinogenicity of arsenic on the lungs might be cell type-specific. The association of cell type with inhalation exposure supported a closer connection with adenocarcinoma than squamous cell carcinoma. Guo *et al.* (2004) speculate that the mechanism of carcinogenesis in the lung could differ depending on the route of exposure, and that inhalation exposure may not be as relevant for squamous and small cell carcinoma induction.

ATSDR (2000) notes that there have been occasional reports of non-respiratory tract cancer types (*e.g.*, bone, stomach, colon, nasal, liver skin cancer) that have been potentially associated with inhalation exposure to inorganic arsenic, but evidence is weak, and statistically significant associations could not be demonstrated.

At the time this report was prepared, there were no studies identified in the available scientific literature regarding the human carcinogenicity of organic arsenicals by the inhalation route.

A1-3.4.4 Human Studies - Oral

Summary results for some human carcinogenicity studies are presented in Table A1.6.

The epidemiological literature, consisting of case-control studies, ecological cohort studies, prospective and retrospective analyses, indicates an association between chronic oral exposure to arsenic (mainly *via* drinking water) and cancer incidence. The most sensitive target tissue is considered to be the skin, based on incidences of squamous cell carcinoma, basal cell carcinoma and intraepidermal carcinoma, but concerns are also indicated for internal cancers of the liver (angiosarcoma and carcinoma), the urogenital system (bladder, kidney, prostate), and the lung, including cancer of the nasal cavity (Tseng *et al.,* 1968; Zaldivar, 1974; Lander *et al.,* 1975; Tseng, 1977; Zaldivar *et al.,* 1981; Cebrian *et al.,* 1983; Luchtrath, 1983; Chen *et al.,* 1985; 1986; 1988a,b, 1992; Brown *et al.,* 1989; Gibb and Chen, 1989; *et al.,* 1989; 1995; Wu *et al.,* 1989; Chen and Wang, 1990; Bates *et al.,* 1995; Chen and Lin, 1994; Chiou *et al.,* 1995; Ferreccio *et al.*, 2000; Chiou *et al.*, 2001). Several of these studies are discussed in greater detail below.

Studies of exposure to arsenical compounds *via* drinking water have failed to observe a significant relationship between arsenic exposure and skin cancer incidence (*e.g.*, Southwick *et al.,* 1983; Morton *et al.,* 1976; Wong *et al.,* 1992). Reasons for the absence of a significant relationship in these studies are unknown; Abernathy *et al.* (1996) suggested that exposures may have been too low or of short durations,

or that the study designs might not have been sufficiently sensitive. Assessment of the association between arsenic exposure and skin cancer risk has been hampered by the low case fatality rate of non-melanoma skin cancer, and mortality studies are likely to markedly underestimate the incidence of the disease (WHO-IPCS, 2001).

A large number of studies of arsenic exposure *via* drinking water and skin cancer incidence or mortality have been conducted on Taiwanese populations.

Tseng *et al.* (1968) and Tseng (1977) conducted a cross-sectional study of 40,000 Taiwanese chronically exposed to arsenic-contaminated drinking water with 7,500 persons drinking water relatively free of arsenic. They found an arsenic exposure-related excess of skin cancer, an increased incidence of Blackfoot disease, hyperpigmentation and keratosis, and possible vascular problems. Several weaknesses of this study have been cited, including poor nutrition in the study population, possible genetic susceptibility, potential exposures to arsenic from other sources, and possible bias in examiners (U.S. EPA, 1998). Despite the shortcomings, the U.S. EPA (1998) relied on the Tseng studies (1968; 1977) to derive an oral slope factor of 0.0015 $(\mu g\ As/kg\ body\ weight/day)^{-1}$, corresponding to a drinking water unit risk of $5x10^{-5}$ $(\mu g\ As/L)^{-1}$.

Several follow-up studies of the Taiwanese population have shown an increase in fatal internal organ cancers as well as an increase in skin cancer. Chen *et al.* (1985) found that the standard mortality ratios (SMR) and cumulative mortality rates for cancers of the bladder, kidney, skin, lung and liver were significantly greater in the Blackfoot disease endemic area of Taiwan when compared with age adjusted rates for the general population of Taiwan. Blackfoot disease and these cancers were all positively associated with high levels of arsenic in drinking water. In the endemic area, SMRs were greatest in villages that used only artesian well water (which was high in arsenic), relative to villages that partially or completely used surface well water (which was low in arsenic). A dose-response relationship could not be established for this study (Chen *et al.,* 1985).

A retrospective case-control study showed a significant association between duration of high arsenic well water consumption and cancers of the liver, lung and bladder (Chen *et al.,* 1986). Approximately 90% of the lung and bladder cancers, and roughly 70% of the liver cancers in the registry were histologically or cytologically confirmed. The control group consisted of 400 persons living in the same area without any of these cancers, and was frequency_matched with the cases by age and sex. Standardized questionnaires determined the history of artesian well water use, socioeconomic variables, disease history, dietary habits, and lifestyle. For the cancer cases, the age-sex adjusted odds ratios were increased for bladder (3.90),

lung (3.39), and liver (2.67) cancer for persons who had used artesian well water for 40 or more years, when compared with controls who had never consumed the artesian well water. Similar findings were obtained in a 15 year cohort study of 789 patients with Blackfoot disease (Chen *et al.,* 1988b). In this study, increased mortality from cancers of the liver, lung, bladder and kidney were observed among Blackfoot disease patients when compared with the general population in the endemic area, or when compared with the general population of Taiwan. The use of multiple logistic regression analysis to adjust for other risk factors including cigarette smoking, did not markedly affect the exposure-response relationships or odds ratios.

Wu *et al.* (1989) reported a significant dose-response relationship between arsenic levels in artesian well water and age-adjusted mortality rates from cancers at all sites, as well as cancers of the bladder, kidney, skin, lung, liver and prostate. The study area and population evaluated in this study consisted of 42 southwestern coastal Taiwanese villages where residents have used water high in arsenic from deep artesian wells for more than 70 years. An ecological study of cancer mortality rates and arsenic levels in drinking water in Taiwan communities utilizing artesian wells corroborated the association between arsenic levels and mortality from internal cancers (Chen and Wang, 1990).

Chen *et al.* (1992) analysed cancer mortality data from an arsenic-exposed population to compare the risk of various internal cancers, and to evaluate risks by the Armitage-Doll model for males and females (Wu *et al.,* 1989). Arsenic levels in drinking water ranged from 0.010 to 1.752 mg/L. The study population had 898,806 person-years of observation and 202 liver cancer, 304 lung cancer, 202 bladder cancer and 64 kidney cancer deaths. The study population was split into four groups according to median arsenic level in well water (*i.e.,* < 0.10 ppm, 0.10 to 0.29 ppm, 0.30 to 0.59 ppm and 0.60+ ppm), and was also stratified by age (*i.e.,* < 30 years, 30 to 49 years, 50 to 69 years and 70+ years). Mortality rates increased significantly with age for all cancers. Significant dose-response relationships were observed between arsenic levels and mortality from cancer of the liver, lung, bladder and kidney in most age groups (both males and females). Chen *et al.* (1992) also used the Armitage-Doll model to calculate cancer potency indexes for internal organs based on the excess lifetime risk of developing cancer from oral intake of 10 µg As/kg body weight/day. Assuming a linear dose extrapolation curve, the lifetime risk at 10 µg As/kg body weight/day could be adjusted to determine an RsD at a one-in-one-million level of risk, yielding RsD values ranging from 0.00083 to 0.0024 µg As/kg body weight/day for males, and from 0.00059 to 0.0028 µg As/kg body weight/day for females. Several confounding factors that were unaccounted for in the Chen *et al.* (1992) study included the source of cancer mortality data, the assumptions regarding

lifetime exposure levels, and the presence of other chemicals (Byrd *et al.,* 1996). The U.S. EPA (1998) did not consider this study adequate for determination of a unit risk factor.

Chiou *et al.* (1995) studied the incidence of cancers in 263 Blackfoot disease patients in a region of endemic arseniasis in Taiwan, in comparison to a group of 2,293 healthy people. Using Cox's proportional hazards regression analysis, a significant relationship was observed between arsenic exposure and cancers of the lung and bladder, as well as cancers of all sites, when age, sex and smoking status was controlled. The authors reported that both Blackfoot disease and smoking were associated with an increased incidence of cancer, after adjustment for cumulative arsenic exposure.

Hsueh *et al.* (1995) observed a significant dose-response relationship between skin cancer prevalence and chronic arsenic exposure in residents in Taiwanese villages in which arseniasis was endemic. Exposure was indexed by duration of residence in the area, duration of consumption of high-arsenic artesian well water, average arsenic exposure, and CAE. The overall prevalence of skin cancer was as high as 6.1%, showing an increase with age. In an evaluation of risk factors for skin cancer, it was observed that chronic carriers of hepatitis B surface antigen with liver dysfunction had an increased prevalence. Similarly, malnourishment, indexed by a high consumption of dried sweet potato as a staple food, was also significantly associated with an increased prevalence of arsenic-induced skin cancer (Hsueh *et al.,* 1995).

An ecological study of the incidence of cancer in 243 Taiwan townships, in relation to drinking water concentrations was conducted by Guo *et al.* (1997). Age, smoking and "urbanization" were controlled for in this study. A positive association was reported for exposure to water with high arsenic concentrations and transitional cell carcinomas of the bladder, kidney, and ureter, all urethral cancer combined, and adenocarcinomas of the bladder (males only). An association between the urbanization index and transitional cell carcinomas was also observed. Guo *et al.* (1997) interpreted the results as indicating arsenic may induce cell type-specific carcinogenicity.

Tsai *et al.* (1999) studied sex-specific mortility due to several cancer and noncancer causes in southwest Taiwan in an area which formerly had high arsenic concentrations in drinking water. Standard Mortality Ratios were calculated in two ways: i) using all of Taiwan as a comparison population, and ii) using a regional referent group. This study was essentially an update of the ecological study by Chen *et al.* (1985). Statistically significantly elevated mortalities were observed from cancer of the lung, larynx, oesophagus, stomach, small intestine, colon, rectum, liver, nose, larynx, lung, bone, prostate, bladder, kidney and skin, as well as from lymphoma and leukaemia. Mortality from nasopharyngeal, buccal or

pharyngeal cancer was not elevated. The NRC (2001) stated that the findings from this study may validate Taiwanese results that had been previously rejected by the U.S. EPA (*e.g.*, due to depressed nutritional status of study population).

Morales *et al.* (2000) modelled the arsenic exposure–response relationships for lung, bladder, and liver cancer using data from the Blackfoot disease-endemic area. Exposure groupings were based on individual well-water arsenic concentrations for each village. It was found that depending on the model used, and whether or not a comparison population was used in the analysis, risk estimates varied widely. However, regardless of the modelling approaches used, the authors reported excess lung and bladder cancer risks at exposure concentrations <50 µg/L.

Chiou *et al.* (2001) conducted a study of 8,102 subjects in the Lanyang basin region of Taiwan. The subjects were followed from time of enrollment (1991-1994) until 1996. The authors reported an exposure-dependent increase in the risk of bladder cancer in the following exposure categories: 10 to 50, 50 to 100, and >100 µg As/L in drinking water. The relative risks of transitional cell carcinoma in these exposure categories were 1.9, 8.2 and 15.3, respectively (all results were adjusted for age, sex and smoking). A notable difference in this study from earlier Taiwanese studies is that estimates of individual (rather than village average) drinking water arsenic concentrations were used, and incidence rather than mortality was the health endpoint.

Drinking water studies that have taken place in countries other than thoes located on the Indian Sub-continent or Tiawan are described in the following paragraphs.

A prevalence study of skin lesions was conducted in two towns in Mexico (Cebrian *et al.,* 1983). In one town, 296 persons were exposed to drinking water with 0.4 mg/L arsenic and a similar group was exposed to drinking water containing 0.005 mg/L. The higher exposure group had an increased incidence of palmar keratosis, skin hyperpigmentation and hypopigmentation, and four types of skin cancers. However, the association between skin cancer and arsenic exposure is weak because of the small number of cases, small cohort size, and short duration of followup. In addition, there was no unexposed group in either town. Other studies have found no excess skin cancer incidence in U.S. residents consuming relatively high levels of arsenic in drinking water; however, the numbers of exposed persons were low (Morton *et al.,* 1976; Southwick *et al.,* 1981).

In a case-control study in Utah, Bates *et al.* (1995) did not find an overall association of inorganic arsenic in drinking water (up to 160 µg As/L) with risk of bladder cancer in exposed populations. The incidence

of bladder cancer in the smoking sub-population of the exposure group indicated a positive trend; this was interpreted as supporting the findings of other studies of synergism between smoking and arsenic exposure.

A cohort study of an arsenic-polluted area in Japan was evaluated in several studies (Tsuda *et al.,* 1987, 1989, 1990, 1995). The arsenic contamination was mainly in groundwater and originated from a factory producing arsenic trisulfide. These studies investigated 467 residents living in the vicinity of the factory. Most recently, Tsuda *et al.* (1995) analyzed mortality in 443 of these original residents from 1959 to 1992, and identified as having used water from wells analysed for arsenic content in 1959. The mortality rates in this group were compared to that of the Niigata prefecture (a population of approximately 2.5 million). Deceased subjects were placed in three categories based on 1959 arsenic drinking water concentrations: < 50 µg/L, 50 to 990 µg/L and > 1 mg/L. The number of deaths from all causes in this cohort was 105 (1,00.5 expected) and the number of deaths from all cancers was 34 (SMR = 148). A significantly elevated mortality from lung cancer was observed in the highest exposure group (SMR = 1569). The total number of lung cancer cases was 9, of which 8 occurred in the highest exposure category. Three urinary tract cancers were also observed in the highest exposure category, which represents an excess on the basis of expected numbers for kidney and bladder cancer combined for the whole of Japan. It was concluded that a strong association exists between arsenic-induced lesions first identified in 1959, and subsequent mortality from lung cancer. However, analysis of groups divided by smoking patterns indicated that it is more likely that the increased incidence of lung cancer was attributable to smoking habits rather than exposure to arsenic. In addition, the authors noted several shortcomings of this study, including smoking habits, small sample size, and a short latency period of 5 years, which limits the significance of the findings. Tsuda *et al.* (1995) examined the potentially confounding incidence of smoking in the cohort, and concluded that the cancer rates suggest a synergistic relationship between smoking and arsenic exposure.

Hopenhayn-Rich *et al.* (1996a) investigated bladder cancer mortality for the years 1986 to 1991 in 26 counties of Cordoba, Argentina. Rates for all of Argentina were used as the standard for comparison. Several areas of Argentina have had high exposures to arsenic from naturally contaminated drinking water, particularly the eastern region of the province of Cordoba. Bladder cancer SMRs were consistently higher in counties with documented arsenic exposure. The clear trends found in this Argentina population with different genetic composition and a high-protein diet support the findings in Taiwan of dose-response relation between ingestion of inorganic arsenic from drinking water and bladder cancer. In a

later study of this region, Hopenhayn-Rich *et al.* (1998) found that the mortality from skin cancer showed a negative association with arsenic exposure in men but a positive association in women.

In a study in Chile, Smith *et al.* (1998) examined mortality rates from bladder, lung, kidney, skin cancer over 1989 to 1993 in a region where drinking water arsenic levels had been high, and compared these data to age-adjusted mortality rates from the rest of Chile (using mortality data from 1991 and census data from 1992 for the age distribution). This study followed up on earlier work by Rivara *et al.* (1997). Smoking habits were apparently accounted for as data were available from a national survey in the two largest cities in the region. The SMR for lung cancer in the region with elevated arsenic drinking water concentrations was 380 in men, and 310 in women. Mortality from chronic obstructive pulmonary disease was not elevated; and while smoking data were limited, it did not appear to be a confounder. The SMR for bladder cancer was 600 in men, and 820 in women. For skin cancer SMRs of 770 in men, and 320 in women were reported.

Buchet and Lison (1998) studied mortality from cancer and other diseases in an ecological study in northern Belgium, where the population is exposed to arsenic from a number of non-ferrous smelters. The study covered deaths that occurred between 1981 and 1991, based on national statistics. In the area with highest exposure, the drinking-water concentration of arsenic was between 20 and 50 µg/L. An intermediate exposure area was defined as < 20 µg As/L, and a low exposure area was defined as < 5 µg As/L. Local mortality rates were used as the reference. No relationship was found between arsenic exposure, and mortality from cancer of the lung, kidney or bladder in this study.

A hospital-based case–control study in Chile of 151 lung cancer cases and 419 referents (167 with cancer and 242 with other diseases) was conducted by Ferreccio *et al.* (1998; 2000). Drinking-water arsenic concentration was assessed based on records from municipal water companies covering the years 1950 to 1994, and information on residence, health and employment history from a questionnaire. The odds ratio for lung cancer (adjusted for age, sex and smoking), was related to drinking-water arsenic levels in the five exposure strata evalated, and was statistically significant in the highest exposure category.

In an ecological study in Victoria, Australia, Hinwood *et al.* (2003) investigated the cancer incidence from 1982 to 1991 in 22 different areas. Population information was derived from the 1986 census, and areas were assigned water arsenic concentrations based on their postal codes. Median water arsenic concentration in the high-exposure areas varied between 13 and 1,077 µg/L. Standardized incidence rates (SIRs) were below 120 for most cancers, including lung and bladder. However, the SIRs for prostate and breast cancer, as well as for melanoma and chronic myeloid leukaemia, were slightly elevated. For liver

cancer there was a statistically significant deficit. The authors noted though that the crude nature of the exposure assessment in this study may have resulted in some misclassifications, and potential underestimation of risk.

In a case–control study in Finland, Kurttio *et al*. (1999) studied the relationship between arsenic in drinking water and the incidence of kidney and bladder cancer over 1981 and 1995 in a cohort of people who had used drilled wells as their drinking water source between 1967 and 1980. The arsenic exposure history was reconstructed from questionnaire data on residence and analysis of arsenic in the wells in 1996, with the assumption that the arsenic content had remained constant over the years. The study population consisted of 61 cases of bladder cancer and 49 cases of kidney cancer, and an age- and sex-matched reference group of 275 people For the cases, arsenic levels in the water were low (median 0.14 µg/L; range: < 0.05 to 64 µg/L). For the control group, arsenic levels were below 4.5 µg/L. Cases of bladder cancer tended to have a higher arsenic exposure during years 3 to 9 before diagnosis, which reached statistical significance in the highest-dose group. A weak relationship was observed between bladder cancer incidence and arsenic exposure when a latency period of 10 years was applied. No relationship was observed between arsenic exposure and kidney cancer.

Ferreccio *et al.* (2000) conducted a case-control study of lung cancer incidence in an area of northern Chile with a history of high arsenic concentrations in drinking water. Detailed exposure history for each case was used to calculate odds ratios to estimate the risk of exposure to several concentrations of arsenic in drinking water relative to a referent concentration of 0 to 10 :g/L. The odds ratios were adjusted for age, socioeconomic status, smoking and working in a copper smelter. The study found an association between arsenic exposure through ingestion and lung cancer incidence. Suggestive evidence of synergistic interaction between smoking and exposure to arsenic in drinking water was also found.

Tucker *et al.* (2001) found a dose response relationship for skin cancer and arsenic in drinking water in a cross-sectional study conducted in Inner Mongolia. Two exposure models were used in the data analysis, peak arsenic concentration and cumulative arsenic dose (CAD), and a dose response relationship was found using both. Limitations of the study were the small number of skin cancer cases (n = 8), the lack of data on the time of peak exposure and the CAD exposure metric itself which groups persons with very different exposure histories together.

Several studies and reviews have questioned the relevance of the Tiawanese dataset for the North American population (U.S. EPA, 2007; Lamm et. al., 2004; Brown and Chen, 1995). For example,

Lamm et. al. (2004) considered the relationship between arsenic exposure through drinking water and bladder cancer mortality. County specific mortality ratios were considered for 133 counties across the U.S. where the primary source of drinking water was groundwater. No arsenic-related increase in bladder cancer mortality was found over an exposure range of 3 to 60 ug/L.

A1-3.4.5 Uncertainties

The studies accepted by the U.S. EPA as the basis for the oral slope factor (Tseng *et al.,* 1968; Tseng, 1977) have been the subject of scrutiny and criticism by a number of investigators.

Concerns raised include: the adequacy of the model used by U.S. EPA and the accuracy and reliability of the exposure data (Brown *et al.,* 1997a, 1997b); a number of host and environmental factors among the Taiwanese that are not applicable elsewhere (Carlson-Lynch *et al.,* 1994); a possible threshold for arsenic carcinogenicity and nonlinearities in the dose-response curve (Abernathy *et al.,* 1996; Slayton *et al.,* 1996); differences in health and nutrition between Taiwan and the United States that might increase cancer risk in Taiwan (Beck *et al.,* 1995); the possibility that arsenic is an essential nutrient at lower doses (NRC, 1999); uncertainty regarding the amount of water consumed daily by Taiwanese males (U.S. EPA, 1998); and the possibility of significant exposure of the Taiwanese subjects to arsenic from sources other than the well water (Chappell *et al.,* 1997).

The selection of exposure groups based on average concentrations of arsenic in Taiwanese village wells is noted as a key source of uncertainty in these studies (Brown *et al.,* 1997a; Chappell *et al.,* 1997). Villages were represented by the average well water concentration, even though there was a large variation in arsenic concentration across individual wells. This precludes any ability to assess individual rates of exposure to those who developed cancers because of the lack of linkage with well water concentrations of arsenic, and well usage rates. The considerable variation in arsenic concentrations within the wells was recognized by Tseng *et al.* (1968), but was not addressed in the exposure estimation. Furthermore, the concentrations of arsenic in the wells was measured in the early 1960s, and for many villages, only 2 to 5 analyses were conducted on well water, and for other villages only one analysis was performed. Also, tap water was supplied to many areas after 1966, and the arsenic-containing wells were only used in dry periods, which would greatly limit arsenic exposures. In addition, because of the study design, particular wells used by subjects with skin cancer could not be identified and arsenic intake could only be assigned at the village level (U.S. EPA, 1998).

Brown *et al.* (1997a) considered the above limitations, together with the sensitivity of the cancer risk estimate to the model used, and concluded that the Tseng studies are not suitable for dose-response assessment, and for extrapolating to lower exposure levels.

Another key source of uncertainty lies in the assumption that all exposure to arsenic was derived from drinking water, when in fact, the diet and other environmental sources may have provided significant additional exposure (Brown and Abernathy, 1997; Chappell *et al.,* 1997). Brown and Abernathy (1997) concluded that the inclusion of dietary intake of arsenic in the cancer risk estimate would have significantly reduced the Maximum Likelihood Estimate. Mushak and Crocetti (1995) had also discussed this aspect of the Tseng studies. Referring to work by Pyles and Woolson (1982) and the OMEE (Weiler, 1987), Mushak and Crocetti (1995) noted that proportions of inorganic arsenic species in vegetables, and specifically potatoes are less than 10%, meaning that dietary exposures to inorganic arsenic would likely be insignificant. However, herbal medicines and teas are widely consumed in Taiwan, and may represent a significant, albeit poorly characterized source of exposure to inorganic arsenic (Espinoza *et al.,* 1995; Ernst, 1998).

Concomitant exposures to other chemicals are also key confounding factors in the Tseng studies. Byrd *et al.* (1996) suggested that humic acids, for which elevated concentrations in drinking water are correlated to that of arsenic, may interfere with the cancer incidence rates that attributed to arsenic. Chan and Huff (1997) also considered humic acid to be a potentially confounding factor in these studies, observing that humic acid is mutagenic and produces symptoms similar to those of Blackfoot disease, although the occurrence of arsenic-related cancers has been reported in the absence of humic acid exposure.

The importance of nutritional status, as well as the actual nutritional status of the study population, has also been debated in the literature. In general, poor nutritional status can lead to increased susceptibility to the toxic action of many chemicals. In the case of arsenic, there may be an additional role of nutrition in resistance or susceptibility to carcinogenicity. As the biomethylation of arsenic has been postulated to play a role in its genotoxicity and carcinogenicity, the status of the methyl donor pool, which is dependent on dietary intake of proteins and amino acids such as cysteine and methionine, may play a significant role in susceptibility to arsenic carcinogenicity. Indeed, Hsueh *et al.* (1995) found that malnutrition, indexed by a high consumption of dried sweet potato as a staple food, was a risk factor for skin cancer. However, Smith *et al.* (1995) reviewed the Taiwanese intake of protein, and found it adequate by current standards. Beck *et al.* (1995), in rebuttal, pointed out that current standards dictate intakes required for normal bodily processes, and may not be adequate to methylate an excessive and sustained intake of arsenic.

A1-3.4.6 Validation of the Dose-Response Curve at Low Exposure Rates

The rates of exposure in the studies that provide the basis for the cancer risk estimates are very high relative to the rates of exposures expected in most situations related to general environmental exposures. Therefore, the validity of the use of these data in extrapolating cancer risks from high to low exposures assuming a linear dose-response curve (*i.e.,* that risk increases with exposure in a linear fashion) must be examined.

To date, there remains considerable uncertainty over the actual carcinogenic risks from arsenic at low concentrations and the carcinogenic mode of action. NRC (2001) concluded that the available mode-of-action data on arsenic do not provide a biological basis for using either a linear or nonlinear extrapolation at this time. Furthermore, estimated numbers of cancer cases based on the cancer potency estimates would be difficult to detect by current epidemiological methods. It remains possible that the estimates of cancer risk associated with various arsenic intakes are overestimates. Eastern Research Group (1997) convened an Expert Panel on Arsenic Carcinogenicity, under contract to the U.S. EPA. The Expert Panel concluded that epidemiological studies clearly demonstrate that arsenic is a human carcinogen *via* the oral and inhalation routes. However, there is unlikely to be a single mode of action for arsenic carcinogenicity. The panel agreed that arsenic and its metabolites do not appear to directly interact with DNA, and that, for each of the biologically plausible modes of action, the dose-response relationship would either show a threshold or would be nonlinear. It was acknowledged though, that at very low doses the dose-response curve might appear linear with a very shallow slope that is virtually indistinguishable from a threshold. Further elaboration on the dose-response relationship at low exposure levels is provided in the following paragraphs.

While a large number of studies indicate an increased risk of various cancers with increasing arsenic drinking water concentrations, it must be recognized that there are several studies of exposure *via* drinking water that did not observe a significant relationship between exposure and cancer incidence (*e.g.*, Southwick *et al.,* 1983; Morton *et al.,* 1976; Wong *et al.,* 1992; Bates *et al.,* 1995). The lack of a correlation between arsenic exposure and cancer in these studies contrasts a number of studies of cancer incidence in populations experiencing higher exposures. Although reasons for the absence of a significant relationship in these studies is not known, Abernathy *et al.* (1996) suggested that exposures may have been too low or of short durations, or the study designs may not have been sufficiently sensitive. Bates *et al.* (1995) suggested that this discrepancy may be due to bias in exposure estimation or

simply due to chance. However, these studies may also be an indication that the extrapolation of cancer risks from high exposures to low exposures is not valid.

Several authors have examined the dose-response curves of cancer incidence and exposure levels, in order to determine if the extrapolation of Tseng *et al.* (1968) is supported at low exposure levels. Rudel *et al.* (1996) reviewed dose-response curves from genotoxicity studies and concluded that for most endpoints (*e.g.*, clastogenicity, chromosomal aberrations excepting sister chromatid exchange), there was a sublinear or threshold dose-response. Thus, it was concluded that use of a linear dose-response curve to extrapolate to low doses would overestimate risks. Guo and Valberg (1997) examined the validity of the use of the U.S. EPA cancer slope factor (CSF) in prediction of risks at low exposure levels. Using a likelihood analysis, the results of multiple epidemiological studies of skin cancer incidence and arsenic exposure *via* drinking water in countries around the world were analyzed. The authors observed that at drinking water concentrations between 170 and 270 µg As/L, the CSF overestimated skin cancer risks. At concentrations under 170 µg As/L, the epidemiological data were concluded to be inadequate to evaluate the CSF. These authors also reported that even with a pooled sample size of 195,000, the statistical analysis was unable to detect a difference in cancer incidence in "exposed" populations (up to 270 µg As/L in drinking water) *versus* baseline cancer rates. In a similar analysis, Valberg *et al.* (1998) examined the results of four North American studies to evaluate the use of the CSF in extrapolation to low dose exposures. Again, the CSF was found to overpredict the incidence of skin cancer in populations exposed to less than 400 µg As/L. Valberg *et al.* (1998) noted several limitations which may have affected the outcome of this analysis, including: the assumption that individuals were exposed for a chronic duration, exposure was estimated at a population level, not an individual level, uncertainties in the measurement of arsenic in drinking water, and uncertainties with regard to age distributions (*i.e.,* the CSF best predicts cancer incidence in older age groups).

Several recent studies and reviews have questioned the relevance of the Tiawanese dataset for the North American population (U.S. EPA, 2007; Lamm et. al., 2004; Brown and Chen, 1995). For example, Lamm et. al. (2004) considered the relationship between arsenic exposure through drinking water and bladder cancer mortality. County specific mortality ratios were considered for 133 counties across the U.S. where the primary source of drinking water was groundwater. No arsenic-related increase in bladder cancer mortality was found over an exposure range of 3 to 60 ug/L.

In contrast, Hertz-Picciotto and Smith (1993) observed that the dose-response relationship for lung cancer risks associated with inhalation exposure to arsenic indicates a supralinear dose-response curve. Based on

a review of six occupational epidemiological studies, a steeper slope was observed in the low dose range, indicating that extrapolation from high dose to low dose would underestimate lung cancer risks. Several reasons were offered to explain this observation. Because occupational studies formed the basis for this evaluation, it is possible that at higher exposure levels, use of protective gear by workers would reduce actual exposure levels. This has also been postulated to be a potentially confounding factor by Enterline (1997). Also, differences in types of exposures and forms of arsenic experienced at higher exposure levels may affect absorption from the lung. Concomitant exposures to other potentially cancer-causing chemicals may have inflated the baseline cancer risk in some of the studies as well. Smoking has been proposed to have a synergistic effect on arsenic carcinogenicity, with a greater synergism at lower arsenic exposure levels (*e.g.,* at residential exposures as opposed to occupational exposures) (Pershagen, 1985). Thus, differences in cigarette smoking in the different study groups may have affected cancer rates. It is also possible that the "healthy worker effect" may influence the shape of the dose-response curve for inhaled arsenic. In order to evaluate the potential impact of the "healthy worker effect", Arrighi and Hertz-Picciotti (1996) examined the effect of controlling for this effect on cancer risk estimates. It was found that controlling for this variable did not significantly affect the dose-response curve, but it did yield stronger associations.

Analyses performed on a subcohort at the Tacoma smelter indicate strong evidence of confounding by year of initial hire, with a non-linear exposure-response relationship evident only among workers hired before 1940 (Viren and Silvers, 1999). For workers hired after 1940, a linear dose-response curve was evident. Similarly, re-analysis of the Anaconda smelter cohort (Lubin *et al.,* 2000) also indicated a linear relationship, and suggested that the apparent non-linearity might be due to overestimation of risk in the high exposure groups (due to highly exposed workers wearing protective devices).

A1-3.5 Mechanism of Action (Weight-of-Evidence)

A1-3.5.1 Non-carcinogenic Effects

Non-carcinogenic toxicity of arsenic is attributed to interactions of inorganic arsenic, specifically As^{III}, with various tissues in the body. A number of studies in mice and rats have shown that arsenic compounds induce metallothionein (Albores *et al.,* 1992; Hochadel and Waalkes 1997; Kreppel *et al.,* 1993; Maitani *et al.,* 1987). The potency of arsenic compounds in inducing metallothionein parallels their toxicity [*i.e.*, $As^{+3} > As^{+5} > MMA^{V} > DMA^{V}$] (WHO-IPCS, 2001; ATSDR, 2005), although only a small percentage of administered arsenic is actually bound to metallothionein (Albores *et al.,* 1992; Kreppel *et al.,* 1994; Maitani *et al.,* 1987). It has been proposed that metallothionein binding might be a protective

measure against arsenic toxicity (NRC, 1999), but as discussed above, there was no difference between the sensitivity of metalothionein knockout mice and metalothionein competent mice with respect to arsenic exposure by the oral route (Ng *et al.,* 1998, 1999).

Pentavalent arsenic (As^V) has long been known to act as a phosphate analog. At the biochemical level, arsenate can substitute for phosphate in critical biochemical reactions which can lead to such effects as uncoupled oxidative phosphorylation in mitochondria (Gresser, 1981), and inhibition of glycolysis by competing with phosphate to form the dysfunctional compound 1-arseno-3-phosphoglycerate, rather than 1:3-diphosphoglycerate (Mayes, 1983).

A1-3.5.2 Role of Arsenic in Carcinogenicity

As trivalent inorganic arsenic (As^{III}) has greater reactivity and toxicity than the pentavalent form, it is generally believed that the trivalent form is the carcinogen (WHO-IPCS, 2001). However, the determination of the specific arsenic form(s) responsible for carcinogenic activity remains a matter of some dispute, partially related to the fact that the mechanism of carcinogenic action of arsenic has not been fully determined (WHO-IPCS, 2001; Tchounwou *et al.,* 2003). Some recent studies have suggested that trivalent MMA^{III} may be a potential contributor to arsenic-induced carcinogenicity (Bernstam and Nriagu, 2000; Yu *et al.,* 2000; Kitchin, 2001), as humans produce more of this metabolite than animals, and some epidemiology studies have associated higher levels of MMA^{III} with increased odds ratios for developing skin cancer. Trivalent DMA^{III} has also been suggested as a potential key arsenic metabolite associated with carcinogenesis (Kitchin, 2001).

Eastern Research Group (1997) convened an Expert Panel on Arsenic Carcinogenicity, under contract to the U.S. EPA. The Expert Panel concluded that epidemiological studies clearly demonstrate that arsenic is a human carcinogen *via* the oral and inhalation routes. However, there is unlikely to be a single mode of action for arsenic carcinogenicity. The panel also agreed that arsenic and its metabolites do not appear to directly interact with DNA.

The genotoxicity database is equivocal with respect to demonstrating a clear mechanism of action for arsenic-induced carcinogenesis. While studies of the *in vitro* clastogenicity of arsenic species indicated inorganic forms have a greater potency in some studies, other studies indicate that both inorganic and organic species are capable of inducing chromosomal aberrations (See Section A1-2.3). Moore *et al.* (1994) suggested that since arsenic exerts its genotoxicity by causing chromosomal abberations, it may

act in a latter stage of carcinogenesis as a progressor, rather than as a classical initiator or promotor (Moore *et al.,* 1994).

Studies of various arsenic species have yielded mixed results with regard to induction of DNA damage, which can be considered to be indicative of cancer initiation. Following oral gavage to rats, neither arsenite nor arsenate resulted in DNA damage (Brown *et al.,* 1997b). Yamanaka *et al.* (1989a,b; 1991; 1995; 1996) reported that oral administration of DMA to mice at high doses resulted in DNA strand breaks or DNA-protein cross-links in the lungs. Similarly, Brown *et al.* (1997b) reported the induction of DNA damage in the lungs of rats following oral administration of DMA^V, even at more moderate dose levels. This DNA damage was attributed to production of dimethylarsenic peroxyl radicals, hydroxyl radicals and superoxide radicals (Yamanaka *et al.,* 1989a,b).

DMA^V has been reported to act as a promoter of carcinogenicity, based on enhanced tumourigenesis in the kidney, bladder, liver, thyroid gland, and lung (Shirachi *et al.,* 1983; Murai *et al.,* 1993; Yamanaka *et al.,* 1995; Yamamoto *et al.,* 1995; Wanibuchi *et al.,* 1996). Following oral administration to rats, sodium arsenite was observed to increase hepatic ornithine decarboxylase (ODC), a marker for promotion of carcinogenesis, and hepatic heme oxygenase activity, an indicator of changes in cell redox potential (Brown and Kitchin, 1996). In a similar study, Brown *et al.* (1997b) studied the impact of sodium arsenate (As^V), MMA^V and DMA^V on six biochemical parameters. DMA^V and MMA^V were reported to cause a decrease in alanine aminotransferase and glutathione, and an increase in cytochrome p450 content (also a marker for promotion of carcinogenicity), and DMA^V also caused a decrease in ODC. Arsenate had no effect on the biochemical markers for cancer promotion. Based on these findings, Brown *et al.* (1997b) suggested that DMA^V is an active promoter of multistage carcinogenesis, while As^{III} also shows some evidence of promotion capability.

Arsenic may act as a comutagen, cocarcinogen or progressor (Barrett *et al.,* 1988; Chan and Huff, 1997; Rossman *et al.,* 2002). Brown and Chu (1983c) proposed that arsenic is a late-stage carcinogen, acting during progression, after completion of initiation and promotion by other causal agents, and before malignant cells are detectable. The role of arsenic as a progressor may be related to its impacts on gene amplification and cell proliferation, which would function to promote the proliferation and growth of transformed cells (Chan and Huff, 1997). Arsenic could also act by enhancing DNA damage or enhancing the inhibition of DNA repair processes (Chan and Huff, 1997; Wiencke *et al.,* 1997; Yager and Wiencke, 1997; Rossman *et al.,* 2002). Based on the results of epidemiological studies, smoking has been reported to potentiate arsenic carcinogenicity (Pershagen, 1985; Bates *et al.,* 1995; Chiou *et al.,*

1995; Tsuda *et al.,* 1995). Administration of arsenic has also been reported to enhance tumour growth and to decrease latency in mice (Schrauzer and Ishmael, 1974; Kerkvliet *et al.,* 1980; Shirachi *et al.,* 1983; Yamamoto *et al.,* 1995), and to transform benign tumour cells to malignant tumour cells (Barrett and Lee, 1992).

Some of the leading theories regarding the mode of carcinogenic action for arsenic compounds are described and discussed in the following section.

A1-3.5.2.1 Theories on Mode of Action

Significant research into arsenic's mode of carcinogenic action has been conducted over the past several years. While substantial progress has been made regarding arsenic's possible mode(s) of action, a scientific concensus has not yet been reached (Kitchin, 2001; Snow et al., 2005). Some of the major theories regarding arsenic's carcinogenic mode of action are discussed in the following sections. It should be recognized that many of these theories contain aspects that relate to other theories; thus, it is possible that multiple processes occuring either sequentially or simultaneously could contribute to the development of cancer in arsenic-exposed individuals.

Perturbation of Normal Methylation Processes

As discussed in Section 1.3, the primary metabolic fate of inorganic arsenic entering the body is methylation. Independent of species-specific toxic potency, and the effects of methylation on tissue concentrations of inorganic arsenic *versus* MMA^V and DMA^V, the process of methylation itself, and disturbance of the normal balance of methyl donors and receptors, has been proposed to have significant effects which could lead to genetic damage and carcinogenicity (Mass and Wang, 1997a).

Several studies have noted alterations in normal patterns of methylation following exposure to arsenic. Mass and Wang (1997b) reported hypermethylation (increased methylation) of cytosines in the region of the p53 tumour suppressor gene in a human adenocarcinoma cell line in *in vitro* cell systems exposed to arsenic. Hypermethylation of DNA is theorized to result from inhibition of methyltransferases, with subsequent decreases in use of the methyl donor, and overmethylation of native methylation substrates. Hypermethylation may affect gene expression, and has been reported to cause inhibition of certain genes, such as the p16 tumour suppression gene (Gonzalez-Zulueta *et al.,* 1995; Herman *et al.,* 1995).

It has been postulated that with excessive arsenic exposures, the subsequent depletion of the methyl donor s-adenosylmethionine might cause a decrease in the methylation of DNA routinely undertaken in DNA

repair activities (Chan and Huff, 1997; Mass and Wang, 1997b). The resultant hypomethylation of DNA due to the depleting effects of methyl donors by arsenic could lead to fragility of DNA and subsequent initiation or promotion of cancer. As other known hypomethylating agents are also clastogens [*e.g.,* 5-azacytidine, ethionine (Collins and Meyers, 1987; Perticone *et al.,* 1987; Meehan *et al.,* 1990)], this mechanism could lead to the DNA strand breakage observed by Yamanaka *et al.* (1989a,b). Zhao *et al.* (1997) found that the DNA of sodium-arsenite-transformed rat liver TRL 1215 cells was globally hypomethylated and the effect was both dose and time-dependent. The authors propose that DNA hypomethylation results in aberrant gene expression.

If this mechanism has a role in arsenic carcinogenicity, then individual characteristics, such as methylation activity and nutritional status (*i.e.,* adequacy of the methyl donor pool) would affect individual susceptibility to arsenic. In addition, genetic polymorphism for methylation capacity, as has been reported in the human population related to ethnicity, age, and previous exposure status (Chappell *et al.,* 1997), could affect individual susceptibility to arsenic. Goering *et al.* (1999) suggest that the unique metabolism of arsenic in different individuals may explain the apparent non-linear threshold response that has been observed in human populations exposed to inorganic arsenic.

Incorporation into Native Enzymes or DNA

Chan and Huff (1997) suggested that due to its similarity to phosphate, arsenic (as As[V])could, by mis-identification as phosphate, become incorporated into DNA during replication. The resultant bond would be weaker than the normal phosphodiester bond, resulting in structural instability in the DNA. This effect would lead to the observed genotoxic endpoints attributed to arsenic (clastogenicity, chromosomal aberrations, sister chromatid exchange, and micronuclei formation). However, because As^{V} is the form similar to phosphate, and thus of a form that is readily incorporated in to bodily tissues (*e.g.*, into bones), this theory does not explain why As^{III} is more active at inducing chromosomal aberrations than is As^{V} (*e.g.,* Kochhar *et al.,* 1996; Moore *et al.,* 1997a; Oya-Ohta *et al.,* 1996).

Alternately, the similarity of arsenic to phosphate or sulphate may result in the incorporation of arsenic into enzymes, with subsequent impairment of normal enzyme activity (Chan and Huff, 1997). For example, Mitchell *et al.* (1971) suggested that arsenic may inhibit mitochondrial energy-linked functions through competition with phosphate during oxidative phosphorylation, thereby uncoupling the reaction, and by forming a complex with lipoic acid cofactors and subsequent inhibition of NAD-linked substrates, as has been observed by Crane and Lipmann (1953).

Trivalent arsenic (As^{III}) is known to interact with tissues through binding to sulfhydryl groups such as glutathione (Vahter and Marafante, 1988), resulting in elevated concentrations in sulfhydryl-rich tissues such as hair and nails. This affinity may also result in the incorporation of arsenic into DNA or enzymes (Rudel *et al.,* 1996; Chan and Huff, 1997). Arsenic has been shown to inhibit DNA repair enzymes (*e.g.*, the ligase I and II enzymes), which contain essential sulfhydryl groups (Rudel *et al.,* 1996). Subsequent decreases in DNA repair might be responsible for DNA amplification and cell transformation (Rudel *et al.,* 1996). Experimental inhibition of the DNA repair enzymes, ligase I and II, has been shown to result in clastogenicity (Li and Rossman, 1989), a genotoxic effect associated with arsenic. A recent study by Andrew *et al.* (2003) reported an association between biomarkers of arsenic exposure (particularly toenail concentrations) and the expression of DNA repair genes in an eastern U.S. population. The authors indicate that their findings are consistent with the hypothesis that inhibition of DNA repair capacity is a potential mechanism for the co-carcinogenic activity of arsenic.

Formation of Reactive Oxygen Species / Oxidative Stress

Several investigators have demonstrated that DMA^{V} is capable of inducing DNA damage in lung tissue such as strand breaks or cross-links (Yamanaka *et al.,* 1989a,b; 1991; 1995; 1996, 2001a; Brown *et al.,* 1997b). It has been postulated that this damage is induced through active oxygen species (superoxide anion radicals and hydroxyl radicals) produced by the reaction of molecular oxygen and dimethylarsine (a metabolite of DMA) (Chan and Huff, 1997). Non-genotoxicity studies have also found that inorganic arsenic compounds induce formation of reactive oxygen species, such as hydroxyl radical, in various tissues (*e.g.*, Garca-Chavez *et al.,* 2003).

Liu *et al.* (2003) found that arsenite (As^{III})-induced oxidative stress promotes telomere attrition, chromosome end-to-end fusions, and apoptotic cell death. Embryos with shortened telomeres, from late generation telomerase-deficient mice, showed an increased sensitivity to arsenic-induced oxidative damage, suggesting that telomere attrition somehow mediates arsenic-induced apoptosis. However, arsenite did not produce chromosome end-to-end fusions in telomerase RNA knockout mouse embryos, even though damaged telomeres and disrupted embryo viability occurred. The authors suggest that these findings may explain why arsenic can initiate oxidative stress and telomere erosion, leading to apoptosis in some cases, and chromosome instability and carcinogenesis in others.

Many other recent studies have reported that both inorganic and organic arsenic compounds induce oxidative stress in various human cell lines (including cancer cell lines), human-hamster hybrid cell lines,

and isolated DNA (Li *et al.,* 2002a,b; Kessel *et al.,* 2002; Schwerdtle *et al.,* 2003; Nesnow *et al.,* 2002). These studies provide support for the theory that reactive oxygen species play an important causal role in the genotoxicity of arsenic compounds in mammalian cells. Evidence of increased levels of 8-oxo-2'-deoxyguanosine levels in *in vivo* studies further supports the theory that reactive oxygen species play a critical role in the development of genotoxicity and carcinogenicity (*e.g.*, Yamanaka *et al.,* 2001a; Wei *et al.,* 2002).

Recently, Pi *et al.* (2002) conducted a cross-sectional study in Wuyuan, Inner Mongolia, China, to investigate the relationship between chronic arsenic exposure from drinking water and oxidative stress in humans. Thirty-three residents drinking tube-well water with high concentrations of inorganic arsenic (mean = 0.41 mg/L) for roughly 18 years, comprised the high-exposure group, while 10 residents exposed to much lower concentrations of arsenic in their drinking water (mean = 0.02 mg/L) comprised the low-exposure group. The activity for superoxide dismutase (SOD) in blood did not differ significantly between the two groups, but the mean serum level of lipid peroxides (LPO) was significantly elevated in the high-exposure group. The elevated serum LPO concentrations were correlated with blood levels of inorganic arsenic and its methylated metabolites. LPO levels also showed an inverse correlation with nonprotein sulfhydryl (NPSH) levels in whole blood. The high-exposure group had mean blood NPSH levels 57.6% lower than the low-exposure group, which were inversely correlated with the concentrations of inorganic arsenic and its methylated metabolites in blood. This study demonstrates that chronic human exposure to arsenic in drinking water results in induction of oxidative stress, as indicated by reduction in NPSH and an increase in LPO.

Gene Amplification/Expression

There is increasing evidence to indicate that arsenic acts as a promoter by modulating the signaling pathways responsible for cell growth (Simeonova and Luster, 2002). By means of some of the mechanisms discussed above, arsenic has been shown to cause gene amplification and cell proliferation (Lee *et al.,* 1985; 1988). It has been postulated that in cases of arsenic-induced promotion, the genes upon which this activity is directed are involved in carcinogenicity (*e.g.,* oncogenes) (Barrett and Lee, 1992; Chan and Huff, 1997). This hypothesis is supported by the observation that oncogene expression has been reported to be amplified in human and animal tumours (Chan and Huff, 1997). Gene amplification could also lead to clastogenicity and malignant transformation through inhibition of DNA repair and stimulation of cell division (Rudel *et al.,* 1996; Chan and Huff, 1997). Rudel *et al.* (1996) proposed a mechanism involving the induction of heat shock proteins (produced in every cell following

stress [Welch and Suhan, 1986]) from arsenic exposure. Rudel *et al.* (1996) pointed out that at least 1 heat shock protein is involved with the gene(s) for certain DNA repair enzyme(s), and that perturbation of the heat shock protein could lead to amplification of that gene, and thus to interference with normal DNA repair. However, the reason for the induction of these heat shock protein, and their biological implication is not fully understood at present (WHO-IPCS, 2001). Chan and Huff (1997) suggested that the formation of apurinic or apyrimidic sites in DNA as a result of disturbance of excision repair processes could lead to DNA single strand breaks and DNA protein cross-links.

Interaction with Other Chemicals through Concomitant Exposures

Arsenic may act as a comutagen, cocarcinogen, promoter or progressor, when individuals are exposed to arsenic simultaneous to other chemicals with the capacity for carcinogenic activity (Byrd *et al.,* 1996; Chan and Huff, 1997; Rossman *et al.,* 2002). It has been suggested that arsenic could enhance DNA damage through inhibition of DNA repair processes caused by such agents as ultraviolet radiation or alkylating agents (Chan and Huff, 1997; Wiencke *et al.,* 1997; Yager and Wiencke, 1997; Rossman *et al.,* 2002). The role of arsenic as a progressor may be related to its impacts on gene amplification and cell proliferation, which would function to promote the growth of transformed cells (Chan and Huff, 1997). Arsenic has also been reported to be capable of transformation of benign tumour cells to malignant tumour cells (Barrett and Lee, 1992). Byrd *et al.* (1996) suggested that populations might be exposed to arsenic and a proximal carcinogen whose concentrations correlated to that of arsenic, in this scenario, arsenic would act as a promoter of various tumour types. The dependence on co-exposure to an initiator of cancer was thought to provide a reason for the wide range of tissues-specific cancers with which arsenic has been associated.

Effects of Nutritional Status

In general, poor nutritional status can lead to increased susceptibility to the toxic action of many chemicals. In the case of arsenic, there may be an additional role of nutrition in resistance or susceptibility to carcinogenicity. The methylation of arsenic following absorption has two general effects:

The conversion from inorganic species to MMA^V and DMA^V, with subsequent impacts on altered toxic potency; and,

The impact on normal methylating activity in the body, especially of DNA and enzymes, as discussed above.

As stated earlier, As^{III} has a high affinity for sulfhydryl groups. The binding to such groups in DNA repair enzymes could lead to gene amplification. Alternately, binding to non-essential sulfhydryl groups, such as those provided in the diet, could provide a substitute for sulfhydryl binding of arsenic, thereby protecting the essential sulfhydryl groups. Therefore, the supply of sufficient levels of sulfhydryl groups and methyl donors (*e.g.*, proteins and amino acids such as cysteine and methionine), may play a significant role in susceptibility to arsenic carcinogenicity (Chan and Huff, 1997).

The theory of adverse effect by interference with sulphydryl groups is supported by several studies in the literature. Vahter and Marafante (1987) studied the impact of a methionine/ cysteine reduced diet on the methylating activity of rabbits, and reported a significant decrease in methylation of inorganic arsenic following a 25% reduction in dietary intake of the amino acids. In an epidemiological study of cancer incidence in a Taiwanese village, Hsueh *et al.* (1995) found that malnutrition, indexed by a high consumption of dried sweet potato as a staple food, was a risk factor for skin cancer.

The importance of nutrition in maintaining adequate levels of methyl donors and sulfhydryl groups has been proposed as a reason for increased cancer risks in subsets of the Taiwanese population, in comparison to those observed in North American populations (and thus for the over-prediction of risks of skin cancer in North American populations exposed to arsenic in drinking water). However, Smith *et al.* (1995) reviewed the Taiwanese intake of protein, and found it adequate by current standards. Beck *et al.* (1995), in rebuttal, pointed out that current standards dictate intakes required for normal bodily processes, and may not be adequate to methylate an excessive and sustained intake of arsenic.

Other Possible Mechanisms

Burns *et al.* (1994) proposed that arsenic-induced immunosuppression may result in reduced natural immune surveillance, which would normally repair or remove transformed and damaged cells prior to tumourigenesis. A number of other studies have suggested that such processes as suppression and/or stimulation of transcription and growth factors, and suppression of p53 may play a role in genotoxicity (WHO-IPCS, 2001; Kitchin, 2001). Rossman and Wang (1999) suggest that the ubiquitin system is targeted by arsenic, resulting in alterations that may result in genotoxicity and carcinogenicity.

Snow et al. (2005) have proposed a low dose adaptive (protective) response by arsenic (known as a hormetic response, characteristic of many agents that induce oxidative stress). A mechanistic model for arsenic carcinogenesis based on a hormesis type of effect would predict a sub-linear (threshold) low dose risk for carcinogenesis. The threshold dose where toxicity outweighs protection is hard to predict and would require information on the the form (metabolite) and concentration of arsenic responsible for changes in gene regulation in the target tissues.

Resistance to Genotoxicity

Chinese hamster lung V79 cells have been isolated that are resistant or hypersensitive to the cytotoxic effects of arsenite (Wang and Rossman, 1993; Wang *et al.,* 1996). However, several human cell lines derived from keratinocytes, fibroblasts or tumour cells lack the inducible arsenic-resistance response (Rossman *et al.,* 1997), even though the human cells were as sensitive to the cytotoxic effects of arsenite as the Chinese hamster lung cells. This lack of inducibility of resistance may partially explain why animals are less sensitive to the carcinogenic effects of arsenic than humans (WHO-IPCS, 2001).

A1-3.6 Consideration of Sensitive Subpopulations

Individual differences, whether resulting from genetically-based differences in biochemistry, nutritionally-based differences in metabolism, or from differences in concomitant chemical exposure profiles, could result in subpopulations of greater or lesser susceptibility to the toxic impacts of arsenic. For example, while the role of methylation as a detoxification process for inorganic arsenic is of some debate, there is evidence in the literature that indicates certain human subpopulations have varied capacities for arsenic methylation, dependent on ethnicity, age, gender, and smoking status.

Considerable research has focused on investigating whether younger members of the population are at greater risk of arsenic-induced toxicity than adults. To date available data are too limited to draw any firm conclusions as to whether arsenic poses a greater relative risk to younger members of the community or not (Watanabe *et al.,* 2003).

A1-3.7 Exposure Limits

The following organizations were consulted to select exposure limits for arsenic; the U.S. EPA; MOE; ATSDR; Health Canada; RIVM; NRC; WHO; and, OEHHA.

The following paragraphs relate to inorganic arsenic species only, as all regulatory TRVs that exist for arsenic have been developed from data on inorganic arsenic exposure. However, as discusssed in previous sections, some recent studies suggest that certain organic arsenicals (such as pentavalent MMA and DMA) may be of similar or greater toxic potency than inorganic arsenic species. In a recent review of the toxicokinetics and toxicology of methylated arsenicals, Cohen *et al*. (2006) note that the animal carcinogenicity data for MMA^V and DMA^V are equivocal. These authors also state that the metabolism and disposition of MMA^V and DMA^V, when formed endogenously during the metabolism of inorganic arsenic, differs from the metabolism and disposition of these methylated species when exposure is exogenous. Furthermore, the trivalent arsenicals that known to be cytotoxic and indirectly genotoxic *in vitro* are formed in negligible amounts in organisms exposed exogenously (ingestion) to MMA^V or DMA^V due to low cellular uptake and limited metabolism of these compounds. Cohen *et al*. (2006) conclude that at anticipated environmental exposures to MMA^V and DMA^V, carcinogenic risk to humans is unlikely. In a science issue paper produced by the U.S. EPA Office of Pesticide Programs on a mode of carcinogenic action for DMA^V (cacodylic acid) (U.S. EPA OPP, 2005), it is also noted that there are differences in methylation efficiency and cellular uptake between direct exposure to DMA^V and exposure to inorganic arsenic, with subsequent metabolism to DMA^V and other methylated species. The U.S. EPA paper also notes that direct exposure to DMA^V results in the production of fewer arsenical metabolites relative to metabolism that occurs following direct exposure to inorganic arsenic. Thus, exposure to inorganic arsenic results in a more complex mixture of metabolites and transformation products. The U.S. EPA paper also states that there is presently insufficient evidence to establish pentavalent MMA and DMA species as the ultimate carcinogenic forms of inorganic arsenic. Rather, it is likely that several inorganic and organic arsenical species may be involved in various modes of action in different target tissues. For DMA^V, U.S. EPA OPP (2005) suggests that this substance is a threshold carcinogen with a carcinogenic mode of action that is non-linear. As such, a reference dose has been proposed using benchmark dose modelling.

Exposure limits derived by the U.S. EPA were selected for use in this assessment. The interpretation of the resultant risk estimates must consider the limitations and uncertainties inherent in these exposure limits. The U.S. EPA derives exposure limits for both threshold and non-threshold effects when data is available. The RfD and RfC are based on the assumption that a threshold exists for certain toxic non-carcinogenic effects. In general, the RfD (or RfC) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The U.S. EPA (1998) has classified arsenic as a Group A carcinogen (human carcinogen). Based on the weight-of-evidence of carcinogenicity in humans by more than one route of exposure, inorganic arsenic compounds are classified by Health Canada (1992) as "carcinogenic to humans" (Group 1). Inorganic arsenic compounds are classified by IARC in Group 1 (carcinogenic to humans) on the basis of sufficient evidence for carcinogenicity in humans and limited evidence for carcinogenicity in animals (IARC, 1987).

A1-3.7.1 Oral Exposure Limits

Non-Carcinogenic (Threshold) Effects

The U.S. EPA (1993) calculated an oral RfD of 0.3 µg As/kg body weight/day based on the epidemiological studies of chronic exposure to arsenic through drinking water (Tseng *et al.,* 1968; Tseng, 1977). Critical effects were hyperpigmentation, keratosis, and possible vascular complications at a lowest-observable-adverse-effects-level of 14 µg As/kg body weight/day. The RfD was based on a NOAEL of 0.8 µg As/kg body weight/day, with the application of an uncertainty factor of 3 to account for both lack of data on reproductive toxicity in humans, and for differences in individual sensitivity. The U.S. EPA (1993) noted some limitations of the studies, in that the exposure levels were not well-characterized (particularly from foods) and other contaminants were present. Also, there was not a clear consensus among U.S. EPA scientists on the oral RfD, and arguments were made for alternate values that are within a factor of 2 or 3 of the currently recommended RfD value (*i.e.,* 0.1 to 0.8 µg/kg/day) (U.S. EPA, 1993). New data that could possibly impact on the recommended RfD for arsenic will be evaluated by the U.S. EPA Work Group as it becomes available. Confidence in the chosen principal study and the resulting oral RfD is considered medium. MOE (1996) adopted 0.3 µg As/kg body weight/day, based on information provided on IRIS in 1993, as the chronic RfD as part of the Guideline for Use at Contaminated Sites in Ontario.

The ATSDR (2000) chronic minimal risk level (MRL) for inorganic arsenic is based on the same studies as the U.S. EPA oral RfD, and uses the same choice of critical effect, NOAEL and uncertainty factors.

Health Canada (1996) has recommended a provisional tolerable daily intake (PTDI) of 2 µg As/kg body weight/day based on technical reports form annual meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA); however, this PTDI was not used in this assessment.

RIVM (Baars *et al.,* 2001) derived a tolerable daily intake (TDI) of 1 µg/kg/day for critical effects on the skin in humans. This value is based on a NOAEL of 2.1 µg/kg/day that was derived by Vermeire *et al.* (1991) from the World Health Organization provisional maximum tolerable weekly intake (PTWI) of organic arsenic (15 mg/kg bw/week for adults weighing 70 kg). This PTWI was derived from a LOAEL of 100 µg arsenic/L in drinking water in a human epidemiological study, assuming a daily intake of drinking water of 1.5 L/day. RIVM used an uncertainty factor of 2 to compensate for observation errors in the epidemiological study. Thus, the TDI was derived as follows: (100 µg arsenic/L x 1.5 L/day) / (70 kg) / (2) = 1 µg/kg/day.

For the purposes of the current assessment, the more conservative exposure limit from U.S. EPA (1998) of 0.3 µg As/kg body weight/day, was selected as the oral exposure limit for non-carcinogenic effects.

Carcinogenic (Non-threshold) Effects

Arsenic exposure *via* the oral route was considered by the U.S. EPA to be carcinogenic to humans, based on the incidence of skin cancers in epidemiological studies examining human exposure through drinking water (Tseng *et al.,* 1968; Tseng, 1977). Based on the application of a linear-quadratic mathematical model to the data from these studies, the U.S. EPA (1998) calculated an oral slope factor of 0.0015 (µg As/kg body weight/day)$^{-1}$. The slope factor (SF) is based on the assumption that carcinogenic effects do not have a threshold (*i.e.,* dose-response relationship is linear to zero exposure). It was assumed that the Taiwanese individuals had a constant exposure from birth, and that males consumed 3.5 L drinking water per day, and females consumed 2.0 L per day. Doses were converted to equivalent doses for U.S. males and females based on differences in body weights and differences in water consumption and it was assumed that background skin cancer risk in the U.S. population would be similar to the Taiwanese population. The multistage model with time was used to predict dose-specific and age-specific skin cancer prevalence rates associated with ingestion of inorganic arsenic; both linear and quadratic model fitting of the data were conducted. The SF of 0.0015 (µg As/kg body weight/day)$^{-1}$, corresponding to an RsD of 0.00067 µg As/kg body weight/day for an acceptable risk level of one-in-one million, was adopted as the oral exposure limit for carcinogenic effects of arsenic for this assessment.

Recently, there has been concern on the part of regulators regarding the applicability of the arsenic cancer potency estimates for cancers at other sites (specifically bladder cancer) in setting exposure limits for arsenic. The National Research Council (NRC) (1999; 2001) has recently re-evaluated drinking water criteria for the United States, based on bladder cancer incidence data in the Taiwanese population as

presented in Wu *et al.* (1989), Chen *et al.* (1992) and Smith *et al.* (1992). NRC (1999; 2001) emphasized that the evaluation of cancer potency factors for bladder cancer has been limited by the amount and the quality of data available for use in the linear model. While the bladder cancer value would yield a greater cancer potency than that based on skin cancer, these data are still limited by many of the same problems as the potency factor for skin cancer, including large uncertainty of total daily exposure to inorganic arsenic (*i.e.,* the poor linkage between water concentrations of arsenic and individual exposure, and lack of data on arsenic intake from food), concomitant exposures to other chemicals and carcinogens (which would be especially important if arsenic is a cancer promoter), and differences in nutritional and health status between Taiwanese and North American populations. Because the intended use of the cancer potency factor is in the estimation of risk to a particular population in comparison to a "background" or "typical" population, and risks for both will be assessed with the same methodologies and the same exposure limit, the use of the skin cancer potency factor is considered acceptable and conservative.

MOE (1996) selected 0.00175 (µg As/kg body weight/day)$^{-1}$ as the oral cancer potency factor as part of the Guideline for Use at Contaminated Sites in Ontario, based on information provided on IRIS in 1993. This number was considered dated and not relevant to the current study.

Health Canada (1992) developed a TD_{05} for ingested arsenic by modifying the quantitative risk assessment prepared by the U.S. Environmental Protection Agency (U.S. EPA, 1998) to predict the dose associated with a 5% increase in the prevalence of skin cancer, incorporating background prevalence rates for Canadians. The estimates for potency to induce a 5% increase in skin cancer prevalence were 906 and 844 µg/L for men and women, respectively. Rounding 844 µg/L to 840 µg/L, the dose associated with this drinking water concentration is 18 µg/kg/day (assuming consumption of 1.5 L of water per day and a 70 kg body weight).

A1-3.7.2 Inhalation Exposure Limits

Non-cancer (Threshold) Effects

Health Canada did not derive a risk estimate for non-cancer effects due to inhalation exposure since carcinogenicity is considered the critical endpoint. ATSDR did not derive an inhalation minimal risk level (MRL) as this agency considers that the only chronic duration study by the inhalation pathway did not clearly establish a NOAEL, and there were no adequate supporting studies (TERA-ITER, 2003). The U.S. EPA has also not established an inhalation RfC or RfD for arsenic.

RIVM (Baars *et al.,* 2001) derived a tolerable concentration in air (TCA) of 0.001 mg/m^3 based on human occupational studies by Blom *et al.* (1985) and Lagerkvist *et al.* (1984). RIVM notes that lung cancer can occur in humans at concentrations greater than 0.01 mg/m^3; however, RIVM also indicates that the mechanism for lung tumours is not directly genotoxic, and that a threshold exists for this effect. Therefore, RIVM chose to call the value a TCA instead of a cancer risk value, and applied an uncertainty factor of 10 to account for intrahuman variability.

The OEHHA (2000) derived an acute 4 hour Reference Exposure Level (REL) of 0.19 µg As/m^3 based on the study by Nagymajtenyi *et al.* (1985). A cumulative uncertainty factor of 1,000-fold (10-fold each for use of a LOAEL, interspecies extrapolation and intraspecies differences in sensitivity) was applied to the study LOAEL (which was adjusted to reflect only the arsenic component of arsenic trioxide). The OEHHA (2000) also used this same study as the basis for their chronic REL. This was accomplished by using the average experimental exposure for the LOAEL group (determined to be 33 µg As/m^3) and applying a cumulative uncertainty factor of 1,000 (10-fold each for use of a LOAEL, interspecies extrapolation and intraspecies differences in sensitivity) to yield a chronic REL of 0.03 µg As/m^3. According to the OEHHA (2000), route-to-route conversion of the LOAEL in the key study indicates that this chronic REL should also be protective of adverse effects that have been observed in studies with oral exposures, either in food or drinking water. Also, OEHHA considers that had available human data been used instead of animal data in the REL derivation, a similar value would have been obtained. Thus, the chronic REL from animal data is believed to be protective of potential adverse health effects in humans.

Cancer (Non-threshold) Effects

The U.S. EPA (1998) considers arsenic to be a non-threshold carcinogen. Based on this assumption, the U.S. EPA (1998) calculated an inhalation unit risk of 0.0043 (µg As/m^3)$^{-1}$, based on studies by Brown and Chu (1983a,b,c), Lee-Feldstein (1983), Higgins *et al.* (1982), and Enterline and Marsh (1982) which indicated increased lung cancer mortality of exposed populations. A geometric mean was obtained for data sets obtained with distinct exposed populations (Anaconda smelter and ASARCO smelter), and then the final estimate was the geometric mean of those two values. It was assumed that the increase in age-specific mortality rate of lung cancer was a function only of cumulative exposures. The unit risk was converted to a slope factor of 0.015 (µg As/kg body weight/day)$^{-1}$ assuming a 70 kg adult breathes 20 m^3/day.

Health Canada developed a TC_{05} for inhaled arsenic based on lung tumour data presented in the large studies of workers at the Tacoma smelter (Enterline *et al.,* 1987b), the Anaconda smelter (Higgins *et al.,* 1986) and the Ronnskar smelter (Jarup *et al.,* 1989). Based on these data, the TC_{05}'s for inhaled arsenic were 7.8, 10 and 51 μg/m^3 for the Anaconda, Tacoma and Ronnskar smelter workers, respectively. The most conservative of these values (7.8 μg/m^3) is the TC_{05} recommended by Health Canada.

To compare the Health Canada and U.S. EPA risk values, both can be converted to a risk-specific concentration (RsC) at a specified risk level (*e.g.*,1 in 100,000 (1E-5) risk level). The U.S. EPA value can be expressed as an RsC by dividing 1 x 10^{-5} (0.00001) by the unit risk of 0.0043 (μg/m^3)$^{-1}$ (and an additional factor of 1,000 to convert to mg/m^3) to yield a RsC value of 0.0000023 mg/m^3. Health Canada's TC_{05} can be divided by 5,000 to represent a 1 in 100,000 risk level of 0.0000016 mg/m^3. It should be noted that the TC_{05} is computed directly from the dose-response curve within or close to the experimental range, while the EPA risk estimate is the upper 95% confidence limit on the low-dose extrapolation. Nonetheless, both jurisdictions values are similar when expressed as an RsC.

RIVM (Baars *et al.,* 2001) derived a tolerable concentration of arsenic in air (TCA) of 0.001 mg/m^3 based on lung tumours in smelter workers. As RIVM considers that the mechanism for lung cancer is not directly genotoxic, a threshold is believed to exist for this effect. Therefore, RIVM chose to call the value a TCA, instead of a cancer risk value, and applied an uncertainty factor of 10 to account for intrahuman variability. However, RIVM indicates that the TCA is protective of lung cancer effects.

The OEHHA (2002) derived a unit risk of 0.0033 (μg/m^3)$^{-1}$ and an inhalation slope factor of 0.012 (μg/kg/day)$^{-1}$. Both values were based on lung cancer incidence data from human occupational studies in smelter workers (Enterline *et al.,* 1987). OEHHA (2002) used a relative risk model and adjusted for interaction with tobaco smoking to arrive at these values.

The World Health Organization (WHO, 2000) has estimated that the unit risk for arsenic-induced lung cancer (risk estimate for lifetime exposure to a concentration of 1 μg/m^3) is 1.5 x 10^{-3}.

The Ontario Ministry of the Environment and Energy (MOE, 1994) recommended a 24-h RfC of 0.3 μg As/m^3 (equivalent to 0.1 μg As/kg body weight/day assuming a 70 kg adult breathes 23 m^3/day); however, this acute exposure limit was not used in this assessment.

It has been suggested that because exposures to air-borne arsenic would be mediated by inhalation of particulate matter, and since a higher proportion of particulate matter would be respirable in occupational

settings as compared to environmental exposures, the inhalation potency of arsenic based on occupational studies is likely overestimated for exposures associated with environmental contamination.

A1-3.7.3 Dermal Exposure Limit

No regulatory dermal exposure limits for arsenic compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF_{dermal}). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF_{dermal} = relative absorption factor for dermal exposure (%).

AF_{dermal} = the fraction of the applied chemical absorbed through the skin.

AF_{oral} = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

A1-3.8 Critical Summary

Table A1.1 summarizes the arsenic exposure limits selected for the current study.

Table A1.1 Summary Table of Exposure Limits for Arsenic

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Cancer (Non-threshold) Effects					
Oral	0.0015 $(\mu g/kg/day)^{-1}$	SFo	skin cancer	Tseng *et al.*, 1968; Tseng, 1977	U.S. EPA (1998)
Inhalation	0.0043 $(\mu g/m^3)^{-1}$	IUR	lung cancer	Brown and Chu (1983a,b,c); Lee-Feldstein (1983); Higgins *et al.*, (1982); Enterline and Marsh (1982)	U.S. EPA (1998)
	0.015 $(\mu g/kg/day)^{-1}$	SFi			
Dermal	NA		NA	NA	NA
Non-cancer (Threshold) Effects					
Oral	0.3 µg/kg/day	RfD	hyperpigmentation, keratosis, and possible vascular complications	Tseng *et al.*, 1968; Tseng, 1977	U.S. EPA (1993)
Inhalation	0.03 $\mu g/m^3$	Chronic REL	increased fetal malformations	Nagymajtenyi *et al.* (1985)	OEHHA (2000)
Dermal	NA		NA	NA	NA

NOTES:
NA = not available; SFo = oral slope factor; IUR = inhalation unit risk; SFi = inhalation slope factor; RfD = reference dose; REL = reference exposure level.

A1-4.0 SUMMARY TABLES FOR ARSENIC TOX PROFILE

Table A1.2 Animal Systemic Toxicity

Species	Test Compound	Duration/ Conditions	Toxicity Value	Endpoint(s)	Reference
Inhalation Studies					
mice	arsenic trioxide	3 h, 5 d/wk, for 1, 5 or 20 days	NOAEL = 123 µg/m^3	mortality and pulmonary bactericidal activity	Aranyi *et al.,* 1985
albino rats (♀)	arsenic trioxide	3 months, continuous	NOAEL = 1.3 µg/m^3	hematological changes; histological changes in the brain, bronchi, and liver; changes in conditioned reflexes, and changes in muscle stimulation efficiency	Rozenshtein, 1970
Oral Studies					
mice	sodium arsenate	28 days	NOAEL = 3 µg/kg BW/day	No treatment-related effects on food and water consumption or weight gain	Hughes and Thompson, 1996
	sodium arsenate	28 days	LOAEL = 300 µg/kg BW/day	hepatic vacuolar degeneration, significantly lower hepatic non-protein sulfhydryl levels, decreased plasma glucose and increased plasma creatinine	
rhesus monkeys	arsenic trioxide	12 months	NOAEL = 3,700 µg/kg BW/day	lack of neurological changes	Heywood and Sortwell, 1979
dogs	inorganic arsenic in diet	2 years	NOAEL = 1,200 µg/kg BW/day LOAEL = 3,100 µg/kg BW/day	lack of adverse effects decreased body weight gain and reduced survival	Byron *et al.,* 1967

Table A1.3 Human Systemic Toxicity

Arsenic Compound	Duration/ Conditions	Concentration or Dose	Effects/Endpoints	Reference
Inhalation exposure studies				
inorganic arsenic	2 months	NOAEL = 110 µg/m^3	nausea and anorexia in 1 worker only; no effects in other workers	Ide and Bullough, 1988
arsenic trioxide	8 to 40 years; case-control study	360 µg/m^3 (average)	reduced peripheral nerve conduction velocity; minor neurological and electromyographic abnormalities	Blom *et al.,* 1985
sodium arsenite	chronic (duration not specified); cross-sectional study	mean air levels ranging from 384 to 1,034 µg/m^3	pigmented skin with hyperkeratinization and multiple warts	Perry *et al.,* 1948

Table A1.3 Human Systemic Toxicity

Arsenic Compound	Duration/ Conditions	Concentration or Dose	Effects/Endpoints	Reference
arsenic trioxide	not specified	mean air concentrations ranging from 5 to 14 µg/m^3	dermatitis in 11 workers	Mohamed, 1998
Oral exposure studies				
inorganic arsenic	2 to 3 weeks in diet	subchronic LOAEL = 50 µg/kg body weight/day	cardiovascular effects, gastrointestinal irritation, haematological effects, hepatic effects, and dermal effects	Mizuta *et al.,* 1956
inorganic arsenic	chronic durations	when oral ingestion exceeds 10 µg/kg/day	generalized hyperkeratosis, formation of hyperkeratotic warts, hyperpigmentation interspersed with small areas of hypopigmentation on the face, neck, and back	ATSDR, 2005
inorganic arsenic	longer term exposures	30 to 100 µg/kg body weight/day	muscular weakness, paresthesia, electromyographic abnormalities, functional denervation, decreased sensitivity to stimuli, peripheral neuropathy, and neurobehavioural abnormalities	ATSDR, 2005
inorganic arsenic	not specified	> 1,000 µg/L in drinking water	50% frequency of EMG abnormalities	Hindmarsh *et al.,* 1977
inorganic arsenic	long term (not specified)	5 µg/kg body weight/day	Fatigue, headache, dizziness, insomnia, nightmare, and numbness of the extremities	Lianfang and Jianzhong, 1994
inorganic arsenic	chronic	0.1 to 15 mg/L-year and >15.0 mg/L-year	odds ratios of 6.6 and 10.1 for diabetes mellitus, respectively	Lai *et al.,* 1994
inorganic arsenic	Chronic	cumulative As exposures of 0.1 to 19.9 mg/L and >20 mg/L	odds ratios for diabetes mellitus and hypertension of 2.77 and 4.28, respectively	Tseng *et al.,* 1996
inorganic arsenic	chronic lifetime	median As conc of < 0.1, 0.1 to 0.34, 0.35 to 0.59, and >0.6 mg/L 0.1 to 9.9 mg/L, 10.0 to 19.9 mg/L, and >20 mg/L	cumulative ischaemic heart disease mortalities of 3.4, 3.5, 4.7, and 6.6%, respectively relative risks of 2.46, 3.97, and 6.47, respectively	Chen *et al.,* 1996
inorganic arsenic	chronic	households consuming well water with As concentration of 0, 0.1 to 50.0, 50.1 to 299.9, and >300 µg/L	odds ratios of 1.0, 3.4, 4.5, and 6.9 for cerebrovascular disease, respectively	Chiou *et al.,* 1997
inorganic arsenic	chronic lifetime	mean DW concentration of 780 µg/L	elevated standard mortality ratios for ischaemic heart disease, cardiovascular disease, diabetes mellitus	Tsai *et al.,* 1999

Table A1.3 Human Systemic Toxicity

Arsenic Compound	Duration/ Conditions	Concentration or Dose	Effects/Endpoints	Reference
inorganic arsenic	chronic	cumulative arsenic exposure of >17mg/L-years	relative risk for developing type II diabetes mellitus of 2.1	Tseng *et al.*, 2000
inorganic arsenic	chronic	>20 µg/L	elevated standard mortality ratios for cardiovascular diseases	Engel and Smith, 1994
inorganic arsenic	chronic	median DW conc of 14 to 166 µg/L	reduced mortality from cardiovascular and ischaemic heart diseases	Lewis *et al.*, 1999
inorganic arsenic	chronic	>800 µg/L	8.3% prevalence of keratosis in females and 10.7% in males; females and males had 11.5 and 22.7% prevalence of hyperpigmentation, respectively	Mazumder *et al.*, 1998
inorganic arsenic	chronic	BD_{01} = 2.5 µg/kg body weight/day	estimated using benchmark dose modelling approach and data from Mazumder *et al.*, 1998	OEHHA, 2000
inorganic arsenic	chronic	>1,000 µg/L	skin lesion prevalence of 37% in males and 25% in females	Tondel *et al.*, 1999
inorganic arsenic	chronic	<500, 500 to 1,000 and >1,000 :g/L	odds ratios of 1.2, 2.2 and 2.5, respectively	Rahman *et al.*, 1999

DW = drinking water

Table A1.4 Animal Reproductive/Developmental Toxicity

Species	Test Compound	Test Duration/Conditions	Toxicity Value	Endpoint(s)	Reference
Inhalation Studies					
♀ mice	arsenic trioxide	four hours per day on days 9 to 12 of gestation	LOAEL = 28.5 mg/m³	significantly increased fetal malformations	Nagymajtenyi *et al.*, 1985
♂ hamsters and rats	arsenic	intratracheal administration twice a week for 6 to 8 weeks	NOAEL = 1300 µg/kg body weight/day	no significant reproductive effects	Omura *et al.*, 1996a,b
♀ rats	arsenic trioxide	whole body inhalation; six hours per day, beginning fourteen days prior to mating and continuing throughout mating	NOAEL = 2.6 mg/m^3 NOAEL = 8.3 mgm^3	maternal toxicity developmental toxicity	Holson *et al.*, 1999; 2000
Oral Exposure Studies					
mice	sodium arsenite	3 generations (drinking water exposure)	NOAEL = 1 mg/kg body weight/day	no observed adverse reproductive or developmental effects	Schroeder and Mitchener, 1971
rats	unspecified arsenic solution	7 months, including pregnancy	NOAEL = 25 µg/kg body weight/day	no significant embryotoxic effects in offspring	Nadeenko *et al.*, 1978

Table A1.4 Animal Reproductive/Developmental Toxicity

Species	Test Compound	Test Duration/Conditions	Toxicity Value	Endpoint(s)	Reference
mice	arsenic acid	2 generations (diet)	maternal NOAEL = 100 mg/kg body weight/day fetal NOAEL = 20 mg/kg body weight/day	lack of maternal toxicity lack of fetal toxicity	Hazleton Laboratories, 1990
mice	MMA	dosed prior to mating, and during pregnancy	55 mg/kg body weight/day	reduced litter numbers; decreased fertility of males	Prukop and Savage, 1986
mice and rabbits	arsenic acid	gestational days six through 15 (mice); gestational days six through 18 (rabbits)	maternal LOAEL (mice) = 7.5 mg/kg body weight/day NOAEL = 7.5 mg/kg/day NOAEL = 0.75 mg/kg/day	decreased body weight developmental toxicity in mice maternal toxicity and developmental toxicity in rabbits	Nemec et al., 1998; OEHHA, 2000
rats	arsenic trioxide	gestational day 9 to 25 (gavage)	maternal LOAEL = 3.8 mg/kg body weight/day developmental NOAEL = 15.2 mg/kg body weight/day	mortality, food consumption, changes in fecal elimination lack of fetal toxicity	Stump *et al.*, 1999
rats	arsenite	gestation day 15 or postnatal day 1, until 4 months old; then, once pups were weaned, they continued receiving the same solution as their drinking water	36.7 mg/L (in drinking water)	rats exposed from gestational day 15 showed increased spontaneous locomotor activity and both groups showed increased numbers of errors in a delayed alternation task	Rodriguez *et al.*, 2001

Table A1.5 Animal Carcinogenicity

Species	Test Compound	Duration/Conditions	Toxicity Value	Endpoint(s)	Reference
Oral Studies					
cynomolgus monkey	sodium arsenate	5 d/wk for 15 years	0.1 mg/kg diet	no malignant tumours	Thorgeirsson *et al.,* 1994
mice (female)	sodium arsenate	26 months	500 µg As/L (in drinking water) (0.07 to 0.08 mg As/kg b.w.)	treatment-related increase in tumours (GI tract, lung, liver, spleen, skin and reproductive system)	Ng *et al.,* 1998; 1999
mice	sodium arsenite	gestational days 8 through 18	0, 42.5 or 85 mg/L sodium arsenite in drinking water	12, 38 and 61% incidence of hepatocellular carcinoma in male offspring at 0, 42.5 and 85 mg/L dose levels, respectively; dose-dependent increase in adrenal tumour incidence in male offspring; 8, 26 and 38% incidence of ovarian tumours in female offspring; 0, 4, 21% incidence of lung carcinoma in female offspring	Waalkes *et al.,* 2003
rats	DMA^V	2-year bioassay	0, 12.5, 50 or 200 mg/L in drinking water	bladder tumours observed in 26 and 39% of rats in 50 and 200 mg/L dose groups, respectively, at weeks 97 to 104	Wei *et al.,* 2002
rats (male)	MMA^V	2-years	0, 50 or 200 ppm in drinking water	no clinical, mortality, haematological or serum biochemistry effects; no excess tumour development	Shen *et al.,* 2003

Table A1.6 Human Carcinogenicity

Test Compound	Duration/ Conditions	Toxicity Value	Endpoint(s)	Reference
Inhalation Studies				
copper smelter occupational exposures	cohort of male copper smelter workers employed for >1year between 1940 and 1964	estimated mean TWA exposures of 0.054 and 0.157 mg As/m^3 in low and high exposure groups, respectively	SMRs for respiratory cancer mortality = 189.4 over 1941-1976 for the total cohort,, 227.7 and 291.4 for low and high exposure groups, respectively	Enterline and Marsh, 1982
copper smelter occupational exposures	cohort of male copper smelter workers employed for >1year between 1938 and 1956; and before 1957	estimated TWA exposures for 1943 to 1958 were 0.29, 0.58 and 11.3 mg As/m^3 for light, medium and heavy exposure categories	SMR for respiratory cancer mortality = 285 between 1938 and 1977; 155 for a slightly different cohort	Lee-Feldstein 1986; Lubin *et al.,* 2000
copper smelter occupational exposures	cohort of male copper smelter workers employed for ≥ 3 years from 1946 to 1976 at one of eight smelters	smelter-average exposure concentrations were 0.007 to 0.013 mg As/m^3 at seven smelters, and 0.069 mg As/m^3 at the Utah smelter	SMR for lung cancer mortality from 1949 to 1980 was significantly increased only at the Utah smelter (SMR=226.7)	Enterline *et al.,* 1987b
copper smelter occupational exposures	cohort of male copper smelter workers employed for >3 months between 1928 and 1967	estimated TWA exposures of 0.05, 0.2 and 0.4 mg As/m3 in the low, medium and high exposure groups, respectively	concentration dependent, significant increases in lung cancer mortality for all exposure groups; overall SMR for lung cancer mortality through 1981 = 372	Jarup et al., 1989
Oral Studies				
arsenic-contaminated drinking water	cross-sectional study of chronically exposed Taiwanese	slope factor = 0.0015 (µg As/kg bw/day)-1, unit risk = 5 x 10-5 (µg As/L)-1	excess skin cancer (as well as increased incidence of blackfoot disease, hyperpigmentation and keratosis)	Tseng et al., 1968; Tseng, 1977 slope factor and unit risk derived by U.S. EPA (1998)
arsenic-contaminated drinking water	various studies with the chronically exposed in region of endemic arseniasis in Taiwan	-----	significant dose-response relationships for internal cancers (including liver, lung kidney, prostate and bladder cancers)	Chen *et al.,* 1985, 1986, 1988a; Chiou *et al.,* 1995; Tsai *et al.,* 1999; Wu *et al.,* 1989
arsenic-contaminated drinking water	various studies with the chronically exposed in region of endemic arseniasis in Taiwan	-----	no significant relationship between arsenic exposure and skin cancer incidence	Southwick *et al.,* 1983; Morton *et al.,* 1976; Wong *et al.,* 1992
arsenic-contaminated drinking water	study of residents from endemic arseniasis region in Taiwan	-----	significant dose-response relationship between skin cancer prevalence and chronic arsenic exposure	Hsueh *et al.,* 1995
arsenic-contaminated	study in SW coastal Taiwan	RsD (1-in-1 million level of risk) = 0.00083 to	various internal cancers	Chen et al., 1992

Table A1.6 Human Carcinogenicity

Test Compound	Duration/ Conditions	Toxicity Value	Endpoint(s)	Reference
drinking water		0.0024 µg As/kg bw/day for males, 0.00059 to 0.0028 µg As/kg bw/day for females		
arsenic-contaminated drinking water	ecological study of cancer incidence in Taiwan	-----	positive association between exposure to high arsenic water; and transitional cell carcinomas of the bladder, kidney and ureter, all urethral cancers and adenocarcinomas of the bladder (in males)	Guo *et al.,* 1997
arsenic-contaminated drinking water	study of cancer incidence in blackfoot disease endemic area of Taiwan	<50 µg As/L	excess lung and bladder cancer risks	Morales *et al.,* 2000
arsenic contaminated drinking water	study of bladder cancer incidence in Lanyang basin region of Taiwan	10-50, 50-100 and >100 µg As/L	relative risks of transitional cell carcinoma of the bladder = 1.9, 8.2 and 15.3, respectively	Chiou *et al.,* 2001
arsenic-contaminated drinking water	prevalence study of skin lesions in two Mexican towns with high and low arsenic levels in drinking water	high: 0.4 mg/L low: 0.005 mg/L	increased incidence of palmar keratosis, skin hyperpigmentation and hypopigmentation, and four skin cancers in high exposure group relative to low	Cebrian et al., 1983
arsenic-contaminated drinking water	case-control study in Utah	max. concentration in drinking water = 160 µg As/L	no overall association of arsenic in drinking water with risk of bladder cancer; positive trend in the smoking subpopulation	Bates *et al.,* 1995
arsenic-contaminated drinking water	cohort study of an area of Japan with arsenic polluted groundwater	<50, 50 to 990 and >1000 µg As/L	slightly elevated total mortality (105 vs. 100.5 expected); elevated deaths from all cancers (SMR=148); significantly elevated mortality from lung cancer in highest exposure group (SMR=1,569)	Tsuda *et al.,* 1995
arsenic-contaminated drinking water	investigation of bladder cancer mortality in Cordoba, Argentina	-----	consistently higher bladder cancer SMRs in counties with documented arsenic exposure	Hopenhayn-Rich *et al.,* 1996a
arsenic-contaminated drinking water	study of mortality rates from bladder, ling, kidney and skin cancer in a region of Chile with formerly high arsenic levels in drinking water	-----	SMRs for lung cancer = 380 and 310 for men and women, repectively; SMRs for bladder cancer = 600 and 820; SMRs for skin cancer = 770 and 320; mortality from COPD was not elevated	Smith *et al.,* 1998
arsenic-contaminated	ecological study in northern Belgium	20 to 50, <20-5 and <5 µg As/L	no relationship between arsenic exposure and mortality from lung,	Buchet and Lison, 1998

Table A1.6 Human Carcinogenicity

Test Compound	Duration/ Conditions	Toxicity Value	Endpoint(s)	Reference
drinking water	with high, intermediate and low arsenic levels in drinking water		kidney or bladder cancers	
arsenic-contaminated drinking water	hospital-based case-control study in Chile	-----	statistically significant elevated risk of lung cancer in the highest exposure category	Ferreccio *et al.*, 1998, 2000
arsenic-contaminated drinking water	ecological study in Victoria, Australia	median concentration in high exposure areas = 13 to 1077 µg As/L	SIR < 120 for most cancers including lung and bladder; slightly elevated SIRs for prostate and breast cancers, melanoma and chronic myeloid leukemia; statistically significant deficit in liver cancer	Hinwood *et al.*, 1999
arsenic-contaminated drinking water	case-control study in Finland	concentration for cases = < 0.05 to 64 µg As/L; for controls <4.5 µg As/L	bladder cancer patients in highest-dose group had significantly higher exposure in the 3 to 9 years prior to diagnosis	Kurttio et al., 1999
arsenic-contaminated drinking water	cross-sectional study in Inner Mongolia	-----	dose-response relationship between skin cancer incidence and exposure	Tucker *et al.*, 2001

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Sudbury Area Risk Assessment
Volume II

Appendix A2:

Detailed Toxicological Profile of Cobalt

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A2: DETAILED TOXICOLOGICAL PROFILE OF COBALT

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APPENDIX A2 DETAILED TOXICOLOGICAL PROFILE OF COBALT

SUMMARY

The purpose of the toxicological profile of cobalt is to: i) outline the most relevant toxicological and epidemiological information on this substance; ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the cobalt exposure limits selected for use in the human health risk assessment of the Sudbury area. The toxicological review of cobalt is based primarily on secondary sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for cobalt. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Cobalt

In the environment, cobalt exists in oxidation states +2 and +3 and is usually combined with other elements such as copper, nickel, manganese, oxygen, sulphur and arsenic (ATSDR, 2001; MOE, 2001a). Cobalt emitted into the atmosphere by combustion sources occurs primarily as the oxide form (Schroeder *et al*., 1987). Studies of cobalt in water generally indicate that free cobalt (Co2+), and cobalt carbonates and bicarbonates will likely dominate in freshwater systems (ATSDR, 2001). In waters that contain high concentrations of organic matter, cobalt can be almost completely complexed (ATSDR, 2001). The form of cobalt in soil or sediment is influenced greatly by the concentration of chelating/complexing agents, pH, and redox potential (Eh). Cobalt adsorbs rapidly and strongly to soil and sediment in which it associates with metal oxides, crystalline minerals, and organic matter (ATSDR, 2001).

Absorption and Toxicokinetics of Cobalt

The gastrointestinal absorption of cobalt in humans varies considerably (*i.e*., from 1 to 97% of the administered dose), and is influenced by the type and dose of cobalt compound, as well as the nutritional status of the subjects (Harp and Scoular, 1952; Smith *et al*., 1972; Sorbie *et al*., 1971; Valberg *et al*., 1969). Soluble forms of cobalt are more efficiently absorbed than less soluble compounds (Kreyling *et al*. 1986).

Inhalation exposure to cobalt occurs as the particulate-bound metal. Absorption of cobalt *via* the inhalation route depends greatly on particle size, with particles >2 μm in diameter tending to deposit in the upper respiratory tract, and smaller particles entering the lower respiratory tract (ATSDR, 2001). The smaller particles have the greatest probability of being absorbed into the bloodstream.

No quantitative estimates for the dermal absorption of cobalt were identified in the literature reviewed. However, it is believed to be low.

Essentiality

Cobalt is an essential micronutrient in humans and most other organisms, as it is a required element in vitamin B12, and is also associated with the regulation of several cofactors and enzymes, and the production of erythropoeitin (Lison, 1996). The Recommended Dietary Allowance (RDA) for vitamin B12 is 2.4 μg/day for adults, which corresponds to 0.1 μg/day of cobalt (ATSDR, 2001). Due to its essentiality, cobalt occurs in many tissues of individuals with no known occupational or environmental exposure, with the highest concentrations occurring in the liver, where vitamin B12 is stored (ATSDR, 2001). Adverse health effects will typically occur only at doses that exceed the daily nutritional requirements for cobalt.

Toxicology of Cobalt

Adverse effects of cobalt in humans have been observed following elevated exposures *via* the inhalation, oral and dermal routes. Effects in humans following inhalation exposure include: lung effects (respiratory irritation, fibrosis, asthma, pneumonia, wheezing, rhinitis, pharyngitis, dyspnea, alveolitis, "hard metal disease"), cardiovascular effects (cardiomyopathy), liver and kidney congestion, ocular effects (conjunctivitis, optic nerve atrophy), skin effects (eczema and erythema), progressive hearing loss, altered thyroid hormone metabolism, and weight loss (ATSDR, 2001). Observed effects following ingestion of soluble forms of cobalt include polycythemia (increased red blood cell production), cardiomyopathy, gastrointestinal effects, visual disturbances, allergic responses, and thyroid effects. The effects of dermal exposure to cobalt include allergic dermatitis and sensitization (ATSDR, 2001).

Generally similar effects have been reported in experimental animals as in humans. Cobalt-induced effects that appear unique to experimental animals include neurobehavioural changes, altered immunological responses, abnormal sperm, and more severe respiratory tract effects than reported in humans (which reflect the higher doses incurred in animal studies relative to human studies). The

respiratory effects reported in animals include: necrotizing inflammation, degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, bronchiolar regeneration, peribronchiolar and septal fibrosis, epithelial hyperplasia in the alveoli, hyperplastic lesions of the nasal epithelium, and emphysema.

No studies were identified regarding reproductive or developmental effects in humans following inhalation, oral, or dermal exposure to cobalt. Developmental effects have not been observed in human fetuses from mothers who were given cobalt orally, as a means of counteracting decreases in hematocrit and hemoglobin levels that can occur during pregnancy (ATSDR, 2001). Limited data exists on the reproductive/developmental toxicity of cobalt compounds in experimental animals. The major effects reported in these studies include testicular atrophy, stunted growth and decreased survival of newborn pups, and increased length of estrous cycle.

Cobalt does not appear to cause cancer in humans *via* inhalation, oral, or dermal exposure routes (ATSDR, 2001). While there is a consistent finding of increased risk of respiratory tract cancer in workers co-exposed to both cobalt and tungsten carbide (*i.e.*, "hard metal" workers), the exposure conditions experienced by these workers would not be expected to occur in the ambient environment. This calls into question the relevance of these findings to the general population. In a recent review of genotoxicity and carcinogenicity studies published between 1991 and 2001, Lison *et al*. (2001) concluded there was no evidence for genotoxic or carcinogenic activity of cobalt in humans. However, a recent study by Hengstler *et al*. (2003) reports that co-exposure to cadmium, cobalt and lead may cause genotoxic effects even at concentrations below current regulatory limits, and that the cancer hazard of cobalt exposure may be underestimated, especially when individuals are co-exposed to cadmium or lead. This hypothesis has not yet been substantiated by other studies identified in the scientific literature.

No studies were located in the literature reviewed regarding carcinogenic effects in animals after oral or dermal exposure to cobalt. Inhalation exposure studies in animals have shown mixed results, with some studies showing a lack of tumours (Wehner *et al*., 1977; Steinhoff and Mohr, 1991) and other studies showing dose-related increased in tumours (NTP, 1998). There is limited evidence that suggests cobalt may predispose lung tissue to the carcinogenic effects of other chemicals (Steinhoff and Mohr, 1991). A number of regulatory bodies presently classify cobalt compounds as carcinogens in animals, but the evidence for classifying cobalt as a human carcinogen has been considered inadequate or limited by these agencies, with the exception of workers exposed to "hard metal" (cobalt with tungsten carbide).

Exposure Limits

ATSDR, U.S. EPA, MOE, WHO and RIVM were the regulatory agencies consulted to select exposure limits for cobalt.

A number of regulatory agencies have derived health-based exposure limits for cobalt. MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits). Exposure limits used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO), RIVM (the Netherlands) and the MOE itself.

The U.S. EPA derived exposure limits for both threshold and non-threshold. It is noted that these values are provisional; and have been withdrawn by the U.S. EPA. It is also noted that U.S. EPA Regions III, VI, and IX have adopted these values.

For the current assessment, cobalt has not been considered a non-threshold carcinogen by the inhalation or oral routes of exposure. There is inadequate information available from oral studies to determine whether or not cobalt is carcinogenic *via* this route. IARC classifies cobalt compounds as "possibly carcinogenic to humans" and ACGIH classifies cobalt in category A3 - confirmed animal carcinogen with unknown relevance to humans. Furthermore, under the old 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), cobalt is classified as group B1 (Probable Human Carcinogen), based on limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals, as evidenced by increased incidence of alveolar/bronchiolar tumors in both sexes of rats and mice (U.S. EPA, 2002). Under the U.S. EPA (1999) cancer guidelines, cobalt is considered likely to be carcinogenic to humans (U.S. EPA, 2002). Health Canada currently has no TRVs for cobalt and has not classified cobalt compounds as to their carcinogenicity.

Oral Exposure Limits

The most sensitive indicators of the effects of cobalt following oral exposure appear to be the related to an increase of hemoglobin in both humans and animals and the elicitation of dermatitis in sensitized individuals (ATSDR, 2001). The U.S. EPA (2002) have reported an oral RfD for cobalt of 20 μg/kg/day. The exposure limit is based upon a study conducted by Duckham and Lee (1976) which demonstrated an increased level of hemoglobin in anemic patients treated therapeutically at a level of 0.18 mg/kg/d. The oral RfD was calculated by dividing this LOAEL by 10 (three to account for the use of a LOAEL, three

for deficiencies in the database, primarily the use of a sub-chronic study; uncertainty factor rounded to 10). It is noted that these values are provisional; however review of the primary studies used in the derivation of these provisional values has indicated that they are based on sound science and standard regulatory policy.

ATSDR (2001) derived an oral intermediate-duration MRL of $1x10^{-2}$ mg cobalt/kg/day. The MRL is based on a LOAEL of 1 mg cobalt/kg/day for polycythemia as reported in a study by Davis and Fields (1958). These authors exposed six male volunteers to 120 or 150 mg/day of cobalt chloride (~1 mg Co/kg/day) for up to 22 days. Exposure resulted in the development of polycythemia in all six patients, with 16 to 20% increases in red blood cell numbers above pre-treatment levels. Oral MRL values were not derived by ATSDR for acute or chronic exposure to cobalt. An acute MRL was not derived because the reported effects in animals were serious and occurred at levels above those reported in the few available human oral studies. No chronic oral studies were available for humans or animals. . The ATSDR (2001) MRL has been selected for the current study. While dated, these study results are consistent with those observed in more recent studies such as Duckham and Lee (1976).

RIVM (Baars *et al.,* 2001) derived a tolerable daily intake (TDI) of 1.4×10^{-3} mg/kg-day based on a LOAEL of 0.04 mg/kg-day for cardiomyopathy in humans after intermediate oral exposure (Morin *et al.,* 1971). RIVM used an uncertainty factor of 30 (three for intra-human variation and 10 for extrapolation to a NOAEL) to yield the TDI.

Inhalation Exposure Limits

The U.S. EPA (2002) have reported an inhalation RfD for cobalt of 5.7×10^{-6} mg/kg/d based on an RfC of 2.0×10^{-5} mg/m^3. The exposure limit is based upon an epidemiological study which showed a NOAEL of 0.0053 mg cobalt/m^3 and a LOAEL of 0.015 mg cobalt/m^3 for decreases in forced vital capacity (FVC), forced expiratory volume in one second (FEV1), forced expiratory flow between 25 and 75% of the FVC (MMEF), and mean peak expiratory flow rate (PEF) in diamond polishers (Nemery *et al.,* 1992). The RfC of 2.0×10^{-5} mg/m^3 was derived by adjusting the NOAEL of 0.0053 mg/m^3 for intermittent exposure (8 hours/24 hours x 5 days/7 days), and dividing by an uncertainty factor of 100 (3 to account for exposure duration that may have been subchronic in some workers, 3 for a lack of inhalation developmental toxicity studies and a multi-generation reproduction study, and 10 for human variability). While these factors yield a cumulative uncertainty factor of 90, the U.S. EPA rounded up to 100 in this case. ATSDR (2001) also used the Nemery *et al.* (1992) study to develop its inhalation MRL, but only

applied a 10-fold safety factor to the time-adjusted NOAEL, resulting in the derivation of a less conservative limit of $1x10^{-4}$ mg cobalt/m^3. WHO (2006) have also established a tolerable concentration for inhaled cobalt of of $1x10^{-4}$ mg/m^3, and is based on a NOAEL of 0.0053 mg cobalt/m^3 in diamond polishers (Nemery *et al.,* 1992).

Overall, the weight-of-evidence indicates that cobalt does not cause cancer in humans by the inhalation, oral, or dermal exposure routes; however, the U.S. EPA (2002) has classified cobalt as a group B1 Probable Human Carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals following inhalation exposure. The U.S. EPA (2002) has also derived an inhalation unit risk for cobalt of $2.8x10^{-3}$ $(\mu g\ Co/m3)^{-1}$ based on tumourigenic effects (alveolar and bronchiolar) in rats and mice (Bucher *et al.,* 1999; NTP, 1998) which equates to a inhalation slope factor of 9.8 $(mg/kg/d)^{-1}$. No other identified regulatory agencies have derived exposure limits for cobalt based on carcinogenic endpoints. There appears to be a consistent increased risk of respiratory tract cancer in workers co-exposed to both cobalt and tungsten carbide (*i.e.,* "hard metal" workers). However, the exposure conditions experienced by "hard metal" workers would not be expected to occur in the ambient environment. The U.S. EPA (2002) IUR and slope factor derivation process did not consider studies in which there was co-exposure to tungsten carbide.

RIVM (Baars et al., 2001) derived a tolerable concentration in air (TCA) of 0.0005 mg/m^3, based on a LOAEL of 0.05 mg/m^3 for interstitial lung disease in humans (Sprince et al., 1988). An uncertainty factor of 100 (10 for extrapolation from a LOAEL and a factor of 10 for intrahuman variability) was applied to the LOAEL to yield the TCA. Medium reliability is suggested for this TCA by RIVM (Baar *et al*, 2001).

Dermal Exposure Limit

No regulatory dermal exposure limits for cobalt were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, is applied to dermal exposure

estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

Criteria Summary

The following table summarizes the cobalt exposure limits selected for the current study.

Summary of Toxicological Criteria Selected For Cobalt in the HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	10 µg/kg/day	RfD	polycythemia	Davis and Fields (1958)	ATSDR (2001)
Inhalation	0.5 µg/m^3	RfC	interstitial lung disease	Sprince et al. (1988)	RIVM (Baar *et al*, 2001)
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

NOTES:
NA = not available; RfD = reference dose; RfC = reference concentration; SFi = inhalation slope factor; IUR = inhalation unit risk;
PPRTV = Provisional Peer Reviewed Toxicity Values.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

References

For a complete list of references see Section A2-5.0 of the detailed toxicological profile on the accompanying CD.

A2-1.0 CHEMICAL CHARACTERIZATION AND GENERAL OVERVIEW

Cobalt is a transition metal that occurs naturally in the environment, accounting for 0.001% of the earth's crust. It has properties similar to iron and nickel. In the environment, cobalt exists in oxidation states +2 and +3 and is usually combined with other elements such as copper, nickel, manganese, oxygen, sulphur and arsenic (ATSDR, 2001; MOE, 2001a). Some natural sources and sinks of cobalt include soil, water, rocks, plants, animals, dust, volcanic eruptions and forest fires. Anthropogenic sources of cobalt include burning of fossil fuels and sewage sludge, phosphate fertilizers, mining and smelting of cobalt-containing ores and industrial emissions from processing of cobalt-containing compounds (ATSDR, 2001).

Cobalt also exists as unstable radioactive isotopes. For example Cobalt 60 (^{60}Co) is used in treating patients in nuclear medicine, sterilizing medical equipment, and scientific research. Small amounts of cobalt may be released into the environment from radioactive waste and nuclear power plant operations (ATSDR, 2001). The general population is typically not significantly exposed to radioactive forms of cobalt. However, workers at nuclear facilities and nuclear waste storage sites may be exposed to higher levels of radioactive cobalt (ATSDR, 2001). The primary form of cobalt that exists in the environment is the stable form of cobalt (^{59}Co). Therefore, the unstable radioactive forms of cobalt are not assessed further.

It is generally assumed that the cobalt species emitted into the atmosphere by combustion sources is the oxide form (Schroeder *et al.,* 1987). In natural waters, many factors influence the speciation and fate of cobalt including the presence of organic ligands (cobalt readily adsorbs to humic acids, EDTA *etc.*), the presence and concentration of major anions (chloride, hydroxide, carbonate, bicarbonate, sulphate), pH and redox potential (Eh). Modelling studies of cobalt speciation in water have produced inconsistent results but generally indicate that free cobalt (Co^{2+}), and cobalt carbonates and bicarbonates will likely dominate in freshwater systems (ATSDR, 2001). In waters that contain high concentrations of organic matter, cobalt can be almost completely complexed (ATSDR, 2001).

The speciation of cobalt in soil or sediment depends on the properties of the soil or sediment, and is influenced greatly by the concentration of chelating/complexing agents, pH, and redox potential (Eh). Cobalt adsorbs rapidly and strongly to soil and sediment in which it is associated with metal oxides, crystalline minerals, and organic matter (ATSDR, 2001). In some soils and sediments Co^{2+} may be oxidized to Co^{3+} by manganese oxides (ATSDR, 2001).

A2-2.0 FRACTION ABSORBED *VIA* DIFFERENT ROUTES

A2-2.1 Oral

The gastrointestinal absorption of cobalt in humans varies considerably (*i.e.,* from 1 to 97% of the administered dose), and is influenced by the type and dose of cobalt compound, as well as the nutritional status of the subjects (Harp and Scoular, 1952; Smith *et al.,* 1972; Sorbie *et al.,* 1971; Valberg *et al.,* 1969). Carson *et al. (*1987) reported that when administered as an inorganic salt, cobalt absorption in humans ranges from 1 to 44%. Greater amounts of cobalt tend to be absorbed when the subjects are deficient in iron (Sorbie *et al.,* 1971; Valberg *et al.,* 1969; Reuber *et al.,* 1994). Increasing oral doses of cobalt does not result in increased absorption but has been found to result in decreased fractional absorption (Houk *et al.,* 1946; Kirchgessner *et al.,* 1994; Taylor, 1962). This suggests that the gastrointestinal absorption of cobalt may be saturable. Soluble forms of cobalt are more efficiently absorbed than less soluble compounds (Kreyling *et al.,* 1986).

In a number of rat studies, the gastrointestinal absorption of cobalt chloride was found to range from 13 to 34% (Ayala-Fierro *et al.,* 1999; Barnaby *et al.,* 1968; Hollins and McCullough, 1971; Kirchgessner *et al.,* 1994; Taylor, 1962). The less soluble cobalt oxide particles have been reported to be poorly absorbed when administered orally, with absorption ranging from 1 to 3% (Bailey *et al.,* 1989; Collier *et al.,* 1989; Patrick *et al.,* 1989). Studies with rats and guinea pigs have found that absorption is 3- to 15-fold greater in younger animals than in adults (Naylor and Harrison, 1995). Absorption of soluble cobalt compounds has been found to be greater in rats (13 to 34%) than in dairy cows (1 to 2%) and guinea pigs (4 to 5%) following oral exposure (ATSDR, 2001).

RAIS (2004) provides a human gastrointestinal absorption factor of 80% for cobalt.

A2-2.2 Inhalation

The deposition pattern for inhaled cobalt-containing particles in the respiratory tract is related to particle size, with particles >2 μm in diameter tending to deposit in the upper respiratory tract, and smaller particles entering the lower respiratory tract (ATSDR, 2001). Fractional deposition of inhaled cobalt oxide particles in humans was found to vary from approximately 50% for particles with a mean geometric diameter of 0.8 μm, to approximately 75% for particles with a mean diameter of 1.7 μm (Foster *et al.,* 1989). Fractional deposition varies considerably with subject age, breathing patterns and the particle size.

Roughly 84% of inhaled cobalt oxide was found to be distributed throughout the body of guinea pigs after 24 hours (Stokinger, 1981). Studies in hamsters suggest that 30% of an inhaled dose of cobalt oxide is absorbed (Barceloux, 1999).

A2-2.3 Dermal

Volunteers that placed their right hands in a box filled with hard metal dust (containing ~5 to 15% cobalt metal and 85 to 95% tungsten carbide) for 90 minutes showed an order of magnitude increase in urinary cobalt levels, which remained elevated for 48 to 60 hours (Scansetti *et al.,* 1994). Radiolabelled cobalt has been reported to be absorbed through intact human skin (Carson *et al.,* 1987). However, no quantitative estimates for the dermal absorption of cobalt were identified in the literature reviewed. RAIS (2004) provides a human dermal absorption factor for cobalt of 0.1%.

Inaba and Suzuki-Yasumoto (1979) reported that the absorption of cobalt chloride through intact skin of guinea pigs was very small (<1%), while absorption through abraded skin was almost 80% by 3 hours after exposure. A study in hamsters (Lacy *et al.,* 1996) also reported a low amount of absorption of cobalt through unabraded skin.

A2-3.0 TOXICOLOGY

Adverse effects of cobalt have been observed following exposure *via* the inhalation, oral and dermal routes. The effects in humans following inhalation exposure to cobalt include lung effects (respiratory irritation, fibrosis, asthma, pneumonia, wheezing), cardiovascular effects (cardiomyopathy), liver and kidney congestion, ocular effects (congestion of the conjunctiva), and weight loss. Observed effects of ingesting cobalt sulphate or cobalt chloride include cardiomyopathy, gastrointestinal effects, visual disturbances, and thyroid effects. The effects of dermal exposure to cobalt include cobalt dermatitis and sensitization (ATSDR, 2001).

The primary target organ systems for the effects of stable cobalt in humans are the respiratory system following inhalation exposure and the cardiac and hematopoietic systems following oral exposure (ATSDR, 2001). Dermatitis is the most common effect following dermal cobalt exposure in humans, and is believed to occur due to an allergic reaction (ATSDR, 2001).

Exposure to radiation from cobalt radionuclides may also produce adverse effects in humans, similar to those produced by exposure to other gamma radiation-emitting isotopes (ATSDR, 2001). However, radiological effects are beyond the scope of this review and are not discussed here. The potential radiological health effects resulting from exposure to radiosotopes of cobalt are discussed in considerable detail in ATSDR (2001).

A2-3.1 Essentiality

Cobalt is an essential micronutrient in humans, as it is a required element in hydroxycobalamin (*i.e.*, vitamin B_{12}). Vitamin B_{12} is a coenzyme in many enzymatic reactions. Vitamin B_{12} is also involved in some enzymes that facilitate hematopoiesis; thus, deficiency of vitamin B_{12} can lead to pernicious anemia (ATSDR, 2001). Cobalt is also associated with the regulation of several cofactors and enzymes, and is involved in the production of erythropoeitin (Lison, 1996). The Recommended Dietary Allowance (RDA) for vitamin B_{12} is 2.4 µg/day for adults, which corresponds to 0.1 µg/day of cobalt (ATSDR, 2001). Due to its essentiality (as vitamin B_{12}), cobalt is found in many tissues of individuals with no known occupational or environmental exposure, such as the liver, muscle, heart, stomach, kidney, and bones (ATSDR, 2001). The highest concentration of cobalt is found in the liver, where vitamin B_{12} is stored (ATSDR, 2001).

A2-3.2 Systemic Toxicity

A2-3.2.1 Human Studies

Oral

Oral administration of inorganic cobalt (frequently in the form of cobalt chloride, $CoCl_2$) has been used clinically to induce polycythemia (red blood cell production) for the treatment of anemia associated with a disease state, pregnancy and to offset blood loss incurred during haemodialysis. In general, the side effects of the treatment of anemia with cobalt chloride have been reported to be of little long term toxicological significance. Immediate effects, including nausea and vomiting, have been reported in some haemodialysis patients at doses of 50 mg $CoCl_2$/day over a two to three-month period (Bowie and Hurley, 1975; Curtis *et al.,* 1976). Bowie and Hurley (1975) also reported transitory loss of hearing in three of 14 patients at 0.041 mg Co/kg body weight/day; assessment of thyroid and liver function indicated no abnormalities in any of the patients. Side effects in anemia patients have been reported to be minimal at 300 mg $CoCl_2$/d for 14 days (Berk *et al.,* 1949). Polycythemia, without biologically significant side effects, was observed in anemic patients at doses of 0.16 to 0.5 mg Co/kg body weight/day for up to 32 weeks (Davis and Fields, 1958; Duckham and Lee, 1976; Taylor *et al.,* 1977). Doses of 0.5 to 0.6 mg Co/kg body weight/day were associated with symptoms of gastric intolerance (Holly, 1955). Oral intake of 75 to 100 mg of cobalt chloride daily by pregnant women was associated with a slight decrease in maternal hemoglobin; no other toxic effects were noted (Barceloux, 1999).

In the Davis and Fields (1958) study, six men, ages 20 to 47, were exposed to a daily dose of cobalt chloride, administered as a 2% solution diluted in either water or milk, for up to 22 days. Five of the six men received 150 mg cobalt chloride per day for the entire exposure period, while the sixth was started on 120 mg/day and later increased to 150 mg/day. Blood samples were obtained from the subjects by daily punctures of fingertips at least two hours after eating, and at least 15 hours after the last dose of cobalt. Blood samples were analyzed for red blood cell counts, hemoglobin percentage, leukocyte counts, reticulocyte percentages, and thrombocyte counts. It was reported that exposure to cobalt resulted in the development of polycythemia in all six subjects, with 16 to 20% increases in red blood cell numbers above pre-treatment levels. Polycythemic erythrocyte counts returned to normal nine to 15 days post-exposure. Hemoglobin levels were also increased by cobalt treatment, though to a lesser extent than the erythrocyte values, with increases of six to 11% over pretreatment values. In five of the six subjects, reticulocyte levels were also elevated, reaching at least twice the pre-treatment values. Thrombocyte and total leukocyte counts did not deviate significantly from pre-treatment values.

Other studies have also reported increased levels of erythrocytes following oral treatment of anemic patients with 0.16 to 1.0 mg cobalt/kg/day daily as cobalt chloride for periods of 3 to 32 weeks (Duckham and Lee, 1976; Taylor *et al.,* 1977).

While cobalt treatment is generally well tolerated in anemic subjects, there may be certain individuals that are deficient in their ability to eliminate this element, and that may be at greater risk of adverse health effects. Curtis *et al.* (1976) reported that among 23 individuals receiving about 0.03 mg Co/kg body weight/day for two to three-months, ine subject later died of cardiac arrest. The authors considered it possible that this death was related to cobalt-induced cardiomyopathy, which was based on tissue analyses indicating higher tissue concentrations of cobalt in this subject, relative to the other patients. This abnormally high retention of cobalt was associated with renal dysfunction in this subject. The authors suggest that renally-impaired patients may be predisposed to possible adverse effects of cobalt treatment. Bowie and Hurley (1975) also noted variation in cobalt tissue concentrations amongst hemodialysis patients.

Another source of data on the potential toxic effects of oral intake of cobalt in humans is the "beer-cobalt" incidents observed in certain populations of Canada, the United States, and Belgium. In the 1960's, cobalt was added to beer as a foam stabilizer (this does not occur anymore), and it was discovered that 43% of a population of people ingesting large quantities of beer (*i.e.,* eight to 30 pints per day, which was associated with cobalt ingestion of 0.04 to 0.14 mg/kg body weight/day) later developed cardiomyopathy characterized by mild congestive heart failure, low cardiac output, and metabolic acidosis, and death in some instances with heavy drinkers (*e.g.,* Alexander, 1969, 1972; Morin *et al.,* 1971). U.S. EPA (1997) noted that the cardiomyopathy may have been due to pre-existing cardiac and hepatic conditions due to alcohol abuse, and to poor nutritional habits. Seghizzi *et al.* (1994) also concluded that an interaction between poor nutrition, alcohol abuse and cobalt exposure may have been responsible for the observed cardiomyopathies.

Cobalt is also well known to function as a hapten, resulting in the generation of antibodies against cobalt-protein complexes. Allergic dermatitis has been reported in some cobalt-sensitized people following oral challenges with cobalt. In eczema patients (hands) challenged orally with 1 mg cobalt as cobalt sulfate, once per week for three weeks (0.014 mg/kg/day), a flare-up of the eczema occurred. This was considered to be a positive allergic response to cobalt (Veien *et al.,* 1987). Exposure levels associated with sensitization to cobalt have not been established (ATSDR, 2001).

Inhalation

Chronic bronchial obstruction caused a decrease in ventilatory function in humans exposed to 0.038 mg Co/m^3 as "hard metal" dust for six hours (Kusaka *et al.,* 1986). "Hard metal" is a metal alloy with a tungsten carbide and cobalt matrix that is used to make cutting tools due to its hardness and resistance to high temperature (ATSDR, 2001).

Gennart and Lauwerys (1990) reported a significant decrease in ventilatory function in diamond polishers, relative to controls, who were exposed to 135.5 and 15.2 µg Co/m^3, respectively, for greater than five years. Nemery *et al*. (1992) conducted a cross-sectional study of cobalt exposure and respiratory effects in diamond polishers. Exposure occurred mainly from the generation of airborne cobalt from the use of cobalt-containing polishing discs. The study groups consisted of 194 polishers working in 10 different workshops, and were divided into control, low-, and high-exposure groups. Exposure was measured by the use of personal samplers. The low exposure group (n=102) was exposed to an average air concentration of 5.3 µg cobalt/m^3, while the high dose group (n=92) was exposed to an average of 15.1 µg cobalt/m^3. It was noted that there was considerable overlap in the total range Workers in the high-exposure group also had significantly reduced lung function compared to controls and the low-exposure group. Lung function test results in the low-exposure group did not differ from controls. A NOAEL of 5.3 µg cobalt/m^3 was identified by ATSDR (2001).

Numerous studies have associated long-term occupational exposure to a variety of cobalt compounds with bronchial asthma in a small proportion of workers (ATSDR, 2001). The risk of asthma has been estimated to increase by as much as five-fold when cobalt air concentrations are greater than or equal to 100 µg/m^3 (Roto, 1980). Although the mechanism of action has not yet been determined, there is strong evidence to suggest that cobalt is a causative agent for occupational asthma (Cugell, 1998). A number of studies have indicated that the cause of cobalt-induced asthma may be an allergic reaction to cobalt-containing dusts (Kusaka *et al.,* 1989, 1990; Shirakawa *et al.,* 1988; 1989). Cobalt is known to function as a hapten, resulting in the generation of antibodies against cobalt-protein complexes. Although the minimum exposure level associated with cobalt sensitization has not been established, sensitization has been demonstrated in hard metal workers with work-related asthma who have experienced prolonged occupational exposure (less than three years) to levels ranging from 0.007 to 0.893 mg cobalt/m^3 (Shirakawa *et al.,* 1988, 1989). The causative role of cobalt in asthma is supported by evidence of affected workers showing partial or complete remission of asthma when removed from the source of exposure, after wearing respirators or after the installation of exhaust ventilation systems (Kusaka *et al.,*

1990). Chronic exposure to cobalt dusts may also lead to the development of a moderate obstructive syndrome, possibly the result of non-specific irritation (Kusaka *et al.,* 1986).

In a case control study investigating the associations between respiratory, cardiac and cancer conditions in workers at automotive machining plants, Park (2001) found that asthma (OR=3.0, 95% CI=0.90-10.0) and heart diseases (OR=3.1, 95% CI=1.26-7.6) increased with employment years in tool grinding. Tool grinders could be exposed to hard metal dusts (including cobalt), and tool grinding has been linked with hard metal disease and cardiomyopathy. Small, non-significant increases in non-malignant respiratory disease and ischemic heart disease were also observed in the tool grinders.

Several studies have investigated the effects of cobalt inhalation in workers in the cemented carbide industry, which uses cobalt in its processes. Respiratory disease with symptoms ranging from shortness of breath and coughing to permanent disability or death in a very few cases has been reported in some workers (Miller *et al.,* 1953; Lundgren and Ohman, 1954). In addition, interstitial lung disease (hard-metal disease) and "airways disease" have been reported in tungsten carbide workers (Sjogren *et al.,* 1980), and in a male worker polishing disks containing cobalt (Nemery *et al.,* 1990). Occupations associated with hard metal disease include: maintenance and re-sharpening of hard metal tools; production of hard metals; diamond tooling (grinding wheels and stone saws); work utilizing cemented tungsten-carbide (oil well drilling sites); armoured plate production (tanks, naval ships); and, manufacture and maintenance (grinding/polishing) of cutting tools and loops for fishing poles (Harding, 2003).

Swennen *et al.* (1993) performed a cross-sectional study on 82 workers in a cobalt refinery. Workers were examined for cobalt in blood and urine, various erythropoietic variables, thyroid metabolism, pulmonary function, skin lesions, and serum enzymes. The concentrations of cobalt in blood and urine after the shifts were significantly correlated with cobalt air concentrations. Exposed workers complained more frequently ($p<0.05$) of dyspnoea and wheezing and had significantly more skin lesions (eczema, erythema) than control workers. There was a dose-effect relationship between reductions in forced expiratory volume and intensity of cobalt exposure (as assessed by measurement of cobalt in blood or urine). The mean cobalt air concentration was reported to be 125 $\mu g/m^3$, and ranged from one to as high as 7,700 $\mu g/m^3$). The authors also reported slightly, but significantly, decreased levels of red blood cells and total hemoglobin in the exposed workers.

Other adverse effects which have been associated with chronic occupational exposure to cobalt include fibrosis, coughing, wheezing, conjunctivitis, rhinitis, pharyngitis, dyspnea, alveolitis, pneumonia, allergic dermatitis, cardiomyopathy, ischemic heart disease, polycythemia, progressive hearing loss, atrophy of

the optic nerve, and altered thyroid hormone metabolism (Cugell, 1998; Meecham and Humphreys, 1991; Barborik and Dusek, 1972; Kennedy *et al.,* 1981; Lison, 1996; Prescott *et al.,* 1992). These effects have been observed in cobalt refinery workers, hard metal workers, diamond polishers and plate painters at cobalt air concentrations ranging from 0.007 to 0.893 mg cobalt/m^3 (exposure from 2 to 17 years) (ATSDR, 2001).

Several recent studies (Verougstraete *et al.,* 2004; Linna *et al.,* 2003; 2004) have looked at the respiratory health of workers exposed to cobalt compounds in modern occupational environments. Findings included an association between cobalt exposure, smoking and declining FEV_1 over time (Verougstraete *et al.,* 2004), limited evidence of cobalt induced asthma among cobalt production workers (Linna *et al.,* 2003) and some association between cobalt exposure and cardiac funtion effects (Linna *et al.*, 2004).

Dermal

Dermatitis is a common reported result of dermal exposure to cobalt in humans, and has been verified in a large number of studies (ATSDR, 2001). Through the use of patch tests and intradermal injections, it has been demonstrated that cobalt-induced dermatitis is likely caused by an allergic reaction to cobalt. Contact allergic dermatitis was reported in 22 of 223 (9.9%) nurses who were tested with a patch test of 1.0% cobalt chloride (Kie□-∠wierczy│ska and Kr□cisz, 2000). Exposure levels associated with the development of dermatitis have not been clearly established.

A2-3.2.2 Animal Studies

Oral

An 8-week study of rats administered cobalt chloride in drinking water resulted in a dose- and time-related increase in erythrocyte numbers (Stanley *et al.,* 1947). An apparent NOAEL of 0.6 mg cobalt/kg/day and a LOAEL of 2.5 mg cobalt/kg/day were identified by ATSDR (2001) from this study. Significantly increased erythrocyte (polycythemia), hematocrit, and hemoglobin levels were also found in experimental animals in longer-term studies at cobalt doses of > 0.5 mg/kg/day (Brewer 1940; Davis 1937; Domingo *et al.,* 1984; Holly, 1955; Krasovskii and Fridlyand 1971; Murdock, 1959).

Four to five months of exposure to 10 to 18 mg Co/kg/d as cobalt chloride, resulted in renal injury (alteration of renal proximal tubules) in rats (Holly, 1955; Murdock, 1959). Increased liver weight (17%) was also reported in rats exposed to 10 mg cobalt/kg/day (as cobalt chloride) for five months (Murdock, 1959).

In an experiment designed to simulate conditions leading to beer-cobalt cardiomyopathy in humans, guinea pigs were administered 20 mg cobalt/kg/day as cobalt sulfate by gavage either alone, or in combination with ethanol for five weeks (Mohiuddin *et al.,* 1970). Observed effects included cardiomyopathy, which was characterized by abnormal EKGs, as well as increased heart weights, lesions of the pericardium, myocardium, and endocardium, and disfigured mitochondria. Alcohol did not intensify the cardiac effects in this study.

Longer-term exposures (two to three months) of rats to 26 to 30.2 mg cobalt/kg/day as cobalt sulfate in the diet, or as cobalt chloride in the drinking water, produced such cardiac effects as degenerative heart lesions and increased heart weight (Grice *et al.,* 1969; Domingo *et al.,* 1984).

A decrease in immunological reactivity (manifested by a decline in phagocytic activity), was reported in rats following six to seven months of treatment with ≥0.5 mg cobalt/kg and above, as cobalt chloride (Krasovskii and Fridlyand, 1971). Other effects reported in this study included a significant increase in the latent reflex period at ≥ 0.5 mg cobalt/kg, and a pronounced neurotropic effect (disturbed conditioned reflexes) at 2.5 mg cobalt/kg.

In rats exposed to 4.96 mg cobalt/kg/day as cobalt chloride for 30 days in drinking water, changes in sympathetically-mediated contractile activity of isolated rat vas deferens were noted (Mutafova-Yambolieva *et al.,* 1994). Rats exposed to 6.44 mg cobalt/kg/day as cobalt nitrate in drinking water showed an increased sensitivity and decreased maximal response to a cholinergic agonist (Vassilev *et al.,* 1993). In rats exposed to 20 mg cobalt/kg/day as cobalt chloride for 57 days in drinking water, an enhanced behavioral reactivity to stress was observed (animals were less likely to descend from a safe platform to an electrified grid) (Bourg *et al.,* 1985). In addition, rats exposed to this dose in the diet for 69 days showed a slower rate of lever pressing relative to controls, but no changes in behavioral reactivity to stress were observed (Nation *et al.,* 1983).

Inhalation

Signs of pulmonary disease were observed in miniature swine following inhalation of a cobalt metal powder mixture at air concentrations of 0.1 and 1.0 mg/m^3 for six hours/day, five days/week for three months (Kerfoot *et al.,* 1975). Animals exhibited a significant decrease in lung compliance and an increase in the amount of collagen in the central areas of the pulmonary alveolar septa. These effects occurred in a concentration-related manner with the lung compliance values reported as 35.5, 23.3 and

19.8 cm^3/cm H_2O for the control, low and high concentrations, respectively. In addition, electrocardiogram abnormalities were noted at 0.1 mg/m^3.

Female rats exposed to cobalt metal powder (99.87% pure; median particle size four μm) by intratracheal instillation at doses of 0.006, 0.03 and 0.06 mg/kg body weight/day for a period of 4 months displayed no mortality and no outward signs of toxicity (Lasfargues *et al.,* 1995).

NTP (1991), also cited as Bucher *et al. (*1990) exposed groups of F344/N rats and $B6C3F_1$ mice of each sex to a 99% pure cobalt sulfate heptahydrate aerosol six hours per day, five days per week, for either 16 days or 13 weeks.

In the 16-day studies, all rats and mice exposed to the highest concentration of 200 mg/m^3 died. Partial survival occurred in animals exposed to 50 mg/m^3. Degeneration of the olfactory epithelium and necrotizing inflammation occurred in the nose of all rats and mice that died, as well as in all animals exposed to 50 mg/m^3. Necrotizing inflammation was observed in the larynx and trachea of rats and mice at concentrations as low as 5 mg/m^3, and similar lesions were observed in the bronchi at concentrations of 50 mg/m^3 or higher. Regenerative and inflammatory lesions, including peribronchial and septal fibrosis in the lung, occurred in both rats and mice exposed to 50 mg/m^3.

In the 13-week studies, rats and mice were exposed to 0, 0.3, 1, 3, 10, and 30 mg/m^3 of cobalt sulfate heptahydrate. All rats, all female mice, and all but two male mice exposed to the highest concentration survived to the end of the study. Rats and mice of the 30 mg/m^3 group lost weight during the first week of exposure but then gained weight at the same rate as controls. Lung weights were increased relative to controls in rats exposed at concentrations as low as one mg/m^3, and in mice exposed to 10 mg/m^3 or higher. Polycythemia was observed in rats but not in mice. Sperm motility was decreased in mice exposed to 3 mg/m^3 or higher (lower concentrations were not evaluated for this endpoint), and increased numbers of abnormal sperm were found in mice of the 30 mg/m^3 group. Testis and epididymal weights were also decreased in mice of this group. Respiratory tract lesions reported in the 13-week studies in rats and mice included degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, necrosis, inflammation in the nose and larynx, bronchiolar regeneration, peribronchiolar and septal fibrosis, and epithelial hyperplasia in the alveoli of the lung. The most sensitive tissue was the larynx, with squamous metaplasia observed in both rats and mice at the lowest tested concentration of 0.3 mg/m^3. This concentration can be considered a LOAEL. A NOAEL could not be determined in this study as the effects occurred at all tested concentrations.

Exposure of rats and mice to aerosols of cobalt sulfate at concentrations from 0.11 to 1.14 mg cobalt/m^3 for 2 years resulted in a range of inflammatory, fibrotic, and proliferative lesions in the respiratory tract of male and female rats and mice (Bucher *et al.,* 1999; NTP 1998). Squamous metaplasia of the larynx occurred in both rats and mice at exposure concentrations of 0.11 mg cobalt/m^3 and higher, with severity of the lesions increasing with exposure concentration. Hyperplastic lesions of the nasal epithelium were noted in rats exposed to 0.11 mg/m^3 and higher, and in mice at concentrations of 0.38 mg/m^3 and higher. Both male and female rats had greatly increased incidences (>90% incidence) of alveolar lesions at all tested exposure levels, including inflammatory changes, fibrosis, and metaplasia. Similar changes were seen in mice at all exposure levels as well, although the lesions were less severe than those in rats.

A series of studies investigating the lung responses of rabbits to a number of cobalt compounds was undertaken by Johansson *et al.* (1983; 1986; 1987). Rabbits exposed to 0.4 and 2.0 mg/m^3 soluble $CoCl_2$ for one and four months showed an increased number of alveolar macrophages and increased macrophage activity. Rats and rabbits exposed to a range of mixed cobalt oxides (0.4 to 9.0 mg/m^3) six hours/day, five days/week for 14 to 16 weeks displayed lesions in the alveolar region of the respiratory tract, characterized by nodular accumulation of type II cells, abnormal accumulation of enlarged, vacuolated macrophages, and interstitial inflammation (Kyono *et al.,* 1992; Johansson *et al.,* 1986; 1987).

Guinea pigs that were sensitized to cobalt by repeated dermal application and then exposed to 2.4 mg cobalt/m^3 as cobalt chloride showed pulmonary inflammatory changes (*i.e.,* altered BAL fluid recovery, increased neutrophils and eosinophils following BAL) relative to controls (Camner *et al.,* 1993).

Lifetime exposure of hamsters to 7.9 mg/m^3 of cobalt oxide resulted in emphysema (Wehner *et al.,* 1977).

Increased levels of hemoglobin and increased numbers of basophils and monocytes were observed in rats and guinea pigs, but not dogs, exposed to 9 mg cobalt/m^3 as cobalt hydrocarbonyl for 3 months (Palmes *et al.,* 1959). Weight loss was noted to have occurred in dogs, but not in rats or guinea pigs.

A2-3.3 Reproductive/Developmental

A2-3.3.1 Human Studies

No studies were identified regarding reproductive effects in humans following inhalation, oral, or dermal exposure to cobalt. No obvious developmental effects have been observed in human fetuses from mothers who were given cobalt orally, as a means of counteracting decreases in hematocrit and

hemoglobin levels that often occur during pregnancy (ATSDR, 2001). No studies were located regarding developmental effects in humans following inhalation or dermal exposure to cobalt.

As mentioned, cobalt has been given to pregnant women to raise hematocrit and hemoglobin levels, which may become depressed during pregnancy. Holly (1955) reported no obvious birth defects in human fetuses following treatment of women with 0.6 mg Co/kg/d for 90 days during the final trimester of pregnancy. Maternal toxicity was also minimal in this study as a small percentage of treated mothers complained of gastric intolerance.

A2-3.3.2 Animal Studies

Only limited data exist on the reproductive/developmental toxicity of cobalt compounds in experimental animals. Testicular degeneration and atrophy have been reported in rats exposed to oral doses ranging from 13.25 to 58.9 mg cobalt/kg/day, as cobalt chloride, for durations of two to three months in either the diet or drinking water (Corrier *et al.,* 1985; Domingo *et al.,* 1984; Mollenhauer *et al.,* 1985; Nation *et al.,* 1983; Pedigo and Vernon 1993). Similar effects on the testicles have been reported in mice exposed to 43.4 mg cobalt/kg/day as cobalt chloride for 13 weeks in drinking water (Anderson *et al.,* 1992; 1993).

Oral exposure of female rats to cobalt chloride at a dose of 5.4 mg cobalt/kg/day, or 21.8 mg cobalt/kg/day, from gestation day 14 through lactation day 21, resulted in stunted growth and decreased survival of newborn pups (Domingo *et al.,* 1985). No teratogenicity was reported. Maternal toxicity also occurred at these tested doses and included reduced body weight and food consumption and altered hematological parameters.

No effects on fetal growth or survival were found following exposure of rats to 24.8 mg cobalt/kg/day as cobalt chloride during gestation days six to 15 (Paternian *et al.,* 1988). Similarly, in mice, exposure to 81.7 mg cobalt/kg/day as cobalt chloride, during gestation days eight to 12 was reported to produce no adverse effects on fetal growth or mortality in mice (Seidenberg *et al.,* 1986).

Testicular atrophy was reported in rats, but not mice, exposed to 19 mg/m^3 cobalt sulfate for 16 days (Bucher *et al.,* 1990; NTP, 1991). In a 13 week study by the same authors, mice exposed to 1.14 mg/m^3 cobalt sulfate showed a decrease in sperm motility, and testicular atrophy occurred at 11.4 mg/m^3. This study also found a significant increase in the length of the estrous cycle in female mice exposed to 11.4 mg/m^3 for 13 weeks (Bucher *et al.,* 1990; NTP 1991).

A2-3.4 Carcinogenicity

A2-3.4.1 Human Studies

Overall, cobalt has not been shown to cause cancer in humans by the inhalation, oral, or dermal exposure routes (ATSDR, 2001). A consistent finding of increased risk of respiratory tract cancer in workers co-exposed to both cobalt and tungsten carbide (*i.e.,* "hard metal" workers) has been reported. However, the exposure conditions experienced by "hard metal" workers would not be expected to occur in the ambient environment. This calls into question the relevance of these findings to general population exposures to cobalt.

A recent study concluded that increased risk of lung cancer is associated with occupational exposure to hard metal dust, but not with exposure to cobalt dusts alone (De Boeck *et al.,* 2003). These authors evaluated the genotoxicity of metal carbide: cobalt mixtures (94:6), and found that the genotoxicity of cobalt was enhanced by tungsten carbide, niobium carbide and chromium carbide, but not by molybdenum carbide, which has a large particle size. A possible mechanism of the increased mutagenicity of these mixtures relative to cobalt alone is enhanced short-term formation of active oxygen species. In an earlier study, De Boeck *et al*. (2000) assessed the genotoxic effects of cobalt as an indicator of carcinogenic risk in workers employed at hard metal plants and cobalt refineries. Both sets of workers were exposed to approximately 20 µg Co/m^3. The authors found no significant increase in genotoxic effects in cobalt-exposed *versus* unexposed workers. There was also no significant difference in genotoxic effects between workers exposed to cobalt only, and those exposed to hard metal dust; however, genotoxic markers were elevated in smokers who were exposed to hard metal dust. The authors concluded that hard metal workers who are also smokers may be at particular risk, and warrant closer study.

In a review of genotoxicity and carcinogenicity studies published between 1991 and 2001, Lison *et al*. (2001) concluded there was no evidence for genotoxic or carcinogenic activity of cobalt ions in humans. For cobalt metal, they noted some *in vitro* evidence of genotoxicity in human lymphocytes, but no evidence of carcinogenic potential. For "hard metal" particles, they reported *in vitro* evidence of genotoxicity, and evidence of carcinogenic activity in exposed humans.

Mur *et al.* (1987) reported an increase in deaths due to lung cancer in workers exposed to cobalt. The standardized mortality ratio (SMR) was 4.66. However, the number of cases was small. In a study of a subgroup within the cohort that controlled for date of birth, age at death, and smoking habits, it was found

that 44% (four workers) in the group exposed to cobalt and 17% (three workers) in the group not exposed to cobalt died of lung cancer. However, the difference was not statistically significant and it was noted that workers were also exposed to, arsenic and nickel, as well as cobalt. Furthermore, the non-neoplastic lung diseases commonly found in cobalt-exposed workers were not reported in this study. Deficiencies of this study include no adjustments for smoking habits, and the exposure levels of cobalt were not reported. A follow-up study of this cohort (Moulin *et al.,* 1993) reported no significant increases in mortality due to respiratory or circulatory diseases. Similarly, there was no increase in the SMR for lung cancer in exposed workers, relative to controls. A slightly elevated SMR for lung cancer was observed in maintenance workers (SMR=1.80), but it was not statistically significant.

Lasfargues *et al. (*1994) reported on mortality rates in a cohort of 709 male workers in a French hard metal plant, using the national rates for French males as the control group. The overall mortality rates in the workers did not differ from controls, but there was a significant increase in mortality due to cancer of the trachea, bronchus, and lung (SMR=2.13). Smoking alone did not account for the lung cancer excesses, but its influence could not be entirely ruled out in this study.

In a study by Berg and Burbank (1972) no correlation was found between cancer mortality and trace metals such as cobalt, in water supplies in the United States. The concentration of cobalt in water was reported to range from 1 to 19 μg/L.

A retrospective cohort study by Tüchsen *et al. (*1996) investigated the incidence of lung cancer associated with exposure to cobalt in 872 women occupationally exposed to cobalt-aluminate spinel in two Danish porcelain factories. The workers were compared to a reference cohort of 520 women not exposed to cobalt, who also worked in these two factories over a five-year period. No significant differences in cancer mortality between exposed and unexposed workers were observed. A slight increase in lung cancer incidence was found in both the exposed and reference group, compared to the background incidence rate for all Danish women. The exposed group had only a slightly higher lung cancer incidence than the reference group (RR = 1.2). Measured cobalt air concentrations ranged from non-detectable to approximately 200 $\mu g/m^3$, with one extremely high concentration (861 $\mu g/m^3$) reported in one year. The authors acknowledged that the study was based on a small number of subjects and recommended a follow-up study once data for another five-year period was available.

Moulin *et al*. (1998) examined 5,777 male and 1,682 female workers who were occupationally exposed to cobalt and tungsten carbide. The mortality rate of these workers (who were exposed to cobalt at air

concentrations ranging from 39 to 169 μg Co/m^3) was significantly elevated relative to the national average. In a subgroup of this cohort, it was reported that after 10 years of simultaneous exposure to cobalt and tungsten carbide, a significant increase in lung cancer risk was observed in 61 exposed workers relative to 180 controls. The results of this study did not change when adjustments were made for smoking and co-exposure to other carcinogens; however, occupational risk was noted to be greatest among smokers (Moulin *et al.,* 1998).

Wild *et al*. (2000) reported no increase in total mortality in a cohort of 2,214 male hard metal workers, who were employed for at least three months. However, there was a significant increase in lung cancer mortality. After adjusting for smoking and co-exposure to other known carcinogens, the risks increased with exposure scores.

A recent study by Hengstler *et al*. (2003) reported that combined effects of cadmium, cobalt and lead on DNA-single strand break induction is more than additive and may even be more than multiplicative. The authors suggest that co-exposure to cadmium, cobalt and lead may cause genotoxic effects even at concentrations below current regulatory limits, and that cobalt may be a stronger human carcinogen than cadmium. They concluded that the cancer hazard of cobalt exposure may be underestimated, especially when individuals are co-exposed to cadmium or lead. The authors' hypothesis has not yet been substantiated by other studies identified in the scientific literature.

A2-3.4.2 Animal Studies

No studies were located in the scientific literature regarding carcinogenic effects in animals after oral exposure to cobalt.

Wehner *et al.* (1977) exposed hamsters to 10 μg cobalt oxide (CoO) per litre of air for seven hours/day, five days/weeks for the animal's lifetime. No increased incidence of tumours was observed. However, pulmonary changes such as interstitial fibrosis, granulomas, hyperplasia of alveolar cells, and emphysema were reported.

NTP (1998), also cited as Bucher *et al*. (1999), exposed groups of 50 male and 50 female rats to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 of cobalt sulfate heptahydrate, for six hours per day, five days per week, for 105 weeks. Survival and body weights remained similar to controls for the duration of the study.

Incidences and severities of proteinosis, alveolar epithelial metaplasia, granulomatous alveolar inflammation, and interstitial fibrosis were markedly greater in all exposed groups of male and female rats than in controls. The incidence of alveolar epithelial hyperplasia in all groups of exposed males, and in females exposed to 3.0 mg/m^3 were significantly greater than those in the controls, as were the incidences of squamous metaplasia in the 1.0 mg/m^3 females, and atypical alveolar epithelial hyperplasia in the 3.0 mg/m^3 females. In the 3.0 mg/m^3 males, the combined incidence of alveolar/ bronchiolar neoplasms (adenoma and/or carcinoma) was significantly greater than in controls. In female rats exposed to 1.0 or 3.0 mg/m^3, the incidences of alveolar/bronchiolar neoplasms were also significantly greater than those in the control group and exceeded the NTP historical control ranges. Squamous cell carcinoma was observed in one female from each of the 1.0 mg/m^3 and 3.0 mg/m^3 groups. The incidences of benign, complex, or malignant pheochromocytoma (combined) in 1.0 mg/m^3 males and in 3.0 mg/m^3 females were found to be significantly greater than those in the controls, and also exceeded the historical control ranges. In addition, the following non-neoplastic effects were reported. Hyperplasia of the lateral wall of the nose, atrophy of the olfactory epithelium, and squamous metaplasia of the epiglottis were observed in all exposed groups (males and females), and the severities of these lesions increased with increasing exposure concentration. The incidence of squamous metaplasia of the lateral wall of the nose and metaplasia of the olfactory epithelium were increased in both the 3.0 mg/m^3 males and females.

The same NTP study team also conducted an experiment in which groups of 50 male and 50 female mice were exposed to aerosols containing 0, 0.3, 1.0, or 3.0 mg/m^3 cobalt sulfate heptahydrate, for six hours per day, five days per week, for 105 weeks. The survival of exposed males and females was similar to that of controls. Mean body weights of male mice in the 3.0 mg/m^3 group were lower than controls from week 96 until the end of the study. The mean body weights of all exposed groups of female mice were generally greater than those of the controls from week 20 onward. Incidences of diffuse histiocytic cell infiltration in 3.0 mg/m^3 males, and of focal histiocytic cell infiltration in the 3.0 mg/m^3 females were significantly elevated, relative to controls. The incidences of alveolar/bronchiolar neoplasms in 3.0 mg/m^3 males and females were also significantly greater than those in the controls. The combined incidences of alveolar/bronchiolar adenoma or carcinoma, and the incidences of alveolar/bronchiolar carcinoma in the 3.0 mg/m^3 males and females, as well as the incidence of alveolar/bronchiolar adenoma in 3.0 mg/m^3 females, exceeded the NTP historical control ranges. Incidences of atrophy of the olfactory epithelium in 1.0 and 3.0 mg/m^3 males and females, and hyperplasia of the olfactory epithelium in 3.0 mg/m^3 males and females, were significantly higher than in controls. Squamous metaplasia of the larynx was observed in all exposed groups of mice of both sexes. It was also noted that male mice showed a pattern of non-

neoplastic liver lesions, along with silver-staining helical organisms within the liver that was deemed characteristic of an infection with *Helicobacter hepaticus*. Past NTP studies have demonstrated that such infections can result in an increased incidence of hemangiosarcoma in the liver of male mice. In this study, incidences of hemangiosarcoma were increased in exposed groups of male mice. This infection was a key confounding variable in this study, but the NTP team believed that incidences of lesions at other sites were not likely to have been significantly impacted by the infection.

The overall conclusions of the NTP study were as follows. There was some evidence of carcinogenic activity of cobalt sulfate heptahydrate in male F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms. Marginal increases in incidences of pheochromocytomas of the adrenal medulla may also have been related to exposure to cobalt sulfate heptahydrate. There was clear evidence of carcinogenic activity in female F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytomas of the adrenal medulla in groups exposed to cobalt sulfate heptahydrate. There was also clear evidence of carcinogenic activity of cobalt sulfate heptahydrate carcinogenicity in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

In another carcinogenicity study, intratracheal instillation of cobalt oxide (CoO) failed to increase tumour incidence in rats; however, an increased incidence of bronchoalveolar proliferation was reported in treated animals (Steinhoff and Mohr, 1991). In a second experiment, the authors reported a higher incidence of squamous cell carcinomas in the lungs of female rats treated with CoO and then benzo[a]pyrene (B[a]P, a known carcinogen), *versus* rats treated with B[a]P only.

The International Agency for Research on Cancer (IARC, 2003) recently revaluated its 1991 classification of cobalt and cobalt compounds as *possibly carcinogenic to humans* (Group 2B), with *inadequate evidence* of carcinogenicity in humans, and *sufficient evidence* for the carcinogenicity of cobalt metal powder in experimental animals. In light of new data, cobalt metal with tungsten carbide (*i.e.,* "hard metal") has been classified as *probably carcinogenic to humans* (Group 2A) on the basis of *limited evidence* in humans for increased risk of lung cancer and *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulphate and of cobalt metal powder. Cobalt metal in the absence of tungsten carbide has been reclassified as *possibly carcinogenic to humans* (Group 2B) given *inadequate* evidence of carcinogenicity from exposure to cobalt without tungsten carbide, and *sufficient evidence* in experimental animals for the carcinogenicity of cobalt sulphate and of cobalt metal powder. The American Conference of Governmental Industrial Hygienists (ACGIH, 2002) classifies cobalt as

Group A3 (animal carcinogen). This classification is applied to agents that are "carcinogenic in experimental animals at a relatively high dose, by route(s) of administration, at site(s), of histologic type(s), or by mechanisms(s) that are not considered relevant to worker exposure." The U.S. EPA has no cobalt file at present in the Integrated Risk Information System (IRIS) database. The U.S. EPA have classified cobalt as a group B1 Probable Human Carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals following inhalation exposure. Health Canada has not classified cobalt compounds as to their carcinogenicity.

A2-4.0 EXPOSURE LIMITS

ATSDR, U.S. EPA, MOE, WHO and RIVM were the regulatory agencies consulted to select exposure limits for cobalt.

For the current assessment, cobalt has not been considered a non-threshold carcinogen by the inhalation or oral routes of exposure. There is inadequate information available from oral studies to determine whether or not cobalt is carcinogenic *via* this route. IARC classifies cobalt compounds as "possibly carcinogenic to humans" and ACGIH classifies cobalt in category A3 - confirmed animal carcinogen with unknown relevance to humans. Furthermore, under the old 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), cobalt is classified as group B1 (Probable Human Carcinogen), based on limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals, as evidenced by increased incidence of alveolar/bronchiolar tumors in both sexes of rats and mice (U.S. EPA, 2002). Under the U.S. EPA (1999) cancer guidelines, cobalt is considered likely to be carcinogenic to humans (U.S. EPA, 2002). Health Canada currently has no TRVs for cobalt and has not classified cobalt compounds as to their carcinogenicity.

Inhalation Exposure Limits

While the weight-of- available evidence indicates that cobalt does not cause cancer in humans by the inhalation, oral, or dermal exposure routes; the U.S. EPA has classified cobalt as a group B1 Probable Human Carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals following inhalation exposure. The U.S. EPA (2002) have derived an inhalation unit risk for cobalt of $2.8x10^{-3}$ $(\mu g\ Co/m3)^{-1}$ based on tumourigenic effects (alveolar and bronchiolar) in rats and mice (Bucher *et al.,* 1999; NTP, 1998) which equates to a inhalation slope factor of 9.8 $(mg/kg/d)^{-1}$. No other identified regulatory agencies have derived exposure limits for cobalt based on carcinogenic endpoints. While there appears to be a consistent increased risk of respiratory tract cancer in workers co-exposed to both cobalt and tungsten carbide (*i.e.,* "hard metal" workers), the exposure conditions experienced by "hard metal" workers would not be expected to occur in the ambient environment. The U.S. EPA (2002) IUR and slope factor derivation process did not consider studies in which there was co-exposure to tungsten carbide.

The U.S. EPA reports an inhalation RfC of 0.02 $\mu g/m^3$ (U.S. EPA, 2002). It is noted that these values are provisional; and have been withdrawn. It is also noted that U.S. EPA Regions III, VI, and IX have adopted these values.

The Ontario Ministry of the Environment (MOE, 2001b) has developed a health-based half-hour Point of Impingement Limit and a health-based 24-hour Ambient Air Quality Criterion for cobalt of 0.3 and 0.1 μg/m^3, respectively.

ATSDR (2001) has derived an MRL for chronic inhalation exposure to cobalt. The chronic inhalation MRL is 1x10^{-4} mg cobalt/m^3, and is based on a NOAEL of 0.0053 mg cobalt/m^3 and a LOAEL of 0.015 mg cobalt/m^3 for decreases in forced vital capacity (FVC), forced expiratory volume in one second (FEV1), forced expiratory flow between 25 and 75% of the FVC (MMEF), and mean peak expiratory flow rate (PEF) in diamond polishers (Nemery *et al.,* 1992). The study in diamond polishers was considered a well-conducted study in humans, and was selected as the critical study for the derivation of a MRL as it examined a human population and defined a NOAEL. The chronic inhalation MRL was derived by adjusting the NOAEL of 0.0053 mg/m^3 for intermittent exposure (eight hours/24 hours x five days/seven days), and dividing by an uncertainty factor of 10 (for human variability). ATSDR notes that this MRL may not be protective for individuals already sensitive to cobalt. An acute inhalation MRL was not derived as the threshold was not well defined for human effects, and animal studies reported effects that occurred at levels above those reported in the few available human studies (ATSDR, 2001). An intermediate-duration MRL was also not derived because available studies did not examine the dose-response relationship at low doses. ATSDR considers that the chronic inhalation MRL should be protective of intermediate exposures as well. WHO (2006) have also established a tolerable concentration for inhaled cobalt of of 1x10^{-4} mg/m^3, and is based on a NOAEL of 0.0053 mg cobalt/m^3 in diamond polishers (Nemery *et al.,* 1992).

RIVM (Baars et al., 2001) derived a tolerable concentration in air (TCA) of 0.0005 mg/m^3, based on a LOAEL of 0.05 mg/m^3 for interstitial lung disease in humans (Sprince et al., 1988). An uncertainty factor of 100 (10 for extrapolation from a LOAEL and a factor of 10 for intrahuman variability) was applied to the LOAEL to yield the TCA. Medium reliability is suggested for this TCA by RIVM (Baar *et al*, 2001).

Oral Exposure Limits

The U.S. EPA reports an oral RfD for cobalt of 20 μg/kg/day and an inhalation RfC of 0.02 μg/m^3 (U.S. EPA, 2002). It is noted that these values are provisional; however review of the primary studies used in the derivation of these provisional values has indicated that they are based on sound science and standard regulatory policy. It is also noted that U.S. EPA Regions III, VI, and IX have adopted these values

In the derivation of the Guideline for Use at Contaminated Sites in Ontario (MOE, 1996), MOE utilized an oral RfD of 60 μg/kg/day for cobalt.

ATSDR (2001) derived an oral intermediate-duration MRL of 10 ug cobalt/kg/day. The MRL is based on a LOAEL of 1 mg cobalt/kg/day for polycythemia as reported in a study by Davis and Fields (1958). These authors exposed six male volunteers to 120 or 150 mg/day of cobalt chloride (~1 mg Co/kg/day) for up to 22 days. Exposure resulted in the development of polycythemia in all six patients, with 16 to 20% increases in red blood cell numbers above pre-treatment levels. Oral MRL values were not derived by ATSDR for acute or chronic exposure to cobalt. An acute MRL was not derived because the reported effects in animals were serious and occurred at levels above those reported in the few available human oral studies. No chronic oral studies were available for humans or animals. The ATSDR (2001) MRL has been selected for the current study. While dated, these study results are consistent with those observed in more recent studies such as Duckham and Lee (1976).

RIVM (Baars *et al.,* 2001) derived a tolerable daily intake (TDI) of 1.4 ug/kg-day based on a LOAEL of 0.04 mg/kg-day for cardiomyopathy in humans after intermediate oral exposure (Morin *et al.,* 1971). RIVM used an uncertainty factor of 30 (three for intra-human variation and 10 for extrapolation to a NOAEL) to yield the TDI.

Dermal Exposure Limits

No regulatory dermal exposure limits for cobalt compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

A2-4.1 Criteria Summary

Table A2.1 summarizes the cobalt exposure limits selected for the current study.

Table A2.1 Summary Of Toxicological Criteria Selected For Cobalt in the HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	10 µg/kg/day	RfD	polycythemia	Davis and Fields (1958)	ATSDR (2001)
Inhalation	0.5 µg/m^3	RfC	interstitial lung disease	Sprince et al. (1988)	RIVM (Baar *et al*, 2001)
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

NOTES:
NA = not available; RfD = reference dose; RfC = reference concentration; SFi = inhalation slope factor; IUR = inhalation unit risk;
PPRTV = Provisional Peer Reviewed Toxicity Values.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

Uncertainties in Selected Cobalt Exposure Limits

It is important to recognize that the selected oral toxicological criterion for cobalt is based on a provisional value derived by U.S. EPA (2002), that has been withdrawn. While this toxicity values was considered the most scientifically defensible one that is currently available, there is uncertainty associated with all cobalt PPRTVs that is important to acknowledge. The key areas of uncertainty regarding the cobalt toxicity values are summarized below (U.S. EPA, 2002). It is also important to recognize that the areas of uncertainty noted below apply equally to other available regulatory exposure limits for cobalt compounds.

- While there is evidence of allergic responses in cobalt-sensitized workers available data provide no information on the dose-response relationship of cobalt sensitization, nor is a NOAEL for the elicitation of the allergic response in humans defined by the available studies.
- There is some evidence documenting interrelationships between cobalt and nickel sensitization, such that people sensitized by nickel may have an allergic reaction following cobalt exposure. However, information on this endpoint is not sufficient to quantify.
- U.S. EPA (2002) notes that confidence in the critical study for the oral RfD is low-to-medium, as it examined a small number of subjects over a subchronic rather than chronic duration. However, it is believed that a sensitive endpoint in a group of sensitive humans was considered in the RfD derivation. The U.S. EPA also notes that confidence in the supporting database is medium, as there supporting studies in both anemic and normal humans, and in animals. However, there are no chronic oral data available and only limited data exists on developmental effects.
- No studies exist that investigated developmental effects after inhalation exposure to cobalt.
- No oral or inhalation exposure multi-generation reproduction studies were located.
- Confidence in the key study that the RfC was derived from is low. Reasons include the fact that this study used a cross-sectional design that investigated only respiratory endpoints, the control group was studied more than one year after the exposed population was studied, one study group was exposed to iron and diamond dust in addition to cobalt (and possibly to asbestos in the past), there was no discussion of duration of exposure. Confidence in the supporting database is medium, as the critical endpoint is well supported by other studies in both humans and animals.

- The precise mechanism of action for cobalt carcinogenicity has not been determined, although a number of potential mechanisms have been identified, with the most likely mechanism being cobalt-induced oxidative stress.
- While available human studies are suggestive of a possible association between cobalt and respiratory tumors, these studies have a number of limitations such as small sample size, inadequate exposure assessment, concurrent exposure to other chemicals, which makes them inappropriate for assessing the carcinogenic potential of cobalt.
- There are no oral studies that investigated the carcinogenic potential of cobalt.
- Available genotoxicity and mutagenicity studies are limited and equivocal with respect to supporting the carcinogenicity of cobalt.

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SARA GROUP

Sudbury Area Risk Assessment
Volume II

Appendix A3:

Detailed Toxicological Profile of Copper

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A3 DETAILED TOXICOLOGICAL PROFILE OF COPPER

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APPENDIX A3 DETAILED TOXICOLOGICAL PROFILE OF COPPER

SUMMARY

The purpose of this toxicological profile of copper is to: i) outline the most relevant toxicological and epidemiological information on this substance; ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the copper exposure limits selected for use in the human health risk assessment of the Sudbury area. The toxicological review of copper is based primarily on secondary sources, such as Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for copper. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Copper

Copper (CAS RN: 7440-50-8) is a group IB metal with four oxidation states (0, I, II and III) (ATSDR, 2004). Copper compounds in the environment are most commonly cupric, or copper (2+), compounds (WHO, 1998). Cupric ions form complexes easily, and are usually found in the environment complexed with organic ligands (WHO, 1998). Metallic copper is sparingly soluble in water, salt solutions and mildly acidic solutions, while most cupric compounds and complexes are highly soluble in water (WHO, 1998).

In natural waters, a very small percentages of copper occur as Cu^{2+}; rather, most copper is adsorbed to suspended particles or complexed with various inorganic or organic ligands (*e.g.,* hydroxide, carbonate, chloride, fulvic and humic acids) (WHO, 1998). In air, copper occurs in the particulate phase. In sediments and soils, copper also tends to occur in the particulate phase, either as a constituent of minerals or adsorbed to oxide surfaces or organic matter (WHO, 1998).

Absorption

Copper is readily absorbed from the stomach and small intestine in both humans and experimental animals, although there appears to be species differences with respect to the site of maximal absorption

(ATSDR, 2004). After nutritional requirements for copper are met (copper is an essential micronutrient; see Section A3.1), there are several physiological mechanisms that generally act to prevent excess copper absorption. For example, much of the excess copper absorbed into gastrointestinal mucosal cells will be bound to metallothionein (ATSDR, 2004). This bound copper is then excreted when the cells are sloughed off. Copper that is not bound to metallothionein may be stored in the liver or incorporated into bile and excreted in the feces (ATSDR, 2004). Average oral absorption efficiencies for copper range from 24 to 60% in presumably healthy adults (ATSDR, 2004).

No studies were identified which investigated copper absorption following human inhalation exposure. Only a few studies were identified that evaluated copper absorption in animals following inhalation exposure, none of which provide quantitative estimates of copper absorption from the respiratory tract; however, there is evidence that copper is absorbed from the inhalation route.

Copper is poorly absorbed through intact skin. Less than 6% of copper deposited on *ex vivo* human skin samples was absorbed (Pirot *et al.,* 1996a,b). These authors also noted that copper chloride was absorbed to a higher extent than copper sulphate (Pirot *et al.,* 1996a). RAIS (2004) cites a dermal absorption factor of 0.001 (or 0.1%) for copper compounds.

Essentiality

Copper is an essential trace element that is naturally present in all environmental media (air, water, soil, sediments), as well as all biota and all foods consumed by humans. The primary source of copper in humans is the diet. It is estimated that the typical daily copper intake from food is around 1.0 to 1.3 mg/day for adults (ATSDR, 2004). The World Health Organization (WHO, 1998) reports that total daily intake of copper in adult's ranges between 0.9 and 2.2 mg, with most studies indicating daily copper intakes at the lower end of this range. WHO (1998) notes that intakes may occasionally exceed 5 mg/day. In some cases, drinking water may also make a substantial additional contribution to the total daily copper intake, particularly if corrosive waters remain in copper pipes for prolonged periods. Other common environmental routes of exposure, such as inhalation and dermal uptake, are insignificant relative to oral consumption of dietary items. For example, inhalation adds approximately 0.0003 to 0.002 mg/day from dusts and smoke (WHO, 1998). Dermal absorption contributes even less to total daily copper intake.

Among the essential roles of copper in the body are incorporation into at least 30 metalloenzymes involved in such biochemical processes as hemoglobin formation, iron metabolism, carbohydrate metabolism, catecholamine biosynthesis, cellular respiration, free radical defenses, neurotransmitter

function, connective tissue biosynthesis (cross-linking of collagen, elastin, and hair keratin) (ATSDR, 2004; WHO, 1998). In a number of these enzymes, copper is an essential co-factor required for enzyme function, while in others, copper confers an appropriate structure for catalytic activity. No other elements are known to be able to substitute for copper in these enzymes (WHO, 1998). Copper is also essential in the maturation of neutrophils (Percival, 1995), and plays an important role in the regulation of gene transcription (Dameron *et al.,* 1991; Zhou and Theil, 1991; Gralla *et al.,* 1991; Carry *et al.,* 1991; Jungmann *et al.,* 1993).

As copper is an essential element, its uptake, metabolism and excretion are physiologically regulated, and most tissues of the body have measurable amounts of copper associated with them.

Toxicology of Copper

For many metals that are essential trace elements, including copper, it must be recognized that there are potential human health risks associated with intakes that are too low (deficiency) as well as intakes that are too high (toxicity). For essential elements, there is a U-shaped dose-response curve that indicates a risk of adverse health effects from both deficiency (at low intakes) and toxicity (at high intakes). In assessing the potential risk from such substances, it is important to define an intake range that will prevent both deficiency and toxicity for the general population (WHO, 1998).

In general, acute toxicity due to ingestion of copper is rare in humans and is typically a consequence of the contamination of beverages, or accidental or deliberate poisoning attempts. The most commonly reported effects following acute oral exposure to soluble copper compounds are signs of gastrointestinal distress. This is a sensitive indicator of oral intake of copper that exceeds nutritional requirements (ATSDR, 2004). Such effects are typically reported following ingestion of water or other beverages containing >3,000 µg copper / L. The most prevalent signs and symptoms of acute oral copper toxicity are nausea, vomiting, abdominal pain, and diarrhea. Such effects typically occur shortly after ingestion and do not persist following cessation of exposure (ATSDR, 2004). The major symptoms of acute copper intoxication in experimental animals include reduced body weights, hepatotoxicity, renal toxicity, and death (at high doses) (ATSDR, 2004; WHO, 1998; CCME, 1997). Acute toxicity data are limited to mostly oral studies. The liver and kidney appear to be the major target organs for acute copper toxicity in experimental animals.

Chronic human oral exposure to copper has been associated mainly with liver effects, although there are some reports of effects on blood, kidney, brain and other organs (Gaetke and Chow, 2003). However, the

primary target of chronic copper toxicity appears to be the liver. This is supported by a number of animal studies as well (WHO, 1998; ATSDR, 2004; CCME, 1997). It is important to recognize though, that with the exception of several defined syndromes (*e.g.,* Wilson's disease, Indian childhood cirrhosis (ICC), idiopathic copper toxicosis (ICT)), liver effects are rarely reported in humans (ATSDR, 2004). The available human studies of copper effects on the liver suggest that liver effects are unlikely to occur at lower doses than gastrointestinal effects, following longer term oral exposures. Thus, gastrointestinal effects would be expected to be the most sensitive indicator of both acute and long term copper toxicity *via* the oral route.

The subchronic and chronic toxicity of copper compounds has not been well characterized in experimental animals through any route of exposure (WHO, 1998). However, the limited data available suggests that gastrointestinal, liver and kidney effects may occur following subchronic or chronic copper exposure.

In humans, copper is a known respiratory irritant. Workers exposed to copper dusts and various airborne copper salts report a number of symptoms that are consistent with upper respiratory tract irritation, including: eye, nose and throat irritation, coughing, sneezing, chest pain, and runny nose (Askergren and Mellgren, 1975; Suciu *et al.,* 1981; OEHHA, 1999).

In general, irritation or allergic skin reactions to copper appear to be rare in humans (CCME, 1997).

There is extremely limited data available regarding reproductive or developmental effects of copper in humans and animals following inhalation, oral or dermal exposure. There is presently no evidence to suggest that copper compounds are carcinogenic in humans or animals (WHO, 1998; ATSDR, 2004; U.S. U.S. EPA, 2004; TERA, 2004). There are no data available on the genotoxicity of copper in humans exposed *via* oral, inhalation or dermal routes. The existing genotoxicity database suggests that copper is a clastogenic agent, and some studies have shown that exposure to copper can result in DNA damage; however, point mutation assay results are mixed and inconclusive (ATSDR, 2004). Overall, the database on mutagenicity and genotoxicity of copper compounds is limited and equivocal, and considerably more research is required to determine whether or not copper is mutagenic and/or genotoxic to mammals (including humans) *in vivo.*

Exposure Limits

The following organizations were consulted to select exposure limits for copper: Health Canada; the U.S. EPA; ATSDR; WHO; MOE; JECFA; Health and Welfare Canada; RIVM; and, the National Academy of Science.

Current Ministry of Environment (MOE) guidelines discourages the development of *de novo* toxicological criteria (exposure limits). Toxicological criteria used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO) and the MOE itself.

A number of regulatory agencies have derived health-based exposure limits for copper compounds, as described below:

Oral Exposure Limits

JECFA (1982) derived a provisional maximum tolerable daily intake (PMTDI) for copper of 0.05 to 0.5 mg/kg body weight/day, which was set equivalent to the provisional daily dietary requirement, rather than an intake associated with an adverse health effect. This value remains as the current PMTDI recommended by JECFA. Health Canada (1996) reported a provisional tolerable daily intake (PTDI) of 0.05 to 0.5 mg/kg body weight/day for copper that is based on this JECFA PMTDI value.

Health and Welfare Canada (HWC, 1990) had estimated safe and adequate dietary copper requirements for a few age classes based on mass balance studies. These safe and adequate requirements were used as the basis for deriving conservative TDIs by Health Canada. For children aged three to 10 years, it was determined that 0.05 to 0.1 mg/kg body weight/day was the safe and adequate range for daily copper intake. For adults, 0.03 mg/kg body weight/day was estimated as the safe and adequate copper intake rate. These values were used by Health Canada in the derivation of human health soil quality guidelines for copper. Health Canada (2003a) reports an oral TDI of 0.03 mg/kg body weight/day that is based on the adult TDI originally recommended by HWC (1990). In the derivation of the *Guideline for Use at Contaminated Sites in Ontario* (MOE, 1996), MOE utilized an oral RfD of 60 µg/kg/day for cobalt.

The U.S. EPA IRIS database contains no oral exposure limits for copper compounds (U.S. EPA, 1988). U.S. EPA Region III (2004), Region VI (2004) and Region IX (2003) all report an oral RfD of around 0.04 mg/kg/d, which was originally derived by the U.S. EPA for preparation of the Health Effects

Assessment Summary Tables (HEAST). The source of this oral RfD of 0.04 mg/kg body weight/day is as follows. The U.S. EPA (1987) developed a drinking water criterion of 1.3 mg copper/L, based on adverse effects such as vomiting, nausea, and diarrhea in humans following acute consumption of copper in drinking water (as reported in studies by Wyllie, 1957; Semple *et al.,* 1960; Chuttani *et al.,* 1965). From this drinking water value, U.S. EPA (HEAST) estimated an oral RfD of 0.04 mg/kg/day [(1.3 mg/L x 2 L/d) / 70 kg] (ORNL, 2004).

ATSDR (2002) has developed an acute duration oral Minimal Risk Level (MRL) for copper of 0.02 mg/kg/day. This MRL has also been adopted by ATSDR as the intermediate duration MRL. ATSDR (2002) considers that available data are inadequate to derive a chronic duration oral MRL. The acute-duration oral MRL is based on gastrointestinal effects reported in the Pizarro *et al.* (1999) study. To estimate total copper exposure, the dose of copper from drinking water (0.0272 mg Cu/kg/day) in this study was added to the reported average dietary copper intake of copper (0.0266 mg Cu/kg/day). This yielded a total copper exposure level of 0.0538 mg Cu/kg/day, which was considered a NOAEL for gastrointestinal effects. The NOAEL was then divided by an uncertainty factor of three (to account for inter-human variability) to yield the acute oral MRL. ATSDR (2002) notes that this MRL accounts for dietary exposure as well as environmental contamination.

RIVM (Baars *et al.,* 2001) noted that copper is an essential nutrient, with a minimum daily requirement of 0.02 to 0.08 mg/kg-day (as reported by WHO, 1996). It was determined that a TDI for copper cannot be lower than the levels required for nutrition essentiality. Thus, RIVM based a TDI on the typical daily intake of the population which was shown to be 0.02 to 0.03 mg/kg/day on average, with a range of 0.003 to 0.1 mg/kg/day and an upper limit of 0.14 mg/kg/day (Slooff *et al.,* 1989). This latter upper limit daily intake value (0.14 mg/kg-day) was selected as the TDI by RIVM.

The National Academy of Science (IOM, 2000) has derived an acceptable Upper Intake Level (UL) based on the NOAEL of 10 mg/day based on Pratt *et al.* (1985). IOM (2000) considered this NOAEL to be protective of the general population and felt that no further uncertainty factor was warranted. This decision is supported by the large database of human information indicating no adverse effects in the 10 to 12 mg/day and a paucity of observed liver effects from copper exposure in humans with normal copper homeostasis. This NOAEL results in an acceptable upper limit of approximately 140 µg/kg/day for adults (10 mg/day ÷ 70 kg). There was insufficient data to establish unique ULs for any other age group (similar sensitivity for all ages) (IOM, 2000). Health Canada has indicated that in 2005/2006, the agency will

officially be using adopt ULs as toxicity reference values for all essential elements (Roest and Petrovic, Health Canada, Personal Communication) for contaminated sites human health risk assessments.

It is important to recognize that all available regulatory oral exposure limit values for copper are similar in magnitude, and are based on either typical daily intakes, or intakes associated with gastrointestinal distress. Copper doses at, or below any of these values would not be expected to result in adverse health effects under conditions of continuous lifetime daily exposure. It is also important to recognize that all oral exposure limits, regardless of their basis, lie within the range of typical estimated daily dietary intakes, and/or recommended nutritional requirements when the body weight of various human age classes is taken into account (*e.g.,* if a 70 kg adult is assumed, the Health Canada TDI of 0.03 mg/kg body weight/day equates to a daily intake of 2.1 mg Cu/day).

Health Canada (2003b) and the U.S. EPA (2003) have set aesthetic objectives for copper in drinking water of 1.0 mg/L. Aesthetic objectives do not have a toxicological basis, but are established based on objectionable taste, colour and/or staining characteristics. The MOE (2003) has adopted the Health Canada aesthetic objective for copper in drinking water.

Inhalation Exposure Limits

Identified regulatory inhalation exposure limits for copper compounds are described below.

OEHHA (1999) derived an acute reference exposure level (REL) for a 1-hour exposure of 0.1 mg/m^3. This acute REL is considered protective against mild adverse effects. The REL was derived based on studies by Gleason (1968), and Whitman (1957; 1962) which investigated metal fume fever in workers. A NOAEL of 1 mg/m^3 was identified from these studies. The NOAEL was mainly based on the report of Whitman (1957) indicating that exposure to copper dust was detectable by taste, but that no other symptoms occurred following exposure to 1 to 3 mg/m^3 for an unspecified short duration. Given that the exposure duration was not clearly stated in these studies, no extrapolation to a one hour concentration could be conducted. Rather, the NOAEL was assumed to be applicable to a one hour exposure. A cumulative uncertainty factor of 10 was applied to the NOAEL (for intraspecies uncertainty) to yield the acute REL. Given the limitations of the existing data, OEHHA suggests that re-evaluation of the acute REL for copper be conducted when better methods or data are available. OEHHA did not derive a chronic REL for inhalation exposure to copper.

The MOE (2001) reports a 24-hr Ambient Air Quality Criterion (AAQC) of 50 µg/m^3 for copper, based on health concerns. No supporting rationale for this AAQC was identified in available MOE publications.

RIVM derived a tolerable concentration in air (TCA) of 0.001 mg/m^3 based on a NOAEC of 0.6 mg/m^3 for lung and immune system effects in rabbits from a short-term toxicity study by Johansson *et al.* (1984). RIVM used an uncertainty factor of 100 (10 each for intra- and interspecies variability), and adjusted for continuous exposure (5/7 x 6/24) to yield the TCA.

ATSDR (2002) considers available data on the toxicity of inhaled copper inadequate for derivation of acute, intermediate, or chronic duration inhalation MRLs.

The U.S. EPA has not derived inhalation exposure limits for any copper compound (EPA, 1988).

Dermal Exposure Limits

No regulatory dermal exposure limits for copper compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF$_{dermal}$). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

Criteria Summary

The following table summarizes the exposure limit selected for the current study

Summary of Toxicological Criteria Selected For The HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	140 µg/kg/day	UL	liver damage	Pratt *et al.,* 1985	IOM, 2000; Health Canada, 2005
Inhalation	1 µg/m^3	TCA	subchronic NOAEC (respiratory and immunological effects) in rabbits	Johansson *et al.,* 1984	RIVM (Baars *et al.,* 2001)
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

NOTES:
NA = not available
TCA = tolerable concentration in air; UL = Upper Intake Level.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

References

For a complete list of references, see Section A3-5.0 of the detailed toxicological profile on the accompanying CD.

A3-1.0 INTRODUCTION

This profile is not intended to provide a comprehensive review of the available toxicological and epidemiological literature on copper compounds. Rather, the purpose of this copper toxicological profile is to: i) outline the most relevant toxicological and epidemiological information on copper compounds ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the exposure limits selected for use in the human health risk assessment of the Sudbury area. This toxicological profile is based primarily on secondary information sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent relevant scientific literature.

Copper (CAS RN: 7440-50-8) is a group IB metal with four oxidation states (0, I, II and III) (ATSDR, 2004). Copper compounds in the environment are most commonly cupric, or copper (2+), compounds (WHO, 1998). Cupric ions form complexes easily, and are usually found in the environment complexed with organic ligands (WHO, 1998). Metallic copper is sparingly soluble in water, salt solutions and mildly acidic solutions, while most cupric compounds and complexes are highly soluble in water (WHO, 1998).

In natural waters, very small percentages of copper occur as Cu^{2+}; rather, most copper is adsorbed to suspended particles or complexed with various inorganic or organic ligands (*e.g.,* hydroxide, carbonate, chloride, fulvic and humic acids) (WHO, 1998). In air, copper occurs in the particulate phase. In sediments and soils, copper also tends to occur in the particulate phase, either as a constituent of minerals or adsorbed to oxide surfaces or organic matter (WHO, 1998).

Copper is an important metal with many uses due to several key properties, including high thermal and electrical conductivity, alloying ability, malleability and resistance to corrosion (ATSDR, 2004). It is used primarily as copper metal, or as one of the common alloys (brass, bronze, gun metal, Monel metal) (ATSDR, 2004). Copper metal and alloys are used for plumbing, building wire, telecommunications, power utilities, in-plant equipment, air conditioning, automotive electrical, automotive non-electrical, business electronics, and industrial valves and fittings (ATSDR, 2004). Copper sulphate is the most important copper compound in industry. Principal uses of copper sulphate include fungicides, algicides and nutritional supplements in agriculture; industrial metal finishing, mineral froth flotation, wood preservation, and water treatment (ATSDR, 2004).

Copper is present naturally in the environment within several minerals, and as metallic copper (ATSDR, 2004). It may be released to the environment naturally *via* volcanic eruption, burning, decomposition of organic material and sea spray (WHO, 1998). Major anthropogenic sources of copper to the environment include smelters, iron foundries, power stations, combustion sources such as municipal incinerators, mining operations and sewage sludge application to land (WHO, 1998; ATSDR, 2004; CCME, 1997).

A3-2.0 FRACTION ABSORBED *VIA* DIFFERENT ROUTES

A3-2.1 Oral

Copper is readily absorbed from the stomach and small intestine in both humans and experimental animals, although there appears to be species differences with respect to the site of maximal absorption (ATSDR, 2004). After nutritional requirements for copper are met (copper is an essential micronutrient; see Section A3.1), there are several physiological mechanisms that generally act to prevent excess copper absorption. For example, much of the excess copper absorbed into gastrointestinal mucosal cells will be bound to metallothionein (ATSDR, 2004). This bound copper is then excreted when the cells are sloughed off. Copper that is not bound to metallothionein may be stored in the liver or incorporated into bile and excreted in the feces (ATSDR, 2004).

Average oral absorption efficiencies for copper range from 24 to 60% in presumably healthy adults (ATSDR, 2004). RAIS (2004) cites an oral absorption factor or 0.3 (or 30%) for copper compounds. Turnlund *et al.* (1989) reported that copper absorption *via* the oral route is dependent on the amount of copper in the diet; when a low copper diet was given (0.78 mg/day), copper absorption was 55.6%, whereas it was 36.3% from the same diet that was supplemented with copper (1.68 mg/day), and was only 12.4% from the same diet that was high in copper (7.53 mg/day). From this, it appears that copper absorption in adults is saturable and that the percentage absorbed decreases with an increasing level of dietary copper. These authors also suggested that copper intakes of approximately 0.8 mg/day are adequate to sustain the body's copper balance. Recent i*n vitro* studies provide further evidence that copper uptake into intestinal cells is a saturable process (Arredondo *et al.,* 2000).

When absorbed from the gastrointestinal tract, copper is typically in either an ionic form, or is bound to amino acids. There are numerous factors that can influence both the rate and extent of copper absorption from the gastrointestinal tract of humans and experimental animals, including: the amount of copper in the diet, pH of the gastrointestinal tract, competition and interactions with various other chemicals in the

diet such as zinc, iron, cadmium, ascorbic acid, sulphates, proteins, amino acids, phytate and fibre, carbohydrates, molybdenum, manganese, and selenium (ATSDR, 2004; WHO, 1998).

A3-2.2 Inhalation

No studies were identified which investigated copper absorption following human inhalation exposure. Only a few studies were identified that evaluated copper absorption in animals following inhalation exposure, none of which provide quantitative estimates of copper absorption from the respiratory tract.

While quantitative data do not appear to exist, there is evidence that copper is absorbed from the inhalation route. For example, Batsura (1969) showed that copper oxide particles are able to penetrate the epithelial cells of alveoli in rats. In this study, copper oxide was observed in alveolar capillaries 3 hours after rats were exposed to a welding dust aerosol generated from pure copper wires. Hirano *et al.* (1990) found that the half-time of copper sulfate in the lungs was roughly 7.5 hours after intratracheal instillation of 20 µg copper/rat. In a later study, Hirano *et al.* (1993) reported that copper oxide particles were cleared from the lungs with a half-time of 37 hours in rats dosed by intratracheal instillation.

A3-2.3 Dermal

Copper is poorly absorbed through intact skin. Less than 6% of copper deposited on *ex vivo* human skin samples was absorbed (Pirot *et al.,* 1996a,b). These authors also noted that copper chloride was absorbed to a higher extent than copper sulphate (Pirot *et al.,* 1996a). RAIS (2004) cites a dermal absorption factor of 0.001 (or 0.1%) for copper compounds.

The available *in vivo* data do not provide quantitative information on the rate or extent of copper absorption through intact skin following dermal exposure of either humans or animals to copper (ATSDR, 2004). Some animal studies have demonstrated that copper can be absorbed dermally when applied with an appropriate vehicle, such as salicylic acid or phenylbutazone (*e.g.,* Beveridge *et al.,* 1984; Walker *et al.,* 1977). In the Walker *et al.* (1977) study, approximately 3.3% of a topically applied saline solution of the copper complex (*bis*[glycinato]copper[II]) penetrated the skin of cats.

A3-3.0 TOXICOLOGY

A3-3.1 Essentiality of Copper

Copper is an essential trace element that is naturally present in all environmental media (air, water, soil, sediments), as well as all biota and all foods consumed by humans. The primary source of copper in humans is the diet. It is estimated that the typical daily copper intake from food is around 1.0 to 1.3 mg/day for adults (ATSDR, 2004). The World Health Organization (WHO, 1998) reports that total daily intake of copper in adult's ranges between 0.9 and 2.2 mg, with most studies indicating daily copper intakes at the lower end of this range. WHO (1998) notes that intakes may occasionally exceed 5 mg/day. In some cases, drinking water may also make a substantial additional contribution to the total daily copper intake, particularly if corrosive waters remain in copper pipes for prolonged periods. Other common environmental routes of exposure, such as inhalation and dermal uptake, are insignificant relative to oral consumption of dietary items. For example, inhalation adds approximately 0.0003 to 0.002 mg/day from dusts and smoke (WHO, 1998). Dermal absorption contributes even less to total daily copper intake.

Among the essential roles of copper in the body are incorporation into at least 30 metalloenzymes involved in such biochemical processes as hemoglobin formation, iron metabolism, carbohydrate metabolism, catecholamine biosynthesis, cellular respiration, free radical defenses, neurotransmitter function, connective tissue biosynthesis (cross-linking of collagen, elastin, and hair keratin) (ATSDR, 2004; WHO, 1998). In a number of these enzymes, copper is an essential co-factor required for enzyme function, while in others, copper confers an appropriate structure for catalytic activity. No other elements are known to be able to substitute for copper in these enzymes (WHO, 1998). Copper is also essential in the maturation of neutrophils (Percival, 1995), and plays an important role in the regulation of gene transcription (Dameron *et al.,* 1991; Zhou and Theil, 1991; Gralla *et al.,* 1991; Carry *et al.,* 1991; Jungmann *et al.,* 1993).

As copper is an essential element, its uptake, metabolism and excretion are physiologically regulated, and most tissues of the body have measurable amounts of copper associated with them. It has been estimated that the whole human body contains 100 to 150 mg copper at any given time (WHO, 1998). All mammals have metabolic mechanisms that maintain copper homeostasis (a balance between metabolic requirements for copper and prevention against accumulation to toxic levels, such that copper levels are generally maintained within a range that avoids both deficiency and excess). Copper homeostasis involves physiological regulation of absorption, cellular uptake, intracellular transport, sequestration/storage,

cellular efflux, and excretion from the body (ATSDR, 2004). Table A3.1 presents a summary of human nutritional requirements for copper.

Table A3.1 Nutritional Requirements for Copper

Agency	Infants	Children	Adults
U.S. Food and Nutrition Board, Institute of Medicine, National Academy of Science (2000)[a]	Adequate Intake 0 to 6 mths: 200 µg/day 7 to 12 mths: 220 µg/day	Estimated Average Requirement 1 to 3 yrs: 260 µg/day 4 to 8 yrs: 340 µg/day 9 to 13 yrs: 540 µg/day 14 to 18 yrs: 685 µg/day 14 to18 yrs (pregnancy): 785 µg/day Recommended Dietary Allowance[b] 1 to 3 yrs: 240 µg/day 4 to 8 yrs: 440 µg/day 9 to 13 yrs: 700 µg/day 14 to 18 yrs: 890 µg/day 14 to 18 yrs (pregnancy): 1,000 µg/day	Estimated Average Requirement 19 to >70 yrs: 700 µg/day 19 to 50 yrs (pregnant): 800 µg/day Recommended Dietary Allowance[b] 19 to >70 yrs: 900 µg/day 19 to 50 yrs (pregnant): 1,000 µg/day

[a] Values are also currently recommended by Health Canada (2004) as Dietary Reference Intakes.
[b] Recommended Dietary Allowance is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of 97 to 98% of healthy individuals in the gender and age group for which it was developed.

As shown in Table A3.1, the recommended copper intake values are within the range of estimated daily intakes. Thus, typical daily intakes of copper would appear to meet nutritional requirements, and would not be expected to result in adverse effects.

However, as with any substance, even essential trace elements, excessive exposures may result in toxicity. There are also certain sensitive sub-populations with genetic defects or other abnormalities in the metabolism of copper that may experience toxicity at levels of exposure that are non-toxic to individuals without these defects. In addition, highly excessive amounts of copper can overwhelm the body's homeostatic regulation of copper intake. Toxicity is likely to occur only when such homoeostatic controls are overwhelmed and/or basic cellular defense or repair mechanisms are impaired. However, this has only been documented to occur in individuals with genetic copper metabolism impairment (*e.g.,* Wilson's disease, Indian childhood cirrhosis, idiopathic copper toxicosis) or cases of intentional or accidental poisoning, where very large amounts of copper were ingested (ATSDR, 2004; WHO, 1998; NRC, 2000).

Threshold levels for copper toxicity in humans have not been firmly established. However, it appears that the main intracellular binding site for copper, metallothionein, becomes saturated with copper before toxicity occurs. As metallothionein is believed to act as an intracellular antioxidant which protects cells from free radicals and reactive oxygen species, the saturation of this protein with copper may result in oxidative stress (WHO, 1998). Copper is able to potentially cause oxidative stress through its ability to cycle between an oxidized state, $Cu(^{2+})$, and reduced state, $Cu(^{+})$. While this ability is key to copper's role in various metalloenzymes, this same property of copper may result in oxidative stress through the generation of superoxide radicals and hydroxyl radicals when converting between the oxidized and reduced states (Camakaris *et al.,* 1999; ATSDR, 2004).

Copper deficiency rarely occurs in humans since most diets have copper in excess of what is required by the body (WHO, 1998). Symptoms of human copper deficiency include anaemia, leucopenia and osteoporosis (ATSDR, 2004). Copper-deficiency is more common in animals, particularly livestock species, and may lead to several different disorders such as anaemia, bone, nerve and cardiovascular disorders, failure of keratinization and reproductive failure (Davis and Mertz, 1987).

A3-3.2 Systemic Toxicity

For many metals that are essential trace elements, including copper, it must be recognized that there are potential human health risks associated with intakes that are too low (deficiency) as well as intakes that are too high (toxicity). For essential elements, there is a U-shaped dose-response curve, that indicates a risk of adverse health effects from both deficiency (at low intakes) and toxicity (at high intakes). In assessing the potential risk from such substances, it is important to define an intake range that will prevent both deficiency and toxicity for the general population (WHO, 1998).

A3-3.2.1 Human Studies

While substantial information on the acute toxicity of copper in humans exists (primarily *via* the oral route), there is only limited data available regarding chronic human toxicity of copper following oral, inhalation or dermal exposure.

A3-3.2.1.1 Oral Exposure Studies

In general, acute toxicity due to ingestion of copper is rare in humans and is typically a consequence of the contamination of beverages, or accidental or deliberate poisoning attempts. There are numerous

documented case reports of intentional or accidental copper ingestion that reported such effects as: severe gastrointestinal upset, jaundice, various liver effects (*e.g.,* centrilobular congestion, necrosis, biliary stasis, elevated serum bilirubin level and aspartate aminotransferase, activity, elevated bile salts, hepatomegaly, altered liver enzyme chemistry), haemolytic anemia, and impaired renal function (WHO, 1998; CCME, 1997; ATSDR, 2004). It appears that acute ingestion of roughly 1,000 times the daily nutritional requirement range for copper, may result in death (Davis and Mertz, 1987; Janus *et al.,* 1989). Gateke and Chow (2003) suggest that the estimated lethal one-time dose of copper in an untreated adult is 10 to 20 g of copper.

The most commonly reported effects following acute oral exposure to soluble copper compounds are signs of gastrointestinal distress. This is a sensitive indicator of oral intake of copper that exceeds nutritional requirements (ATSDR, 2004). Such effects are typically reported following ingestion of water or other beverages containing >3,000 µg copper / L. The most prevalent signs and symptoms of acute oral copper toxicity are nausea, vomiting, abdominal pain, and diarrhea. Such effects typically occur shortly after ingestion and do not persist following cessation of exposure (ATSDR, 2004).

A number of recent studies evaluated the threshold for gastrointestinal effects following oral copper exposure in humans. In studies in which adults ingested a single dose of copper sulphate in water following an overnight fast, nausea occurred at copper concentrations of 4,000 µg/L and higher (Gotteland *et al.,* 2001; Olivares *et al.,* 2001). Araya *et al,* (2001) found that 179 subjects ingesting a single dose of copper sulphate showed a statistically significant increase in the occurrence of nausea only, or nausea, vomiting, diarrhea, or abdominal pain at copper concentrations above 6,000 µg/L; there were no observed adverse effects at 4,000 µg/L. A copper sulphate concentration of 8,000 µg/L or higher, administered in an orange-flavored drink, resulted in nausea (Gotteland *et al.,* 2001). Abdominal pain, nausea, and/or vomiting were reported at drinking water concentrations of 5,000 µg/L copper sulphate or copper oxide, with no gastrointestinal effects reported at 3,000 µg/L (Pizarro *et al.,* 1999; 2001).

In a number of these recent studies, LOAEL and NOAEL values were identified or estimated. Gotteland *et al*. (2001) and Olivares *et al*. (2001) identified a LOAEL for nausea in adults who had fasted overnight, of 0.01 mg Cu/kg/day. Olivares *et al.* (2001) reported a NOAEL of 0.006 mg/kg/day. Pizarro *et al*. (1999; 2001) reported a LOAEL for gastrointestinal effects in subjects exposed to copper sulphate in drinking water for one to two weeks, of 5,000 µg/L (0.07 mg Cu/kg/day). The NOAEL was 3,000 µg/L (0.03 mg Cu/kg/day).

Araya *et al.* (2004) showed that the main determinants of gastrointestinal response to copper intake are the copper concentration and the volume ingested at one time. The dose received and the vehicles are less important, according to these authors. Over the course of their two month study, Araya *et al*. (2004) also found that symptom reporting decreased over time, suggesting an adaptive response to repeated copper exposure.

It is believed that the mechanism for the gastrointestinal effects of acute copper exposure may be due to stimulation of the vagus nerve by copper, which has been demonstrated in a number of studies in monkeys, dogs, and ferrets (ATSDR, 2004). Moreover, a number of animal studies support the gastrointestinal tract as a sensitive target of copper toxicity (*e.g.,* NTP 1993; Haywood 1980; Haywood and Comerford, 1980).

Chronic human oral exposure to copper has been associated mainly with liver effects, although there are some reports of effects on blood, kidney, brain and other organs (Gaetke and Chow, 2003). However, the primary target of chronic copper toxicity appears to be the liver. This is supported by a number of animal studies as well (WHO, 1998; ATSDR, 2004; CCME, 1997).

It is important to recognize though, that with the exception of several defined syndromes (*e.g.,* Wilson's disease, Indian childhood cirrhosis (ICC), idiopathic copper toxicosis (ICT)), liver effects are rarely reported in humans (ATSDR, 2004). These three syndromes in particular, have been most frequently associated with adverse effects of copper on the liver. However, there are a number of other disease conditions that are also associated with predisposing affected individuals to copper-induced hepatotoxicity. These various diseases and conditions are described in WHO (1998).

It is generally believed that Wilson's disease, Indian childhood cirrhosis, and possibly idiopathic copper toxicosis are all caused, at least in part, by an underlying genetic susceptibility to copper toxicity. Wilson's disease is an autosomal recessive genetic disorder with a worldwide occurrence of 1 in 30,000 (Scheinberg and Sternlieb, 1996). It is characterized by high levels of copper in the liver and low levels of serum ceruloplasmin. It is considered unlikely that the Wilson's disease is related to exposure to high levels of copper because reducing dietary copper intake does not prevent the development of the disease (Scheinberg and Sternlieb, 1996). ICC is a type of liver cirrhosis typically seen in infants and young children living in rural areas of the Indian subcontinent. This disease has a high rate of occurrence among susceptible blood relatives (Pandit and Bhave 1996; Tanner 1998). In families where a child has ICC, up to 22% of the siblings are also affected. To date, a causal relationship between high copper intake and ICC has not been firmly established, although ATSDR (2002) believes there is strong evidence to support

an association. Like Wilson's disease, ICT is believed to be caused by an autosomal-recessive inherited defect in copper metabolism, combined with excessive dietary copper exposure (Muller *et al.,* 1998). ICT is typically characterized by an early age of onset of clinical symptoms, followed by a rapid progression and death within 2 weeks to 11 months. It is common to find very high copper levels in the liver, and various abnormal biochemical markers in the liver such as altered activity and concentrations of aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time (ATSDR, 2004). There is also often marked evidence of panlobular and pericellular fibrosis associated with a mild inflammatory infiltrate, ballooning degeneration of hepatocytes, and an abundance of Mallory bodies. While there is suggestive evidence that environmental copper exposure is the causative agent of ICT, there is also evidence that an increased genetic susceptibility to copper toxicity may be the underlying cause (ATSDR, 2004). For example, Muller *et al*. (1996) provided suggestive evidence that the disease is transmitted in an autosomal recessive mode.

Further details on these conditions, as well as other genetic or pre-existing physiological conditions which predispose individuals to copper toxicity can be found in ATSDR (2004), NRC (2000) and WHO (1998).

Overall, the hepatotoxicity of copper has not been well studied in healthy humans that do not have a genetic susceptibility to copper (ATSDR, 2004). Olivares *et al*. (1998) identified a NOAEL of 0.315 mg Cu/kg bw/day for liver effects in infants exposed to copper sulphate in drinking water for nine months. In a cross-sectional study of infants consuming food made with tap water containing 0.8 to 4.2 mg Cu/L, Zietz *et al*. (2003) found no correlation between aminotransferase levels (indicators of liver cell injury) and copper concentration or exposure. Estimated daily copper intake of the infants was generally between 0 and 1.75 mg Cu/day, with one high outlier above 2.5 mg Cu/day. No symptoms of liver damage were found in any of the examined infants. An earlier study of seven adults receiving capsules containing 0.14 mg Cu/kg/day as copper gluconate, showed no significant alterations in serum aspartate aminotransferase, alkaline phosphatase, serum gamma glutamyl transferase, or lactate dehydrogenase activities (Pratt *et al.,* 1985). There were also no reported alterations in hematocrit level or mean corpuscular volume in these individuals.

These available human studies of copper effects on the liver suggest that liver effects are unlikely to occur at lower doses than gastrointestinal effects, following longer term oral exposures. Thus, gastrointestinal effects would be expected to be the most sensitive indicator of both acute and long term copper toxicity *via* the oral route.

A3-3.2.1.2 Inhalation Studies

In humans, copper is a known respiratory irritant. Workers exposed to copper dusts and various airborne copper salts report a number of symptoms that are consistent with upper respiratory tract irritation, including: eye, nose and throat irritation, coughing, sneezing, chest pain, and runny nose (Askergren and Mellgren, 1975; Suciu *et al.,* 1981; OEHHA, 1999). Other effects, such as metallic or sweet taste, and nausea have been reported in workers exposed to copper fumes at levels between 1 and 3 mg/m^3 for short periods of time (Whitman, 1962; 1957).

Occupational inhalation exposure to copper fumes, often in welding or smelting operations, has been reported to result in "metal fume fever", which is typically characterized by headache, dyspnea, dryness of the mouth and throat, chills, fever, nausea, myalgia, coughing, metallic taste in the mouth and muscle aches, and usually begins four to eight hours following exposure to various metal oxides, including copper oxide (OEHHA, 1999). Symptoms and signs of this condition cease within 24 to 36 hours post-exposure (ATSDR, 2004). Symptoms consistent with metal fume fever have been reported by workers exposed to airborne copper dust at concentrations of 0.03 to 0.12 mg/m^3 (Gleason, 1968). Armstrong *et al.* (1983) reported upper respiratory tract irritation, in conjunction with "metal fume fever" symptoms in factory workers exposed to copper fumes for one to 10 hours as a result of cutting pipes that contained copper.

Longer term occupational studies of human exposure to copper generally involved co-exposure to various other substances. This makes it difficult to assess the potential health effects of copper exposure in these studies. As such, the usefulness of the available data on vineyard sprayers, mining and smelting workers, and pulp and paper workers, in determining the effects of long term inhalation exposure to copper compounds, is greatly diminished (WHO, 1998).

Suciu *et al.* (1981) studied 75 to 100 workers who were involved in grinding and sieving copper dust, for a three year period. During the first year of operation, the workers were exposed to 434 mg Cu/m^3, which declined in subsequent years, such that by year three, the air concentration was 111 mg Cu/m^3. Lung radiographs revealed linear pulmonary fibrosis, and in some cases, nodulation. Other effects reported in this study included hepatomegaly, seven cases of enlargement of the sella turcica, nonsecretive hypophyseal adenoma, arterial hypertension, 'red facies', headache, vertigo, drowsiness, and impotence in 16% of workers. It must be recognized however that the significance of all findings in this study is difficult to interpret because a control group was not used. Furthermore, there were other limitations to this study including possible confounding due to exposure to arsenical compounds, lack of a dose-

response evaluation, inadequate reporting of study design, and no statistical analysis of the data (CCME, 1997).

Copper is widely believed to be the causal agent in the occupational disease known as "vineyard sprayer's lung". This disease condition is reported in vineyard workers spraying an anti-mildew agent containing that contains 1 to 2.5% copper sulfate neutralized with hydrated lime (ATSDR, 2004). The condition was first described in humans by Pimentel and Marquez (1969). However, the available data on this disease consists almost entirely of medical case reports with no concentration-response information. Commonly reported symptoms include intra-alveolar desquamation of macrophages, formation of histiocytic and noncaseating granulomas containing inclusions of copper, and healing of lesions in the form of fibrohyaline nodules, that is similar to those found in silicosis (Pimentel and Marquez, 1969; Plamenac *et al.,* 1985). Higher incidences of abnormal columnar cells, squamous metaplasia without atypia, copper containing macrophages, eosinophilia, and respiratory spirals were reported in the sputum of both smoking and non-smoking vineyard sprayers, while rural workers from the same geographic region, who did not work in the vineyards did not display these effects (Plamenac *et al.,* 1985).

A3-3.2.1.3 Dermal Studies

In some susceptible individuals, exposure to copper metal (such as in jewellery) has produced itching dermatitis (Saltzer and Wilson, 1968). Allergic contact dermatitis has also been observed in individuals following a patch test using a copper penny and/or a copper sulfate solution (Barranco, 1972; Saltzer and Wilson, 1968). However, in general, irritation or allergic skin reactions to copper appear to be rare in humans (CCME, 1997). In most studies reporting a dermal effect of copper, the exposure concentrations leading to an effect are typically poorly characterized (WHO, 1998). Furthermore, it is possible in some studies that there was contamination of the tested copper mixtures with nickel - a known contact allergen.

A3-3.2.1.4 Other Studies

High serum copper concentrations have been associated with an increased risk of coronary heart disease in some studies (Ford, 2000; Medeiros *et al.,* 1991; WHO, 1998). However, in a review of the available studies, WHO (1998) noted that causal interpretation of these data is difficult for the following reasons: i) cardiovascular disease might have affected serum copper levels; thus changes in serum copper may be a consequence rather than a cause of cardiovascular diseases; ii) the possibility that elevated serum copper levels are the result of preclinical disease could not be ruled out in these studies; and, iii) information on vitamin C, iron status and other nutrients that can alter serum copper levels were not provided in these

studies. Overall, the role of elevated serum copper in the etiology of cardiovascular disease remains a matter of controversy (WHO, 1998).

A3-3.2.2 Animal Studies

The major symptoms of acute copper intoxication in experimental animals include reduced body weights, hepatotoxicity, renal toxicity, and death (at high doses) (ATSDR, 2004; WHO, 1998; CCME, 1997). Acute toxicity data are limited to mostly oral studies. The liver and kidney appear to be the major target organs for acute copper toxicity in experimental animals. The subchronic and chronic toxicity of copper compounds has not been well characterized in experimental animals through any route of exposure (WHO, 1998). However, the limited data available suggests that gastrointestinal, liver and kidney effects may occur following subchronic or chronic copper exposure.

A3-3.2.2.1 Short-term Oral Studies

Acute oral LD_{50}'s in various experimental animals exposed to a variety of copper compounds range from 15 to 1,600 mg/kg body weight/day (CCME, 1997; WHO, 1998).

An early study (Boyden *et al.,* 1938) reported increased mortality in rats fed a diet containing 4,000 ppm of copper (equivalent to a dose of 133 mg Cu/kg/day), for one week. Deaths were associated with severe anorexia. Weanling rats exposed to 300 mg Cu/kg/day as Cu(II) in the diet died after two weeks exposure (Haywood, 1985). The deaths were attributed to extensive centrilobular necrosis in the liver.

A set of studies of short-term copper toxicity in rats and mice was reported by Hebert *et al.* (1993). In a 15-day feeding study in rats, in which oral doses of copper (II) sulfate pentahydrate were administered in the diet (up to 305 mg Cu/kg body weight per day), there were no clinical signs of toxicity, although weight gain was reduced at doses above 194 mg Cu/kg body weight per day. Upon histopathological examination, there were changes in the forestomach reported at doses above 45 mg Cu/kg body weight per day, kidney effects at doses above 93 mg Cu/kg body weight per day, and changes in the liver and bone marrow at doses above 194 mg Cu/kg body weight per day. A NOEL of 23 mg Cu/kg body weight per day was identified by the authors. In a similar study with mice, using the same dosing regimen, it was found that mice were less sensitive than rats to copper. Weight gain was only reduced in females at the highest tested dose of 781 mg Cu/kg body weight/day. The only effects observed upon histopathological examination of the liver, kidneys and forestomach were hyperplasia and hyperkeratosis in the forestomach at 197 mg Cu/kg body weight/day and higher (in males), or greater than or equal to 216 mg

Cu/kg body weight per day in females. NOEL's of 92 and 104 mg Cu/kg body weight/day were identified for male and female mice, respectively.

Forestomach effects in treated animals consist of irritation of the gastric mucosa, and have only been observed in rodent studies where copper(II) sulfate was administered in the diet (WHO, 1998). These effects have not been observed in drinking water studies. It is believed that these gastric mucosa irritation effects are due to the dissociation of copper sulphate in the stomach which forms sulfuric acid (WHO, 1998).

In similar studies that utilized drinking water instead of diet as the exposure medium, Hebert *et al.* (1993) reported various clinical signs of toxicity and deaths occurring at doses of 31 mg Cu/kg body weight per day. However, these effects were largely attributed to dehydration, as a result of the poor palatability of the drinking-water. Despite this confounding due to dehydration, the authors established a NOEL in females of 26 mg Cu/kg body weight per day for kidney effects, while in males there was histopathological evidence of kidney damage even at the lowest dose tested of 10 mg Cu/kg body weight per day. The authors also reported reduced water consumption, body weight, and various clinical signs of toxicity at doses of 58 to 62 mg Cu/kg body weight per day and higher in mice. However, as in rats, this result was also believed to have been confounded by dehydration of the treated animals, caused by an objectionable taste of the water and a refusal to drink.

NTP (1993) reported on two week toxicity studies of cupric sulfate pentahydrate in male and female F344/N rats and B6C3F_1 mice using the drinking water and dosed feed routes. The test animals were evaluated for hematology, clinical chemistry, urinalysis, reproductive toxicity, tissue metal accumulation, and histopathology. In the drinking water studies, groups of five rats and five mice per sex were administered cupric sulfate at concentrations ranging from 300 to 30,000 mg/L for 15 days. One female rat, one male mouse, and three female mice in the 3,000 ppm groups, as well as all rats and mice in the 10,000 and 30,000 ppm groups died before the end of this study. The surviving animals in the 3,000 ppm groups showed reduced weight gain or weight loss. It was noted that water consumption in the three highest dose groups of both species was reduced by more than 65%. Clinical signs in these groups were attributed to dehydration. The only gross or microscopic change that could be attributed to cupric sulfate was an increase in the size and number of cytoplasmic protein droplets in the epithelium of the renal proximal convoluted tubule in male rats within the 300 and 1,000 ppm groups. In the 2-week dosed feed studies, groups of five rats and five mice per sex were fed diets containing 1,000 to 16,000 mg/kg of cupric sulfate. No chemical-related deaths were reported in any dose group. Relative to controls, rats and

mice in the two highest dose groups showed reduced body weight gains which were attributed to decreased feed consumption. Hyperplasia with hyperkeratosis of the squamous epithelium on the limiting ridge of the forestomach was seen in rats and mice of both sexes. This lesion was more severe in rats than in mice. Inflammation of the liver was observed in rats in the 8,000 and 16,000 ppm groups. Depletion of hematopoietic cells was noted in rats of both sexes in bone marrow tissue (8,000 ppm and 16,000 ppm groups), and the spleen (16,000 ppm group). The kidneys of both male and female rats in the 4,000, 8,000, and 16,000 ppm groups showed an increased number and size of protein droplets in the epithelia of the renal cortical tubules.

A3-3.2.2.2 Short-term Inhalation Studies

An LC_{50} value for inhalation exposure of rabbits to copper (II) hydroxide (physical form and duration were not specified) was reported to be >1,303 mg Cu/m^3 (Tomlin, 1994).

Guinea-pigs exposed to copper (II) oxide aerosol at 1.6 mg/m^3 (median diameter of approximately 0.03 µm) for 1 h showed significant reductions in tidal volume, minute volume and lung compliance, both during and after exposure (Chen *et al.,* 1991).

Drummond *et al.* (1986) reported mild respiratory effects in hamsters and mice exposed to airborne copper sulfate. In hamsters, decreased cilia beating was observed at 3.3 mg Cu/m^3 for three hours. In mice, alveolar thickening was observed at 0.12 mg Cu/m^3 for three hours/day, five days/week for one to two weeks (Drummond *et al.,* 1986). The severity of this effect increased with the duration of exposure. These authors also conducted an immunological challenge study in copper sulphate-exposed mice. Increased mortality and decreased survival time were observed when mice were challenged by an aerosol of *Streptococcus zooepidemicus* following inhalation exposure to 0.56 mg Cu/m^3 for three hours, or 0.13 mg Cu/m^3, for three hours/day, five days/week for two weeks (Drummond *et al.,* 1986). There was also evidence of decreased bactericidal activity in mice exposed to 3.3 mg Cu/m^3, for three hours to *S. zooepidemicus*, or 0.12 mg Cu/m^3 for three hours/day, five days/week for two weeks following exposure to an aerosol of *Klebsiella pneumonia* (Drummond *et al.,* 1986).

Another immunological challenge study was conducted by Skornik and Brain (1983). These authors investigated the effects of copper sulfate aerosols on respiratory defense mechanisms in male hamsters. Pulmonary macrophage phagocytic rates were measured by determining the *in vivo* uptake of radioactive colloidal gold one, 24, or 48 hours after a single four hour inhalation exposure to 0, 0.3, 3.2, 4.0, 5.8 and 7.1 mg Cu/m^3. It was found that when hamsters were exposed for four hours to greater than or equal to

3.2 mg Cu/m^3, macrophage endocytosis was significantly reduced (relative to controls) by one h after exposure. This effect was dose-dependent. By 24 hour post-exposure, the percent of gold ingested by pulmonary macrophages remained depressed but to a lesser degree than at one hour post-exposure. By 48 hours post-exposure, the rate of macrophage endocytosis in hamsters returned to control levels except in those animals exposed to 3.2 and 5.8 mg Cu/m^3. The authors identified a NOEL of 0.3 mg Cu/m^3.

In rabbits exposed to 0.6 mg Cu/m^3 as copper chloride for six hours/day, five days/week for four to six weeks, the only reported histological alteration in the lungs was a small increase in alveolar type II cell volume density (Johansson *et al.,* 1984). The authors did not consider this an adverse effect.

A3-3.2.2.3 Short-term Dermal Studies

A number of studies of skin irritation in experimental animals have found copper compounds to generally be non-irritating (CCME, 1997; ATSDR, 2004). Dermal LD_{50} values for copper compounds are limited but indicate a low dermal toxicity. For example, an LD_{50} value of >1,124 mg/kg/day in rats was reported for copper (II) oxysulphate, while a dermal LD_{50} of >2,058 mg/kg/day was reported in rabbits for copper (II) hydroxide (NIOSH, 1993; Tomlin, 1994).

A3-3.2.3.4 Subchronic or Chronic Oral Studies

Liu and Mederios (1986) reported a significant increase in hemoglobin levels, and systolic blood pressure in rats exposed to 14 mg Cu/kg/day as copper carbonate in the diet, for 15 to 20 weeks. Other studies reported decreased hemoglobin levels in animals exposed to higher copper doses. For example, hemoglobin levels decreased in rats dosed with greater than 66 mg Cu/kg/day for 20 to 90 days (*e.g.,* Kumar and Sharma 1987; NTP, 1993), and in pigs exposed to greater than 24 mg Cu/kg/day for a period of 48 to 54 days (Suttle and Mills, 1966a,b; Kline *et al.,* 1971).

NTP (1993) reported on 13 week toxicity studies of cupric sulfate pentahydrate that were conducted in male and female F344/N rats and $B6C3F_1$ mice by the dosed feed route. Animals were evaluated for hematology, clinical chemistry, urinalysis, reproductive toxicity, tissue metal accumulation, and histopathology. Groups of 10 rats per sex received diets containing 500 to 8,000 ppm cupric sulfate, and groups of 10 mice per sex received diets containing 1,000 to 16,000 ppm cupric sulfate for 92 days. The oral dose estimates ranged from 32 to 551 mg/kg per day for rats, and 173 to 4157 mg/kg per day for mice. There were no chemical-related deaths observed in either rats or mice, and no obvious clinical signs of cupric sulfate toxicity were noted. The terminal mean body weights were lower (relative to controls)

for both rats and mice in the 4,000 ppm or greater groups. Mice showed a dose-related decrease in liver weights. The hematological, clinical chemistry and urinalysis investigations of rats showed variable changes that were generally restricted to the 4,000 ppm and 8,000 ppm groups. Increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in male and female rats indicated hepatocellular damage. Males also had increases in 5'-nucleotidase and bile salts. Rats also showed decreases in mean cell volume, hematocrit, and hemoglobin, which suggested development of a microcytic anemia. However, simultaneous increases in reticulocyte numbers suggested a compensatory response to the anemia by bone marrow tissue. Increased urinary glucose, N-acetyl-ß-D-glucosaminidase and aspartate aminotransferase suggested renal tubule epithelial damage. There were dose-related increases in copper in all male rat tissues examined (liver, kidney, plasma, and testis). These increases in copper were accompanied by increased zinc in the liver and kidney. Plasma calcium levels were significantly reduced in the 4,000 and 8,000 ppm groups. In the 8,000 ppm group, plasma magnesium was significantly increased relative to controls. In the 4,000, 8,000 and 16,000 ppm groups, the rats displayed hyperplasia and hyperkeratosis of the forestomach, inflammation of the liver, and an increased number and size of protein droplets in the epithelial cytoplasm and the lumina of the proximal convoluted tubules. These effects were similar to those seen in the two-week dosed feed study. Both the incidence and severity of these lesions were dose related. Transmission electron microscopy of the livers of rats of both sexes showed increases in the number of secondary lysosomes in hepatocytes of the periportal area.

In male and female mice within the 4,000 ppm or higher dose groups, there was a dose-related increase in hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach. Only minimal positive staining for copper was present in the liver and was limited to occurrence in the high-dose (16,000 ppm) male and female groups. In rats, the NOAEL for kidney effects was 1,000 ppm (in feed) for males and 500 ppm for females, while the NOAEL for liver inflammation was 1,000 ppm for males and 2,000 ppm for females. The NOAEL for forestomach effects was identified to be 1,000 ppm. Mice were much less sensitive than rats to cupric sulfate. The primary target tissue in mice was the epithelium of the limiting ridge of the forestomach. The NOAEL for forestomach hyperplasia and hyperkeratosis in mice was 2,000 ppm cupric sulfate in the feed.

In a 90 day study with rats and mice reported by Hebert *et al.* (1993), copper (II) sulfate pentahydrate was administered in the feed up to a dose of 138 mg Cu/kg body weight per day in rats, and 1,000 mg Cu/kg body weight per day in mice. In rats of both sexes, there were no overt signs of toxicity other than a dose-related reduction in growth. This growth reduction was statistically significant in male and female rats at 67 and 138 mg Cu/kg body weight per day, respectively, and in male and female mice at 97 and 267 mg

Cu/kg body weight per day). These dose levels were identified as LOAELs by the authors. NOAELs (based on growth) for mice were identified to be 44 and 126 mg Cu/kg body weight/day in males and females respectively. Upon histopathological examination, there was hyperplasia and hyperkeratosis in the forestomach in both rats and mice (starting at 34 mg Cu/kg body weight per day in rats and 187 to 267 mg Cu/kg body weight per day in mice). There were also liver and kidney effects in the rats only (at doses at and above 67 mg Cu/kg body weight per day). The liver and kidney effects consisted of inflammation of the liver and degeneration of the kidney tubule epithelium. Rats also displayed reduced spleen iron levels, and haematological changes indicative of microcytic anaemia at doses of 34 mg Cu/kg body weight per day and higher. The NOEL in rats was reported to be 17 mg Cu/kg body weight per day.

Rats fed on a high copper diet (500 mg/kg) had liver damage in the form of multi-focal hepatitis, often surrounding necrotic foci, after 3, 6 and twelve months exposure. No body weight effects or reduced food consumption was observed (Aburto *et al.,* 2001).

Based on a review of a number of studies investigating copper-induced liver toxicity in rats, ATSDR (2002) has suggested that there are three phases of copper toxicity in the rat.

In the first phase, copper levels increase in the liver, with minimal to no damage to hepatic tissues. As the liver concentrations increase, inflammation and necrosis occurs. Following this, copper levels in the liver begin to decrease and the parenchymal tissue begins to regenerate. At this particular point, the animal develops a tolerance to copper. This was demonstrated in a study which found that after a 15 week exposure to 320 mg Cu/kg/day, a subsequent 3-week exposure to 640 mg Cu/kg/day did not cause adverse effects (Haywood and Loughran, 1985). This contrasts the severe hepatocellular necrosis observed in rats on a control diet for 15 weeks, followed by a three week exposure to 640 mg Cu/kg/day (Haywood and Loughran 1985). At exposures to 550 mg Cu/kg/day, regeneration of the parenchymal tissue ceased to occur and rats develop chronic hepatitis (Haywood, 1985; Haywood and Loughran, 1985).

It appears that the renal toxicity of copper in rats also has a predictable pattern (Haywood 1980, 1985; Haywood *et al.,* 1985a,b). For example, no treatment-related effects were observed in rats exposed to 300 mg Cu/kg/day (as dietary copper sulfate) for one to two weeks. However, eosinophilic droplets were observed in the epithelial cell cytoplasm of the proximal convoluted tubules in rats exposed to 450 mg Cu/kg/day for two weeks. The number of these droplets increased with increasing exposure duration. Exposure to 100 to 280 mg Cu/kg/day for three to five weeks produced necrosis and degeneration of proximal tubule cells. By 9 weeks exposure, extensive desquamation of the epithelial cells of the

proximal convoluted tubules was noted in rats exposed to 180 mg Cu/kg/day. There is conflicting evidence regarding the degree to which regeneration of the proximal tubules may occur over the course of dietary copper exposure (ATSDR, 2004).

Two drinking water studies focusing on immunological endpoints were conducted in mice exposed to several concentrations of copper sulfate for 8 weeks (Pocino *et al.,* 1990), or copper chloride for periods of three to five, or eight to 10 weeks (Pocino *et al.,* 1991). The mice underwent several tests to assess immune function, including: *in vitro* lymphoproliferative responses to *Escherichia coli* lipopolysaccharide (LPS), and concanavalin A (Con A); induction and evaluation of antibody response to sheep red blood cells, evaluation of autoantibody production; and induction and elicitation of delayed-type hypersensitivity response (Pocino *et al.,* 1991 study only). At the lowest tested dose (13 mg Cu/kg/day as copper chloride), there was impaired cellular immunity (indicated by reduced proliferative response to LPS), and impaired humoral immunity (indicated by decreased autoantibody production). Impaired performance on the other immune function tests was observed at doses equal to or greater than 26 mg Cu/kg/day as copper chloride, and 24 mg Cu/kg/day as copper sulfate.

Massie and Aiello (1984) reported a 15% decrease (relative to controls) in the lifespan of mice exposed to 42.5 mg Cu/kg/day as copper gluconate in drinking water over their lifetime. It was also found that a dose of 4.2 mg/kg/day did not significantly affect lifespan, although it did result in reduced body weights relative to controls.

A3-3.3 Reproductive/Developmental Toxicity

A3-3.3.1 Human Studies

There is extremely limited data available regarding reproductive or developmental effects in humans following inhalation, oral or dermal exposure to copper.

Aschengrau *et al.* (1989) reported no association between the risk of spontaneous abortion and copper exposure in a population of Massachusetts women exposed to drinking water concentrations of >1 mg Cu/L from 1976 to 1978.

Copper is well known to impair sperm mobility in various mammals (including humans) (*e.g.*, Holland and White, 1980; 1982; Simonik *et al.,* 1990). In fact, copper is used in intrauterine birth control devices (IUDs) to exploit this spermatotoxic effect (CCME, 1997). While some researchers have expressed

concerns over the possible transfer of copper to the fetus, if pregnancy occurs during IUD use, there have been no reports of teratogenic effects associated with the use of IUDs that contain copper (CCME, 1997).

A3-3.3.2 Animal Studies

There is some limited evidence suggesting that exposure to copper compounds can affect reproduction/development in experimental animals.

Aulerich *et al.* (1982) reported that reproductive performance, as assessed by the length of gestation, number of kits whelped, and average kit weight, was not adversely affected in mink fed a diet containing up to 12 mg Cu/kg/day as copper sulfate. There were also no statistically significant alterations in newborn mortality rates, relative to controls, although a slight trend towards increased kit mortality between birth and 4 weeks of age in the offspring of mink exposed to 6 or 13 mg Cu/kg/day was noted. The incidence of kit mortality was 9, 12, 19, 38, and 32% in the 1, 6, 3, 6, and 13 mg Cu/kg/day groups, respectively.

In the NTP (1993) study, cupric sulfate produced no adverse effects on any of the reproductive parameters measured in male or female rats or mice. The rats and mice in this study were exposed to copper-dosed feed containing 500 to 16,000 mg Cu/kg diet.

Haddad *et al.* (1991) reported delayed growth and development in the offspring of rats exposed to 130 mg Cu/kg/day as copper sulfate in the diet for seven weeks prior to mating, and during the gestation period. Specific effects observed included significant decreases in mean somite number, crown-rump length, and yolk sac diameter in 11.5 day old embryos. In 21.5 day old fetuses and newborns, there was delayed ossification in the cervical and cauda vertebrae, sternum, metacarpals, forelimb phalanges, metatarsals, and hindlimb phalanges.

Lecyk (1980) exposed mouse dams to doses ranging from 26 to 208 mg Cu/kg/day as copper sulfate in the diet. At 208 mg/kg/day, there was a decreased mean litter size and decreased fetal body weights. At 26 and 52 mg/kg/day, there was stimulation of embryonic development. Doses of 78 mg/kg/day and greater caused an increase in foetal mortality, a decrease in fetal body weight and skeletal malformations. Control mice showed no reproductive or developmental abnormalities. It is unclear if maternal toxicity may have contributed to the observe effects as maternal toxicity was not documented in the study. A developmental NOAEL of 52 mg/kg body weight/day was identified from this study.

Llewellyn *et al.* (1985) reported that an oral dose of 130 mg/kg body weight/day (as copper acetate) produced significantly increased testis weights in exposed rats.

In a study with rams, Gamcik *et al.* (1990) found that 15 mg/kg body weight/day of either copper oxide or copper sulphide, administered in feed for 50 days, reduced sperm activity, and caused sperm malformations by 40 days (copper oxide). The same effects were noted by 50 days for copper sulphide. By day 50, various clinical signs of copper intoxication were evident (*e.g.*, reduced body weight, staggers, reduced libido *etc.*). The sperm effects did not occur in control animals, nor were the other clinical signs of toxicity evident. Liver, but not testes copper concentrations were significantly elevated in treated versus control animals at the end of the experiment. Copper is well known to impair sperm mobility in various mammals (Simonik *et al.,* 1990; CCME, 1997).

A3-3.4 Carcinogenicity

A3-3.4.1 Human Studies

There is presently no evidence to suggest that copper compounds are carcinogenic in humans (WHO, 1998; ATSDR, 2004; U.S. EPA, 2004; TERA, 2004).

While there are a number of occupational epidemiology studies that have examined cancer risk among copper smelter workers, the excess cancer risks reported in these studies has not been attributed to copper exposure; rather, the risks are most frequently attributed to arsenic exposure. In a large historical prospective study of 3,550 men working for at least 1 year in the tank house of nine copper refineries in the USA (where copper is the major substance workers are exposed to), there was no statistically significant evidence of an increased risk of cancer (Logue *et al.,* 1982).

Recently, Theophanides and Asastassopoulou (2002) presented a scheme in which above normal copper concentrations may induce growth proliferation and cancer through oxidative damage to DNA with hydroxyl radicals produced from conversions between Cu^{+} and Cu^{2+} ions. However, there remain no studies in the literature that demonstrate any association between human exposure to copper and increased risk of cancer.

A3-3.4.2 Animal Studies

The available studies investigating the carcinogenicity of copper compounds laboratory rodents give no indication that inorganic copper salts are carcinogenic. However, the short durations of these studies, the

low levels of exposure, the small sample sizes used the limited extent of histopathological examination, and inadequate reporting of methods and results limits the conclusions that can be reached from these studies (WHO, 1998). Furthermore, there are no animal cancer studies in the literature that have investigated inhalation exposures to copper compounds (ATSDR, 2004). Thus, the available animal data is widely considered inadequate to determine whether or not copper compounds possess carcinogenic activity. It is interesting to note that a number of studies have reported that the administration of copper compounds either inhibited the development of tumours induced by well-known carcinogens, such as dimethylnitrosamine and *p*-dimethylaminoazobenzene, or increased the length of the latency period (WHO, 1998).

There are no data available on the genotoxicity of copper in humans exposed *via* oral, inhalation or dermal routes. The existing genotoxicity database suggests that copper is a clastogenic agent, and some studies have shown that exposure to copper can result in DNA damage; however, point mutation assay results are mixed and inconclusive (ATSDR, 2004). Overall, the database on mutagenicity and genotoxicity of copper compounds is limited and equivocal, and considerably more research is required to determine whether or not copper is mutagenic and/or genotoxic to mammals (including humans) *in vivo*.

A3-4.0 EXPOSURE LIMITS

The following organizations were consulted to select exposure limits for copper: Health Canada; the U.S. EPA; ATSDR; WHO; MOE; JECFA; Health and Welfare Canada; RIVM; and, the National Academy of Science.

A number of regulatory agencies have derived health-based exposure limits for copper compounds, as described below. It should be noted that current MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits). Exposure limits used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, ATSDR, World Health Organization (WHO), and the MOE itself.

A3-4.1 Oral Exposure Limits

Identified regulatory oral exposure limits for copper compounds are described below.

JECFA (1982) derived a provisional maximum tolerable daily intake (PMTDI) for copper of 0.05 to 0.5 mg/kg body weight/day, which was set equivalent to the provisional daily dietary requirement, rather than an intake associated with an adverse health effect. This value remains as the current PMTDI recommended by JECFA. Health Canada (1996) reported a provisional tolerable daily intake (PTDI) of 0.05 to 0.5 mg/kg body weight/day for copper that is based on this JECFA PMTDI value.

Health and Welfare Canada (HWC, 1990) had estimated safe and adequate dietary copper requirements for a few age classes based on mass balance studies. These safe and adequate requirements were used as the basis for deriving conservative TDIs by Health Canada. For children aged three to 10 years, it was determined that 0.05 to 0.1 mg/kg body weight/day was the safe and adequate range for daily copper intake. For adults, 0.03 mg/kg body weight/day was estimated as the safe and adequate copper intake rate. These values were used by Health Canada in the derivation of human health soil quality guidelines for copper. Health Canada (2003a) reports an oral TDI of 0.03 mg/kg body weight/day that is based on the adult TDI originally recommended by HWC (1990). In the derivation of the Guideline for "Use at Contaminated Sites in Ontario" (MOE, 1996), MOE utilized an oral RfD of 60 µg/kg/day for cobalt.

The U.S. EPA IRIS database contains no oral exposure limits for copper compounds (EPA, 1988). U.S. EPA Region III (2004), Region VI (2004) and Region IX (2003) all report an oral RfD of around 0.04 mg/kg/d, which was originally derived by the U.S. EPA for preparation of the Health Effects Assessment

Summary Tables (HEAST). The source of this oral RfD of 0.04 mg/kg body weight/day is as follows. The U.S. EPA (1987) developed a drinking water criterion of 1.3 mg copper/L, based on adverse effects such as vomiting, nausea, and diarrhea in humans following acute consumption of copper in drinking water (as reported in studies by Wyllie, 1957; Semple *et al.,* 1960; Chuttani *et al.,* 1965). From this drinking water value, U.S. EPA (HEAST) estimated an oral RfD of 0.04 mg/kg/day [(1.3 mg/L x 2 L/d) / 70 kg] (ORNL, 2004).

ATSDR (2002) has developed an acute duration oral Minimal Risk Level (MRL) for copper of 0.02 mg/kg/day. This MRL has also been adopted by ATSDR as the intermediate duration MRL. ATSDR (2002) considers that available data are inadequate to derive a chronic duration oral MRL. The acute-duration oral MRL is based on gastrointestinal effects reported in the Pizarro *et al.* (1999) study. To estimate total copper exposure, the concentration of copper in the drinking water (0.0272 mg Cu/kg/day) in this study was added to the reported average dietary copper intake of copper (0.0266 mg Cu/kg/day). This yielded a total copper exposure level of 0.0538 mg Cu/kg/day, which was considered a NOAEL for gastrointestinal effects. The NOAEL was then divided by an uncertainty factor of three (to account for inter-human variability) to yield the acute oral MRL. ATSDR (2002) notes that this MRL accounts for dietary exposure as well as environmental contamination.

RIVM (Baars *et al.,* 2001) noted that copper is an essential nutrient, with a minimum daily requirement of 0.02 to 0.08 mg/kg-day (as reported by WHO, 1996). It was determined that a TDI for copper cannot be lower than the levels required for nutrition essentiality. Thus, RIVM based a TDI on the typical daily intake of the population which was shown to be 0.02 to 0.03 mg/kg/day on average, with a range of 0.003 to 0.1 mg/kg/day and an upper limit of 0.14 mg/kg/day (Slooff *et al.,* 1989). This latter upper limit daily intake value (0.14 mg/kg-day) was selected as the TDI by RIVM.

The National Academy of Science (IOM, 2000) has derived an acceptable Upper Intake Level (UL) based on the NOAEL of 10 mg/day based on Pratt *et al*. (1985). IOM (2000) considered this NOAEL to be protective of the general population and felt that no further uncertainty factor was warranted. This decision is supported by the large database of human information indicating no adverse effects in the 10 to 12 mg/day and a paucity of observed liver effects from copper exposure in humans with normal copper homeostasis. This NOAEL results in an acceptable upper limit of approximately 140 µg/kg/day for adults (10 mg/day ÷ 70 kg). There was insufficient data to establish unique ULs for any other age group (similar sensitivity for all ages) (IOM, 2000). Health Canada has indicated that in 2005/2006, the agency will

officially be using adopt ULs as toxicity reference values for all essential elements (Roest and Petrovic, Health Canada, Personal Communication) for contaminated sites human health risk assessments.

It is important to recognize that all available regulatory oral exposure limit values for copper are similar in magnitude, and are based on either typical daily intakes, or intakes associated with gastrointestinal distress. Copper doses at, or below any of these values would not be expected to result in adverse health effects under conditions of continuous lifetime daily exposure. It is also important to recognize that all oral exposure limits, regardless of their basis, lie within the range of typical estimated daily dietary intakes, and/or recommended nutritional requirements when the body weight of various human age classes is taken into account (*e.g.,* if a 70 kg adult is assumed, the Health Canada TDI of 0.03 mg/kg body weight/day equates to a daily intake of 2.1 mg Cu/day).

Health Canada (2003b) and the U.S. EPA (2003) have set aesthetic objectives for copper in drinking water of 1.0 mg/L. Aesthetic objectives do not have a toxicological basis, but are established based on objectionable taste, colour and/or staining characteristics. The MOE (2003) has adopted the Health Canada aesthetic objective for copper in drinking water.

For the purposes of this risk assessment an oral RfD of 140 µg/kg/day was selected (IOM, 2001, Health Canada, 2005).

A3-4.2 Inhalation Exposure Limits

Identified regulatory inhalation exposure limits for copper compounds are described below.

OEHHA (1999) derived an acute reference exposure level (REL) for a 1-hour exposure of 0.1 mg/m^3. This acute REL is considered protective against mild adverse effects. The REL was derived based on studies by Gleason (1968), and Whitman (1957; 1962) which investigated metal fume fever in workers. A NOAEL of 1 mg/m^3 was identified from these studies. The NOAEL was mainly based on the report of Whitman (1957) indicating that exposure to copper dust was detectable by taste, but that no other symptoms occurred following exposure to 1 to 3 mg/m^3 for an unspecified short duration. Given that the exposure duration was not clearly stated in these studies, no extrapolation to a one hour concentration could be conducted. Rather, the NOAEL was assumed to be applicable to a one hour exposure. A cumulative uncertainty factor of 10 was applied to the NOAEL (for intraspecies uncertainty) to yield the acute REL. Given the limitations of the existing data, OEHHA suggests that re-evaluation of the acute

REL for copper be conducted when better methods or data are available. OEHHA did not derive a chronic REL for inhalation exposure to copper.

The MOE (2001) reports a 24-hr Ambient Air Quality Criterion (AAQC) of 50 µg/m^3 for copper, based on health concerns. No supporting rationale for this AAQC was identified in available MOE publications.

RIVM derived a tolerable concentration in air (TCA) of 0.001 mg/m^3 based on a NOAEC of 0.6 mg/m^3 for lung and immune system effects in rabbits from a short-term toxicity study by Johansson *et al.* (1984). RIVM used an uncertainty factor of 100 (10 each for intra- and interspecies variability), and adjusted for continuous exposure (5/7 x 6/24) to yield the TCA.

ATSDR (2002) considers available data on the toxicity of inhaled copper inadequate for derivation of acute, intermediate, or chronic duration inhalation MRLs.

The U.S. EPA has not derived inhalation exposure limits for any copper compound (EPA, 1988).

For the purposes of this risk assessment an inhalation TCA of 1 µg/m^3 derived by RIVM (Baars *et al*., 2001) was selected.

A3-4.3 Dermal Exposure Limits

No regulatory dermal exposure limits for copper compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF_{dermal}). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF_{dermal} = relative absorption factor for dermal exposure (%).

AF_{dermal} = the fraction of the applied chemical absorbed through the skin.

AF_{oral} = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

A3-4.4 Criteria Summary

Table A3.2 summarizes the exposure limit selected for the current study.

Table A3.2 Summary of Toxicological Criteria Selected For The HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	140 µg/kg/day	UL	liver damage	Pratt *et al.,* 1985	IOM, 2000; Health Canada, 2005
Inhalation	1 µg/m^3	TCA	subchronic NOAEC (respiratory and immunological effects) in rabbits	Johansson *et al.,* 1984	RIVM (Baars *et al.,* 2001)
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

NOTES:
NA = not available
TCA = tolerable concentration in air; UL = Upper Intake Level.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

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Sudbury Area Risk Assessment
Volume II

Appendix A4:

Detailed Toxicological Profile of Lead

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A4: DETAILED TOXICOLOGICAL PROFILE OF LEAD

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APPENDIX A4: DETAILED TOXICOLOGICAL PROFILE OF LEAD

SUMMARY

The purpose of this toxicological profile of lead is to: i) outline the most relevant toxicological and epidemiological information on this substance; ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the lead exposure limits selected for use in the human health risk assessment of the Sudbury area. The toxicological review of lead is based primarily on secondary sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for lead. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Lead

Lead is a naturally occurring metallic element and occurs in a variety of minerals, often in close association with zinc (CCME, 1999). Common lead-containing minerals include galena (lead sulphide), anglesite (lead sulphate), and cerussite (lead carbonate) (Schoof, 2003). While most of the lead found in the environment is the result of anthropogenic activities, there are significant natural sources as well, including volcanoes, forest fires, sea spray, and weathering of lead-containing minerals (Environment Canada, 1996).

Lead is persistent in both water and soil. The different forms of lead in the environment are governed by factors such as temperature, pH, and the presence of humic materials. Elemental lead occurs rarely in the ambient environment; rather, the most common form of lead in the environment is Pb^{2+}. Lead is largely associated with suspended solids and sediments in aquatic systems, and it occurs in relatively immobile forms in soil. In aerobic soils, lead compounds undergo weathering and may become more stable over time (the most prevalent lead compounds in aerobic soils tend to be lead carbonates, hydroxides, or carbonate-hydroxide complexes (Leita and De Nobili, 1991; Lindsay, 1979)). In anaerobic soils, much of the sulphate in soil is reduced to sulphide, thus the predominant form of lead in these soils is lead sulphide - a highly stable, insoluble and relatively non-reactive lead species. There is no conclusive evidence as to which forms of lead in soil have the greatest relative bioavailability to organisms, as this is influenced greatly by pH, cation exchange capacity, the presence of ligands that can bind lead, and soil

organic matter content. It is generally believed however, that the amount of free Pb^{2+} in solution best represents what is actually bioavailable to organisms (CCME, 2002).

Particulate-bound lead emitted from mining operations, smelters, and combustion sources occurs primarily in the form of lead-sulfur compounds such as $PbSO_4$, $PbO{\cdot}PbSO_4$, and PbS (U.S. EPA, 1986). In the ambient atmosphere, lead exists primarily in the form of particulate-bound $PbSO_4$ and $PbCO_3$, and is deposited onto soil and water surfaces in this form (ATSDR, 2007).

Absorption

The gastrointestinal absorption of lead varies depending on a number of factors including speciation, solubility, particle size (if lead is in a matrix like food or soil), the exposure medium (*e.g.,* food, water, soil), and the age and physiological state of the exposed individual (*e.g.,* fasting or fed, nutritional status – particularly with respect to calcium and iron).

It has been suggested that the absolute oral bioavailability of soluble lead in human children is 50% (Alexander *et al*., 1974; Ziegler *et al*., 1978). Absolute oral bioavailabilities for soluble lead are lower in adults. Commonly reported estimates of oral lead bioavailability from the diet are 7 to 15% for adults, and 40 to 53% in infants and children (Schoof, 2003).

Many studies have investigated the oral bioavailability of lead, when ingested in a soil matrix. It is well established that the highest lead concentrations occur in the smaller particle size ranges in soils. This has important implications for lead exposure in young children as it is the smaller particles (*i.e.,* <100 µm in diameter) that are most readily ingested, inhaled and that adhere most readily to skin surfaces. In general, the available animal studies have indicated that absorption of lead from soil varies depending on the source of the lead, ranging from near zero to greater than 50% absolute bioavailability (Schoof, 2003). Several studies have demonstrated that the gastrointestinal absorption of lead in a soil matrix is lower than for dissolved lead (Freeman *et al*., 1992; Freeman *et al*., 1994; Casteel *et al*., 1997; U.S. EPA 1996a,b,c).

In the U.S. EPA IEUBK model (U.S. EPA, 1994a,b; 2002c), it is assumed that 50% of an oral dose of lead is absorbed from food or water, while 30% of lead in soil is assumed to be absorbed in the gastrointestinal tract. Thus, the default assumption for relative oral bioavailability for lead in a soil matrix, compared to soluble forms of lead in food or water is 60% [30 / 50%] (U.S. EPA, 1994a,b; 2002c). This value compares well to what has generally been observed in soil bioavailability studies with

rats and swine (Schoof, 2003). On average, the results of these studies support the use of a default assumption that 30% of an oral lead dose is absorbed from soil (*i.e.,* relative oral bioavailability of 0.6).

As airborne lead is bound to particulate matter, the absorption of lead in the lungs first depends on the deposition of particles within the respiratory tract. The rate of deposition of particulate airborne lead in the lower respiratory tract of adult humans is approximately 30 to 50%; however, this can vary substantially depending on particle size and ventilation rate (U.S. EPA, 1986). Only particles less than 250 um in mass mean aerodynamic diameter would be expected to deposit in the lower respiratory tract. The World Health Organization (WHO, 1984) estimated that approximately 20 to 60% of the total amount of lead inhaled is deposited in the lung, with the majority of this amount being absorbed. Others have also reported that once deposited in the lower respiratory tract, particulate lead is almost completely absorbed (U.S. EPA, 1986; Morrow *et al*., 1980). Children inhale comparatively more lead than adults because of a number of modifying factors, including differences in metabolic rates and airway dimensions (ATSDR, 2007).

Limited information is available regarding the dermal absorption of lead in humans. Dermal absorption of inorganic lead compounds is widely reported to be much lower than absorption by inhalation or oral routes of exposure. The extent of dermal absorption is dependant on the form of lead. Skin absorption of lead acetate was estimated to approach 0.06% while up to 8% absorption was reported for the organo-lead compound, tetraethyl lead (Moore *et al*., 1980).

Essentiality

Lead is not known to be an essential micronutrient in humans or other mammals.

Toxicology of Lead

The toxic effects of lead in humans are widely believed to be the same regardless of the route of entry, and are correlated to PbB in the vast majority of studies (ATSDR, 2007). Thus, to be consistent with the majority of the available literature on lead toxicology and epidemiology, the following toxicological summary focuses on effects that are associated with, or correlated with PbB concentrations. It is important to recognize that PbB levels reflect recent lead exposure as well as historical lead exposures that have redistributed in the blood through hematopoiesis (blood cell synthesis) in bone marrow (ATSDR, 2007). PbB concentrations are also difficult to relate to exposure doses (*i.e.,* mg/kg/day) as occupationally exposed populations are primarily exposed to lead through inhalation (with some lead

ingestion), whereas the general population (particularly children) is exposed to lead primarily *via* the oral route (with some lead inhalation).

The effects from chronic exposure to lead on humans and experimental animals are primarily neurological, renal, hematological, reproductive, and developmental (ATSDR, 2007; CDC, 1991). Well-characterized human health effects include neurotoxicity and renal effects, which can be severe at blood lead levels greater than 120 µg/dL (U.S. EPA, 1986). Numerous epidemiological studies have reported associations between blood and bone lead levels and cardiovascular effects on adults; however, there is debate as to whether there is a causal relationship (ATSDR, 2007; U.S. EPA, 2006, 2007). Anemia is also commonly reported, resulting from reduced hemoglobin production and damage to erythrocytes. A PbB concentration of 40 µg/dL in children is associated with heme synthesis impairment and other effects contributing to clinial symptoms of anemia (Schwartz et al., 1990; U.S. EPA, 2007). A 10% probability of anemia (hematocrit <35%) was associated with 20 µg/dL PbB at one year of age (Schwartz *et al.* 1990). Severe lead exposure in children (PbB above 380 µg/dL) can cause coma, convulsions, and even death. Clinical signs of lead toxicity, generally manifested as neurotoxicity and anemia, are evident at PbB levels of 70 µg/dL and greater (IARC, 2004). The most commonly reported and well-studied effects of environmental lead exposure are adverse effects on neurological function and neurobehavioural development in children, and reduced growth rate. However, it remains unclear if lead causes such effects in adults since extensive research on this topic has not been conducted (U.S. EPA, 2004; 2006). Decreases in cognition in older adults may occur but is not associated to PbB levels but rather to increased bone Pb levels indicating that cumulative exposure may be an important factor (U.S. EPA, 2006). Also, effects occurring as a result of occupational exposure can include but are not limited to: fatigue, irritability, inability to concentrate, as well as effects on motor speed, dexterity, sensory impairment and amytrophic lateral sclerosis (U.S. EPA, 2006). The effects in children often manifest as decreased IQ and memory, decreased gestation period, retarded growth rate, and are typically accompanied by blood enzyme level changes, and may occur at blood lead levels so low as to be essentially without a clearly discernible threshold for effects (U.S. EPA, 2004). The U.S. EPA (2004) also notes that many health effects associated with environmental lead exposure occur without overt signs of toxicity, and can manifest themselves over a relatively short period of time (before the usual term of chronic exposure can occur).

New studies demonstrate associations between neurological, haematological, immune, cardiovascular, and renal effects and PbB levels ≤10 µg/dL (CDC, 2005; U.S. EPA, 2006, 2007). While the issue of whether or not a threshold exists for the cognitive effects of lead in children continues to be debated, there

is consistent information from the available lead health effects literature indicating that PbB levels > 10 µg/dL are linked to decreased intelligence and impaired neurobehavioral development (WHO, 1995; U.S. EPA, 2004, 2006; Lanphear *et al*., 2005; CDC, 2004, 2005; Tellez-Rojo *et al.,* 2006; ATSDR, 2007) in children. The current state of opinion held by several agencies is that no "safe" threshold for PbB can be identified (CDC, 2005; U.S. EPA, 2006, 2007). In the absence of a definitive toxicological threshold, effects at blood levels ≤10 µg/dL cannot be ruled out, and there is an emphaisis on preventative measures at lower PbB concentrations (CDC, 2005).

Given the vast amount of literature available on the human health effects of lead exposure, only selected epidemiological studies that investigated the neurotoxicity and other well characterized effects of lead are summarized in this profile. For further details, readers of this profile are encouraged to consult ATSDR (2007) and WHO (1995), as well as the primary scientific literature.

There are eight cohort studies that contribute to the understanding of effects of exposure to lead: Boston (Bellinger *et al*., 1992), Cleveland (Ernhart *et al*., 1989), Cincinnati (Dietrich *et al*., 1993), Rochester (Canfield *et al*., 2003a) in the U.S., Sydney (Cooney *et al*., 1989) and Port Pirie (Wigg *et al*., 1988; Baghurst *et al*., 1992) in Australia, Kosovo (Wassermann *et al*., 1997; Factor-Litvak *et al*., 1999) in former Yugoslavia, and Mexico (Schnaas *et al*., 2000). Individually, these studies provide clear evidence of lead effects, and together they provide a more powerful data set for analysis of the contributions of confounding factors, period of vulnerability for lead exposure, and quantitative relationships between lead exposure and selected effects.

Lanphear *et al*. (2005) pooled the data from seven of the eight international cohorts mentioned above (all but the Sydney, Australia cohort) which fit criteria of similar methodology (longitudinal cohort), similar exposure measures (PbB at one or more ages), and similar outcome measurements (Full Scale IQ) based on similar instruments to measure outcomes, and covariate collection. This pooled analysis used data supplied by the authors for each cohort, and is to date, the most comprehensive analysis that attempts to quantify the relationship between lead exposure, relevant outcomes and most sensitive period for impacts. The pooled analysis demonstrated a lead-related intellectual deficit among children who had maximal PbB below 7.5 µg/dL. No evidence of a threshold for no discernable effect could be determined.

Tellez-Rojo et al. (2006) conducted a prospective study compared PbB levels which were < 10 µg/dL to indices of mental development (MDI) and psychomotor development (PDI) in infants which were 12 and 24 months of age. The results showed that both the MDI and PDI for infants at those ages were inversely

related to PbB levels, therefore making the case that 10 µg/dL should not be associated with the threshold level at which adverse effects do not occur.

The U.S. based Advisory Committee on Childhood Lead Poisoning and Prevention (ACCLPP) has concluded, using the evidence from the longitudinal studies and other supporting research, that there is a negative association between PbB levels <10 µg/dL and adverse health effects (IQ decrements and possibly other effects) (ACCLPP, 2004). They also note that there is a steeper slope in the PbB-IQ dose-response curve, supported by the Lanphear pooled analysis, at lower PbB levels. They are careful to note that there are still some reservations about a direct causal relationship but that the weight-of-evidence does favor a relationship (ACCLPP, 2004). At very low exposure levels it should be expected that variability between individuals should limit the sensitivity of a single measure to properly characterize effects responsible for intellectual deficits (ACCLPP, 2004). There remains the question as to what level of PbB indicates that a measured change in IQ is not different from the variations of the measurement tool.

Wilson *et al.* (2005) has carried out a secondary analysis of the Lanphear *et al*. (2005) pooled data and of Canfield *et al*. data (2003a) which indicates that a change in IQ is not statistically different from normal when PbB level are less than 4 µg/dL. This calculation is concordant with other epidemiologic data to date.

Cross sectional studies from the 1970s to the 1990s indicate that lead exposure measured in a variety of ways (*e.g.,* PbB at various ages) has a relationship to decrements in IQ, school performance and some measures of attention, as a continuum from PbB of 10 µg/dL to 40 µg/dL (Banks *et al*., 1997). A review and meta-analysis of a number of cross sectional and prospective cohort studies, which investigated neurobehavioural aspects of lead neurotoxicity in children, was conducted by Winneke and Krämer (1997). Based on meta-analyses conducted on the data from both cross sectional and prospective studies, it was concluded that a doubling of PbB concentration from 10 to 20 µg/dL is associated with an average loss of one to three IQ points. Similar conclusions were reached by Pocock *et al.* (1994), WHO (1995), and ATSDR (2007). In addition to decreased IQ, some neuropsychological findings in lead-exposed children suggest that part of the impairment resembles performance deficits that are characteristic of children with attention deficit disorders (Winneke and Krämer, 1997).

It is important to recognize that the epidemiological studies investigating the relationship between PbB levels and fetal and child neurotoxity are hampered by the complexity of mental developmental processes, and the questionable sensitivity and significance of IQ tests in detecting subtle differences in

neuropsychologic performance. It is also important to recognize that PbB concentrations reflect the absorbed dose of lead, and that interpretation of PbB data depends on knowing the history of exposure to lead. This is because lead is stored in bone tissue within the body, and has a long half-life. Thus, in situations where an individual has not experienced chronic lead exposure, the PbB concentrations would reflect recent lead exposures. However, PbB concentrations can also reflect both recent and past exposures to lead (for example, when lead in bone is mobilized within the body during deossification, and if lead exposure is intermittent in nature). A consequence of this is that biological effects may differ for populations or individuals with the same PbB concentrations since different exposure timescales may be involved.

Effects on reproduction/development due to elevated levels of Pb have been observed from various occupational studies and have been review by ATSDR, 2005 and U.S. EPA, 2006. However, much of these studies are inconclusive in that they are limited by confounding factors such as lack of exposure data, co-exposure to other chemicals and inadequate control of other risk factors (e.g., socioeconomic). In males, reproductive effects are measured via semen quality, time to pregancy and reproduction history, however, semen quality has shown to be the endpoint with the most conclusive evidence that can be associated with Pb exposure. Effects on the female reproductive system are limited and inconclusive. Lastly, many of the development studies that have been performed have led to limited conclusions on the effects of Pb.

The U.S. EPA (2004) has maintained its previous cancer classification for lead compounds as B2 - probable human carcinogen, based on sufficient animal evidence of kidney tumours, but inadequate human evidence. The U.S. EPA has determined that an estimate of carcinogenic risk from oral exposure (such as a slope factor) using standard methods would not adequately describe the potential risk for lead compounds. The U.S. EPA's Carcinogen Assessment Group made this determination given the current lack of understanding on various toxicological and toxicokinetic characteristics of lead.

IARC (2004) classified inorganic lead compounds as probably carcinogenic to humans (Group 2A), based on limited evidence for carcinogenicity in humans and sufficient evidence for carcinogenicity in experimental animals. The IARC evaluation considers the evidence of carcinogenicity in humans and experimental animals, as well as other data relevant to the evaluation of carcinogenicity and its mechanisms. For example, IARC (2004) noted that while there appears to be little evidence that lead is directly genotoxic, it may be indirectly genotoxic as a result of oxidative stress effects caused by the

formation of reactive oxygen species. The IARC Working Group does not typically provide quantitative estimates of any chemical's carcinogenic risk.

Health Canada has not formally classified lead compounds with respect to their carcinogenic potential.

The OEHHA (California Environmental Protection Agency Office of Environmental Health Hazard Assessment) considers lead compounds human carcinogens as they have derived both oral and inhalation slope factors and unit risks for lead. However, at this time, no other regulatory agencies, other than OEHHA, are known to have derived regulatory exposure limits for lead that are based on carcinogenic effects.

Exposure Limits

As described below WHO, Health Canada, RIVM, MOE, ATSDR, U.S. EPA and OEHHA were the organization consulted to select exposure limits for lead. It should be noted that current MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits) when health based exposure limits are available from major health agencies. Exposure limits used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO), and the MOE itself.

Although the toxicological database for lead is large, the majority of human effects data is expressed as a PbB concentration, rather than a dose or concentration in an environmental medium. In addition, there are inadequate empirical data for demonstrating a threshold for the health effects of lead. In fact, many consider lead a non-threshold toxicant, indicating that any epsoure to lead leads to possible efects. Given these limitations, many regulatory agencies have not derived conventional exposure limits such as RfDs, TDI's or MRLs, and advocate that exposure to lead should be minimized. In order to utilize the wealth of literature relating human PbB concentrations to health effects, such agencies (*e.g.,* ATSDR, U.S. EPA) have developed models or other approaches to relate environmental lead exposure to PbB levels. This is described further in Section A4-5.0. In addition, environmental quality guidelines for lead have also been developed with a different approach than is used for most other chemicals. Instead of developing exposure limits based on no- or low-effects-levels observed in test organisms following controlled exposures, lead guidelines are typically back calculated from a critical PbB concentration (usually 10 µg/dL, as recommended by CDC, 2004, 2005; U.S. EPA, 2004; CEOH, 1994). A blood lead level of ≥10

ug/dl is a level of concern in an individual and is indicative of elevated exposure and possible harm to health.

Although recent scientific data indicate associations between intellectual performance and other health effects in children and PbB levels extending below 10 µg/dL, it appears that major agencies (MOE, 2007; U.S. EPA, 2002, 2006, 2007; CDC, 2004, 2005) acknowledge that a clear threshold for protection of neurological and other deleterious impacts in children and adults has not yet been identified. In the absence of a definitive toxicological threshold, effects at PbB levels ≤10 µg/dL cannot be ruled out, and there is an emphaisis on preventative measures (CDC, 2005). In addition, derivation of acceptable exposure levels is complicated by numerous confounding factors that influence lead toxicity, including socioeconomic status, pre-existing lead body burdens, age, health status, nutritional status and lifestyle factors such as alcohol consumption and tobacco smoke (environmental tobacco smoke has been associated with elevated PbB). As a result, CDC (2004, 2005) has recommended that the PbB level of concern should remain at their 1991 (CDC, 1991) recommended level of 10 µg/dL. The decision for not lowering the PbB level of concern below 10 µg/dL is based on the following (CDC, 2004,2005):

- Lack of effective clinical interventions to lower PbB levels for children with levels less than 10 µg/dL or to reduce the risks for adverse developmental impacts; and,
- Inaccuracy inherent in laboratory analytical testing of PbB levels in children; and,
- No evidence of clear threshold for neurological impacts in children and as such, a decision to lower the PbB level of concern would be "arbitrary" and "provide uncertain benefits".

Health Canada's current blood intervention level (10 µg/dL) is not to be interpreted as an acceptable level of exposure below which no adverse health effects shall occur (Wilson *et al.,* 2005). It is designed to indicate the need for mitigation of risk for individuals already exposed. Health Canada is currently undergoing a review of their PbB population intervention level established by CEOH in 1994.

A value that is the basis for many jurisdiction's exposure limits is the tolerable daily intake (TDI) of 3.57 µg/kg/day derived by the WHO. The TDI was derived based on a "provisional tolerable weekly intake" (PTWI) of 25 µg of lead per kg of body weight recommended by FAO/WHO (1993), and reaffirmed by WHO (1999), for all age groups. This PTWI value was in turn based upon technical reports presented at annual meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as well as upon epidemiological studies associating lead exposure with neurological effects in infants and children. The PTWI represents the permissible or tolerable human weekly intake that is unavoidable as a result of consuming typical foods. The PTWI was based on metabolic studies of infants which indicated that if

daily lead intakes equal approximately 5 µg/kg/day then PbB levels remained at a fairly constant low level within the body. Daily intakes of lead in the range of 3 to 4 µg/kg body weight/day by infants and children were not associated with an increase in blood lead concentrations (WHO, 1995; Ziegler *et al.*, 1978; WHO, 1987). The PTWI of 25 µg/kg/week was converted to a provisional tolerable daily intake (PTDI) of 3.57 µg/kg/day by dividing the PTWI by seven (days in a week). The TDI for lead accounts for exposure from all sources, via all routes, and is considered protective of all humans, including infants and children.

Health Canada (2004) has adopted 3.6 µg/kg body weight/day as the provisional TDI for lead, and the Canadian Council of Ministers of the Environment (CCME) and Health Canada use this value as the basis for derivation of soil and drinking water guidelines that are protective of human health. By definition, a TDI represents an exposure that is without adverse health effects (Health Canada, 2004). The defensibility of this has been questioned in light of recent evidence which advocates the consideration of lead as non-threshold toxicant (Wilson *et al.*, 2005). In the Netherlands, RIVM (Baars *et al*., 2001) has also derived a TDI of 3.6 µg/kg body weight/day, based on the provisional PTWI of 25 µg/kg/week derived by the FAO/WHO (1993).

The MOE (1994) recommended an intake of concern for populations (IOC_{pop}) of 1.85 µg/kg/day in order to minimize the predicted number of children with individual blood lead levels of concern. Subclinical neurobehavioural and developmental effects were the critical effects appearing at the lowest levels of exposure (MOE, 1994). The intake of concern for individuals (IOC_{ind}) was based on a Lowest Observed Adverse Effect Level (LOAEL) in infants and young children of 10 µg/dL PbB divided by an intake/PbB slope factor of 0.21 µg Pb per DL PbB per µg/day. This resulted in an IOCind of 3.7 µg/kg/day for a 13 kg child (0.5 to 4 yrs). To derive the IOC_{pop} an uncertainty factor of 2 was applied to the IOC_{ind}, which resulted in a daily intake of 1.85 µg/kg/day (MOE, 1994). This value is based on the same research as the other agencies limits discussed elsewhere. In the *Guideline for Use at Contaminated Sites in Ontario* (MOE, 1996), MOE adopted the oral RfD of 1.85 µg/kg/day for lead. As it is based on an internal PbB concentration, this IOC_{pop} applies to lead exposure received from all sources, via all routes. This IOC_{pop} does not represent a "safe level of exposure" rather it is an intake-level-of-concern in the population that is not considered to be enterely safe or acceptable since MOE considers lead to be a non-threshold toxicant and thus any level of exposure is associated with a level of risk. More recently (MOE, 2007), the MOE has summarizes the scientific basis for air quality guidelines and standards developed by the U.S. Environmental Protection Agency (US EPA), the California Environmental Protection Agency (Cal/EPA), New Zealand, Australia, United Kingdom and the World Health Organization. Following this

review, MOE selected the Cal/EPA's (2001) derivation of their lead air guideline as the most appropriate approach on which to base an updated air standard for lead in Ontario (24-hour AAQC of 0.5 ug/m^3). Cal/EPA's guideline is based on an air concentration associated with a 5% probability of exceeding the BLL of concern.

Two regulatory agencies that are typically leaders in the development of chemical exposure limits (ATSDR and U.S. EPA) have not derived any exposure limits for lead compounds; rather, they have developed alternate approaches that relate environmental lead exposure to PbB levels.

ATSDR (2007) did not derive any minimal risk levels (MRLs) for lead due to the lack of a clear threshold for health effects and the need to consider multi-media routes of exposure. However, ATSDR has developed guidance for employing media-specific slope factors to integrate exposures from various pathways for site-specific risk assessments.

The U.S. EPA IRIS database does not recommend oral or inhalation RfD (or RfC) for lead due to high levels of uncertainty, and because lead is considered a non-threshold toxicant (U.S. EPA, 2004). The U.S. EPA believes that the effects of lead exposure, particularly changes in blood enzyme levels, and children's neurodevelopment, may occur at blood levels so low as to be essentially without a threshold. As further support for not deriving an RfD or RfC, the U.S. EPA (2004) states that current knowledge of lead toxicokinetics suggests that risk values derived by standard procedures (such as an oral RfD) would not be representative of the potential risk, due to difficulties in attempting to account for pre-existing body burdens of lead, and certain lifestages when stored lead may be mobilized within the body. For example, lead is well known to be stored in bone tissue, and its mobilization from bone varies greatly with age, health status, nutritional state, physiological state (pregnant, lactation, menopause *etc*.).

Alternatively, the U.S. EPA has developed the Integrated Exposure Uptake Biokinetic Model (IEUBK) as a means of predicting the occurrence of blood lead concentrations above 10 µg/dL in children. This model is used to determine the contribution of lead from all media to PbB (U.S. EPA, 2004). The IEUBK model predicts the geometric mean PbB concentration for a child exposed to lead in various media (or a group of similarly exposed children). The model can also calculate the probability that the child's PbB exceeds 10 ug Pb/dL (P10). Preliminary remediation goals (PRGs) for lead are generally determined with the model by adjusting the soil concentration term until the P10 is below a 5% probability (U.S. EPA, 2003). In addition, an Adult Lead Model was developed by the U.S. EPA Superfund Program for when adult exposures to lead are of concern, especially in the case of pregnant women (www.epa.gov/superfund/programs/lead/adult.htm). The model equations were developed to calculate

cleanup goals such that there would be no more than a 5% probability that fetuses exposed to lead would exceed a blood lead (PbB) concentration of 10 µg/dL.

There are no non-cancer inhalation exposure limits for lead. However, the FAO/WHO TDI of 3.57 µg/kg/d accounts for lead exposure from all sources and is considered protective of all humans, including infants and children. The MOE IOC_{pop} of 1.85 µg/kg/day is also considered protective of multimedia lead exposure (MOE, 1994).

Only one agency was identified as having developed quantitative toxicity estimates based on the carcinogenicity of lead (*i.e.,* OEHHA, 2002). The U.S. EPA did not derive any exposure limits based on carcinogenic endpoints as its Carcinogen Assessment Group concluded that the uncertainties associated with lead pharmacokinetics and factors affecting the absorption, release, and excretion of lead (*i.e.,* age, health, nutritional status, body burden, and exposure duration) preclude the development of a numerical estimate to predict carcinogenic risk (U.S. EPA, 2004). Thus, the U.S. EPA believes that the current limited knowledge of lead toxicokinetics suggests that a carcinogenic risk estimate derived by standard procedures would not adequately represent the true potential risk.

The OEHHA (2002) reports an inhalation unit risk factor of 1.2 E-5 $(\mu g/m^3)^{-1}$, an inhalation slope factor of 0.042 $(mg/kg/day)^{-1}$, and oral slope factor of 0.0085 $(mg/kg/day)^{-1}$. All values were originally calculated by OEHHA (2002) from rat kidney tumor incidence data (the Azar *et al.*, 1973 study) using a linearized multistage procedure. The studies by Azar *et al*. (1973) and Koller *et al*. (1986) were considered to represent the best available tumour dose-response data for use in quantitative cancer risk assessment. The derivation of the unit risk and slope factors is described in detail within OEHHA (2002). In summary, rat kidney tumour data was extrapolated to humans by means of the best fitting linearized multistage model (*i.e.,* GLOBAL86), conversion of rat doses to human equivalent doses (HED), the use of standard human receptor parameters, and assumptions related to the inhalation and oral bioavailability of lead.

No regulatory dermal exposure limits for lead compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in a human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose

absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).
AF*dermal* = the fraction of the applied chemical absorbed through the skin.
AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

Criteria Summary

The following table summarizes the exposure limits selected for lead in the current study.

Exposure Limits Selected for Lead in the HHRA

Route of Exposure	Exposure Limit	Toxicological Basis	Reference	
			Study	Regulatory
Non-cancer (Threshold) Effects (IOC_{POP})				
Oral	1.85 µg/kg/day	Neurological effects in children	Weight-of-evidence, numerous studies	MOE, 1994; MOE, 1996
Inhalation				
Dermal				
Cancer (Non-threshold) Effects				
Oral	NA	NA	NA	NA
Inhalation[1]	NA	NA	NA	NA
Dermal	NA	NA	NA	NA

NOTES:
NA = not available; IOC_{POP} = intake of concern (population).
[1] While OEHHA considers lead to be a carcinogen via the inhalation route, no other identified regulatory agency shares this opinion. As such, lead is not considered a carcinogen in the current HHRA.

References

For a complete list of references, see Section A4-6.0 of the detailed toxicological profile on the accompanying CD.

A4-1.0 INTRODUCTION

This profile is not intended to provide a comprehensive review of the available toxicological and epidemiological literature on lead compounds. Rather, the purpose of the lead toxicological profile is to: i) summarize the most relevant toxicological and epidemiological information on this substance; ii) outline any recent information that may challenge previous findings; and iii) provide supporting rationale for the lead exposure limits selected for use in the human health risk assessment of the Sudbury area.

The following toxicological review of lead is based primarily on secondary sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature. The U.S. EPA and the CDC (Centre for Disease Control) have both conducted recent literature searches and updated their toxicological profiles for lead. Neither of these agencies found any new information to change their previous findings.

The following toxicological review focuses entirely on inorganic lead compounds as these are the predominant forms of lead in the environment.

As lead is an extremely well-studied chemical, there is a wealth of data available regarding various forms of lead toxicity in both humans and animals. This toxicological review does not attempt to summarize or capture the findings from all studies. It is strongly recommended that readers consult detailed regulatory agency reviews like ATSDR (2007) and WHO (1995) should additional information be desired on topics addressed in this review, or should information be desired on topics not addressed in this review.

Lead is a naturally occurring metallic element and occurs in a variety of minerals, often in close association with zinc (CCME, 2002). Common lead-containing minerals include galena (lead sulphide), anglesite (lead sulphate), and cerussite (lead carbonate) (Schoof, 2003). While most of the lead found in the environment is the result of anthropogenic activities, there are significant natural sources as well, including volcanoes, forest fires, sea spray, and weathering of lead-containing minerals (Environment Canada, 1996).

Due to several desirable properties, including the ability to resist corrosion, lead has historically been used in a wide variety of products in both industrial applications and residential uses (*i.e.,* paint, petroleum, smelters, batteries, *etc.*). Due to this prevalence of use, and its persistence in the environment, there is an abundance of data for exposed human populations.

Lead is persistent in both water and soil. The different forms of lead in the environment are governed by factors such as temperature, pH, and the presence of humic materials. Elemental lead occurs rarely in the ambient environment; rather, the most common form of lead in the environment is Pb^{2+}. Lead is largely associated with suspended solids and sediments in aquatic systems, and it occurs in relatively immobile forms in soil. In aerobic soils, lead compounds undergo weathering and may become more stable over time (the most prevalent lead compounds in aerobic soils tend to be lead carbonates, hydroxides, or carbonate-hydroxide complexes (Leita and De Nobili, 1991; Lindsay, 1979)). In anaerobic soils, much of the sulphate in soil is reduced to sulphide, thus the predominant form of lead in these soils is lead sulphide - a highly stable, insoluble and relatively non-reactive lead species. There is no conclusive evidence as to which forms of lead in soil have the greatest relative bioavailability to organisms, as this is influenced greatly by pH, cation exchange capacity, the presence of ligands that can bind lead, and soil organic matter content. It is generally believed however, that the amount of free Pb^{2+} in solution best represents what is actually bioavailable to organisms (CCME, 2002).

Particulate-bound lead emitted from mining operations, smelters, and combustion sources occurs primarily in the form of lead-sulfur compounds such as $PbSO_4$, $PbO{\cdot}PbSO_4$, and PbS (U.S. EPA, 1986). In the ambient atmosphere, lead exists primarily in the form of particulate-bound $PbSO_4$ and $PbCO_3$, and is deposited onto soil and water surfaces in this form (ATSDR, 2007).

A4-2.0 FRACTION ABSORBED VIA DIFFERENT ROUTES

A4-2.1 Oral

The gastrointestinal absorption of lead varies depending on a number of factors including speciation, solubility, particle size (if lead is in a matrix like food or soil), the exposure medium (*e.g.,* food, water, soil), and the age and physiological state of the exposed individual (*e.g.,* fasting or fed, nutritional status – particularly with respect to calcium and iron).

For example, less than 10% of lead may be absorbed when ingested with a meal, but this absorption efficiency may increase to around 60 to 80% when ingested after a subject has fasted (ATSDR, 2007). It is suggested that the reduced absorption of lead when ingested with a meal is due to the presence of substantial amounts of calcium and phosphates in the food (Blake *et al*., 1983; Blake and Mann, 1983; Heard and Chamberlain, 1982).

It has been suggested that the absolute oral bioavailability of soluble lead in human children is 50% (Alexander *et al*., 1974; Ziegler *et al*., 1978). Absolute oral bioavailabilities for soluble lead are lower in adults. Commonly reported estimates of oral lead bioavailability from the diet are 7 to 15% for adults, and 40 to 53% in infants and children (Schoof, 2003).

There is limited human evidence to suggest that the gastrointestinal absorption of lead may be higher in children than in adults (ATSDR, 2007). In animals, there are a number of studies that demonstrate an age-dependency for gastrointestinal absorption of lead. For example, rat pups absorbed 40 to 50 times more lead *via* the diet than adult rats (Forbes and Reina, 1972; Kostial *et al*., 1978). Pounds *et al*. (1978) found that gastrointestinal lead absorption was 37.9% for young monkeys *versus* 26.4% in adults following a single radiolabeled gavage dose of 6.37 mg lead/kg as lead acetate. The differences in lead absorption between juvenile and adult animals and humans may reflect both dietary differences and physiological differences between the immature and mature gastrointestinal tract (U.S. EPA, 1986). There are no available data on lead absorption in older children and adolescents.

A number of studies have demonstrated that nutritional status can greatly influence gastrointestinal lead absorption. Iron status appears to be a particularly key variable. Children who are iron deficient have higher blood lead concentrations than similarly exposed children who have a nutritionally adequate iron status (Mahaffey and Annest, 1986; Marcus and Schwartz, 1987). Animal studies have provided evidence for the effect of iron deficiency on lead absorption. In rats, iron deficiency increases the

gastrointestinal absorption of lead, possibly due to lead binding to proteins in the intestine that normally bind iron (Barton *et al*., 1978; Morrison and Quatermann, 1987). Calcium status is also a key variable affecting gastrointestinal lead absorption. An inverse relationship has been observed between dietary calcium intake and blood lead concentration in children, which suggests that children who are calcium deficient may absorb more lead than children with an adequate calcium intake (Mahaffey *et al*., 1986; Ziegler *et al*., 1978). Calcium also appears to reduce lead absorption in adults (Blake and Mann, 1983; Heard and Chamberlain, 1982), and in rats (Barton *et al.,* 1978).

A number of studies have noted that blood lead levels can increase during pregnancy, which may reflect the increased absorption of lead and/or increased mobilization of bone lead (Gulson *et al*., 2004; Lagerkvist *et al*., 1996; Schuhmacher *et al*., 1996).

Many studies have investigated the oral bioavailability of lead, when ingested in a soil matrix. It is well established that the highest lead concentrations occur in the smaller particle size ranges in soils. This has important implications for lead exposure in young children as it is the smaller particles (*i.e.,* <100 µm in diameter) that are most readily ingested, inhaled and that adhere most readily to skin surfaces. Some studies have suggested that lead at mining waste sites is of relatively low oral bioavailability than lead found at smelter sites or in urban areas (Hemphill *et al*., 1991; Steele *et al*., 1990). These differences in bioavailability are mainly attributed to speciation and particle size differences. For example, lead-containing soil particles at mining sites are typically in larger particle size ranges and consist of mostly insoluble lead sulfides. However, a site-specific evaluation is usually necessary to ascertain whether soil lead is likely to be of high or low oral bioavailability. The oral bioavailability of lead sulphide is roughly one tenth of that for the highly water soluble lead acetate (Freeman *et al*., 1994). With respect to soil particle size, Barltrop and Meek (197) reported an inverse relationship between diets containing metallic lead of particle sizes <250 µm and gastrointestinal absorption in rats. The authors found 2.3-fold increase in tissue lead concentration when rats ingested an acute dose of 37.5 mg Pb/kg soil with a particle size of <38 µm, relative to a soil particle diameter between 150 and 250 µm. In an *in vitro* test, Healy *et al*. (1982) found that the solubility of lead sulfide in gastric acid was much greater for particles of 30 µm diameter than it was for particles of 100 µm diameter.

Several studies have demonstrated that the gastrointestinal absorption of lead in a soil matrix is lower than for dissolved lead. In immature swine administered oral doses of soil-borne lead (either 75 or 225 µg Pb/kg body weight), the bioavailability was reduced to 50 to 82% of a similar dose of highly water soluble lead acetate (Casteel *et al*., 1997; U.S. EPA 1996a,b,c). Soil lead oral bioavailability estimates

(relative to soluble lead acetate) from animal studies range from 12 to 27% (Freeman *et al*., 1992; Freeman *et al*., 1994). In the latter study, it was also estimated that the absolute bioavailability of lead in the soils from the Freeman *et al*. (1992) study was approximately 2.7%. In general, the available animal studies have indicated that absorption of lead from soil varies depending on the source of the lead, ranging from near zero to greater than 50 percent absolute bioavailability (Schoof, 2003).

As for dissolved lead, the influence of a fed *versus* fasting state is an important determinant of soil lead bioavailability. Maddaloni *et al*. (1998) reported that adult subjects who ingested soil (particle size range less than 250 μm) from the Bunker Hill NPL site absorbed 26% of the lead dose when the soil was ingested in the fasted state, but only 2.5% when the same soil lead dose was ingested with a meal. Similar studies do not appear to have been conducted with infants or children.

The gastrointestinal absorption of lead from water, food or soil may be a saturable process, based on observed nonlinear relationships between uptake and blood lead concentrations in a number of studies (ATSDR, 2007). It is suggested that this may reflect both capacity-limited absorption in the gastrointestinal tract, and capacity-limited binding of lead with red blood cells, once lead has entered the systemic circulation (ATSDR, 2007). However, in a study with immature swine that received either oral doses of lead in soil, or intravenously administered lead, the lead dose: blood lead relationship was non-linear; although, linearity was noted for the relationship between lead dose and lead concentrations in bone, kidney and liver (Casteel *et al*., 1997). The findings from this study suggest that the non-linearity in the lead dose: blood lead relationship may be due to lead-induced effects on some aspect of its toxicokinetics other than absorption (ATSDR, 2007). There is some evidence from mechanistic studies for capacity-limited processes at the level of the intestinal epithelium, which provide support for a non-linear intake-uptake relationship for lead (ATSDR, 2007).

In the U.S. EPA IEUBK model (U.S. EPA, 1994a,b; 2002), it is assumed that 50% of an oral dose of lead is absorbed from food or water, while 30% of lead in soil is assumed to be absorbed in the gastrointestinal tract. Thus, the default assumption for relative oral bioavailability for lead in a soil matrix, compared to soluble forms of lead in food or water is 60% [30 / 50%] (U.S. EPA, 1994a,b; 2002). This value compares well to what has generally been observed in soil bioavailability studies with rats and swine (Schoof, 2003). On average, the results of these studies support the use of a default assumption that 30% of an oral lead dose is absorbed from soil (*i.e.,* relative oral bioavailability of 0.6).

In evaluating the oral bioavailabilty of lead, it is also important to consider behavioural factors. For example, small children's normal hand-to-mouth activity results in higher exposures than older children

or adults would experience. Pica children would incur the highest likely exposures from their deliberate soil and dust ingestion.

A4-2.2 Inhalation

As airborne lead is bound to particulate matter, the absorption of lead in the lungs first depends on the deposition of particles within the respiratory tract. The rate of deposition of particulate airborne lead in the lower respiratory tract of adult humans is approximately 30 to 50%; however, this can vary substantially depending on particle size and ventilation rate (U.S. EPA, 1986). Only particles less than 250 um in mass mean aerodynamic diameter would be expected to deposit in the lower respiratory tract. The WHO, 1984 estimated that approximately 20 to 60% of the total amount of lead inhaled is deposited in the lung, with the majority of this amount being absorbed. Others have also reported that once deposited in the lower respiratory tract, particulate lead is almost completely absorbed (U.S. EPA, 1986; Morrow *et al*., 1980). It is widely believed that most of the lead deposited in the pulmonary region is either absorbed or cleared, since autopsies on human lungs have shown that very little lead is retained in the lung (Barry, 1975).

Children inhale comparatively more lead than adults because of a number of modifying factors, including differences in metabolic rates and airway dimensions (ATSDR, 2007). Xu and Yu (1986) found that fractional deposition in two-year-old children was estimated to be 150% greater than in adults. The lead deposition rate in 10-year old children was estimated by James (1978) to be 60 to 170% greater than that of adults, while Barltrop (1972) estimated that children inhale 40% more lead than adults. The percentage of inhaled lead that is swallowed following clearance from the upper respiratory tract (from the action of the mucociliary apparatus) was reported to be 6% (Chamberlain *et al.,* 1975).

A4-2.3 Dermal

Limited information is available regarding the dermal absorption of lead in humans. Dermal absorption of inorganic lead compounds is widely reported to be much lower than absorption by inhalation or oral routes of exposure.

The extent of dermal absorption is dependant on the form of lead. Skin absorption of lead acetate was estimated to approach 0.06% while up to 8% absorption was reported for the organo-lead compound, tetraethyl lead (Moore *et al*., 1980). A series of studies conducted with human volunteers had shown that lead salts, such as lead chloride and lead nitrate, are rapidly absorbed through the skin when applied in

aqueous solution, resulting in elevated lead concentrations in sweat and saliva, but not blood (Lilley *et al.,* 1988; Florence *et al.,* 1988; Stauber *et al.,* 1994). However, these studies were not able to accurately quantify the amount of lead absorbed or determine which tissues, if any, accumulated lead through the dermal route (Florence *et al.,* 1998). A recent study in which aqueous solutions of lead acetate or nitrate were applied to the skin of mice, found that 0.04% of the administered dose was absorbed through the skin in less than 24 hours (Florence *et al.,* 1998). Increased lead concentrations were detected in mouse skin, muscle, pancreas, spleen, kidney, liver, caecum, bone, heart and brain but not in the blood. The authors concluded that blood lead, the prime index of lead status in human populations, is not a good indicator of dermal exposure.

A4-2.4 Transplacental and Lactational Transfer

The transplacental route of exposure is important to consider when assessing prenatal exposure to lead (ATSDR, 2007). Lead is reported to cross the placental barrier and be present in the umbilical cord at a concentration of 80 to 90% of that in maternal blood (Inouye, 1989). In addition, it has been demonstrated that as much as one-third of the maternal dose can be transferred to the mother's milk during lactation, with subsequent exposure to nursing infants (ATSDR, 2007). Thus, maternal PbB must also be considered as a route of exposure when assessing the pre- and post-natal risks of lead.

A4-3.0 TOXICOLOGY

There is a large database available regarding the health effects of lead in both humans and experimental animals. Lead is a somewhat unique chemical in that there are a large number of reliable studies conducted in human subjects, including children that provide well-characterized data on the health effects of lead. However, most of the available studies do not express health or toxic endpoints as a lead dose; rather, such endpoints have predominantly been expressed in terms of blood lead levels (PbB). It is important to recognize that PbB levels reflect recent lead exposure as well as historical lead exposures that have redistributed in the blood through hematopoiesis (blood cell synthesis) in bone marrow (ATSDR, 2007). It is also important to recognize that PbB comprises a small percentage of the total lead body burden humans; typically, 90% or more of the inorganic lead body burden is stored in bone tissue. This can be a significant source of lead when bone tissue is undergoing significant deossification or demineralization, such as during pregnancy, lactation and menopause (IARC, 2004). PbB concentrations are also difficult to relate to exposure doses (*i.e.,* mg/kg/day) as occupationally exposed populations are primarily exposed to lead through inhalation (with some lead ingestion), whereas the general population (particularly children) is exposed to lead primarily *via* the oral route (with some lead inhalation). Nonetheless, the toxic effects of lead in humans are widely believed to be the same regardless of the route of entry, and are correlated to PbB in the vast majority of studies (ATSDR, 2007). Thus, to be consistent with the majority of the available literature on lead toxicology and epidemiology, the following toxicological summary focuses on effects that are associated with, or correlated with PbB concentrations.

The effects from chronic exposure to lead on humans and experimental animals are primarily neurological, renal, hematological, reproductive, and developmental (ATSDR, 2007; CDC, 1991). The U.S. EPA (2004) notes that many health effects associated with environmental lead exposure occur without overt signs of toxicity, and can manifest themselves over a relatively short period of time (before the usual term of chronic exposure can occur). New studies demonstrate associations between neurological, haematological, immune, cardiovascular, and renal effects and PbB levels ≤10 µg/dL (CDC, 2005; U.S. EPA, 2006, 2007). The current state of opinion held by several agencies is that no "safe" threshold for PbB can be identified (CDC, 2005; MOE, 2007; U.S. EPA, 2006, 2007). In the absence of a definitive toxicological threshold, effects at blood levels ≤10 µg/dL cannot be ruled out (CDC, 2005).

Well-characterized human health effects include neurotoxicity and renal effects, which can be severe at blood lead levels greater than 120 µg/dL (U.S. EPA, 1986). The most commonly reported and well-studied effects of environmental lead exposure are adverse effects on neurological function and

neurobehavioural development in children (U.S. EPA, 2004; 2006). The effects in children often manifest as decreased IQ and memory, decreased gestation period, retarded growth rate, and are typically accompanied by blood enzyme level changes, and may occur at blood lead levels so low as to be essentially without a clearly discernible threshold for effects (U.S. EPA, 2004). While the issue of whether or not a threshold exists for the cognitive effects of lead in children continues to be debated, there is consistent information from the available lead health effects literature indicating that PbB levels > 10 µg/dL are linked to decreased intelligence and impaired neurobehavioral development (Lanphear *et al*., 2005; CDC, 2004; U.S. EPA, 2004; 2006; ATSDR, 2007; WHO, 1995) in children. Contrary to previous studies, Tellez-Rojo et al. (2006) compared PbB levels which were < 10 µg/dL to indices of mental development and psychomotor development in infants which were 12 and 24 months of age and the results demonstrated that both MDI and PDI were inversely related to PbB levels; thereby concluding that 10 µg/dL should not be proposed as the threshold level.

Neurotoxcity in adults remains unclear since there is not an extensive amount of literature on this topic (U.S. EPA, 2006). Decreases in cognition have been associated with bone Pb levels and not with PbB indicating that cumulative exposure is an important factor (U.S. EPA, 2006). Occupational exposure to Pb has been seen to result in fatigue, irritability, lack of concentration and adverse effects on motor speed, dexterity, sensory impairment and to cause amytrophic lateral sclerosis (U.S. EPA, 2006).

Associations between blood and bone lead and cardiovascular effects in adults have been reported; however, there is debate as to whether the relationships are causal (ATSDR, 2007; U.S. EPA, 2006, 2007). Anemia is also commonly reported, resulting from reduced hemoglobin production and damage to erythrocytes. A PbB concentration of 40 µg/dL is associated with heme synthesis and other effects contributing to clinical symptonms of anemia in children (Schwartz et al., 1990; U.S. EPA, 2007). A 10% probability of anemia (hematocrit <35%) was associated with 20 µg/dL PbB at one year of age (Schwartz *et al.* 1990). Severe lead exposure in children (PbB above 380 µg/dL) can cause coma, convulsions, and even death. Clinical signs of lead toxicity, generally manifested as neurotoxicity and anemia, are evident at PbB levels of 70 µg/dL and greater (IARC, 2004).

Studies examining reproduction/development effects due to occupational Pb expsoure has been reviewed by ATSDR, 2005 and U.S. EPA, 2006. Many of the studies are inconclusive due to confounding factors. Effects in males have been seen and in particular, semen quality has shown to be adversely effected with elevated exposure to Pb. Effects on the female reproductive system and development studies are limited, and have led to inconclusive evidence (U.S. EPA, 2006).

A4-3.1 Essentiality

Lead is not known to be an essential micronutrient in humans or other mammals.

A4-3.2 Systemic Effects

A4-3.2.1 Human Studies

Given the vast amount of literature available on the human health effects of lead exposure, only selected epidemiological studies that investigated the neurotoxicity and other well characterized effects of lead are briefly summarized in the following paragraphs. For further details, readers of this profile are encouraged to consult ATSDR (2007) and WHO (1995), as well as the primary scientific literature.

A4-3.2.1.1 Neurotoxicity and Developmental Neurobehavioural Effects

There are eight cohort studies that contribute to the understanding of effects of exposure to lead: Boston (Bellinger *et al*., 1992), Cleveland (Ernhart *et al*., 1986), Cincinnati (Dietrich *et al*., 1993), Rochester (Canfield *et al*., 2003a) in the U.S., (Cooney *et al*., 1989) Sydney and Port Pirie (Wigg *et al*., 1988; Baghurst *et al*., 1992) in Australia, Kosovo (Wassermann *et al*., 1997; Factor-Litvak *et al*., 1999) in former Yugoslavia, and Mexico (Schnaas *et al*., 2000). Individually, these studies provide clear evidence of lead effects, and together they provide a more powerful data set for analysis of the contributions of confounding factors, period of vulnerability for lead exposure, and quantitative relationships between lead exposure and selected effects. The scales have been standardized and are widely accepted as objective evaluations for intelligence and cognitive development in children.

These studies use various measures of lead exposure (single PbB, serial PbB, lifetime PbB exposure burden, *etc*.), standardized measures or outcomes (more detailed tests of neurocognitive function, learning and achievement), and take account of factors which may be associated with lead exposure and measured outcomes (more detail on home environment, parental care and IQ, education, *etc*.). Longitudinal studies examine periods of vulnerability, as well as long-term effects spanning years after infancy, and can incorporate methodological controls to mitigate bias (*i.e.,* blindedness of the examiners to PbB values).

Lanphear *et al*. (2005) pooled the data from seven of the eight international cohorts mentioned above (all but the Sydney, Australia cohort) which fit criteria of similar methodology (longitudinal cohort), similar exposure measures (PbB at one or more ages), and similar outcome measurements (Full Scale IQ) based on similar instruments to measure outcomes, and covariate collection. Covariates considered within the

pooled analysis include: child's sex, birth order, birth weight, maternal education, maternal age, marital status of mother, pre-natal alcohol exposure, pre-natal tobacco exposure, and the HOME inventory assessment. Measures of PbB were determined for several periods: sample taken at the time of the IQ test; maximum PbB concentration; average lifetime PbB concentration (6 months of age to concurrent) and early childhood PbB (mean PbB value from six months to 24 months). The total number of children included in this analysis was 1,581. Data for all 10 covariates were available for the 1,308 children; while 1,333 children had data on the four major covariates selected in the final model from the stepwise regression analysis (maternal IQ, HOME Inventory, birth weight and maternal education).

This pooled analysis used data supplied by the authors for each cohort, and is to date, the most comprehensive analysis that attempts to quantify the relationship between lead exposure, relevant outcomes and most sensitive period for impacts. The pooled analysis demonstrated a lead-related intellectual deficit among children who had maximal PbB below 7.5 µg/dL. No evidence of a threshold for no discernable effect could be determined. For the entire pooled dataset there was an observed decline of 6.2 global IQ points (95% CI 3.8 to 8.6) for an increase in PbB from <1 to 10 µg/dL. The authors acknowledged that the limitations of this analysis included potential for residual confounding (*i.e.,* maternal depression was not assessed; HOME was not carried out at the same age for all children) (Lanphear *et al*., 2005). The strength of the Lanphear *et al*. (2005) pooled analysis rests with the method of analysis, and with the resulting consistency of the relationship between PbB measured with global IQ changes.

Tellez-Rojo et al. (2006) concurred with the evidence in Lanphear *et al*. (2005) for lead a non-threshold neurotoxicant. The study measured mental and psychomotor development (MDI and PDI) in infants of 12 and 24 months living in Mexico City whose PbB levels were < 10 µg/dL and whose PbB levels never rose above the 10 µg/dL level. Results showed that the MDI and PDI were inversely related to PbB levels (decreasing with increasing PbB levels) indicating that 10 µg/dL should not be used as a threshold value.

The U.S. based Advisory Committee on Childhood Lead Poisoning and Prevention (ACCLPP) has concluded, using the evidence from the longitudinal studies and other supporting research, that there is a negative association between PbB levels >10 µg/dL and adverse health effects (IQ decrements and possibly other effects) (ACCLPP, 2004). They also note that there is a steeper slope in the PbB-IQ dose-response curve, supported by the Lanphear pooled analysis, at lower PbB levels (Figure A4-1). They are careful to note that there are still some reservations about a direct causal relationship but that the weight-

of-evidence does favor a relationship (ACCLPP, 2004). At very low exposure levels it should be expected that variability between individuals should limit the sensitivity of a single measure to properly characterize effects responsible for intellectual deficits (ACCLPP, 2004). There remains the question as to what level of PbB indicates that a measured change in IQ is not different from the variations of the measurement tool.

Wilson *et al.*, 2005 has carried out a secondary analysis of the Lanphear *et al*. (2005) pooled data and of Canfield *et al*. (2003a) data which indicates that a change in IQ is not statistically different from normal when PbB level are less than 4 µg/dL. This calculation is concordant with other epidemiologic data to date.

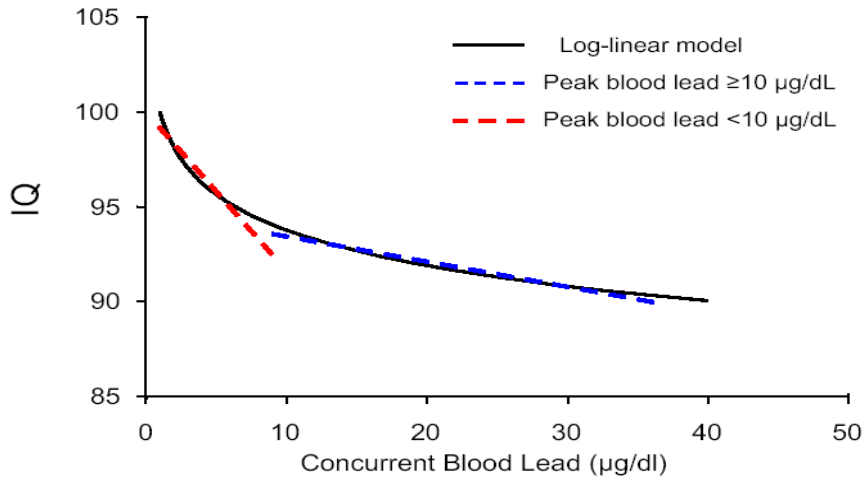

Figure A4-1 Log-linear Model for Concurrent PbB Concentration Along With Linear Models for Concurrent PbB Levels Among Children With Peak PbB Levels Above and Below 10 µg/dL (From Lanphear *et al*., 2005)

Cross sectional studies from the 1970s to the 1990s indicate that lead exposure measured in a variety of ways (*e.g.*, PbB at various ages) has a relationship to decrements in IQ, school performance and some measures of attention, as a continuum from PbB of 10 µg/dL to 40 µg/dL (Banks *et al*., 1997).

A review and meta-analysis of a number of cross sectional and prospective cohort studies, which investigated neurobehavioural aspects of lead neurotoxicity in children, was conducted by Winneke and Krämer (1997). Neurobehavioural deficits in environmentally exposed children were found to occur at PbB concentrations as low as 10 to 15 µg/dL. Based on meta-analyses conducted on the data from both

cross sectional and prospective studies, it was concluded that a doubling of PbB concentration from 10 to 20 µg/dL is associated with an average loss of one to three IQ points. A similar conclusion was reached by Pocock *et al.* (1994), WHO (1995), and ATSDR (2007). In addition to decreased IQ, some neuropsychological findings in lead-exposed children suggest that part of the impairment resembles performance deficits that are characteristic of children with attention deficit disorders (Winneke and Krämer, 1997).

Pocock *et al*. (1994) conducted a systematic review of 26 studies published since 1979 including cross sectional studies of PbB and as well as prospective studies of birth cohorts. The authors conclude that a doubling of PbB from 10 to 20 µg/dL is associated with a mean deficit of full scale IQ of approximately one to two IQ points. However, many limitations of the studies were identified: selection biases; inadequately addressing confounding factors; reverse causality between IQ and lead exposure. No evidence of a threshold associated with PbB could be determined (Pocock *et al*., 1994).

A large number of epidemiological and experimental studies in human subjects from a number of different countries have reported adverse effects on central nervous system functioning at blood lead levels around 10 µg/dL (*e.g.,* Canfield *et al*., 2003a; Canfield *et al*., 2003b; Factor-Litvak *et al*., 1999; Tong *et al*.,1998; Wasserman *et al*., 1997; Bellinger *et al*., 1992; Winneke *et al*., 1990; Lansdown *et al*., 1986; Fulton *et al*., 1987; Fergusson *et al*., 1988; Silva *et al*., 1988; Bergomi *et al*., 1989; Hansen *et al*., 1989; Hatzakis *et al*., 1989; Winneke *et al*., 1990; Lyngbye *et al*., 1990; Needleman *et al*., 1990; Yule *et al*., 1981; Lansdown *et al*., 1986; Hawk *et al*., 1986; Schroeder *et al*., 1985). A brief summary is provided for a selection of these studies below.

It is important to recognize that the epidemiological studies investigating the relationship between PbB levels and fetal and child neurotoxity are hampered by the complexity of mental developmental processes, and the questionable sensitivity and significance of IQ tests in detecting subtle differences in neuropsychologic performance. It is also important to recognize that PbB concentrations reflect the absorbed dose of lead, and that interpretation of PbB data depends on knowing the history of exposure to lead. This is because lead is stored in bone tissue within the body, and has a long half-life. Thus, in situations where an individual has not experienced chronic lead exposure, the PbB concentrations would reflect recent lead exposures. However, PbB concentrations can also reflect both recent and past exposures to lead (for example, when lead in bone is mobilized within the body during deossification, and if lead exposure is intermittent in nature). A consequence of this is that biological effects may differ for populations or individuals with the same PbB concentrations since different exposure timescales may be

involved. Some studies have attempted to address this by using biological markers that relate mainly to chronic exposure such as free erythrocyte protoporphyrin (FEP) and erythrocyte zinc protoporphyrin (ZPP) (ATSDR, 2007).

Brief Summary of a Selection of Epidemiological Studies

In a Rochester cohort of 172 children whose residences were undergoing household lead remediation, tests for venous PbB and outcomes were determined at ages six, 12, 18, 24, 36, 48 and 60 months (Canfield *et al*., 2003a). Investigators were blinded with respect to remediation activity when testing PbB. Sex, birth weight, iron status, mother's IQ and years of education, race, tobacco use during pregnancy, yearly household income and total score for HOME were considered in the statistical analysis. IQ was measured by Stanford Binet Intelligence Scale. Before adjustments, children's IQ was inversely associated with PbB measured at three and five years of age for each 10 μg/dL increase of PbB. After adjustment for nine covariates, significant inverse associations for IQ and all measures of lead exposure were found, with no significant differences in this relationship at different ages. Lifetime average of 1 μg/dL was associated with 0.46 point IQ change (CI = - 0.15 to - 0.76). There was a greater change in IQ at PbBs below 10 μg/dL when compared to levels above 10 μg/dL.

Additional tests (Shape School Task) were used by Canfield *et al*. (2003b) to examine executive function and learning in lead exposed children recruited for a separate longitudinal study in Rochester, New York. Canfield *et al*. (2003b) found that IQ performance in these children was associated with PbB levels and with Shape School Task scores. Results showed weak evidence of an association, but the authors do not dismiss the potential for impaired executive function and impaired colour discrimination linked to PbB levels.

Canfield *et al*. (2003a) results are supported by the Lanphear *et al*.(2005) pooled analysis. The authors also observed greater differences in IQ (per unit of PbB concentration) at low PbB than at the upper ranges. However, it must be noted that in the Canfield *et al*. (2003a) study there were also significant associations between child's IQ and mother's IQ and income, as well as with the child's own characteristics (birth weight).

In a study of middle class families in Boston, Bellinger *et al*. (1992) reported an apparent association between verbal functioning and PbB at 24 months. They also reported an apparent association of non verbal functioning at age ten with PbB at 24 months. Results were interpreted as "without apparent threshold". PbB levels varied from not detected to 25 μg/dL (1.21 μmol/L) in this group of 148 children.

Such a small study population is considered, nevertheless, to have insufficient power to detect significant differences. Given that differences were found, this study provided support for an association of PbB (as a marker of current exposure) at 24 months and both concurrent and long-term intellectual performance in children.

Tong *et al*. (1998) (Port Pierie, Australia cohort) reported that correlations between changing PbB and cognitive functioning was weak and lacked significance. Among children whose PbB levels decreased the most, cognitive scores did not improve relative to the scores of children whose PbB levels declined the least. It was concluded that the cognitive defects associated with exposure to environmental lead in early childhood appeared to be only partially reversed by subsequent decline in PbB. However, the analysis did not provide definitive evidence that the effects of early lead exposure could persist throughout childhood.

Factor-Litvak *et al*. (1999) examined a cohort in Yugoslavia and reported on intellectual function deficits in 577 children who resided in towns with or without a lead smelter. Adjusted losses in intellectual function as PbB increased by 20 µg/dL from 10 to 30 µg/dL were:

- Age 2: 2.5 pts (0.2 to 4.8);
- Age 4: 4.5 pts (2.2 to 4.8); and,
- Age 7: 4.3 pts (3.4 to 5.1).

At ages 4 and 7, performance scales were lower than for verbal sub scales. At age seven, losses in performance and verbal scales with an increase of PbB of 10 to 30 µg/dL were 9.4 pts (5.6 to 13.3). At all ages, associations were greater after adjustment for potential confounders. However, reported behavioural problems were more prevalent in Pristina (non-smelter town; lower socioeconomic status) than in Mitrovica (smelter town; higher socioeconomic status).

The potential role for other influences of lead exposure on childhood learning and behaviour were examined in this cohort by Wasserman *et al*. (1998). Pre-natal and post-natal PbB levels for smelter and non smelter children were similar at all stages of testing except at 36 months of age, when the smelter town children had much higher PbB than the non-smelter children. More educated mothers reported significantly more problems with sub-scales: destructive, aggressive, anxious/depressed and withdrawn behaviour, and sleep problems. Mothers who reported more positive child-rearing experiences were associated with significantly fewer problems on most subscales. Log PbB at three years of age was most

associated with behaviour; but there was no clear pattern of PbB and behaviour type. The authors stated that the magnitude of potential lead effects may be small (2 to 4% of the variance).

Winneke *et al*., 1990 noted that school-aged children in Europe (with PbB concentrations of <5 to 50 µg/dL) were found to have detectable exposure-related behavioural and cognitive effects. No threshold for neurotoxicity could be identified from these data.

Decreased IQ values among children with PbB concentrations ranging from 5.6 to 25 µg/dL have been reported (*e.g.,* Yule *et al*., 1981; Fulton *et al*., 1987; Hatzakis *et al.,* 1989). Cooney *et al*. (1989) reported that at a PbB concentration of 0.25 µg/dL, there were no adverse effects on neurobehavioural development. While this study suggests a threshold for the effect of lead on neurobehavioural parameters, other studies indicate there is no threshold, and the issue remains one of considerable controversy and debate. Several authors suggest a LOAEL of 10 to 15 µg/dL for perinatal PbB concentrations (Wolf *et al*., 1985; Bellinger *et al*., 1987; Dietrich *et al*., 1987; Wigg *et al*., 1988).

Volpe *et al*. (1992) reported that lead exposures resulting in PbB concentration of less than 25 µg/dL are not associated with neurobehavioural deficits in children.

A study of the effect of environmental exposure to lead was conducted on a cohort of 537 children born near a lead smelter, near Port Pirie in South Australia (McMichael *et al*., 1988). Results of this study showed that child development at ages two, three, and four appeared to be inversely related to postnatal PbB concentrations based on the McCarthy Scales of Children's Abilities. Reductions in perceptual performance and memory scores were also reported. The authors cautioned that the data was somewhat equivocal due to the difficulties in defining and controlling for confounding variables and effects. In a follow-up study of this cohort, an increase in PbB concentration from 10 to 30 µg/dL was shown to cause a decrease in the General Cognitive Index score on the McCarthy Scales (which combines scores for verbal, perceptual-performance, and memory and motor performance subscales) (McMichael *et al*., 1992). A significantly stronger inverse relationship between PbB concentrations and children's intelligence scores was observed for girls relative to boys.

Kindergarten-aged children in the vicinity of a battery recycling smelter in Taiwan, with PbB concentrations of 15 to 25 µg/dL, showed a mild but significant decrease in IQ, compared to kindergarten children from a reference area (Wang *et al.* 1998). Average air concentrations in the kindergarten classroom were >10 ug Pb/m^3 and nearby soil samples were as much as 400 times greater than

background lead concentrations. A follow-up study conducted 2.5 years after children moved away from the smelter area showed a significant decrease in PbB concentrations and partial recovery of IQ.

Bellinger *et al*. (1987) conducted a prospective cohort study of children (n=249) living in the Boston area, from birth to two years of age. While postnatal exposure was not associated with detrimental effects, prenatal exposure was reported to impair early cognitive development [assessed using the Bayley Mental Development Index (MDI)]. In a follow-up to the Boston prospective study, Bellinger *et al*. (1991) found that prenatal PbB concentrations >10 µg/dL in cord blood was associated with a slower cognitive development in children up until at least 24 months of age. After 57 months of age, however, prenatal exposure was not related to intelligence test results.

Dietrich *et al*. (1987) investigated the effects of chronic low to moderate fetal lead exposure in lead-hazardous areas of Cincinnati (n=305). A direct relationship between prenatal, umbilical and newborn PbB concentrations and deficits on the Bayley MDI at three and six months was observed. Male infants appeared more sensitive (Dietrich *et al*., 1987). Further study suggested that the neurobehavioural deficits were partly mediated by lead-related reductions in birth weight and gestation. After adjustments were made for confounding variables, there was a statistical significance between postnatal lead blood concentrations of >10 µg/dL and lowered Performance IQ, when compared to children with mean blood concentrations <10 µg/dL (Dietrich *et al*., 1993).

The association between the physical and behavioural characteristics of infants, and maternal and umbilical cord PbB concentrations was investigated among a sample of 42 mother-baby pairs from a heavily industrialized area of Mexico (Rothenberg *et al*., 1989). As maternal lead concentrations at birth increased, the consolability and self-regulating behaviour of the infants was decreased for as long as 30 days after birth. Increased maternal PbB concentrations were also associated with decreased gestational age.

The U.S. EPA (2006) has reviewed several studies (Nordberg et al. 2000; Rhodes et al. 2003; Wright et al. 2003; Weisskopf et al. 2004a; Krieg et al. 2005 etc.) associating exposure of relatively low levels of environmental lead with decreased cognitive performance in older adults. To determine exposure, PbB and bone Pb concentrations were measured. No association between PbB levels with changes in cognitive performance could be determined (Nordberg et al. 2000; Rhodes et al. 2003; Krieg et al. 2005). However, decreased cognition was found to be associated with increased bone Pb levels (Wright et al. 2003; Weisskopf et al. 2004a) suggesting that cumulative exposure may be an important factor (U.S. EPA 2006).

Neurological effects associated with the occupational exposure to Pb in adults have been examined in several studies and summarized by the U.S. EPA (2006). A blood Pb threshold of ≥ 14 µg/dL has been proposed for these effects (U.S. EPA, 2006). Individuals exposed to high lead concentrations in their workplace have exhibited symptoms such as fatigue, irritability, inability to concentrate for prolonged periods of time, as well as neurological disorders with verbal intelligence, memory, and perpetual speed (Baker *et al*., 1990). Exposure to organolead compounds such as trimethyl Pb and tetraethyl Pb have also been linked to decreased manual dexterity and verbal memory/learning (Balbus et al. 1997; 1998; Schwartz et al. 1993; 2000; 2001b; Stewart et al. 1999). Lucchini et al. (2000) found that the PbB threshold for neurological symptoms such as decreased concentration, irritability and muscle pain to be 12 µg/dL. However, studies by Chia et al. (1997) and Osterberg et al. (1997) found no neurological effects despite mean PbB levels of greater than 30 µg/dL. Psychomotor, motor speed and dexterity were shown to decrease with PbB threshold levels of 18 µg/dL (Schwartz et al. 2001a). Other studies shown no effects on neurobehavioral performance with PbB levels < 30 µg/dL, but cumulative Pb exposure was associated with decreased neurobehavioral performance (Lindgreen et al. 1996; Bleecker et al. 1997a; 2005a).

A recent study (Fujimara *et al*., 1998) reported that two male smelter workers with PbB concentrations of 105.6 and 76.5 µg/dL, respectively, showed significantly delayed values for motor and sensory nerve conduction velocities and evoked potentials, relative to controls. A general finding of this study was that peripheral nerves appear to be more sensitive to lead than the central nervous system, possibly due to the blood-brain barrier or impairment of slow axonal transport in peripheral nerves by lead (Yokoyama and Araki, 1992). The WHO (1995) has suggested that subclinical effects of occupational lead exposure occur at 30 µg/dL for delayed nerve conduction velocities, 35 µg/dL for interval variability changes and 40 µg/dL for neurobehavioural changes.

In general, sensory nerve impairment was found to be associated with long term exposure with a PbB threshold of 28 to 30 µg/dL (Chia et al. 1996a; 1996b; Kovala et al. 1997; Yokoyama et al. 1998; Chuang et al. 2000; Bleecker et al. 2005b). At PbB levels between 17 to 20 µg/dL, prolonged visual evoked potentials (VEPs) were found (Abbate et al. 1995). Prolonged latencies in the brainstem auditory pathway were also associated with cumulative PbB levels (Holdstein et al. 1986; Discalzi et al. 1992; 1993; Bleecker et al. 2003). Negative effects on postural sway; a task that requires visual, vestibular and peripheral sensory inputs as well as motor output, was effected by increased PbB levels (Chia et al. 1994a; 1996c; Yokoyama et al 1997; Dick et al. 1999; Ratzon et al. 2000). However, Iwata et al. (2005) characterized a PbB level of 14 µg/dL to have a negative effect on postural sway. PbB levels of ≥ 20

µg/dL were found to compromise the parasympathetic and sympathetic nervous systems (Teruya et al. 1991; Ishida et al. 1996; Niu et al. 2000).

The U.S. EPA (2006) reviewed studies (Armon et al. 1991; Chancellor et al. 1993; Kamel et al. 2002, 2003; Louis et al. 2003; etc.) which examined the potential effects due to previous occupational exposure to elevated levels of Pb. Armon et al. (1991), Chancellor et al. (1993) and Kamel et al. (2002) have found increased risks of developing amytrophic lateral sclerosis (ALS); a neurodegnerative disease, with past occupational exposure to Pb. Kamel et al. (2003) also showed that individuals with the ALAD2 allele (codes for the enzyme aminolaevulinic acid dehydratase; involved in the production of heme); had a 2-fold increased risk of developing ALS compared to individuals who had the ALAD1 allele. Increased risks of essential tremor (ET) of up to 30 times were also associated with the ALAD2 allele (Louis et al. 2003).

While PbB concentrations have been the major indicator of lead exposure in the available human studies, others have investigated lead concentrations in teeth, and associated neurological and behavioural effects in children. Needleman *et al*. (1979) estimated lead exposure from concentrations measured in the dentine of deciduous teeth in a cross-section of first and second graders in two Massachusetts communities (n=270). The neuropsychological performance of each child was evaluated using a number of tests including the Wechsler Intelligence Scale for Children-Revised (WISCR). The high dentine lead children performed significantly worse on the Full Scale and Verbal Subscale of the WISCR, with verbal and auditory processing, attention and classroom behaviour being the most sensitive indicators. In a followup study of this cohort, Needleman *et al*. (1990) reported that the educational success of young adults was significantly inversely associated with the amount of lead in teeth they shed as first and second graders. Teeth lead levels above 20 mg/kg were associated with a seven-fold risk of not graduating from high school, a six-fold risk of having a reading disability, deficits in vocabulary, problems with attention and fine motor coordination, greater absenteeism, and lower class ranking. The authors concluded that the early exposure to lead had an enduring effect on children, well into adolescence. However, it was also found that teeth lead levels did not correspond well to PbB levels. In the initial cohort, the available preschool blood lead levels for highly exposed children averaged 35 µg/dL (Needleman *et al*., 1979).

The Needleman studies have been the subject of some controversy. Criticisms of the statistical analysis include improper control of confounding variables, improper exclusion of data such that groups of cases from the original sample of children tested were systematically excluded, and failure to give adequate consideration to the issue of multiple comparisons in the analysis of a large number of variables.

Furthermore, there has been some difficulty in obtaining copies of the original data for peer review (Ernhart and Scarr, 1991).

A4-3.2.1.2 Hematological Effects

Anemia and altered blood enzyme levels or activity have been commonly correlated to elevated PbB concentrations in the scientific literature. However, hematological effects are less sensitive endpoints for lead toxicity than neurological, neurodevelopmental, and neurobehavioural effects. For anemia, there is a linear relationship with PbB concentrations (Landrigan, 1991).

The mechanism of action for lead-induced anemia is thought to be primarily due to inhibition of the enzyme ferrochelatase, which catalyses the transfer of iron from ferritin into protoporphyrin to form haem. This inhibition results in an increased excretion of coproporphyrin in the urine and accumulation of free erythrocyte protoporphyrin (FEP) (Landrigan, 1991). The U.S. EPA (2007) has stated that elevated FEP is potentially adverse to the health of young children. Elevated delta-amino-levulinic acid and coproporphyrins in the urine have been reported in children and adults with PbB concentrations in the range of 30 to 40 µg/dL (Landrigan, 1991). However, the precise mechanism(s) underlying lead-induced anemia remain unclear. It has been hypothesized that, in addition to impaired haem synthesis and ferrochelatase inhibition, the inhibition of erythropoietin synthesis (a hormone that stimulates production of erythrocytes) may be an important factor leading to lead-induced anemia (Factor-Litvak *et al.* 1998). Erythropoietin is produced mainly in the proximal renal tubule and regulates both steady-state and accelerated erythrocyte production (Erslev and Caro, 1986). Levels of this hormone have been shown to be significantly depressed in pregnant women with moderately elevated PbB concentrations (Graziano *et al.* 1991). A prospective study with children aged 4.5, 6.5, and 9.5 years (n=211, 178, and 234, respectively), found a positive association between PbB and erythropoietin concentrations (Factor-Litvak *et al.,* 1998). The association was strongest in children aged 4.5 years and weakened considerably by 9.5 years. Mean PbB concentrations in the 4.5-year group were 38.9 µg/dL, and were 28.2 µg/dL in the 9.5-year group. However, none of the children in this study showed clinical signs of anemia. The authors concluded that in lead-exposed children, normal hemoglobin maintenance requires hyperproduction of erythropoietin. As children age and continue to be exposed to lead, this compensatory mechanism appears to fail, suggesting a gradual loss of renal endocrine function in association with chronic lead exposure (Factor-Litvak *et al.,* 1998).

Unlike neurological, neurodevelopmental, and neurobehavioural effects, there is believed to be a threshold for lead-induced anemia. Recent studies reported that 40 µg/dL PbB is associated with the impairment of heme synthesis and ethrocyte survival contributing to clinical symotoms of anemia in children (Schwartz *et al.,* 1990; U.S. EPA, 2006; 2007). PbB levels ≥ 40 µg/dL are thought to cause Frank anemia, which is evident only with elevated PbB levels over prolonged periods of (U.S. EPA 2006; 2007). In adults, a level of 50 µg/dL PbB has been associated with heme effects contributing to anemia (U.S. EPA, 2006). Schwartz *et al.* (1990) reported that blood levels associated with anemia increased with age in a model relating blood concentrations to hematocrit measurements in children in a contaminated area. A 10% probability of anemia (hematocrit <35%) was associated with 20 µg/dL PbB and 75 µg/dL PbB at one and five years of age, respectively.

ALAD (aminolaevulinic acid dehydratase) is another critical enzyme involved in heme production, and its inhibition is also associated with the development of anemia (ATSDR, 2007; WHO, 1995). The threshold PbB concentration for increases in FEP and decreased ALAD acitvity is in the range of ~20 to 30 µg/dL in children and adults (Nutrition Foundation, 1982; Landrigan, 1991; Roels and Lauwerys, 1987; Morita *et al.,* 1997). Blood lead levels in this range are sufficient to halve ALAD activity and double FEP levels (U.S. EPA, 2006). Numerous studies report elevated FEP levels above approximately 15 µg/dL PbB in children, and between 15 to 30 µg/dL PbB in adults (U.S. EPA, 2007). Studies of children living in proximity to a lead smelter indicated a threshold PbB concentration of 60 µg/dL or greater for effects on FEP (McNeil *et al*., 1975; Landrigan *et al*., 1976). In general, the inhibition of ALAD activity has beeen reported at levels below 10 µg/dL in children and adults. Chisolm *et al*. (1985) found PbB concentrations as low as 5 µg/dL in association with inhibited ALAD activity in children.

A4-3.2.1.3 Other Effects

Renal effects are believed to be the result of lead-induced inhibition of mitochondrial respiration and phosphorylation (Goyer, 1989). A number of studies indicate an association between lead exposure and adverse renal effects in adults. The NHANES III general population study reported alterations in urinary creatinine rates in hypertensive subjects at a mean PbB concentration of 4.2 µg/dL, and a women's health study reported significant associations between both ceatinine clearance and glomerular filtration rates and PbB at a mean PbB level of 2.2 µg/dL (Akesson *et al*., 2005). In several studies with mean PbB levels <10 µg/dL, changes in creatinine clearance associated with each µg/dL increase in PbB range from 0.2 to 1.8 mL/min (U.S. EPA, 2006). However, low PbB effect levels are confounded with occupational studies documenting nephropathy at higher blood levels ranging at PbB levels starting from >30 to 40 µg/dL

(U.S. EPA, 2006). A threshold for nephrotoxicity has not been determined based on information to-date (U.S. EPA, 2006, 2007).

Chronic lead exposure may result in irreversible kidney-related changes such as interstitial fibrosis, tubular atrophy, and glomerular sclerosis, as well as hypertension and gout (Goyer, 1989; Nolan and Shaikh, 1992; Morgan *et al.,* 1996). Some studies have found evidence of renal tubular dysfunction in children living in the vicinity of lead smelters (Bernard *et al.,* 1995; Verberk *et al.,* 1996).

Numerous epidemiological studies have linked lead exposure with the risk of cardiovascular effects such as hypertension and increased blood pressure in adults; however, there is currently considerable scientific debate as to whether there is a causal relationship between lead exposure and adverse cardiovascular outcomes (ATSDR, 2007; U.S. EPA, 2006, 2007). A critical level of blood PbB causing increased blood pressure has not yet been established (U.S. EPA, 2006). Occupationally exposed populations with PbB levels of between 30 to 120 µg/dL experienced hypertension, increased heart rate and increased blood pressure (Pollock and Ibels, 1986; Weiss *et al*., 1986, 1988; de Kort *et al*., 1987; Marino *et al*., 1989). However, these studies all had small cohort sizes (n<100) and failed to control for at least one confounding factor (ATSDR, 2007). A number of other studies have failed to find a strong correlation between PbB and cardiovascular effects (ATSDR, 2007; WHO, 1995). A critical meta-analysis of 31 studies representing different sex and racial groups reported significant increases in systolic (1.0 mm Hg) and diastolic (0.6 mm Hg) blood pressure associated with doubling of PbB (range 2.3 to 63.8 µg/dL) (Nawrot *et al.*, 2002). Kannel (2000) utilized Nawrot *et al.* (2002) data to estimate risk of blood pressure associated with PbB at the population level. A decrease in PbB from 10 to 5 µg/dL was reported to result in a decrease in the annual number of cardiovascular events (27-39 events per 100,000 persons).

The evidence addressing cardiovascular outcomes in relation to lead exposure in children is limited. Factor-Litvak *et al.* (1996) found marginally significant assocations between increases in systolic (0.054 mm Hg) and diastolic (0.042 mm Hg) blood pressure with each µg/dL increase in PbB in children 9.5 years of age. Clear positive associations between PbB levels and cardiovascular functions were not evident in other studies, and results were confounded by additional factors, such as other sources of contamiant exposure and small smapel sizes (Gump *et al.*, 2005; Chen *et al.*, 2006). Neither a threshold nor a range of PbB concentrations has been identified to-date (U.S. EPA 2006, 2007).

Long-term exposure of lead as measured in bones is also important in assessing cardiovascular effects (U.S. EPA, 2006). Lead concentrations in bone have been associated with high blood pressure in adults. Various studies have reported an increased odds ratio of hypertension with each 10 µg/g increase in bone

Pb over concentratrions ranging from <1 to 96 µg/g (Hu *et al.,* 1996; Korrick *et al.,* 1999; Cheng *et al.,* 2001; Rothenberg *et al.,* 2002). Rothenberg *et al.* (2002) and Glenn *et al.* (2003) reported an increase in systolic blood pressure in adults of ~ 0.75 mm Hg associated with a 10 µg/g increase in bone lead ranging from 1 to 52 µg/g. Implications of childhood exposure were addressed in a study that showed increased blood pressure in adults who lived near active lead smelters as children was associated with elevated bone Pb levels; however, blood lead levels were not linked with the adverse effects Gerr *et al.*, (2002).

Associations between PbB and biomarkers of immune function have been reported in several human studies. Significant associations between biomarkers of humoral immunity (serum IgE levels) in children at PbB levels < 10 µg/dL have been reported (Sarasua *et al.,* 2000; Karmus *et al.,* 2005). The lowest PbB concentration linked elevated IgE in adults is ≥30 µg/dL (Heo *et al.,* 2004). Significant associations between biomarkers of cellular immunity (T-cell abundance) and PbB levels have also been reported at levels < 10 µg/dL in children; however, other studies did not report associations at higher PbB concentrations (Lutz *et al.,* 1999). Evidence that has been reviewed to-date has not warranted a threshold PbB level for immunotoxic effects (U.S. EPA, 2007).

Studies have reported associations between phagocyte and lymphocyte function and PbB levels ranging from 4 to 90 µg/dL in children and adults (U.S. EPA, 2006). In one study, male workers from a storage battery plant (n=25) with a mean PbB concentration of 74.8 µg/dL showed a significant decrease in chemotaxis and random migration of neutrophils, relative to controls, where the mean PbB concentration was 16.7 µg/dL (Undeğer and Basaran, 1998). The results of this study suggest that chronic occupational lead exposure may diminish neutrophil function, which may result in a reduced immunological response to infections.

A4-3.2.2 Animal Studies

The following summary focuses on relevant chronic duration animal studies. Information on acute and subchronic animal studies is available in ATSDR (2007) and WHO (1995). Only oral exposure studies were identified for lead; there were no chronic inhalation or dermal exposure studies found in the available scientific literature. A unique feature of lead toxicology research in animals, compared to most other metals, is that there are extensive data available on various forms of systemic toxicity resulting from pre-natal exposures. These studies are described in this section as the effects investigated are not directly related to reproductive, teratogenic or other typical developmental toxicity parameters or endpoints. Although, it is recognized that the damage leading to these systemic effects occurs during developmental stages.

Subchronic and chronic exposure of rodents to lead has resulted in intranuclear inclusion bodies in the proximal tubular epithelium of the kidney, as well as functional and ultrastructural changes in the kidney mitochondria leading to hyperaminoaciduria, glycosuria and hyperphosphaturia, at PbB concentrations of >70 µg/dL (Nutrition Foundation, 1982).

Hubermont *et al*. (1976) investigated the effects of prenatal exposure to lead on heme metabolism in pups. Lead nitrate was administered at concentrations of 0.009, 0.09, and 0.9 mg lead/kg/day in drinking water to dams before mating, throughout gestation, and during lactation. PbB levels in the dams and pups that received the high dose were 42 and 68 µg/dL, respectively. An increase in free tissue porphyrins and a decrease in blood ALAD activity was observed in the high dose pups, relative to controls. A more recent study (Bogden *et al*., 1995) found an increase in free erythrocyte protoporphyrins in 1 week old rats exposed to lead acetate throughout gestation and seven days of lactation. These effects did not occur in 1day old pups, after similar gestational exposure. Interestingly, the PbB concentration in the 1day old pups was higher (72.5 µg/dL) than in the seven day old pups (51.8 µg/dL). Hayashi (1983) administered lead acetate at 0.7 mg lead/kg/day in the drinking water of rats for the first 18 or 21 days of pregnancy. This treatment resulted in decreased ALAD activity in fetal and maternal erythrocytes, and increased ALAD activity in fetal but not maternal liver. In addition, fetal, but not maternal, hematocrits and hemoglobin levels were decreased in the group treated for 21 days. Fetal PbB levels were 27 µg/dL and 19 µg/dL in the 18-day and the 21-day treated groups, respectively. Maternal PbB levels were approximately 4 µg/dL in both treated and control groups.

Some studies have shown that exposure to lead during critical developmental stages may result in immunotoxicity in the offspring. Nine-week old female Fischer 344 rats were prenatally exposed to lead acetate administered to dams in drinking water at concentrations of 0, 100, 250, and 500 ppm during breeding and pregnancy (Miller *et al.,* 1998). Macrophage cytokine and effector function properties were elevated in the 250 ppm dose group, while cell-mediated immunity was depressed. Interferon levels were decreased in the 500 ppm dose group and serum IgE levels were increased in the 100 ppm group. The authors concluded that maternal exposure to moderate lead concentrations produces chronic immune modulation in F344 rat offspring exposed *in utero*. The dams showed no immune alterations at any of the dose levels tested.

Substantial species differences in sensitivity to the behavioural effects of lead have been clearly demonstrated in laboratory animals, with monkeys being one of the most sensitive species (Nutrition Foundation, 1982). Monkeys demonstrated enhanced agitation at PbB concentrations ranging from 33.1

to 42.9 µg/dL (Levin *et al*., 1988). When PbB concentrations were 11 to 13 µg/dL, three-year old monkeys, exposed to lead from birth, showed impaired responses in discrimination reversal tasks, non-spatial form discrimination and non-spatial colour discrimination (Rice, 1985).

Monkeys with average PbB concentrations of 33.1 to 42.9 µg/dL experienced decreased visual attentiveness (Levin *et al*., 1988). Monkeys showed impaired learning and decreased visual acuity for a variety of tasks at PbB concentrations ranging from less than 40 to 85 µg/dL (Bushnell *et al*., 1977; Bushnell and Bowman, 1979; Rice *et al*., 1979; Rice and Willes, 1979).

Rice (1992a) examined the behavioural effects of lead on newborn monkeys orally dosed with lead acetate to yield a blood concentration plateau of 33 µg/dL. No discrimination reversal impairment, and no differences in behavioural delayed alternation were observed; however, differences in performance were observed in the differential reinforcement of low rate schedule (DRL) behavioural test, and in the monkeys' ability to learn visual discrimination. In further work with monkeys, Rice (1992b) demonstrated that monkeys that were exposed to lead at PbB concentrations of 19 to 36 µg/dL exhibited significant impairment to behaviour. Rats developed signs of hyperactivity at PbB concentrations of 40 to 60 µg/dL (Overmann, 1977) with transient hyperactivity observed at PbB concentrations of 20 to 59 µg/dL.

Golter and Michaelson (1975) reported increased activity level in rats orally administered 1.09 mg lead/day as a lead acetate solution.

Rabe *et al*. (1985) evaluated neurodevelopmental endpoints in the offspring of rats exposed to lead acetate at 448 mg lead/kg/day in drinking water prior to mating, and throughout gestation. Mean PbB levels were 98 µg/dL at day 1 and 20 µg/dL at day 16 in pups from treated dams, and approximately 10 µg/dL at these times in pups from control dams. Body weights were reduced in treated pups relative to controls at birth but not at day 30. Neurobehavioral function (based on surface righting and negative geotaxis reflexes, spatial discrimination, and reversal in T-maze) in treated rats did not differ from the controls, when tested in the pups at day 17.

Rodrigues *et al*. (1993) evaluated open field behavior in 6-month old rats that had been exposed to lead acetate *in utero*, and during lactation, *via* their drinking water. The daily doses of lead were estimated to be 18, 36, and 146 mg/kg/day, which resulted in PbB concentrations of 51, 67, and 169 µg/dL. Lead-treated rats showed an altered activity pattern in the mid and high dose groups that was characterized by increased activity in the open field, and failure to habituate to the test environment.

A number of studies have been conducted on the neurological and neurobehavioural effects of lead in experimental animals, when administered during gestation, and in a number of studies, during postnatal lactation periods as well. Selected studies are briefly summarized below.

Offspring of female Wistar rats orally administered 1.0 mM lead acetate in drinking water displayed a hastened appearance of eye opening, startle reflex and negative geotaxis, while spontaneous alternation performance was delayed relative to controls (Mello *et al.,* 1998).

Nagymajtényi *et al.* (1998) treated rats with 80, 160, and 320 mg/kg body weight lead acetate by gavage during days five to 15 of pregnancy, or days five to 15 of pregnancy and four weeks of lactation, or days five to 15 of pregnancy and four weeks of lactation and eight weeks post-weaning. Male rats exposed to lead during pregnancy (only) displayed an increased hyperactivity and grooming behaviour. Electrophysiological functions in male rats showed dose-dependent changes.

Taylor *et al*. (1982) found that when female rats were exposed to lead acetate (0, 28 and 56 mg lead/kg/day) *via* drinking water, prior to mating and through gestation and lactation, there were no significant differences in the pups' acquisition of a response at 11 days of age. However, there was a significantly slower extinguishing of the response when the reward was no longer provided at 21 days. PbB levels at 21 days were 3.7 µg/dL in controls, 38.2 µg/dL in the low-exposure group, and 49.9 µg/dL in the high-exposure group.

Jett *et al*. (1997) reported impaired learning in a swimming task in rat pups that were administered 250 ppm lead acetate in the diet, starting 10 days prior to breeding and continuing throughout gestation and lactation. The impaired learning was evident at 21 days of age, but not at 56 or 91 days. PbB levels were not provided in this study, but it was noted that lead concentrations in the hippocampus of the treated pups was 41 to 47% lower at 56 and 91 days than it was at 21 days.

A study with monkeys reported prolonged deficits in learning and motor functions, in animals between the ages of three and seven years, that had been exposed to lead *in utero* (Newland *et al*., 1996). During exposure, maternal PbB concentrations ranged from 21 to 70 µg/dL.

Rats exposed to lead during gestation and through postnatal day 28 (during lactation) showed a 30 to 40% reduction in choline acetyltransferase activity (ChAT) in the septum and hippocampus, relative to controls (Bielarczyk *et al.,* 1994). The PbB concentration in the pups was approximately 20 to 22 µg/dL compared with 2 to 3 µg/dL in controls. There was also a reduction in muscarinic cholinergic receptor

binding in the septum, but not in the hippocampus. A followup study found that early lead exposure in rats causes long-lasting cholinergic deficits which induce secondary responses in the hippocampus that resemble compensatory changes (Bielarczyk *et al*., 1996; Bourjeily and Suskiw, 1997).

Sierra *et al*. (1989) found that gestational exposure of guinea pigs to 5.5 or 11 mg lead/kg/day produced dose-dependent alterations of the neuroglial enzymes, glutamine synthetase and glycerol-3-phosphate dehydrogenase. PbB levels in treated dams and fetuses were within the range of 10 to 30 µg/dL lead.

A4-3.3 Reproductive/Developmental Toxicity

A4-3.3.1 Human Studies

The majority of the human data on reproductive toxicity comes from observations in occupational cohorts. Both ATSDR, 2007 and U.S. EPA, 2006 have stated that the majority of these studies are limited by such factors as lack of quantitative exposure data, co-exposure to other chemicals and inadequate or complete lack of controlling for other risk factors (such as family history, socioeconomic status, pre-existing diseases, alcohol, smoking *etc.*).

Many occupational studies also indicate that reproductive effects occur in male workers with high lead blood concentrations (ATSDR, 2007; U.S. EPA, 2006). These studies have measured reproductive effects using endpoints such as semen quality (e.g., sperm count, motility and morphology); time to pregnancy and reproductive history of the male (number of pregnancies fathered) (U.S. EPA, 2006). Lancranjan et al., 1975; Cullen et al., 1984; Assennato et al., 1986; Chowdhury et al., 1986; Lerda, 1992 and Alexander et al., 1996a have all shown that increased occupational exposure to Pb have resulted in decreased sperm counts and where measured, increased amounts of abnormal sperm and decreased motility (U.S. EPA, 2006). In many of these cases, adverse effects have occurred at levels > 40 µg/dL (U.S. EPA, 2006). Bonde et al. (2002) and Hernandez-Ochoa et al. (2005) have shown that decreased sperm counts and adverse effects to morphology and quality were likely to occur at levels > 45 µg/dL. However, other counfounding variables such as socio-economic status, environmental expsosures, cigarette smoking and alcohol consumption need to be accounted for (Hernandez-Ochoa et al. 2005). Benoff et al. (2003a,b) found higher Pb concentrations in the seminal fluid in male partners who were part of a couple undergoing artificial insemination or in vitro fertilization which indicated a possible transfer of Pb from parental exposure to fetal development which led to Hernandez-Ochoa et al. (2005) concluding that the determination of Pb in seminal fluid may be a better indicator of exposure than PbB since adverse effects in sperm could be correlated with higher Pb levels in seminal fluid or in Pb in

spermatozoa. Overall, effects on sperm are observed starting at around 30-40 µg/dL PbB (ATSDR, 2007). However, given the small sample sizes used in these occupational studies, the relevance of these data to the general population is unknown (ATSDR, 2007).

Measuring time to pregnancy was less conclusive (U.S. EPA, 2006). Joffe et al. (2003) showed no connections between high exposure to Pb and time to pregnancy, however using the data from the same study but different analysis, Apostoli et al. (2000) showed an increased time to pregnancy with men who had Pb exposure. Similarly, Shiau et al. (2004) showed similar results, particularly when PbB levels were ≥ 30 µg/dL. Reproductive history (achieving a pregnancy) was monitored but associations with occupation Pb exposure were varied amongst studies. Bonde and Kolstad (1997), used birth registries and employment records to link occupational exposure to Pb (time and place) with birth rates and no associations were made. Lin et al. (1996) and Sallmen et al. (2000) found fewer birth rates with increased PbB. In the Lin et al. (1996) study, effects were seen at levels greater than 25 µg/dL and in the Sallmen et al. (2000) study, levels were divided into three groups, 1.3 to 1.9 µg/dL, 10 to 20 µg/dl and greater than 50 µg/dL.

A limited number of studies have examined the effects of lead on female reproductive function. Studies have mainly addressed the issue of time to pregnancy. One study (Sallmen et al., 1995) showed that time to pregancy did not differ in women who had PbB of < 10 µg/dL, 10-19 µg/dL and ≥ 20 µg/dL, but with PbB levels between 29 to 50 µg/dL there was a slight increase in time to pregnancy. However, the larger PbB levels range had a considerably smaller sample size (U.S. EPA, 2006).

Developmental effects have also been reported in a large number of human studies where there was pre-natal exposure to low levels of lead. The most commonly reported developmental effects include reduced birth weight, reduced gestational age and neurobehavioral deficits or delays. At this time, there is no clear evidence of an association between prenatal lead exposure and major congenital malformations (ATSDR, 2007; U.S. EPA, 2006). The evidence for an association between PbB levels and reduced birth weight and gestational age is inconsistent, and therefore equivocal. Evidence in support of neurobehavioral deficits or delays is much more consistent, with most studies indicating a positive association between low level lead exposure and developmental neurobehavioral effects (ATSDR, 2007). Given the availability of good human data on the developmental toxicity of lead, less recent emphasis has generally been placed on animal studies. However, there is also a wealth of data available on the reproductive and developmental toxicity of lead in experimental animals, and the results are generally in good agreement with those of the human studies (ATSDR, 2007).

All available human studies investigating the reproductive or developmental toxicity of lead compounds have involved multi-route exposure, of which oral exposure has generally dominated (although inhalation was likely a major route in many of the occupational studies). No studies were identified regarding reproductive or developmental effects of lead in humans after specific, single-route exposure *via* oral, inhalation or dermal routes.

Selected relevant human studies which investigated the reproductive or developmental toxicity of lead are briefly described below. Although many of these studies are considered to have been well-conducted by ATSDR (2007), it is important to recognize that a number of studies suffered from various limitations including: lack of actual measures of exposure, possible additional non-occupational exposure to lead, simultaneous exposure to other chemicals, confounding by other known risk factors for developmental toxicity (smoking, alcohol), reliance on hospital records, and failure to account for home and family influences on behavior and development.

McMichael *et al*. (1986) reported that the relative risk of preterm delivery increased more than 4-fold at PbB levels of >14 µg/dL compared with PbB levels below 8 µg/dL. An interesting finding in this study was that the average maternal PbB level at delivery was significantly lower for stillbirths than for live births, and maternal and cord PbB levels were also lower in low-birth-weight pregnancies than in pregnancies with birth weights >2,500 g. Davis and Svendsgaard (1987) suggested that these findings may reflect an increased transfer of lead from mother to fetus, which is supported by the inverse correlations noted between placental lead levels and birth weight, head circumference, and placental weight (Ward *et al*., 1987) and increased levels of lead in the placenta in cases of stillbirth and neonatal death (Wibberlev *et al*., 1977). The latter authors also suggested that such findings may reflect accumulation of lead in the placenta during times of fetal stress.

A cohort of infants from Cincinnati (whose mothers had high PbB concentrations during pregnancy) were followed from birth to age 15 months (Shukla *et al*., 1989). A statistically significant negative correlation between PbB concentrations in infants and growth rate was observed. Infants who had a 10 µg/dL or greater PbB concentration during the three to 15 month age period, were approximately 2 cm shorter in height at 15 months than infants who had a <10 µg/dL PbB concentration, even though both sets of infants were born to mothers with pre-natal blood concentrations greater than 7.7 µg/dL.

Moore *et al*. (1982) conducted a cross-sectional study of 236 mothers and their infants in Glasgow, Scotland, and found reductions in gestational age with increasing cord or maternal PbB lead levels. In 11 cases of premature birth (gestational age <38 weeks), maternal PbB levels averaged 21 µg/dL, and cord

PbB lead levels averaged 17 µg/dL at delivery. The overall geometric mean PbB levels at delivery were 14 µg/dL (maternal) and 12 µg/dL (cord). Statistical analyses showed a significant negative coefficient for length of gestation against log-transformed maternal or cord PbB levels. Birth weight was not associated with PbB lead levels in this study.

In a prospective cohort study (Factor-Litvak *et al*., 1999), prenatal lead exposure and reproductive outcome was investigated in pregnant women from two towns in Kosovo, Yugoslavia (Titova Mitrovica is a lead smelter town, and Pristina is the reference town 25 miles south of Titove Mitrovica with no lead smelter). At mid-pregnancy, 401 and 506 women were recruited from the smelter and reference towns, respectively. Mean PbB concentrations in the smelter and reference towns were 19 and 5.6 µg/dL, respectively. At the time of delivery, mean PbB concentrations were 23.4 and 6.8 µg/dL, respectively. There were no differences found between the two towns with respect to birth weight or length of gestation. In addition, no associations were observed between PbB concentrations (maternal and cord, both at mid-term and time of delivery) and birth weight, length of gestation, or preterm delivery.

As part of the National Health and Nutritional Examination Survey (NHANES III) conducted from 1988 to 1994, 2,186 girls (aged 8 to 18) were sampled for PbB and asked questions about their pubertal development (as measured by pubic hair stage (n=1,986), breast development stage (n=1,986), and age at menarche (n=1,796). The data were adjusted for age, race, smoking status, dietary information, presence or absence of anemia and socioeconomic status (SES). A PbB level of 3 µg/dL was associated with delayed pubertal development in African American, Mexican American and non-Hispanic white American girls (Selevan *et al*., 2003). Higher PbB were associated with statistically significant delays in all pubertal measures among African American girls, and with breast and pubic hair development among Mexican American girls; changes were similar but not significant among non-Hispanic white girls. Higher PbB levels were significantly associated with a higher age at puberty for African American girls, causing delays of three months. A PbB level of 3 µg/dL was also significantly associated with decreased height, but not weight or body-mass index (Selevan *et al*., 2003).

A4-3.3.2 Animal Studies

In general, animal studies support the findings from human studies with respect to reproductive or developmental toxicity of inorganic lead compounds. In extrapolating the data from rodent studies to humans, ATSDR (2007) notes that since a greater proportion of nervous system development takes place post-natally in rodents than in humans, rodent studies that extended exposure into the early postnatal

period are probably more analogous to human prenatal exposure than rodent studies that investigated only prenatal exposure.

Inhalation and oral teratogenicity studies in rats and mice indicate that lead acetate and lead nitrate are not teratogenic following oral or inhalation exposure, although some studies demonstrated evidence of fetotoxicity (ATSDR, 2007).

The U.S. National Toxicology Program has conducted three studies that investigated the reproductive and developmental effects of lead acetate trihydrate in rats and mice. These studies are summarized in the following paragraphs.

NTP (1996) conducted a study of the reproductive effects of lead acetate trihydrate in Sprague-Dawley rats. The study had two phases – phase one was a 28 day exposure period, and phase two had a six month exposure duration. In the phase one study, twenty-six male and sixteen female Sprague-Dawley rats/group were administered either 0 or 0.3% lead acetate trihydrate in the drinking water for at least 28 days. There were no treatment-related effects on body weight, food consumption, mortality, organ weight (other than kidney), clinical signs, or behavior. Mean water consumption values were decreased in the 0.3% group compared to controls. Absolute and relative kidney weights were increased by to 10% in the 0.3% female group, when compared to controls; however there were no treatment-related microscopic lesions observed in the kidneys. Epididymal sperm density and percent abnormal sperm were comparable between the control and treated groups. There were more homogenization-resistant spermatids, both on a per testis, and per mg testis basis, in the 0.3% males relative to controls. The testes were not examined microscopically in the phase 1 study.

In the phase two study, four hundred male and female Sprague-Dawley rats were administered 0, 0.025, 0.05, 0.1, or 0.3% lead acetate trihydrate in the drinking water for up to six months. Male and female body weights were unaffected, except during Week four when the 0.3% male body weights were slightly decreased by 3%. There were no treatment-related differences observed in food consumption values, clinical observations, or mortality. Similar to the phase one study, mean water consumption values decreased with increasing lead concentration although the magnitude of the effect decreased as the exposure duration increased. There were no treatment-related changes noted in either gross observations or organ weight data, with the exception of kidney weights. Treatment-related increases in kidney weights were observed in both sexes but were more prominent in females. Microscopic evaluation of the left kidneys in female rats revealed treatment-related lead inclusions in the nuclei of tubular epithelial cells and tubular epithelial cell megalocytosis. The incidence of these lesions increased in a duration-dependent

manner. For example, more than half the females in the 0.1% group had lead inclusions at 16 and 24 weeks, while almost all the males and females in the 0.3% group had inclusions by 16 and 24 weeks.

No treatment-related changes were reported with respect to sperm motion parameters or testicular spermatid head counts. After one week exposure, the mean percent abnormal sperm values increased by 83 and 72% in the 0.1 and 0.3% males, respectively, when compared to controls, but were comparable in all dose groups when evaluated at four, eight, 16, and 24 weeks. After one week, mean epididymal sperm density was decreased by 25% in the 0.3% group when compared to controls. But, mean epididymal sperm density values were comparable among all dose groups at four, eight, and 16 weeks of treatment, with the exception of an increased (23%) epididymal sperm density count in males of the 0.05% group at week 16. By 24 weeks, the mean epididymal sperm density values were decreased by 23 and 18% in the 0.1 and 0.3% males, respectively, relative to controls.

The study authors concluded that lead acetate trihydrate is not a selective male reproductive toxicant, given the minor effects on sperm density that occurred at doses concomitant with, or greater than, those that produced microscopic kidney changes. A no-observable-adverse-effect level (NOAEL) was not established in this study as the 0.025% group displayed decreases in water consumption. A maximum tolerated dose (MTD) was estimated for the 0.3% dose group, based on decreased water consumption, increased kidney weights, increased incidence of renal intranuclear lead inclusions and renal tubular megalocytosis.

NTP (1985a) tested lead acetate trihydrate for reproductive toxicity in Swiss mice, using the RACB protocol. Exposure levels of 0.5, 1.0, and 2.0% in water were selected for the continuous breeding phase of the study. In the first generation, mortalities were elevated at all tested doses, and in controls. Reproductive toxicity was observed at all dose levels. The number of litters per pair was reduced by 29% and 58% in the mid and high dose groups. The number of live pups per litter was reduced in mid and high dose groups (16 and 55% respectively), while adjusted live pup weight was reduced in all dose groups by 4% (low), 12% (mid), and 16% (high). Cumulative days to litter increased markedly for all litters within the high dose group, and no high-dose pair was able to produce a fifth litter. Even though excessive mortality occurred, a crossover study was performed to attempt to identify the more affected sex, using the control and 0.5% groups. Mated pairs (treated female only) delivered 16% fewer pups/litter than controls. Three of nine females died in this portion of the study. Necropsy of F_0 adult controls and 0.5% group showed that male and female body weight was unaffected, but female brain weight was reduced by around 8%. Female estrous cycles in F_0 animals was unaffected by lead treatment. There were no

observed differences between controls and the 0.5% group in organ weights, or sperm parameters. The assessment of the F_2 generation only used offspring from the low dose group. At the beginning of the cohabitation period, body weights for treated male mice were 12% lower than in controls (6% lower in treated females). The lead-treated mice in this generation delivered 20% fewer live pups/litter than controls, and the pups weighed 7% less than control pups. Pup viability and sex ratios were unaffected by lead treatment. Necropsy results for F_1 adults showed that treated females weighed 6% less than controls, while the pituitary gland weighed 35% less. The estrous cycle did not differ between these groups. In male treated mice, body weight was reduced by 12%, and absolute testis weight was reduced by 11%, both relative to controls. Sperm parameters did not differ between these groups. The NTP study team concluded that the tested drinking water concentrations of lead acetate trihydrate produced mortality and reproductive toxicity. A followup study (NTP, 1985b) at another RACB laboratory was conducted that used lower doses in an attempt to separate the potential reproductive/developmental toxicity of lead from the excess mortality induced by the tested concentrations.

In the NTP (1985b) followup study, lead acetate trihydrate was re-tested for reproductive toxicity using the RACB protocol, at a different laboratory. Given the high mortality rates in the NTP (1985a) study, exposure levels for the followup study were set at 0.125, 0.25, and 0.5% w/v of lead acetate trihydrate in drinking water. Based on measures of water consumption and body weights, these concentrations produced dose estimates of 200, 375, and 700 mg/kg body weight/day. The highest dose caused a slight (around 10%) reduction in water consumption, though body weight was unaffected. In the first generation, oen control female, one low dose female, four middle dose females, and seven females and one male at the high dose died. While these deaths were considered treatment-related, the precise cause of death was not determined. F_0 body weights were not affected by lead consumption. A 7% decrease in adjusted live pup weight was reported in the high dose group, although the number of litters per pair or the number of pups per litter did not differ from controls. No crossover study was conducted since there were no changes in pup number, and only small changes in pup weight occurred. The F_1 mating was conducted with offspring from the control and 0.5% groups. Mortality in these mice was increased at 4 weeks of age by 15 and 30% for males and females, respectively. At the time of F_1 mating, mating and fertility indices were equal between the control and 0.5% groups. F_2 pup body weight was reduced by 16%, relative to controls, although litter size and pup viability did not differ. Necropsy results for the F_1 adults showed that females in the 0.5% group weighed 12% less than controls, brain weight was 7% less than controls, and adjusted spleen weight was 2.2 times greater than in controls. Estrous cycle evaluations were not performed in this study. In the F_2 treated males, body weight was reduced by 11%, and absolute

testis weight was reduced by 9%, relative to controls. Absolute brain and relative kidney weights were reduced by 5% and 13%, respectively, while relative spleen weight was increased 1.7-fold (all relative to controls). Sperm parameters were unaffected by lead treatment. The study authors concluded that this study replicated the mortality and pup body weight effects seen in the first study, while also showing that lead acetate trihydrate causes reproductive toxicity only in the presence of adult mortality or systemic organ weight changes. No NOAEL or LOAEL values were reported from this study.

Hamilton and O'Flaherty (1994) found no alteration in growth rate in weanling female rats continuously exposed to lead in drinking water (250 and 1,000 mg lead/L of lead acetate; 40 and 74 µg/dL PbB) and fed a lead-replete diet. However, their offspring had depressed body weight, decreased tail length growth, increased weanling growth-plate width, disruption of chondrocyte organization, and wider metaphyseal trabeculae. Similar results were reported for mice exposed *via* drinking water by Pinon-Lataillade *et al*. (1995). The exposed off-spring were mated with unexposed mates to determine lead exposure on subsequent generations. The authors reported reduced litter sizes for females and reduced body, testes, epididymis, seminal vesicle and ventral prostate weights in males. Junaid *et al*. (1997) exposed mice to lead acetate by oral gavage to examine ovarian follicular development. Results showed that small, medium and large follicles were significantly affected by the lead and atresia also occurred in the medium follicles.

A series of developmental and reproductive studies were conducted on rats (Ronis *et al*., 1996; Ronis *et al.,* 1998a,b,c) at a range of 0.05 to 0.6% w/v lead acetate in drinking water. A number of effects were observed including for males: decreased secondary sex organ weights (prepubertal exposure) and suppressed prepubertal growth and serum testosterone levels (*in utero* exposure) and decreased birth weights; for females: delayed vaginal opening and disrupted estrus cycling (prepubertal exposure), as well as suppressed prepubertal growth and circulating estradiol levels (*in utero* exposure) and decreased birth weights. The overall conclusion of the four studies is that the mechanism of toxicity for the observed reproductive and developmental effects of lead involves disruption of growth hormone and luteinizing hormone secretion during puberty (Ronis *et al.,* 1998b,c).

The effects of chronic lead exposure on testis ultrastructure were examined in the cynomolgus monkey after oral administration of lead acetate (1,500 ∝g/kg body weight/day) in the following groups: birth to 10 years (lifetime); postnatal day 300 to 10 years (postinfancy); and postnatal 0 to 400 (infancy) (Foster *et al.,* 1998). At age 10 years, circulating blood lead (PbB) concentrations in the lifetime and postinfancy treatment groups was approximately 35 µg/dL. Sertoli and spermatogenic cells of monkeys from the

infancy and lifetime groups revealed injuries, and ultrastructural changes in the testis. Thus, chronic lead exposure resulting in moderate PbB concentrations induced persistent ultrastructural changes in the testis of the cynomolgus monkey.

There have been numerous other animal studies conducted with inorganic lead compounds, using a range of dose levels and exposure durations that investigated various reproductive or developmental toxicity parameters. The reported findings range from no observed adverse effects (*e.g.,* Kristensen *et al*., 1995; Hubermont *et al*., 1976; Fowler *et al*., 1980; Foster *et al*., 1998), to such effects as tissue damage or changes to the ovaries and testes, irregular estrous cycles, testicular atrophy, various sperm parameter changes, impaired spermatogenesis, altered circulating reproductive hormone levels, impaired ovarian-uterine function, sex organ weight changes, delayed sexual maturation, interference with hypothalamic-pituitary endocrine function, increased stillbirths, reduced birthweights, and impaired growth rates in offspring (eg., Hilderbrand *et al*., 1973; Chowdhury *et al*., 1984; Kaushal *et al*., 1996; Franks *et al*., 1989; Grant *et al*., 1980; Sourgens *et al*., 1987; Kimmel *et al*., 1980). In the studies reporting adverse reproductive or developmental effects, the PbB levels were similar to those known to occur within lead-exposed human populations; however, the more severe effects only occurred at PbB concentrations well above 10 µg/dL (around an order of magnitude or higher for the reproductive or developmental effects of greatest severity).

See Section A4-3.2.2 for a discussion on animal neurobehavioral development toxicity, and other manifestations of systemic toxicity (*e.g.,* hematological, renal, immunological *etc*.) that occur as a result of prenatal, and/or early post-natal exposure to lead. Many studies investigating the development effects of lead have focused on these forms of toxicity, particularly neurological and neurobehavioural effects.

A4-3.4 Carcinogenicity

A4-3.4.1 Human Studies

There are a number of epidemiological studies available for occupational cohorts exposed to lead and lead compounds. Two studies did not find any relationship between lead exposure and cancer in exposed workers (Dingwall-Fordyce and Lane, 1963; Nelson *et al*., 1982). However, other studies have reported a positive association between occupational lead exposure and cancer.

Selevan *et al*. (1985) reported excesses of respiratory cancer and kidney cancer in 1987 lead smelter workers. Kidney cancer is considered to be of particular interest as it also been found to occur in

experimental animals dosed with inorganic lead compounds (OEHHA, 2002). The Standardized Mortality Ratio (SMR) was 204 for kidney cancer, but only 6 cases were observed, and the SMR was not statistically significant. An update of this study was conducted by Steenland *et al.* (1992). This update included 11 years of follow-up and 363 additional death records. There were three additional deaths from kidney cancer during this time frame. The updated SMR from kidney cancer was 193, with a total of nine total kidney cancer deaths; however, the SMR remained not statistically significant, relative to controls. However, when the SMR for kidney cancer was calculated for workers with the highest lead exposure (SMR = 239), there was a statistically significant difference noted.

There are two case reports of renal cancer in men occupationally exposed to elevated levels of lead (Baker *et al.*, 1980; Lilis, 1981). In Baker *et al.* (1980), it was reported that the histology in the renal tumor was similar to those observed in lead-exposed animals.

In an epidemiological study of 7,121 deceased California plumbers and pipefitters, Cantor *et al.* (1986) reported an increased cancer incidence, both for types of neoplasms investigated, and for cancers specific to several sites including the respiratory system, kidney, and stomach. This study is confounded though by simultaneous exposure to known carcinogens such as asbestos and chromium. As excess mesotheliomas were observed (which is a well-characterized tumour type that is specific to asbestos inhalation exposure), it was considered likely that asbestos exposure also contributed to the observed increase in stomach and other respiratory system cancers. Smoking may have also confounded the results of this study. However, the authors did not attribute the observed excess kidney cancers to asbestos, chromium, or cigarette smoking, as these are not generally considered causes of kidney cancer.

Copper and Gaffrey (1975) and Copper (1985) found that a group of battery plant and lead smelter workers had increased excesses of cancer, in particular, stomach and lung cancer. There do not appear to be any newer human studies investigating the carcinogenic potential of lead, as U.S. EPA (2004) reported that a recent review of the toxicology literature pertinent to the cancer assessment for inorganic lead and compounds (conducted by an independent contractor in September, 2002), did not identify any critical new studies for lead.

Ades and Kazantzis (1988) studied 4,293 male workers at a zinc-lead-cadmium smelter in Great Britain. There was an effect of lead exposure on lung cancer noted but as lead exposure was highly correlated with co-exposure to arsenic, it is likely that the excess lung cancers were due to arsenic, rather than lead exposure. This study is further limited by its failure to report cigarette smoking rates in the workers that were evaluatd.

Fu and Boffetta (1995) conducted a meta-analysis of the published studies on cancer and workplace exposures to inorganic lead compounds. The studies included in the analysis were the two case reports mentioned above, 16 cohort studies, and seven case-control studies. The meta-analysis showed a small but statistically significant, excess relative risk of overall cancer (RR = 1.11). There were also statistically significant relative risks reported for stomach cancer, (RR = 1.33), lung cancer (RR = 1.29), and bladder cancer (RR = 1.41). The relative risk for kidney cancer did not reach the level of statistical significance in this analysis (RR = 1.19). In a meta-analysis of a subgroup of workers exposed to high lead concentrations, higher relative risks were reported for stomach cancer (RR = 1.50), and lung cancer (RR = 1.42). The authors concluded that while the meta-analysis indicates a relationship between lead exposure and cancer, it is limited by the paucity of information reported in the available studies on potential confounders such as cigarette smoking, dietary habits, and co-exposure to other carcinogens at many of the workplaces studied (Fu and Boffetta, 1995).

In general, all of the available human studies suffer from various limitations including lack of quantitative exposure information, failure to control for smoking, and possible concurrent exposures to various other chemicals, including arsenic and cadmium (U.S. EPA, 2004). In addition, the cancer excesses observed in these studies have been relatively small. There is also no consistency with respect to tumour sites among the available studies, and no dose-response relationships are evident. Given these numerous shortcomings, the U.S. EPA (2004) considers the available human evidence inadequate to reach any conclusions as to whether or not lead is a human carcinogen. The U.S. EPA (2004) also notes some unique features of lead that add further uncertainty to attempting to quantify its carcinogenic risk potential. For example, age, health, nutritional state, body burden, and exposure duration influence the toxicokinetics of lead in complex and poorly understood ways. In addition, the U.S. EPA believes that the current limited knowledge of lead toxicokinetics suggests that a carcinogenic risk estimate derived by standard procedures would not adequately represent the true potential risk. Thus, the Carcinogen Assessment Group of the U.S. EPA recommends that no numerical estimate for lead be derived at this time.

A4-3.4.2 Animal Studies

A number of animal studies have investigated the carcinogenic potential of soluble inorganic lead compounds administered *via* the oral route. The most sensitive and commonly reported endpoint in these studies is the development of bilateral renal carcinoma, although gliomas have been reported in some studies where rats were orally administered lead acetate or lead subacetate. While tumours in other tissues

are occasionally seen, the principal finding in these studies has been kidney tumours. In the majority of studies, the kidney tumours occurred at the highest tested doses. There are no available studies investigating the carcinogenicity of lead compounds *via* the inhalation or dermal exposure routes (ATSDR, 2007). Relevant oral animal cancer bioassays are briefly described below.

One study was identified in which the intratracheal instillation route was utilized. Kobayashi and Okamoto (1974) exposed 20 hamsters to lead oxide *via* this route. No tumors were observed after 10 intratracheal instillations of 1 mg of lead oxide. However, simultaneous administration of lead oxide with benzo[a]pyrene (10 instillations of 1 mg), which by itself also did not cause tumors in this study, did result in lung tumors. The authors suggested that lead might be acting as a promoter or co-carcinogen for benzo[*a*]pyrene-initiated carcinogenicity.

Azar *et al*. (1973) administered 10, 50, 100, or 500 ppm lead, as lead acetate, in the diet to 50 rats/sex/group for 2 years. Control rats received the basal laboratory diet. In a second two-year feeding study conducted concurrently, 20 rats/group were given diets containing 0, 1,000, or 2,000 ppm lead as lead acetate. No renal tumors were reported in the control or treated animals of either sex receiving dietary concentrations of 10 to 100 ppm. Male rats fed 500, 1,000, and 2,000 ppm lead acetate showed an increased renal tumor incidence of 5/50, 10/20, and 16/20, respectively. There was also a renal tumour incidence of 7/20 in females from the 2,000 ppm group. The results of this study should be interpreted with caution however, as a number of key experimental details were not reported, such as potential contamination from lead in the air or drinking water, the strain of rats used, and details on observed clinical signs of toxicity (U.S. EPA, 2004).

Kasprzak *et al*. (1985) fed 1% lead subacetate (roughly 8,500 ppm Pb) to male Sprague-Dawley rats in the diet for 79 weeks. In the surviving rats, 44.8% (13 of 29 surviving rats) had renal tumors by 58 weeks. Four rats had adenocarcinomas, and nine had adenomas. The renal tumours were noted to be bilateral. No renal tumors were observed in the control group.

Koller *et al*. (1986) exposed male Sprague-Dawley rats to lead acetate in drinking water for 76 weeks, at a concentration of 2,600 mg Pb/L. A control group received no lead exposure in drinking water. No kidney tumors were observed among the control rats, while thirrteen of 16 (81%) lead-treated rats displayed renal tubular carcinoma. Three of the tumours had formed by 72 weeks exposure, and the remainder were evident by the end of the study.

Van Esch and Kroes (1969) fed lead acetate at 0, 0.1%, and 1.0% in the diet to 25 Swiss mice/sex/group for a period of two years (a control group received no lead acetate in the diet). There were no renal tumors in the control group. In the 0.1% (1,000 mg/kg) group, there were 6/25 male mice that developed renal tumors (adenomas and carcinomas combined). In the 1.0% group (10,000 mg/kg), only one female developed a renal tumor. In an attempt to explain the lack of a dose-response relationship and inconsistent results, the authors attributed this low tumour incidence to elevated mortality rates. Further details were not available. These authors also reported that hamsters given lead subacetate at 0.5% and 1% in the diet showed no significant increase in renal tumours (Van Esch and Kroes, 1969).

Various other studies have investigated kidney tumour development in chronic animal studies. Schroeder *et al*. (1970) found that no kidney tumours developed in a lifetime rat study where male animals were exposed to lead nitrate in drinking water at a concentration of 25 mg/L. Zawirska and Medras (1968) exposed rats of both sexes to lead acetate in feed for 18 months at a concentration of 3 mg/day initially, then 4 mg/day. Male and female rats had a kidney tumour incidence of 62 and 44%, respectively. Nogueira (1987) fed male rats lead acetate at dietary levels of 0.5 or 1%. No kidney tumours were observed in rats in the 0.5% group, but 90% (9/10) rats in the 1% group developed kidney tumours. Similarly, Boyland *et al*. (1962) found that male rats fed lead acetate in the diet for 12 months, at a concentration of 1%, had a kidney tumour incidence of 94% (15/16 rats). Mao and Molnar (1967) also reported a high kidney tumour incidence in male rats fed lead subacetate in the diet over their lifetime, at a concentration of 1%. The tumour incidence was 78% (31/40 rats).

It is noteworthy that all oral rat studies demonstrating an increased incidence of kidney tumours administered either lead acetate or lead subacetate as the test compound. These are highly soluble forms of lead that do not typically occur in environmental media. There do not appear to be any studies with inorganic lead compounds that are representative of the forms of lead that typically occur in the ambient environment (*e.g.,* lead carbonates, hydroxides, oxides, sulphates, sulphides, *etc*.). Thus, the applicability of the animal carcinogenicity data from studies using lead acetate or subacetate to ambient environmental lead exposure may be questionable. It is of interest that the only oral study in which an inorganic lead salt was investigated (*i.e.,* lead nitrate in Schroeder *et al*., 1970) found that no kidney tumours developed after lifetime exposure to 25 mg/L in drinking water.

A4-3.4.3 Cancer Classifications for Lead Compounds

In its 10th Report on Carcinogens, the U.S. National Institute for Environmental Health Sciences (NIEHS, 2004) recommends that lead acetate and lead phosphate be considered as "reasonably anticipated to be human carcinogens".

The U.S. EPA (2004) has maintained its previous cancer classification for lead compounds as B2 - probable human carcinogen, based on sufficient animal evidence of kidney tumours, but inadequate human evidence. The U.S. EPA has determined that an estimate of carcinogenic risk from oral exposure (such as a slope factor) using standard methods would not adequately describe the potential risk for lead compounds. The U.S. EPA's Carcinogen Assessment Group made this determination given the current lack of understanding on various toxicological and toxicokinetic characteristics of lead.

IARC (2004) classified inorganic lead compounds as probably carcinogenic to humans (Group 2A), based on limited evidence for carcinogenicity in humans and sufficient evidence for carcinogenicity in experimental animals. The IARC evaluation considers the evidence of carcinogenicity in humans and experimental animals, as well as other data relevant to the evaluation of carcinogenicity and its mechanisms. For example, IARC (2004) noted that while there appears to be little evidence that lead is directly genotoxic, it may be indirectly genotoxic as a result of oxidative stress effects caused by the formation of reactive oxygen species. The IARC Working Group does not typically provide quantitative estimates of any chemical's carcinogenic risk.

Health Canada has not formally classified lead compounds with respect to their carcinogenic potential.

The OEHHA (California Environmental Protection Agency Office of Environmental Health Hazard Assessment) considers lead compounds human carcinogens as they have derived both oral and inhalation slope factors and unit risks for lead (see Section A4-4.0). However, at this time, no other regulatory agencies, other than OEHHA, are known to have derived regulatory exposure limits for lead that are based on carcinogenic effects.

A4-4.0 EXPOSURE LIMITS

As described below and summarized in Table A4.1 WHO, Health Canada, RIVM, MOE, ATSDR, U.S. EPA and OEHHA were the organization consulted to select exposure limits for lead. It should be noted that current MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits) when health based exposure limits are available from major health agencies. Exposure limits used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO), and the MOE itself.

Although the toxicological database for lead is large, the majority of human effects data is expressed as a PbB concentration, rather than a dose or concentration in an environmental medium. In addition, there are inadequate empirical data for demonstrating a threshold for the health effects of lead. In fact, many consider lead a non-threshold toxicant, indicating that any exposure to lead leads to possible effects. Given these limitations, many regulatory agencies have not derived conventional exposure limits such as RfDs, TDI's or MRLs, and advocate that exposure to lead should be minimized. In order to utilize the wealth of literature relating human PbB concentrations to health effects, such agencies (*e.g.,* ATSDR, U.S. EPA) have developed models or other approaches to relate environmental lead exposure to PbB levels. This is described further in Section A4-5.0. In addition, environmental quality guidelines for lead have also been developed with a different approach than is used for most other chemicals. Instead of developing exposure limits based on no- or low-effects-levels observed in test organisms following controlled exposures, lead guidelines are typically back calculated from a critical PbB concentration (usually 10 µg/dL, as recommended by CDC, 2004; U.S. EPA, 2004; CEOH, 1994).

Although recent scientific data indicate associations between intellectual performance and other health effects in children and PbB levels extending below 10 µg/dL, it appears that major agencies (CDC, 2004, 2005; MOE, 2007; U.S. EPA, 2002, 2006, 2007) acknowledge that a clear threshold for protection of neurological and other deleterious impacts in children and adults has not yet been identified. The derivation of acceptable exposure levels is complicated by numerous confounding factors that influence lead toxicity, including socioeconomic status, pre-existing lead body burdens, age, health status, nutritional status and lifestyle factors such as alcohol consumption and tobacco smoke (environmental tobacco smoke has been associated with elevated PbB). As a result, CDC (2004, 2005) has recommended that the PbB level of concern should remain at their 1991 (CDC, 1991) recommended level of 10 µg/dL.

The decision for not lowering the PbB level of concern below 10 µg/dL is based on the following (CDC, 2004, 2005):

- Lack of effective clinical interventions to lower PbB levels for children with levels less than 10 µg/dL or to reduce the risks for adverse developmental impacts;
- Inaccuracy inherent in laboratory analytical testing of PbB levels in children; and,
- No evidence of clear threshold for neurological impacts in children and as such, a decision to lower the PbB level of concern would be "arbitrary" and "provide uncertain benefits".

The level of concern is not indictive of a "safe" level of PbB, nor is it a definitive toxicologic threshold. Deleterious effects at PbB levels ≤10 µg/dL cannot be ruled out (CDC, 2005). Rather, the level of concern is designed to trigger preventative activities. Prevention of lead exposure, with the long-term goal of eliminating lead poisoning, targets not only children with PbB ≤ 10 µg/dL, but those with higher PbB as well (CDC, 2005). Primary prevention measures target major lead exposure sources in the United States., including lead in housing (*e.g.,* paint) and nonessential uses of lead, such as those present in various commercial products (*e.g.,* cosmetics, toys, utensils) (CDC, 2005). It is believed that preventative measures will have the greatest level of success in the elimination of lead exposures (CDC, 2005).

Health Canada's current blood intervention level (10 µg/dL) is not to be interpreted as an acceptable level of exposure below which no adverse health effects shall occur (Wilson *et al.,* 2005). It is designed to indicate the need for mitigation of risk for individuals already exposed. Health Canada is currently undergoing a review of their PbB population intervention level established by CEOH in 1994.

A value that is the basis for many jurisdiction's exposure limits is the TDI of 3.57 µg/kg/day derived by the WHO. The TDI was derived based on a PTWI of 25 µg of lead per kg of body weight recommended by FAO/WHO (1993), and reaffirmed by WHO (1999), for all age groups. This PTWI value was in turn based upon technical reports presented at annual meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as well as upon epidemiological studies associating lead exposure with neurological effects in infants and children. The PTWI represents the permissible or tolerable human weekly intake that is unavoidable as a result of consuming typical foods. The PTWI was based on metabolic studies of infants which indicated that if daily lead intakes equal approximately 5 µg/kg/day then PbB levels remained at a fairly constant low level within the body. Daily intakes of lead in the range of 3 to 4 µg/kg body weight/day by infants and children were not associated with an increase in blood lead concentrations (WHO, 1995; Ziegler *et al.*, 1978; WHO, 1987). The PTWI of 25 µg/kg/week was

converted to a PTDI of 3.57 µg/kg/day by dividing the PTWI by seven (days in a week). The TDI for lead accounts for exposure from all sources and is considered protective of all humans, including infants and children.

Health Canada (2004) has adopted 3.6 µg/kg body weight/day as the PTDI for lead, and the CCME and Health Canada use this value as the basis for derivation of soil and drinking water guidelines that are protective of human health. By definition, a TDI represents an exposure that is without adverse health effects (Health Canada, 2004). The defensibility of this has been questioned, as this level is associated with increased PbB levels (Wilson *et al.,* 2005). Recent evidence advocates the consideration of of lead as non-threshold toxicant, and it has been recommended that Health Canada develop guidance for the interpretation of the PTDI for lead (Wilson *et al.,* 2005).

The MOE (1994) recommended an intake of concern for populations (IOC_{pop}) of 1.85 µg/kg/day in order to minimize the predicted number of children with individual blood lead levels of concern. Subclinical neurobehavioural and developmental effects were the critical effects appearing at the lowest levels of exposure (MOE, 1994). The intake of concern for individuals (IOC_{ind}) was based on a Lowest Observed Adverse Effect Level (LOAEL) in infants and young children of 10 µg/dL PbB divided by an intake/PbB slope factor of 0.21 µg Pb per DL PbB per µg/day. This resulted in an IOC_{ind} of 3.7 µg/kg/day for a 13 kg child (0.5-4 yrs). To derive the IOC_{pop} an uncertainty factor of two was applied to the IOC_{ind}, which resulted in a daily intake of 1.85 µg/kg/day (MOE, 1994). This value is based on the same research as the other agencies limits discussed elsewhere. In the Guideline for Use at Contaminated Sites in Ontario (MOE, 1996), MOE adopted the oral RfD of 1.85 µg/kg/day for lead. This IOC_{pop} does not represent a 'safe level of exposure' rather it is an intake-level-of-concern in the population that is not considered to be enterely safe or acceptable since MOE consideres lead to be a non-threshold toxicant and thus any level of exposure is associated with a level of risk. More recently (MOE, 2007), the MOE has summarizes the scientific basis for air quality guidelines and standards developed by the U.S. Environmental Protection Agency (US EPA), the California Environmental Protection Agency (Cal/EPA), New Zealand, Australia, United Kingdom and the World Health Organization. Following this review, MOE selected the Cal/EPA's (2001) derivation of their lead air guideline as the most appropriate approach on which to base an updated air standard for lead in Ontario (24-hour AAQC of 0.5 ug/m^3). Cal/EPA's guideline is based on an air concentration associated with a 5% probability of exceeding the BLL of concern.

Two regulatory agencies that are typically leaders in the development of chemical exposure limits (ATSDR and U.S. EPA) have not derived any exposure limits for lead compounds; rather, they have developed alternate approaches that relate environmental lead exposure to PbB levels.

ATSDR (2007) did not derive any minimal risk levels (MRLs) for lead due to the lack of a clear threshold for health effects and the need to consider multi-media routes of exposure. However, ATSDR has developed guidance for employing media-specific slope factors to integrate exposures from various pathways for site-specific risk assessments. This guidance is described further in Section A4-5.0.

The U.S. EPA IRIS database does not recommend oral or inhalation RfD (or RfC) for lead due to high levels of uncertainty, and because lead is considered a non-threshold toxicant (U.S. EPA, 2004). The U.S. EPA believes that the effects of lead exposure, particularly changes in blood enzyme levels, and children's neurodevelopment, may occur at blood levels so low as to be essentially without a threshold. As further support for not deriving an RfD or RfC, the U.S. EPA (2004) states that current knowledge of lead toxicokinetics suggests that risk values derived by standard procedures (such as an oral RfD) would not be representative of the potential risk, due to difficulties in attempting to account for pre-existing body burdens of lead, and certain lifestages when stored lead may be mobilized within the body. For example, lead is well known to be stored in bone tissue, and its mobilization from bone varies greatly with age, health status, nutritional state, physiological state (pregnant, lactation, menopause *etc*.). Alternatively, the U.S. EPA has developed the Integrated Exposure Uptake Biokinetic Model (IEUBK) as a means of predicting the occurrence of blood lead concentrations above 10 µg/dL in children. This model is used to determine the contribution of lead from all media to PbB (U.S. EPA, 2004). The IEUBK model predicts the geometric mean PbB concentration for a child exposed to lead in various media (or a group of similarly exposed children). The model can also calculate the probability that the child's PbB exceeds 10 ug Pb/dL (P10). Preliminary remediation goals (PRGs) for lead are generally determined with the model by adjusting the soil concentration term until the P10 is below a 5% probability (U.S. EPA, 2003). In addition, an Adult Lead Model was developed by the U.S. EPA Superfund Program for when adult exposures to lead are of concern, especially in the case of pregnant women (www.epa.gov/superfund/programs/lead/adult.htm). The model equations were developed to calculate cleanup goals such that there would be no more than a 5% probability that fetuses exposed to lead would exceed a blood lead (PbB) of 10 µg/dL. The IEUBK model is described further in Section A4-5.0.

There are no non-cancer inhalation exposure limits for lead. However, the FAO/WHO TDI of 3.57 µg/kg/d accounts for lead exposure from all sources and is considered protective of all humans, including infants and children.

Only one agency was identified as having developed quantitative toxicity estimates based on the carcinogenicity of lead (*i.e.,* OEHHA, 2002). The U.S. EPA did not derive any exposure limits based on carcinogenic endpoints as its Carcinogen Assessment Group concluded that the uncertainties associated with lead pharmacokinetics and factors affecting the absorption, release, and excretion of lead (*i.e.,* age, health, nutritional status, body burden, and exposure duration) preclude the development of a numerical estimate to predict carcinogenic risk (U.S. EPA, 2004). Thus, the U.S. EPA believes that the current limited knowledge of lead toxicokinetics suggests that a carcinogenic risk estimate derived by standard procedures would not adequately represent the true potential risk.

The OEHHA (2002) reports an inhalation unit risk factor of 1.2 E-5 $(\mu g/m^3)^{-1}$, an inhalation slope factor of 0.042 $(mg/kg/day)^{-1}$, and oral slope factor of 0.0085 $(mg/kg/day)^{-1}$. All values were originally calculated by OEHHA (1997) from rat kidney tumor incidence data (the Azar *et al.*, 1973 study) using a linearized multistage procedure. The studies by Azar *et al*. (1973) and Koller *et al*. (1985) were considered to represent the best available tumour dose-response data for use in quantitative cancer risk assessment. The derivation of the unit risk and slope factors is described in detail within OEHHA (2002). In summary, rat kidney tumour data was extrapolated to humans by means of the best fitting linearized multistage model (*i.e.,* GLOBAL86), conversion of rat doses to human equivalent doses (HED), the use of standard human receptor parameters, and assumptions related to the inhalation and oral bioavailability of lead.

No regulatory dermal exposure limits for lead compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF_{dermal}). This factor, calculated as follows, is applied to dermal exposure

estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

For the purposes of this risk assessment an oral, inhalation and dermal exposure limit of 1.85 ug/kg/day was selected for lead (MOE, 1996a, MOE, 1994).

A4-4.1 Criteria Summary

Table A4.1 summarizes the exposure limits selected for lead in the current study.

Table A4.1 Exposure Limits Selected for Lead

Route of Exposure	Exposure Limit	Toxicological Basis	Reference	
			Study	Regulatory
Non-cancer (Threshold) Effects (IOC_{POP})				
Oral	1.85 µg/kg/day	Neurological effects in children	Weight-of-evidence, numerous studies	MOE, 1994; MOE, 1996
Inhalation				
Dermal				
Cancer (Non-threshold) Effects				
Oral	NA	NA	NA	NA
Inhalation[1]	NA	NA	NA	NA
Dermal	NA	NA	NA	NA

NOTES:
NA = not available; IOC_{POP} = intake of concern (population).
[1] While OEHHA considers lead to be a carcinogen via the inhalation route, no other identified regulatory agency shares this opinion. As such, lead is not considered a carcinogen in the current HHRA.

A4-5.0 TOOLS FOR RELATING ENVIRONMENTAL LEAD EXPOSURE TO BLOOD LEAD CONCENTRATIONS

The following sections summarize selected tools developed by regulatory agencies that may be used to relate environmental lead exposure to blood lead concentrations.

A4-5.1 ATSDR Slope Factor Approach

In a number of epidemiological that correlated environmental lead exposure with blood lead levels, regression analyses were conducted and slope factors for various media including air, soil, dust, water, and food were calculated (ATSDR, 2007). The ATSDR slope factor approach is based on an integrated exposure regression analysis (described in Abadin and Wheeler, 1993) that attempts to utilize the slope factors from selected epidemiological studies to integrate lead exposures across various exposure pathways, and to provide a cumulative lead exposure estimate expressed as total blood lead. ATSDR (2007) reports air slope factors (µg/dL/µg/m^3) that range from 1.0 to 2.7 across various studies of adults and children. The reported water slope factors range from 0.03 to 0.3 µg/dL/µg/L in infant, children and adult studies. The reported dietary slope factors range from 0.014 to 0.24 µg/dL/µg/day in infant, children and adult studies. Reported slope factors for soil ingestion range from –0.00016 to 0.007 µg/dL/mg/kg in studies with children and adults. For dust ingestion, the reported slope factors range from 0.0002 to 0.0096 in studies with children and adults.

ATSDR (2007) suggests that the following equation, in conjunction with the use of the appropriate slope factors, can provide a total PbB concentration that correlates with the environmental exposures levels for lead in a given area. The equation is as follows:

Total PbB (µg/dL) = [δS x PbS] + [δD x PbD] + [δW x PbW] + [δAO x PbAO]

+ [δ AI x PbAI] + [δF x PbF]

Where,

PbS = soil lead concentration

PbD = dust lead concentration

PbW = water lead concentration

PbAO = outside air lead concentration

PbAI = inside air concentration

PbF = food lead concentration

T = relative time spent

δ = the respective slope factor for each specific media

ATSDR (2007) believes this approach can be a useful screening tool. Its predictive ability has been tested and was concluded to be promising tool for assessing lead-impacted sites (Abadin *et al*., 1997). ATSDR (2007) states that the key strengths of this approach are its simplicity and flexibility to take into account environmental and biological variability between sites through the selection of appropriate slope factors. For example, slope factors from a lead mining area study can be used to address concerns at a mining community. Also, as more refined slope factors become available, they can be adopted in favour of current ones. This approach also enables rapid identification of the major exposure pathways that may be contributing to elevated PbB levels.

A4-5.2 U.S. EPA IEUBK Model

The U.S. EPA's Integrated Exposure, Uptake, and Biokinetic (IEUBK) computer model has the ability to quantify the relationship between environmental lead concentrations in different media (*e.g.,* soil, water, air and food) to PbB levels in children of different ages (U.S. EPA, 1994b; 2002). This model is fully discussed in Chapter 6 (Section 6.6) of the main Report. In addition to the IEUBK model, two other pharmacokinetic models were available for lead. The IEUBK Model and Leggett Model are both considered to be classical multi-compartmental kinetic models (ATSDR, 2007). Only the O'Flaherty model employs physiologically-based parameters to describe lead toxicokinetics. Further descriptions of the Leggett and O'Flaherty models can be found in ATSDR (2007).

A4-5.3 Other Approaches

In addition to ATSDR and U.S. EPA, some other regulatory agencies have also developed tools for relating environmental lead exposure to blood lead concentrations. For example, the California Department of Toxic Substances Control developed the 'LeadSpread' model. (California DTSC, 2004). LeadSpread is a tool that can be used to estimate blood lead concentrations due to exposure to lead *via*

dietary intake, drinking water, soil and dust ingestion, inhalation, and dermal contact. Each pathway is represented by an equation relating incremental blood lead increase to a concentration in an environmental medium, using contact rates and empirically determined ratios. The contributions *via* the five pathways are then summed to yield an estimate of median blood lead concentration resulting from the multi-pathway exposure. Ninetieth, ninety-fifth, ninety-eighth, and ninety-ninth percentile concentrations are estimated from the median by assuming a log-normal distribution with a geometric standard deviation (GSD) of 1.6.

A4-6.0 REFERENCES

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Sudbury Area Risk Assessment
Volume II

Appendix A5:

Detailed Toxicological Profile of Nickel

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A5 DETAILED TOXICOLOGICAL PROFILE OF NICKEL

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APPENDIX A5 DETAILED TOXICOLOGICAL PROFILE OF NICKEL

SUMMARY

This profile is not intended to provide a comprehensive review of the available toxicological and epidemiological literature on nickel compounds. Rather, the purpose of this nickel toxicological profile is to: i) outline the most relevant toxicological and epidemiological information on nickel compounds ii) describe recent information that may challenge previous findings; and iii) provide supporting rationale for the exposure limits selected for use in the human health risk assessment of the Sudbury area. This toxicological profile is based primarily on secondary sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for nickel. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Nickel

Nickel is a transition metal which exists in several oxidation states (most often +2) (Budavari *et al.*, 1989). Nickel increases the hardness of other metals and it used in the production of stainless steel and other alloys (ATSDR, 2005; Chang, 1996). The toxicokinetics and toxicity of nickel are strongly influenced by its form (*e.g.*, metallic, salt, oxide) and solubility. The more soluble nickel compounds include the nickel salts (nickel chloride and nickel sulphate) and nickel nitrate, while less soluble nickel compounds include nickel oxide (*i.e.*, black crystalline form and more stable green form) and nickel sub-sulfide (ATSDR, 2005). In general, the more soluble nickel compounds have a greater toxicity than less soluble forms. However, at the site of deposition, the less-soluble compounds are more likely to be carcinogenic (ATSDR, 2005).

Results of the speciation analyses of environmental media in the Greater Sudbury area (GSA) has indicated that some of the air filters and dust samples collected over the past year likely contain small amounts of nickel subsulphide (Ni_3S_2). It is important to understand that nickel subsulphide differs in toxicity and mode of action from nickel oxide, the form of nickel typically found in the highest quantities within most media sampled in Sudbury (both nickel subsulphide and nickel oxide would be considered smelter emission products). In fact, nickel subsulphide is more toxic *via* inhalation than nickel oxide.

In soils, nickel can occur in a variety of forms of varying solubility, and/or be adsorbed onto organic matter or clay particles. Overall, the solubility, and subsequent bioavailability of nickel is a function of the nickel sources and local soil geochemistry (Schoof, 2003).

Absorption

Ingestion is a major route of exposure for nickel compounds (U.S. EPA, 1986; Nieboer and Nriagu, 1992). The different chemical forms of nickel can significantly influence the amount of nickel that is absorbed from the gastrointestinal tract with the more soluble nickel compounds having higher absorption efficiency (U.S. EPA, 1986; Ishimatsu *et al.*, 1995). For example, metallic nickel, nickel oxide and nickel subsulfide are poorly absorbed when ingested, while nickel sulfate, nickel chloride and nickel nitrate (which are more soluble) have a much higher absorption efficiency (Ishimatsu *et al.*, 1995).

Estimates of nickel bioavailability in humans following ingestion vary from study to study and have been reported to range from approximately 1 to 30% (*e.g.*, Perry and Perry, 1959; ICRP, 1960; Nodiya, 1972; Horak and Sunderman, 1973; Christensen and Lagesson, 1981; Hansen and Tjell, 1981; Owen, 1990; Nielsen *et al.*, 1999; Patriarca *et al.*, 1997). ATSDR (2005) reported that in humans, approximately 27% of nickel was absorbed when given in drinking water, while only about 1% was absorbed when nickel was given with food.

Essentiality

Nickel is an essential trace element in animals, based on reports of nickel deficiency in several animal species (*e.g.*, rats, chickens, cows, and goats) (ATSDR, 2005). Effects of nickel deficiency were observed in the liver and included abnormal cellular morphology, oxidative metabolism, and increases and decreases in lipid levels. In addition, decreases in growth and haemoglobin concentrations and impaired glucose metabolism have also been observed. However, nickel deficiency has never been reported in humans as nickel intake generally exceeds dietary requirements (Anke *et al.*, 1995; Denkhaus and Salnikow, 2002). Nickel is widely considered to be a normal constituent of the diet, with daily intakes ranging from 100 to 300 µg/day (U.S. EPA, 1991a). The functional importance of nickel has not been clearly demonstrated as no enzymes of cofactors that include nickel are known in humans (Denkhaus and Salnikow, 2002). Therefore, the essentiality of nickel in humans has not been confirmed and nickel dietary recommendations have not been established for humans (ATSDR, 2005; Denkhaus and Salnikow, 2002).

Toxicology of Nickel

Only limited data appears to exist on the systemic toxicity of ingested or inhaled nickel in humans. The majority of human studies with nickel compounds have focused on potential carcinogenic effects or dermal allergic responses.

There do not appear to be any studies in the available scientific literature that link ambient environmental exposure to nickel to increased cancer incidence in the general population. However, there are a number of studies which demonstrate that nickel refinery workers exposed by inhalation to various nickel compounds are at a significantly increased risk for cancer of the lung and the nasal cavity, relative to non-occupationally exposed individuals (EC, 2000; WHO, 2000; IARC, 1990; ICNCM; Doll *et al.*, 1990; CEPA, 1994).

Extensive reviews of studies prior to 1990 of the toxicology of nickel and nickel compounds, including animal carcinogenicity and human epidemiological data, were published by the International Agency for Research on Cancer (IARC, 1990), and by the International Committee on Nickel Carcinogenesis in Man (ICNM, 1990; Doll *et al.,* 1990). These reviews examined ten cohorts (roughly 80,000 workers) involved in mining, smelting and refining nickel, as well as workers in nickel related industries (using metallic nickel powder and nickel alloy) (*e.g.,* Inco Limited in Clydach, Wales; Falconbridge Limited in Falconbridge, Ontario; Inco in Huntington, West Virgina; Inco in Copper Cliff and Coniston, Sudbury, Ontario; Inco in Port Colborne, Ontario; Falconbridge in Kristiansand, Norway; Falconbridge in Oak Ridge, Tennessee; Outokumpu Oy in Finland; Societe le Nickel in New Caledonia; and, Henry Wiggin Alloy Co. in Hereford, England). In general, these reviews indicated increased risks for lung and nasal cancers associated with occupational inhalation exposures incurred during high temperature oxidation of nickel matte and nickel-copper matte (*i.e.*, roasting, sintering, calcining), as well as with exposures in electrolytic refining and hydrometallurgy. The greatest risks were observed in workers involved in roasting, sintering and calcining operations, with much smaller risks observed for workers in refineries. However, these increased risks applied to only a small proportion of nickel workers. The reviews also indicated that different nickel compounds have different carcinogenic potencies, and more than one form of nickel may exhibit carcinogenic activity. From these reviews, it was evident that the increased respiratory cancer risk observed in nickel workers was largely attributable to mixtures of oxidic and sulphidic nickel at air concentrations in excess of 10 mg/m^3. There was also evidence that high concentrations of oxidic nickel, in the absence of sulphidic nickel, was associated with increased lung and nasal cancer risks in exposed workers. There was some evidence that soluble nickel species may enhance

the risks associated with the less soluble forms of nickel. Respiratory cancer risks were associated with soluble nickel exposures, but only when there was co-exposure to less soluble forms of nickel, at air concentrations in excess of 1 mg/m^3. There was no evidence that metallic nickel was associated with increased lung and/or nasal cancer risks.

It is important to recognize however, that many of the epidemiological studies reviewed by IARC (1990) and Doll *et al.* (1990) had several limitations. The principal limitation was the lack of data related to concentrations of nickel in air within the facilities that were evaluated. Both Doll *et al.* (1990) and IARC (1990) acknowledged that this is a critical limitation. Thus, it is not possible to determine exposure-response relationships from these studies. In addition, at the time these reviews were conducted, the mechanism underlying the carcinogenicity of the various nickel species was poorly understood. Furthermore, Doll *et al.* (1990) noted that the conclusions of many of the epidemiological studies (with respect to lung tumour incidence) were confounded by a lack of information about the smoking habits of the workers. Finally, it must be recognized that nickel smelter and refinery workers have concomitant exposures to various other substances, including copper, cobalt, arsenic, lead, and sulphuric acid mist, depending on the type of nickel being processed, and the processing methods used. Some of these substances are strongly associated with increased respiratory cancer in workers (*i.e.*, arsenic, sulphuric acid mist). The potential influence of co-exposure to these substances has not been adequately accounted for in any occupational epidemiology study of nickel exposure and cancer incidence.

Since 1990, numerous studies, updates of previous cohort studies, and detailed interpretive reviews of the toxicological and epidemiological literature have been conducted. Key findings and conclusions from these most recent studies and reviews are summarized in the following paragraphs.

Update studies for the Clydach, Wales refinery cohorts have been reported by Easton *et al.* (1992) and Draper *et al.* (1994). Easton *et al.* (1992) confirmed the earlier observation that cancer risks are largely confined to workers employed in the 1930s and before. It is suggested that nasal cancer risk may be due to exposure to soluble nickel and at least one insoluble nickel species, possibly nickel oxide. Draper *et al.* (1994) suggest that the confinement of cancers to mostly pre-1930s workers likely reflects that leaching of nickel matte with an arsenic containing sulfuric acid ceased in the 1920s. Julian and Muir (1996) re-examined cancer incidence in male workers employed by Inco and Falconbridge. A small excess of laryngeal cancer was reported, but only in employees that were not exposed to the sintering process. Underground miners with more than 25 years exposure were shown to have a significant excess of lung and nasal cancers. It was speculated that the high prevalence of smoking among this cohort may have

contributed to observed increased incidence rates for lung cancer. Andersen (1992) and Andersen *et al.* (1996) conducted morbidity updates of the Kristiansand, Norway cohort. The updated analysis showed an excess risk of lung cancer in association with exposure to soluble forms of nickel. The study also suggested a multiplicative effect of smoking and nickel exposure. Arena *et al.* (1998) expanded upon and re-evaluated the cohort of U.S. high alloy nickel workers. The authors observed a statistically significant 13% increased risk for lung cancer, relative to the total U.S. population. However, no significant excess was found when smaller, local referent populations were used as the comparison.

Anttila *et al.* (1998) followed up on an earlier study of lung cancer incidence in workers at a nickel refinery in Harjavalta, Finland (Karjalainen *et al.*, 1992). In this first study, examination of cancer registry data for a cohort of workers followed from 1953 to 1987 revealed an elevated incidence of nasal cancer and a slight increase in lung cancer incidence. Anttila *et al.* (1998) followed up on this study by examining cancer registry data up to the end of 1995. A cohort of 1388 workers employed for at least three months at the copper/nickel smelter and nickel refinery in Harjavalta was studied. Overall cancer incidence in this cohort was similar to the unexposed cohort; both were at expected levels for the Finnish population. A small increase in lung cancer incidence, and an increased risk of nasal cancer in the exposed cohort were observed. Refinery workers were primarily exposed to nickel sulfate at concentrations below 500 $\mu g/m^3$, but exposure to lower concentrations of other soluble nickel compounds also occurred. As the elevated lung and nasal cancer risks were confined to refinery workers, where the primary exposure was to nickel sulfate, the authors concluded that nickel sulfate is likely responsible for the excess cancer risk. An increased risk of stomach cancer in the nickel-exposed cohort was also observed in this study; however, as stomach cancer has not generally been associated with nickel exposure, the authors speculated that this was a chance finding.

Further evidence against an association between stomach cancer and nickel exposure was reported in the results of a negative two-year oral (gavage) carcinogenicity study dosing nickel sulphate hexahydrate (CRL, 2005). In this study, the daily oral administration of nickel sulphate hexahydrate did not produce a dose-related increase in any common tumour type (including stomach) or an increase in any rare tumours.

Pang *et al.* (1996) evaluated cancer mortality rates in a small cohort of English nickel platers with no known occupational exposure to chromium. There was no evidence of an increase in lung or nasal cancers in this cohort. However, there was weak evidence that nickel plating is associated with an excess risk for stomach cancer. TERA (1999) notes that these data are of interest in that this appears to be the only cohort that was exposed almost entirely to soluble nickel salts.

A clear dose-related effect was reported for cumulative exposure to water-soluble nickel and lung cancer in a case-control study of Norwegian nickel-refinery workers (Grimsrud *et al.*, 2002). Smoking was reported to be a low to moderate confounding factor. The authors indicated that although no other dose-dependant increases for other types of nickel were noted, a general increase in risks from other types of nickel could not be excluded. Soluble nickel compounds have also been reported to result in adverse effects on the kidney tubular function in humans albeit at high doses (Vyskocil *et al.*, 1994).

The results of an investigation conducted by Seilkop and Oller (2003, 2005) found that smoking and/or lifestyle factors are related to the marginal increase in lung cancer risks of nickel alloy workers, as opposed to low level exposure of nickel oxide and metallic nickel. Another recent study examined whether nickel content in inhaled smoke from cigarettes might be a supplementary source of nickel exposure to workers in a nickel refinery in Norway (Torjussen *et al.*, 2003). The authors of this study reported that blood plasma and urine concentrations were similar among randomly selected smoking and non-smoking employees. In addition, after smoking the cigarettes ≤ 1.1% of nickel in the mainstream smoke was recovered with most of the tobacco nickel being recovered in the ash (Torjussen, *et al.*, 2003).

Seilkop and Oller (2003, 2005) also estimated safety limits for workers from fitted animal dose-response curves. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.5 to 1.1 mg inhalable nickel oxide/m^3. The authors report that although the animal data for nickel oxide suggest a threshold response for lung cancer, this cannot be concluded with certainty as sampling uncertainty in data make the non-threshold response equally as plausible. They report that the non-linearity of the observed dose-response of nickel oxide is well represented by benchmark dose models (excluding the high dose response).

In a review article, Oller (2002) suggested that in isolation, water soluble nickel compounds are not complete carcinogens. However, they may enhance the carcinogenic risks associated with other compounds when inhaled, if concentrations are large enough to induce chronic lung inflammation (Oller, 2002). Soluble and water-insoluble nickel compound exposures appear to increase respiratory cancer risks at concentrations in excess of 1 and 10 mg Ni/m^3 work place dust, respectively (ICNCM, 1990). By keeping exposure below levels resulting in chronic respiratory toxicity, Oller (2002) suggested that possible tumour-enhancing effects would be avoided. A human equivalent NOAEL for respiratory chronic active inflammation of 0.0027 mg/m^3 for chronic nickel exposure was calculated by ATSDR (2005).

The information from new studies and follow-up studies of existing cohorts generally confirms the conclusions previously made in the IARC (1990) and Doll *et al.* (1990) studies. That is, more than one form of nickel can contribute to an increased incidence of lung cancer. Also, it appears that the role of soluble nickel species as a contributor or enhancer of risks associated with exposure to insoluble nickel compounds is confirmed (*e.g.*, Easton *et al.*, 1992; Anttila *et al.*, 1998; Oller, 2002). It appears most likely that soluble nickel compounds enhance the potential carcinogenicity of insoluble nickel compounds. For example, Pang *et al.* (1996) found no increased incidence of lung or nasal cancer in nickel plating workers exposed almost entirely to soluble nickel compounds. The previous conclusion (Doll *et al.*, 1990) that respiratory cancer risk is primarily related to soluble nickel at concentrations in excess of 1 mg/m^3 and to exposure to insoluble forms at concentrations above 10 mg/m^3, does not appear to be confirmed. Rather, the recent evidence suggests that lower concentrations of both soluble and insoluble nickel may contribute to an increased risk of respiratory tract cancer in exposed workers (EC, 2000).

Additional insights from the more recent suite of studies include two studies that found a small positive association between soluble nickel exposure and stomach cancer (*e.g.*, Pang *et al.*, 1996, Anttila *et al.*, 1998). The possible multiplicative effect of smoking in some studies is also of interest (*e.g.*, Andersen *et al.*, 1996; Julian and Muir, 1996).

A recent review by Sivulka (2005) shows that among occupational cohorts exposed to Ni compounds, the vast majority of affected human tissues were the lung or nasal sinus tissues. Meta-analysis of a large number of studies has shown that kidney, or stomach cancers that have been reported in some nickel cohorts are not related to nickel exposure. Generally metallic nickel has been identified as non-tumourigenic, especially at lower doses. This review does not focus on the subsulphide but on evidence of direct health effects attributable to metallic nickel. The review concludes that exposure to metallic nickel (nickel metal powder) does not appear to significantly increase the risk of respiratory cancer at concentrations that are as high or higher than those observed in current workplace environments.

A comprehensive review of the toxicology of soluble nickel salts (which included detailed evaluation of the available carcinogenicity evidence) has been prepared by TERA (1999). The key findings and conclusions from this review, with respect to the carcinogenic potential of soluble nickel compounds, are summarized below.

- The carcinogenic activity of insoluble nickel salts should not be used to predict the carcinogenic potential of soluble nickel salts, due to differences in tumorigenicity observed in animal studies (*e.g.*, NTP, 1996a,b,c).
- A non-linear dose-response relationship appears to exist for these compounds; although, data are presently insufficient to determine where non-linearities occur in the dose-response curves.
- Using the U.S. EPA's draft guidelines for carcinogen assessment, it was concluded that inhaled and ingested soluble nickel compounds should be classified as "cannot be determined" at this time. Using the current U.S. EPA (1986) guidelines, classification of the carcinogenic potential of soluble nickel compounds would be D - not classifiable as to human carcinogenicity.
- Multiple oral studies of soluble nickel compounds in different experimental animal species do not indicate a cancer-causing potential (however, all studies had design flaws that preclude a definitive conclusion, such as single doses, low dose ranges, high animal mortality, incomplete reporting, *etc*.). Despite these limitations, the oral exposure study results are consistent with those seen after inhalation and parenteral administration of soluble nickel compounds.
- The lack of definitive oral carcinogenicity data for soluble nickel, and absence of oral studies which investigate potential interactions between soluble and insoluble forms of nickel via the oral route, are key areas of scientific uncertainty.
- The evidence for genotoxicity of soluble nickel compounds is mixed and equivocal.
- As animal studies show clear differences in the ability to induce tumors by inhaled soluble and insoluble nickel (*e.g.,* NTP, 1996a,b,c), an animal carcinogenesis study involving co-exposure by inhalation to both soluble and insoluble forms of nickel would be useful in addressing the potential biological interactions between these forms.
- Extensive epidemiology data suggests inhalation exposure to mixed soluble and insoluble nickel salts can cause the development of lung and nasal cancer in humans. However, the possible contribution of soluble nickel exposure to the observed cancer deaths is controversial and poorly understood. Furthermore, interpretation of the data is confounded by co-exposure to other metals and other chemicals (some of which are known carcinogens such as arsenic and inorganic acid mists), limitations in exposure measurements, and smoking (possibly higher rates than in the general population in some cohorts).

- Overall, the available epidemiology data are insufficient to determine whether soluble nickel alone is a human carcinogen. The currently available evidence suggests soluble nickel is not carcinogenic by itself, but may act as an effect modifier, or promoter, which increases the lung and nasal cancer risk associated with exposure to other, less soluble forms of nickel.
- Potential means by which soluble nickel could increase the carcinogenicity of insoluble nickel species and possibly other chemicals, include: production of an inflammatory response and associated enhanced cell proliferation, impaired clearance, and local generation of oxygen radicals. However, it must be recognized that the available data are insufficient to determine which mechanism(s) operate and whether there is a dose-response relationships for this promotion effect of soluble nickel.
- The development of a cancer dose-response curve for soluble nickel for the inhalation route is not recommended at this time.
- It appears that toxicokinetic factors may predispose soluble nickel species to having a low carcinogenic potential. For example, soluble forms are inefficiently taken up by cells by passive diffusion, and the soluble nickel that does enter cells binds preferentially to cellular proteins over DNA, thereby reducing the amount of soluble nickel that is available to react with DNA.

In its evaluation of nickel compounds, IARC (1990) concluded the following with respect to carcinogenicity of nickel compounds. This evaluation has not been updated since 1990.

- There is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry.
- There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys.
- There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulfides.
- There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides and nickel telluride.
- There is inadequate evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulfide and nickel titanate.

The overall IARC classification for nickel compounds is Group 1 – carcinogenic to humans. This was based on the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and other supporting data, as well as the underlying concept that nickel compounds can generate free nickel ions at critical sites in target cells. Metallic nickel was classified as Group 2B - possibly carcinogenic to humans. IARC has not conducted a more recent evaluation since 1990.

Other carcinogen classifications for nickel compounds are as follows. The U.S. EPA classifies nickel refinery dusts and nickel subsulfide as Class A – known human carcinogens. Health Canada (1994) classifies soluble nickel (primarily nickel sulfate and nickel chloride) as Group I, carcinogenic to humans. However, Health Canada only estimated the cancer potency of soluble nickel compounds *via* the inhalation route and did not estimate cancer potency *via* the oral route. Health Canada (1994) classifies metallic nickel as Group VI - unclassifiable with respect to carcinogenicity in humans. Health Canada classifies the groups of oxidic, sulphidic and soluble nickel compounds as Group I, carcinogenic to humans. The U.S. EPA has not evaluated soluble salts of nickel, as a class of compounds, for potential human carcinogenicity, however nickel (various soluble and insoluble compounds) are presently being reassessed under the U.S. EPA IRIS Program.

It should be noted that the IARC, U.S. EPA and Health Canada classifications for nickel compounds do not take into account studies published since the early 1990's or the TERA (1999) review and recommendations.

Exposure Limits

TERA, ATSDR, OEHHA, Health Canada, the U.S. EPA were the organizations consulted to select exposure limits for Nickel. In addition, the inhalation exposure limits developed by Seilkop (2004) were also reviewed as they were considered relevant to the current study given the current state of knowledge related to nickel compounds and their toxicological behaviour.

Oral Exposure Limit

Identified regulatory oral exposure limits for soluble nickel compounds and nickel oxide are described below. No agency has derived oral exposure limits for nickel subsulphide.

ATSDR (2005) considers that there is insufficient data available to derive an acute, intermediate or chronic-duration oral MRL for any nickel compound.

The U.S. EPA (1991a) derived an oral RfD of 20 µg/kg body weight/day for soluble salts of nickel based on rat data from the Ambrose *et al.* (1976) study. This RfD was based on a NOAEL of 5,000 µg/kg body weight/day, for decreased body and organ weights, which corresponded to a dietary concentration of 100 ppm nickel in the diet over the two-year study. The form of nickel administered was nickel sulphate hexahydrate. A cumulative uncertainty factor of 300 was applied to the NOAEL (10 for interspecies extrapolation; 10 to protect sensitive populations; three to account for inadequacies in the available reproductive studies), to yield the oral RfD.

OEHHA (2003a) used the same principal study (*i.e.*, Ambrose *et al.*, 1976) as the U.S. EPA to derive a chronic oral REL of 50 µg/kg body weight/day. The only difference in derivation of the REL *versus* the U.S. EPA oral RfD is the magnitude of the cumulative uncertainty factor that was applied to the study NOAEL (5,000 µg/kg body weight/day). In both cases, the study NOAEL was equivalent to a NOAELHEC (*i.e.*, a NOAEL to a duration-adjusted human equivalent concentration). OEHHA applied a cumulative uncertainty factor of 100 (10 for interspecies uncertainty and 10 for intraspecies uncertainty) to the NOAELHEC to yield the chronic REL.

Also using the Ambrose *et al.* (1976) study as the principal study, Health Canada (1996; 2003) recommends a TDI of 50 µg/kg body weight/day for nickel sulphate. Again, this TDI is based on the NOAEL from Ambrose *et al.* (1976). An overall uncertainty factor of 100 (10-fold for interspecies extrapolation and 10-fold for interspecies variation) was applied to the study NOAEL (considered the human-equivalent NOAEL) to yield the TDI. Unlike the U.S. EPA, Health Canada did not consider an additional uncertainty factor for design limitations in reproductive studies necessary.

Recently, in their review of the toxicology of soluble nickel compounds, TERA (1999) calculated an oral reference dose of 8 µg/kg/day nickel for ingested nickel-soluble salts. Several studies were considered as the basis for the RfD for soluble nickel salts including Vyskocil *et al.* (1994), Ambrose *et al.* (1976), American Biogenics Corp. (1988), Dieter *et al.* (1988), and Smith *et al.* (1993). The most sensitive endpoint was determined to be increased albuminuria (indicating renal glomerular dysfunction) in male and female rats exposed to nickel in drinking water for six months (Vyskocil *et al.*, 1994). Increased albuminuria was observed at sixmonths but not after three months of exposure. In this study, only a single dose was tested (*i.e.*, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day in females). These doses were the LOAEL values for this study, as albuminuria was considered a biologically significant effect. Despite this limitation of single doses, the LOAELs are supported by several other oral studies (TERA, 1999). Other limitations of Vyskocil *et al.* (1994) and the other available oral studies with soluble nickel

compounds noted by TERA (1999) included subchronic durations, lack of comparison to baseline values, small numbers of test animals, considerable variability in responses in both control and exposed groups, uncertainty surrounding biological significance of renal effects, inadequate reporting, and high mortality rates in both treated and control groups. Upon consideration of these issues, TERA (1999) selected Vyskocil *et al.* (1994) as the most appropriate choice for the principal study. As there was substantial variability for albuminuria in male rats, although not statistically significant, the LOAEL of 7.6 mg Ni/kg/day in females was considered the most appropriate study LOAEL for deriving an RfD from. An overall uncertainty factor of 1,000 was applied (*i.e.*, 10 for intrahuman variability, 10 for interspecies extrapolation, and a 10 for subchronic-to-chronic extrapolation, an insufficient toxicological database, and use of a minimal LOAEL) to the LOAEL of 7.6 mg/kg/day to yield the oral RfD of 8 µg/kg body weight/day.

TERA (1999) noted that the nickel doses used in the principal study did not include the nickel present in the diet. Therefore, the RfD represents the dose of nickel in addition to the amount received in food. TERA (1999) considered this oral RfD to agree well with the U.S. EPA oral RfD of 20 µg/kg body weight/day for total nickel exposure, and was within the expected inherent uncertainty surrounding an RfD. In addition, an independent peer review panel, through TERA's ITER Peer Review program has recently approved the oral RfD value. The TERA RfD was not used in this study as reference values derived for all other COC were expressed on a total exposure basis, whereas this value is considered an incremental value. For consistency purposes, the EPA value of 20 µg/kg/day was considered preferable.

Health Canada (1996; 2003) reports a TDI of 1.3 µg/kg body weight/day for nickel chloride. This TDI was obtained by applying a 1,000-fold uncertainty factor (10-fold for use of a LOAEL, 10 fold for interspecies differences and 10 fold for sensitive individuals in the population) to the LOAEL of 1.3 mg/kg/day based on reproductive toxicity in the rat study by Smith *et al.* (1993). It should be noted however, that this study was criticized by the U.S. EPA for lacking a clear dose-response relationship. Since the speciation analysis conducted as part of this study did not indicate the presence of any nickel chloride or related substances, this limit was deemed inappropriate. In addition, it is not expected that nickel chloride would be very stable in the environment (*i.e.*, very soluble in water) nor is it anticipated to be a significant species in the facility emissions.

No regulatory agencies were identified that developed chronic oral exposure limits (RfDs, TDIs, *etc*.) for nickel oxide, with the exception of RIVM in the Netherlands. This agency used the study by Ambrose *et al.* (1976), which used nickel sulphate, to derive a TDI of 50 µg/kg body weight/day for nickel oxide. An

overall uncertainty factor of 100 was applied to the study NOAEL of 5,000 µg/kg body weight/day to yield the TDI. The use of a nickel sulphate study to estimate a nickel oxide TDI appears scientifically questionable however, especially when nickel oxide studies are available in the scientific literature. As such, this TDI was not considered for use in the human health risk assessment of the Greater Sudbury Area.

Other credible regulatory agencies have recently published oral toxicity reference values for nickel. For example, the World Health Organization (WHO, 2007) published a nickel drinking water guidance (DWG) based on studies by Springborn (2000a,b) and Nielsen *et al*. (1999). In the Nielsen *et al*. study, soluble nickel was given to fasted (overnight + 4 hours post-exposure) human subjects at a single test concentration (12 µg/kg bw/day). There were 20 nickel-sensitized female (with hand eczema) and 20 age-matched controls. A LOAEL of 12 µg/kg/day was reported in pre-sensitized subjects (with nickel eczema). Nine of 20 women with pre-existing eczema had exacerbation of this condition. No effects were seen in the controls. WHO used this unbounded LOAEL as the basis for their drinking water guideline. No UF was applied as the LOAEL was from a sensitive human subpopulation.

There are numerous problems with using the Nielsen *et al.* (1999) study as the basis for a nickel oral TRV, including: i) the small numbers of subjects; ii) a single test concentration (an unbounded LOAEL was used with no information available as to whether similar or other effects occur at higher or lower doses); iii) use of fasted subjects which would greatly increase absorption of nickel beyond what would normally occur; and, iv) use of a very sensitive subpopulation that does not reflect the majority of people. Any one of these issues would often preclude a study from consideration as the basis for TRV development. In fact, OEHHA (2001) excluded this study from consideration in their derivation of a public health goal (PHG) for drinking water. Their concern appears to be that the results of this study do not represent the general population, due mainly to use of fasted subjects and a sensitive subgroup predisposed to nickel-induced eczema. Also, other studies seem to contradict Nielsen *et al*. (1999). OEHHA (2001) notes a few studies that demonstrated oral administration of soluble nickel at low doses over time may reduce nickel contact dermatitis. The issues surrounding the use of the Springborn (2000a,b) data are discussed in detail below.

The State of California's Office of Environmental Health Hazard Assessment (OEHHA) published a public health goal (PHG) in 2005 based on consideration of three developmental toxicity studies in rats (*i.e.*, Smith *et al*., 1993 and Springborn Laboratory, 2000a,b), although the actual dose used to derive the PHG is from Springborn Laboratory (2000b). This dose is a reported NOAEL of 1120 µg/kg/day. The

Smith *et al*. (1993) study used drinking water exposures while the Springborn studies used gavage exposures. OEHHA applied a total UF of 1000 (*i.e.*, 10 for interspecies, 10 for intraspecies and 10 to account for potential oral carcinogenicity of soluble nickel) to this NOAEL. The test compound was actually NiSO4 * 6H20. The resulting TRV used by OEHHA to derive the PHG for drinking water was 1.12 µg/kg bw/day. Interestingly, the same TRV could be derived from the Nielsen *et al*. (1999) study if one applied a 10-fold UF to the LOAEL. However, use of this TRV value is questionable given the following issues:

- While there are a number of studies demonstrating development and/or reproductive toxicity in experimental animals, there is no strong evidence in the scientific literature that nickel is a developmental toxicant in humans. One study (Chaschin *et al*., 1994) of workers at a Russian hydromet nickel refinery had increased rates of spontaneous abortions relative to controls. However, these exposures were inhalation only, and confounded by manual labour and heat stress. Interestingly, the estimated dose associated with the health outcome is 20 µg/kg bw/day (*i.e.*, the same value as the current U.S. EPA oral RfD).

- A number of the animal reproduction/developmental studies had design limitations which make the results questionable (*e.g.*, small sample sizes, inappropriate comparisons, non-random matings, other trace metals in diet, inadequate reporting of water intake rates, elevated temperature and humidity during experiments, questionable biological significance of some reported effects). However, the Springborn studies appear to have much fewer design limitations than previous studies.

- Interestingly, Springborn (2000b), which was a more detailed investigation, did not confirm the findings of Springborn (2000a). Springborn (2000a) was a preliminary study, and the dose identified as the LOAEL in the Springborn (2000a) study was identified as the NOAEL in the Springborn (2000b) study (*i.e.*, 10 mg/kg bw/day). This dose was the lowest test dose in the Springborn (2000a) study, and the highest test dose in the Springborn (2000b) study (note that the NOAEL and LOAEL reported by OEHHA reflect adjusting on a relative molar mass basis; 10 mg/kg/day is the actual unadjusted test dose of the administered compound; see the footnote below for further information). OEHHA notes there is uncertainty around the actual effects threshold. To account for this, OEHHA selected the dose that is one dose level below the actual NOAEL in the Springborn (2000b) study as the "new" NOAEL (*i.e.*, 5 mg/kg/bw/day). OEHHA

then adjusted this new NOAEL down to 1.12 mg/kg bw/day through a molar mass ratio procedure.

- Use of a 10-fold UF to protect against oral carcinogenic effects is not well supported by the scientific literature (*i.e.*, there is no strong evidence that nickel is a carcinogen by oral route for any chemical form). All oral animal studies in the literature are negative for carcinogenicity. Injection studies show tumours only in injection site tissues or in one highly susceptible mouse strain. The genotoxicity database on nickel compounds is mixed and equivocal. The basis for OEHHA using this 10-fold UF appear to be two ecological studies (*i.e.*, Isacson *et al*., 1985 and Ling-Wei *et al.*,1988) that reported an association between nickel in drinking water and bladder, nasopharyngeal and lung cancer rates. However, as is typical with ecological studies, these studies cannot show causation. For example, in the Isacson study, only males had the association (may be related to failure to account for confounding factors such as smoking rate or occupation). Even Isacson *et al.* (1985) suggest that nickel is really just an indicator of presence of other chemicals that might be related to the cancer rates. In the Ling-Wei study, there were only 15 subjects and 15 controls. It is the SARA Group's opinion that both studies are too weak to use as basis for this UF.

- While not all regulatory agencies follow this procedure, there was no use of a NOAEL-HEC value in the derivation of this TRV.

With respect to nickel subsulphide, no agency-derived oral exposure limits were identified. Furthermore, there is no evidence that has indicated that orally ingested nickel subsulphide would be carcinogenic. Nickel subsulphide would be expected to rapidly dissociate in the gut, and as such oral exposure to this compound would be negligible.

Inhalation Exposure Limit

Soluble Nickel Compounds

The ATSDR (2005) chronic inhalation MRL (minimal risk level) is 0.00009 mg Ni/m^3. This MRL is considered most appropriate for soluble nickel salts. The MRL was derived from a NOAEL of 0.03 mg/m^3 in rats for respiratory effects (chronic active inflammation and lung fibrosis) (NTP, 1996b). This NOAEL was then adjusted for intermittent exposure (0.03 mg/m^3 x 6/24 x 5/7) and multiplied by the Regional Deposited Dose Ratio (RDDR) of 0.506 (to extrapolate from particle deposition in rats to

deposition in humans), to yield a human-equivalent NOAEL value (NOAELHEC). The NOAELHEC was then divided by a cumulative uncertainty factor of 30 (three-fold for extrapolation from animals to humans and 10-fold for human variability) to yield the chronic MRL.

ATSDR (2005) also calculated an intermediate-duration inhalation MRL (Minimal Risk Level) for nickel of 0.0002 mg/m^3. This MRL is based on a nickel sulfate NOAEL of 0.06 mg/m^3 for lung inflammation (NTP, 1996b). This study NOAEL was adjusted for intermittent exposure (*i.e.*, 0.06 mg/m^3 x 6/24 x 5/7 = 0.01 mg/m^3), and then converted to a NOAELHEC by multiplying the adjusted NOAEL by a regional deposited dose ratio of 0.474. Following this, the MRL was obtained by dividing the NOAELHEC by a cumulative uncertainty factor of 30 (*i.e.*, three for inter-species extrapolation with dosimetric adjustments and 10 for intrahuman variability).

TERA (1999) and Haber *et al.* (2000b) suggest a reference concentration of 0.0002 mg/m^3 for inhaled nickel soluble salts, based on lung fibrosis in male rats, as reported in NTP (1996b). TERA reviewed the quantal data from the NTP (1996b) study on the incidence of respiratory tract lesions and then fit these data to a polynomial mean response regression model and a Weibull power mean response regression model, using the maximum likelihood method. This was followed by benchmark dose modelling. Based on the modelling results, the most sensitive endpoint was lung fibrosis in male rats. The $BMCL_{10}$(HEC) for lung fibrosis in male rats was 0.0017 mg Ni/m^3. An uncertainty factor of 10 was applied to this value to account for intrahuman variability, and yielded the RfC.

OEHHA (1999) derived an acute reference exposure level (REL) for nickel compounds (excluding nickel oxide) of 0.006 mg Ni/m^3. This value was derived using the LOAEL of 0.067 mg/m^3 for decreased forced expiratory volume (>15%) from the study by Cirla *et al.* (1985) involving seven volunteer metal plating workers with occupational asthma. The nickel species in this study was nickel sulphate hexahydrate, and the exposure duration was 30 minutes. OEHHA extrapolated the LOAEL to a one hour concentration, which was 0.033 mg/m^3. A cumulative uncertainty factor of six was applied to this value (based on use of a LOAEL) to yield the acute REL.

OEHHA (2003a) derived a chronic reference exposure level (REL) of 0.00005 mg/m^3 for nickel compounds (except nickel oxide). The principal study was NTP (1996b) and the critical effects were pathological changes in lung, lymph nodes, and nasal epithelium, which included active pulmonary inflammation, macrophage hyperplasia, alveolar proteinosis, fibrosis, lymph node hyperplasia, and olfactory epithelial atrophy. The study NOAEL was 0.03 mg/m^3. The nickel species tested was nickel sulfate hexahydrate. The study NOAEL was adjusted for continuous exposure (multiplied by 6/24 x 5/7)

and then converted to a NOAELHEC by multiplying against a regional deposited dose ratio (RDDR) of 0.29. Following this, the NOAELHEC was divided by a cumulative uncertainty factor of 30 (*i.e.,* three for interspecies uncertainty and 10 for intraspecies uncertainty) to yield the chronic REL.

Health Canada (1996) derived a tolerable concentration (TC) for nickel sulphate of 0.0000035 mg Ni/m^3. This TC was derived based on the LOEL in rats of 0.02 mg Ni/m^3 from the Dunnick *et al.* (1989) study. The LOEL was adjusted for continuous exposure by applying factors of 6/24 and 5/7, and an uncertainty factor of 1,000 was applied (*i.e.*, 10 for intraspecies variation, 10 for interspecies variation, and 10 for a less than chronic study). However, the Dunnick *et al.* (1989) study exposed rats to nickel oxide (a relatively insoluble form) rather than to a soluble nickel form, such as nickel sulphate. Thus, the appropriateness of using a nickel oxide study as the basis for a TC for nickel sulphate is questionable, especially given that nickel sulphate studies exist in the scientific literature. Given this, the TC was not considered for use in the human health risk assessment of the Greater Sudbury Area.

The only jurisdiction identified that classifies soluble nickel salts as human carcinogens is Health Canada. Health Canada (1996; 2003) reports an inhalation slope factor of 3.1 $(mg/kg/day)^{-1}$ and an inhalation unit risk of 0.71 $(mg/m^3)^{-1}$ for soluble nickel salts. These values were calculated from the Health Canada (1996) tumorigenic concentration 5% (TC05) of 0.07 mg/m^3 for soluble nickel (primarily nickel sulfate and nickel chloride), which in turn, was based on Doll *et al.* (1990). It is important to note however, that the TERA (1999) review concluded that the available information on carcinogenicity of soluble nickel salts is inadequate to derive a reliable TC05, slope factor or inhalation unit risk for any soluble nickel compound. Thus, these Health Canada values are not considered reliable estimates of carcinogenic potency for soluble nickel compounds and will not be used in the human health risk assessment of the Greater Sudbury Area. Health Canada (1996; 2003) also reports a TC05 of 0.040 mg/m^3 for combined oxidic, sulphidic and soluble nickel. Given the conclusions of the TERA (1999) review, this TC05 would appear to be equally flawed as the TC05 for soluble nickel compounds; thus, it is not considered a reliable estimate of carcinogenic potency for the combined nickel compounds, and will not be used in the human health risk assessment of the Greater Sudbury Area.

Oller (2002) suggested that in isolation, water soluble nickel compounds are not complete carcinogens. However, they may enhance the carcinogenic risks associated with other compounds when inhaled, if concentrations are large enough to induce chronic lung inflammation (Oller, 2002). By keeping exposure below levels resulting in chronic respiratory toxicity, Oller (2002) suggested that possible tumour-enhancing effects would be avoided. Seilkop (2004) has derived an inhalation unit risk of $1.9x10^{-4}$

$(\mu g/m^3)^{-1}$ for nickel sulphate when exposure is in the presence of a carcinogen. The basis of this unit risk is the incidence of an inflammatory response in the exposed animals.

Interestingly, when the IUR for nickel sulphate (Seilkop, 2004) is converted to a risk-specific concentration (RsC) assuming a target risk level of one-in-one hundred thousand, the RsC is very similar to the OEHHA chronic REL for 'nickel compounds except nickel oxide'. The RsC that corresponds to the nickel sulphate IUR is 0.05 $\mu g/m^3$ (the OEHHA REL is also 0.05 $\mu g/m^3$). Thus, it can be extrapolated from this comparison that although the OEHHA chronic REL is not derived from a cancer endpoint, it would appear to be protective of both non-cancer and potential cancer effects of inhaled soluble nickel.

Nickel Subsulphide

Chronic exposure to nickel subsulphide has been found to result in inflammatory changes in the lungs The chronic MRL derived by ATSDR (0.00009 mg Ni/m^3) is based on the lowest of the LOAELs observed in a series of NTP studies which looked at various forms of nickel. The MRL was based on the most sensitive endpoint and form of nickel studied (sulphate) and as such is considered of protective similar effects following exposure to nickel subsulphide (ATSDR, 2005).

The U.S. EPA considered the following studies in the determination of unit risks for nickel compounds (refinery dust and subsulphide).

A cohort of employees of a nickel refinery in West Virginia who experienced a minimum one year exposure to nickel refinery dusts (containing nickel subsulphide, sulphate and oxide or only nickel oxide) did not show an increased incidence of lung cancer above expected rates (Enterline and Marsh, 1982). Chovil *et al.* (1981) studied a cohort of nickel refinery workers in Ontario, and observed a dose-related trend for the relationship between weighted exposure in years to the incidence of lung cancer. Similarly, a cohort of Welsh nickel refinery workers had elevated risks of cancer compared to the national average; increased rates of nasal cancer were observed in men employed prior to 1920, while this rate was less than the national average for those starting work between 1920 and 1925, and equalled the expected value for those employed after 1925 (Doll *et al.*, 1977). A significantly increased lung cancer-related mortality was observed in employees starting prior to 1925 but not in those starting between the years 1930 to 1944. Magnus *et al.* (1982) conducted a study of men employed at a nickel refinery in Norway, and reported an elevated occurrence of respiratory cancer for nickel- exposed workers compared to expected values, and for workers involved in nickel processing steps compared to non-processing employees.

Each of these epidemiology studies used in the EPA determination of the unit risk associated with nickel, had factors limiting their usefulness for a unit risk calculation. For example, none were able to account for exposures to other chemicals, metals or nickel species (such as nickel subsulphide), that were present in the occupational environment of a nickel refinery. Only one attempted to account for differences between smokers and non- smokers, an important consideration when examining the incidence of lung cancer. Three of the four studies did not provide measurements of airborne nickel concentrations or estimates of worker exposure. The EPA estimated exposures based on information provided in other reports in which concentrations of nickel in the work environment were projected on the basis of the operating procedures used. Other problems included poorly or heterogeneously defined cohorts, poor follow-up success, and no consideration of the role of the latency period for lung cancer. Of the four summarized above, Enterline and Marsh (1982) was the most relevant since estimated exposures were provided, the latent period could be examined, and the effects in refinery workers could be compared to non-refinery workers. However, the mixed exposure to other substances and to cigarette smoke were confounding factors that limit the interpretation of this study.

Based on these studies, the U.S. EPA derived slope factors for nickel refinery dust of $2.4x10^{-4}$ $(\mu g/m^3)^{-1}$ and $4.8x10^{-4}$ $(\mu g/m^3)^{-1}$ for nickel subsulphide. The relevance of these slope factors to the Sudbury environment is questionable given the concomitant presence of multiple nickel species in proportions that will differ from those found within refineries. These slope factor were retained for further consideration, most notably for consideration of the potential presence of nickel sulphide in the ambient environment.

Seilkop and Oller (2003, 2005) have estimated safety limits for workers from fitted animal dose-response curves after accounting for interspecies differences in deposition and clearance, differences in particle size distributions, and human work activity patterns. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.002 to 0.01 mg inhalable nickel subulphide/m^3. Subsequently Seilkop (2004) has derived a non-linear inhalation unit risk of $6.3x10^{-4}$ $(\mu g/m^3)^{-1}$ for nickel subsulphide. This IUR is independent of any specific risk level and can be used to establish 10^{-5} or 10^{-6} acceptable risk levels as appropriate

Nickel Oxide

OEHHA (2003b) derived a chronic REL specifically for nickel oxide of 0.0001 mg/m^3. The principal study was NTP (1996c), and the critical effects considered were pathological changes in lung and lymph

nodes, including active pulmonary inflammation, lymph node hyperplasia, and adrenal medullary hyperplasia (females only). This study identified a LOAEL of 0.5 mg/m^3. The study NOAEL was adjusted for continuous exposure (multiplied by 6/24 x 5/7) and then converted to a NOAELHEC by multiplying against an RDDR of 0.29. Following this, the NOAELHEC was divided by a cumulative uncertainty factor of 300 (*i.e.,* 10 for use of a LOAEL, three for interspecies uncertainty, and 10 for intraspecies uncertainty) to yield the chronic REL.

Health Canada (1996; 2003) recommends a tolerable inhalation concentration (for non-cancer effects) of 0.00002 mg/m^3 for nickel oxide. This TC was derived based from the lowest identified LOEL of 0.02 mg/m^3 reported in Spiegelberg *et al.* (1984). In this study, rats exposed for four months to nickel oxide displayed a dose-related increase in lung granulocyte, lymphocyte, and multinucleated macrophage counts. An uncertainty factor of 1,000 was applied to this LOEL (*i.e.*, 10 for intraspecies variation, 10 for interspecies variation, and 10 for use of a less than chronic study, and minimal effects at the LOEL) to yield the TC.

Seilkop and Oller (2003, 2005) also estimated safety limits for workers from fitted animal dose-response curves. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.5 to 1.1 mg inhalable nickel oxide/m^3. The authors report that although the animal data for nickel oxide suggest a threshold response for lung cancer, this cannot be concluded with certainty as sampling uncertainty in data make the non-threshold response equally as plausible. They report that the non-linearity of the observed dose-response of nickel oxide is well represented by benchmark dose models (excluding the high dose response). Subsequently Seilkop (2004) has derived an inhalation unit risk of 2.3×10^{-5} $(\mu g/m^3)^{-1}$ for nickel oxide. This IUR is independent of any specific risk level and can be used to establish 10^{-5} or 10^{-6} acceptable risk levels as appropriate.

Interestingly, when the IUR for nickel oxide from Seilkop (2004) is converted to a risk-specific concentration (RsC) assuming a target risk level of one-in-one hundred thousand, the RsC is very similar to the OEHHA chronic REL for nickel oxide. The RsC that corresponds to the nickel oxide IUR is 0.4 µg/m^3, while the OEHHA REL is 0.1 µg/m^3. Thus, it can be extrapolated from this comparison that although the OEHHA chronic REL is not derived from a cancer endpoint, it would appear to be protective of both non-cancer and potential cancer effects of inhaled nickel oxide.

Alternate Regulatory Approach

As part of a process to reshape their clean air policy through the generation of new ambient air quality directives, in the late 1990s the European Union established a series of Steering Groups and Working Groups to provide guidance back to the EU on development of appropriate ambient air quality target values. In the development of their proposed target value for nickel, the EU working group based its limit value on three distinct toxicological approaches – one non-cancer and two cancer approaches. The non-cancer approach was based upon non-cancer data on respiratory effects (specifically lung inflammation and fibrosis) of nickel sulphate hexahydrate in a 1996 NTP rodent study. The proposed limit was based upon a LOAEL of 0.06 mg Ni/m^3, a 1000-fold uncertainty factor (*i.e.*, 10-fold for extrapolation from LOAEL to NOAEL, 10-fold for interspecies extrapolation, and 10-fold for intrahuman variability), and a 6-fold correction factor to account for the fact that the animals were not continuously exposed to nickel sulfphate during the study. This yields a limit, based upon non-cancer endpoints, of 10 ng Ni/m^3 (European Commission DG Environment, 2001).

Under the EC classification system, nickel oxide, nickel monoxide, and nickel sulphide are considered "Group 1" (*i.e.*, known human carcinogens), whereas nickel, nickel carbonate, nickel hydroxide, nickel sulphate, and nickel tetracarbonyl are classified as "Group 3" (*i.e.*, possible carcinogens). As such, the EC working group also developed a nickel target value using two different cancer approaches. In the first approach, the EU working group based its first approach upon the International Committee on Nickel Carcinogenesis in Man (ICNCM, 1990) report conclusion that more than one nickel species gives rise to concern with respect to lung and nasal cancer, and that soluble nickel is not carcinogenic in itself but acts so as to increase the overall risk of cancer associated with exposure to other forms of nickel (European Commission DG Environment, 2001).

In the first cancer approach, the EC working group selected three unit risk values (*i.e.*, the U.S. EPA, the Centre d'Etude sur l'Evaluation de la Protection dans le domaine Nucléaire [CEPN], and the WHO) to derive a range of unit risk values of 2.4×10^{-4} to 3.8×10^{-4} (µg Ni/m^3)$^{-1}$. Based upon the observations that nickel subsulphide is very low or absent in ambient air, and that these studies were based upon workplace exposures (*i.e.*, the possibility that nickel refinery workers are exposed to different nickel species than the general public), the EC working group concluded these estimates likely overestimate the true risk by an order of magnitude. This would result in an actual cancer unit risk range of 2.4×10^{-5} to 3.8×10^{-5} (µg Ni/m^3)$^{-1}$, or 0.026 to 0.042 µg Ni/m^3 assuming a 1-in-1,000,000 acceptable cancer risk level (European Commission DG Environment, 2001).

In the second cancer-based method to establishing a target value for nickel, the EU working group used a variety of threshold approaches, based upon three separate reports, which assumed a LOAEL for nickel refinery workers and applied various adjustments (*i.e.*, exposure time, work to lifetime, breathing rate) and uncertainty factors (*i.e.*, LOAEL to NOAEL and adjustments for sensitive subgroups). The three different applications of this threshold approach resulted in a calculated concentration range between 0.006 to 1.1 µg Ni/m^3 (European Commission DG Environment, 2001).

Following an evaluation of these three different approaches, the EU working group proposed a limit value range of 0.01 to 0.05 µg Ni/m^3 (as an annual mean), based upon non-cancer effects. The working group also believed that a limit value in this range can be judged compatible with the objective of limiting excess lifetime cancer risks to not more than one-in-a-million. The majority of the working group proposed a limit value at the lower end of this range, to represent an annual mean of total airborne nickel (European Commission DG Environment, 2001).

Based upon this work, in 2004 the European Parliament adopted a target value for airborne nickel of 20 ng Ni/m^3, or 0.02 µg Ni/m^3, considered protective of both cancer and non-cancer health endpoints (OJEU, 2005).

Dermal Exposure Limit

No regulatory dermal exposure limits for nickel compounds were identified in the literature reviewed for the current assessment.

Due to chemical and experimental variables in addition to individual variables (*e.g.*, differences in susceptibility to nickel, age, gender, integrity of skin), Hostynek (2002) reported that a threshold value for nickel inducing sensitization cannot be developed at this time. Similarly, Hostynek (2002) reported that identifying concentrations which will elicit a reaction in sensitized individuals is also not possible.

No regulatory dermal exposure limits for nickel compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in a human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

With respect to nickel dermal exposure, it is important to recognize that very little nickel penetrates the skin to enter the systemic blood circulation. Rather, nickel tends to penetrate only to outer layers of the skin (primarily the stratum corneum). A number of studies and reviews suggest that ionized nickel compounds do not penetrate intact skin, which results in dermal absorption of nickel being negligible in most circumstances (See Section A5-2.1.3 for further details).

Criteria Summary

The following table summarizes the exposure limits selected for the current study.

Summary Table of Exposure Limits Selected for Nickel

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	20 µg/kg/day	RfD	Decreased body and organ weight (rats)	Ambrose *et al.*, 1976	U.S. EPA, 1991a
Inhalation	0.02 µg/m^3 (total nickel)	RfD	Respiratory effects (lung inflammation and lung fibrosis)	European Commission DG Environment, 2001	OJEU, 2005
Dermal [a]	NA		NA	NA	NA

Summary Table of Exposure Limits Selected for Nickel

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	nickel oxide 2.3×10^{-5} $(\mu g/m^3)^{-1}$ nickel subsulphide 6.3×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Seilkop, 2004	None
	nickel refinery dusts 2.4×10^{-4} $(\mu g/m^3)^{-1}$ nickel subsulphide 4.8×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Extrapolations from epidemiologic datasets from Enterline and Marsh, 1982; Chovil *et al*., 1981; Peto *et al*., 1984; and Magnus *et al*., 1982	U.S.EPA, 1991
	nickel compounds 3.8×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Andersen, 1992; Andersen *et al*., 1996	WHO, 2000
	combined oxidic, sulphidic and soluble nickel 0.04 mg/m^3	TC05[b]	Lung cancer	Doll *et al*., 1990	Health Canada, 1996
Dermal	NA		NA	NA	NA

NOTES:

NA = not available; RfD = reference dose; REL = reference exposure level ; IUR = inhalation unit risk.

[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

[b] Health Canada (2004) converts the TCO5 of 0.04 mg/m^3 to an IUR of 0.0013 $(\mu g/m^3)^{-1}$ by dividing the 0.05/TC05.

References

For a complete list of references, see Section A5-5.0 of the detailed toxicological profile on the accompanying CD.

A5-1.0 INTRODUCTION

Nickel is a transition metal, which exists in several oxidation states (most often +2) (Budavari *et al.*, 1989). Nickel increases the hardness of other metals and it used in the production of stainless steel and other alloys (ATSDR, 2005; Chang, 1996). The toxicokinetics and toxicity of nickel are strongly influenced by its form (*e.g.,* metallic, salt, oxide) and solubility. The more soluble nickel compounds include the nickel salts (nickel chloride and nickel sulphate) and nickel nitrate, while less soluble nickel compounds include nickel oxide (*i.e*., black crystalline form and more stable green form) and nickel sub-sulfide (ATSDR, 2005). In general, the more soluble nickel compounds have a greater toxicity than less soluble forms. However, at the site of deposition, the less-soluble compounds are more likely to be carcinogenic (ATSDR, 2005).

The form of nickel emitted to the atmosphere depends on the source. Complex nickel oxides, nickel sulfate, and metallic nickel are all associated with combustion, incineration, smelting and refining processes (ATSDR, 2005).

Nickel in air is associated with particulate matter, with nickel compounds appearing to distribute primarily into the coarse PM mode (*i.e.*, >PM2.5 to PM10) (MOE, 2004). Recently collected Ontario data on nickel speciation in airborne PM indicates that the dominant nickel species in both urban and specific nickel source-affected areas (such as oil-fired power plants, petroleum refineries, nickel smelters (including those in the Greater Sudbury Area, and nickel refineries) is nickel sulphate (57 to 85%), followed by nickel oxide (6 to 31%), and nickel hydroxide (8 to 23%) (MOE, 2004). Very little or no sulphidic or metallic nickel species were detected in these areas. This is an important finding as the most toxic nickel species are the sulphidic compounds, particularly nickel subsulphide. The lack of detection of sulphidic nickel compounds in ambient air of a number of nickel source-affected areas is a key consideration in the risk assessment of nickel emissions from industrial sources. This finding suggests that risk assessments investigating ambient environmental exposures to nickel should not use toxicity reference values that are based on nickel subsulphide, as this does not appear to be a relevant nickel species that people are exposed to in the ambient environment. Similarly, metallic nickel is another form) of nickel for which there would be negligible ambient environmental exposure. While people are unlikely to be exposed to nickel refinery dust per se in the ambient environment, the nickel species present in nickel refinery dust do occur in the ambient environment (including various soluble nickel salts, nickel subsulphide and nickel oxide), although at much lower concentrations and in quite different proportions than those which occur in nickel refinery dust.

Results of the speciation analyses of environmental media in the Greater Sudbury area (GSA) has indicated that some of the air filters and dust samples collected over the past year likely contain small

amounts of nickel subsulphide (Ni3S2). As discussed below, it is important to understand that nickel subsulphide differs in toxicity and mode of action from nickel oxide, the form of nickel typically found in the highest quantities within most media sampled in Sudbury (both nickel subsulphide and nickel oxide would be considered smelter emission products). In fact, nickel subsulphide is more toxic ***via* inhalation** than nickel oxide.

However, due to the small amounts of material present in the air filters and the small particle size, there was some question as to whether these nickel species were indeed Ni3S2 or a similar looking form (such as millerite, a natural ore form of nickel). Further investigation of this issue is ongoing and this profile will be amended as confirmatory analysis becomes available.

In soils, nickel can occur in a variety of forms of varying solubility, and/or be adsorbed onto organic matter or clay particles. Overall, the solubility, and subsequent bioavailability of nickel is a function of the nickel sources and local soil geochemistry (Schoof, 2003).

In this profile, toxicological data are reviewed on a number of soluble and insoluble nickel compounds. However, one insoluble compound, nickel carbonyl, is not focused on in this profile as it is not expected to occur in environmental media, due to its high reactivity and instability (ATSDR, 2005).

A5-2.0 TOXICOKINETICS

A5-2.1 Absorption

A5-2.1.1 Absorption Following Oral Exposure

Ingestion is a major route of exposure for nickel compounds (U.S. EPA, 1986; Nieboer and Nriagu, 1992). The different chemical forms of nickel can significantly influence the amount of nickel that is absorbed from the gastrointestinal tract with the more soluble nickel compounds having higher absorption efficiency (U.S. EPA, 1986; Ishimatsu *et al*., 1995). For example, metallic nickel, nickel oxide and nickel subsulfide are poorly absorbed when ingested, while nickel sulfate, nickel chloride and nickel nitrate (which are more soluble) have a much higher absorption efficiency (Ishimatsu *et al.,* 1995).

Estimates of nickel bioavailability in humans following ingestion vary from study to study and have been reported to range from approximately 1 to 30% (*e.g.,* Perry and Perry, 1959; ICRP, 1960; Nodiya, 1972; Horak and Sunderman, 1973; Christensen and Lagesson, 1981; Hansen and Tjell, 1981; Owen, 1990; Nielsen *et al*., 1999; Patriarca *et al.,* 1997). ATSDR (2005) reported that in humans, approximately 27% of soluble nickel was absorbed when given in drinking water, while only about 1% of soluble nickel was absorbed when given with food. Similarly, Nielsen *et al. (*1999) reported human absorption of soluble nickel in food to be approximately 1%. Denkhasu and Salnikow (2002) reported that approximately 1 to 2% of ingested nickel is absorbed under normal dietary conditions with unabsorbed nickel being excreted in the feces (Ishimstsu *et al.*, 1995). However, actual absorption of nickel depends greatly upon the concentration and species of nickel in food, as well as the individual's capacity for absorption.

Maximum absorption of soluble nickel compounds has been shown to occur under fasting conditions, with absorption being much lower when administered with food or in water with a meal (Nielsen *et al*., 1999; Horak and Sunderman, 1973; Sunderman *et al*., 1989). After an overnight fast, human subjects orally dosed with 12, 18, and 50 µg/kg body weight of nickel sulfate had absorbed a mean of 27 ∀ 17% of the dose when ingested in water and 0.7 ∀ 0.4 % of the dose when ingested in food (Sunderman *et al*., 1989). In another study, four healthy human subjects (two males; two females) ingested 10 µg/kg body weight nickel as stable ^{62}Ni isotope in water after overnight fasting. The percentage of ^{62}Ni absorbed ranged from 29 to 40% (mean of approximately 33%), based on calculation of the amount of ^{62}Ni excreted in the feces (Patriarca *et al.,* 1997).

In mice, 1 to 10% of nickel was absorbed after oral administration of nickel sulphate hexahydrate or nickel chloride solutions *via* gavage (Nielsen *et al*., 1993). Similarly, an absorption range of 1 to 10% for the gastrointestinal tract of mice, rats and dogs has been reported for nickel sulphate and nickel chloride given in the diet or by gavage (ATSDR, 2005).

There is a paucity of oral bioavailability data for insoluble nickel compounds; however, the absorption of nickel metal, nickel oxide (green) and nickel oxide (black) were estimated to be 0.09%, 0.01% and 0.04%, respectively, based on rat data (Ishimatsu *et al.*, 1995). Absorption of nickel sub-sulfide and nickel sulfide, which are considered slightly soluble compounds, was estimated to be 0.5 to 2.1%. In comparison, the absorption of the soluble nickel compounds in this study (*i.e*., nickel sulfate nickel chloride and nickel nitrate) was estimated to be 10 to 34% (Ishimatsu *et al.*, 1995).

Only limited data are available on the bioavailability of nickel from soil following oral exposure. The oral bioavailability of nickel chloride administered as an aqueous slurry with sandy loam or clay loam soil or in water were compared based on nickel blood concentrations in rats (Griffin *et al.*, 1990). Bioavailability was reduced for the soil bound nickel compared to the nickel chloride in water. The relative bioavailabilities of nickel were respectively 63 and 34% for the sandy and clay loam slurry, while the absolute bioavailabilities were 3 and 1.5%. Simulated gastric experiments conducted on naturally metal enriched topsoils indicate that approximately 0.1 to 2.4% of total ingested nickel is soluble and available for uptake by humans (Lottermoser, 2002). MOE (2002) reported that relative bioavailability estimates ranged from 11 to 28%, with a mean of 19%, for soil from a former nickel refinery site in which the predominant nickel species was nickel oxide (metallic nickel was also present). Given the high variability and site-specificity of nickel solubility and its bioavailability from soil ingestion, site-specific studies on nickel solubility and bioavailability can be highly useful in refining estimates of exposure and risk, and establishing remedial objectives.

Based on values provided by U.S. EPA (1995), RAIS (2004) reports gastrointestinal absorption factors ranging from 20% (for nickel refinery dust and nickel subsulphide) to 27% for soluble salts of nickel.. These values are recommended by RAIS for human health risk assessment.

A5-2.1.2 Absorption Following Inhalation Exposure

Respiratory tract absorption depends on the solubility of the nickel compound, with more soluble compounds having higher degrees of absorption. Reports of nickel absorption *via* inhalation range from 6% to 40% (ATSDR, 2005; Owen, 1990; ICRP, 1960).

It is important to recognize that once deposited in the lung, there are considerable differences in bioavailability of nickel compounds. Soluble nickel compounds such as nickel chloride and nickel sulphate are readily absorbed, while little of the less soluble nickel compounds such as nickel oxide and nickel subsulphide are absorbed in the lungs.

A5-2.1.3 Absorption Following Dermal Exposure

A report of the National Cancer Institute (NCI, 1985) concluded that the absorption of nickel into the blood through skin had not been demonstrated in humans, as nickel uptake was limited to the outer layers of skin (NCI, 1985; U.S. EPA, 1986). A study in which radioactive nickel was applied to occluded human skin reported dermal uptake ranging from 55 to 77% for nickel sulphate (Norgaard, 1955);

however, it could not be determined if the nickel was absorbed into the bloodstream or merely into the deep layers of the skin (ATSDR, 2005). Less than 1% of nickel chloride was absorbed through the skin of guinea pigs within 24 hours (Lloyd, 1980). Denkhaus and Salnikow (2002) reported that because ionized nickel compounds do not penetrate intact skin, dermal absorption of nickel is negligible.

The dermal absorption of radio-labeled nickel in dermatomed male pig skin samples was reported to be enhanced by 20% when applied with a chemical mixture containing phenol, toluene and trichloroethylene compared to application of nickel alone (Turkall *et al.,* 2003). After 16-hours, the total penetration (*i.e.*, the sum of radioactivity bound to skin and in receptor fluid) was reported to be 57.9% (0.4% receptor fluid; 57.6% bound to skin) of the initial dose for nickel and 69.5% (0.7% receptor fluid; 68.9% bound to skin) for the nickel mixture. The authors reported that these results indicate potential health risks from dermal exposure to nickel may be greater when other chemicals are present (Turkall *et al.*, 2003).

Human stratum corneum from dermatomed cadaver leg skin were exposed to aqueous solutions of nickel salts ($Ni(NO_3)_2$, $NiSO_4$, $NiCL_2$ and $Ni(\text{-}OOCCH_3)_2$ at a concentration of 1% Ni^{2+}) *in vitro* as donor solutions (Tanojo *et al.*, 2001). Pure water was used as the receptor fluid. After 96-hours approximately 98% of the dose was unabsorbed and remained in the donor fluid. Approximately ≤1% was found in the stratum corneum while <1% was found in the receptor fluid (Tanojo *et al.,* 2001). Permeability coefficients were calculated and no significant differences were reported among the various nickel salts.

In four men and one woman dermally exposed to nickel salts in methonal on the arm and back, most of the nickel dose applied remained on the skin surface or was adsorbed in the upper most layers of the stratum corneum after 24 hours. However, approximately 1% of the nickel nitrate dose was reported to be found beyond the third stratum corneum strip which the authors reported was indicative of intercellular diffusion (Hostynek *et al*., 2001).

Based on values from U.S. EPA (1995), RAIS (2004) reports dermal absorption factors for nickel compounds of 0.1% for soluble salts, nickel refinery dust and nickel subsulphide.

A5-2.2 Distribution

Once soluble nickel compounds are absorbed into the bloodstream, nickel (as Ni^{2+}) binds to serum albumin and multiple small organic ligands, amino acids or polypeptides, and is transported in plasma, where it distributes and accumulates in various parts of the body (Chang, 1996; Klaassen, 1996).

In non-occupationally exposed individuals, autopsy results indicate that nickel concentrations are highest in the lungs, followed by the thyroid, adrenals, kidney, heart, liver, brain, spleen and pancreas (Rezuke *et al.*, 1987). Following oral exposure, nickel concentrations in humans are generally highest in the kidney, followed by the lung (Haber, 2000a). Occupationally exposed workers have higher lung burdens of nickel than the general population (ATSDR, 2005). Deposition of nickel occurred primarily in the kidney after both short and long-term oral exposure of soluble nickel compounds to various experimental animals (ATSDR, 2005).

Following inhalation exposure, the size of the particle that nickel is associated with will determine where it is deposited in the respiratory system. Larger particles (with an aerodynamic diameter of 5 to 30 μm) will generally stay in the upper respiratory tract (*i.e.,* nose, mouth, throat). Smaller particles tend to be distributed to the lower respiratory tract (aerodynamic diameters of 1 to 5 μm and <0.5 μm) are respectively deposited to the tracheobronchial region and alveoli in the deepest parts of the lungs (ATSDR, 2005; Klaassen, 1996). Less soluble nickel compounds tend to remain in the nasal mucosa (ATSDR, 2005).

No information was identified regarding the distribution of nickel compounds following dermal exposure.

A5-2.3 Metabolism

Little information is available on the metabolism of nickel. In human serum, nickel binds to albumin, L-histidine, and α2-macroglobulin (ATSDR, 2005). Similar binding of nickel to serum proteins occurs in animals. If the resulting nickel-ligand complex is of sufficiently low molecular weight, it can cross biological membranes (ATSDR, 2005).

A5-2.4 Excretion

The dominant excretion pathway for absorbed nickel in both humans and animals is the urine regardless of the route of exposure (ATSDR, 2005; Haber, 2000a; Chang, 1996). In humans, small amounts are also excreted in the bile, sweat and exhaled air (Chang, 1996). Unabsorbed nickel from the diet is excreted in the feces (Chang, 1996; Haber, 2000a).

A5-3.0 HEALTH HAZARD ASSESSMENT

The toxicity of nickel is strongly influenced by its form (*e.g.,* metallic, salt, oxide) and solubility (ATSDR, 2005). Nickel toxicity can manifest itself in a variety of ways including: i) non-cancer respiratory and other disorders resulting from the ingestion or inhalation of nickel compounds; ii) cancer resulting from the inhalation of nickel compounds; iii) allergy or nickel hypersensitivity resulting from contact dermatitis and asthma; and, iv) iatrogenic poisoning which may have occurred historically in persons undergoing hemodialysis, as a result of corrosion of prostheses containing stainless steel, or medical treatments involving nickel-contaminated medications (MOE, 2001). The immune system and kidneys also appear to be target tissues for nickel.

A summary of relevant human health and animal studies are provided below. This summary is not meant to be an exhaustive review of the literature; rather it is meant to describe the critical studies used to derive the exposure limits selected for this assessment, and to provide appropriate supporting scientific documentation.

A5-3.1 Animal Studies

A5-3.1.1 Acute Systemic Toxicity Studies

Dermal acute toxicity studies of nickel compounds indicate a potential for nickel sulphate hexahydrate to cause cutaneous injury and lipid peroxidation in guinea pigs at a dose of 50 mg/kg/day for seven to 14 days (Mathur *et al*., 1992). Nickel sulphate has also been shown to act as a contact allergen in mice (Ikarashi *et al*., 1992).

Acute lung inflammation was observed in rats exposed for six hours/day for 12 days to 0.7 mg/m^3 nickel sulfate and 0.44 mg/m^3 nickel subsulfide (NTP, 1996a,b). These concentrations were the lowest doses tested. The higher LOAELs for mice indicate that they are less sensitive than rats to the acute toxicity of nickel (ATSDR, 2005).

Effects on the lung were reported in rats dosed with 0, 0.1, 0.5 or 5 mg of standard nickel (average diameter of 5 um) and ultra fine nickel (average diameter of 20 nm) by intratracheally injection for three days (Zhang *et al*., 2003). Inflammation in the bronchoalveolar lavage fluid and indicators of lung injury were higher with the ultra fine nickel compared to the standard nickel. The authors concluded that ultra

fine nickel appears to be much more toxic than standard nickel but the mechanism of toxicity is unknown (Zhang *et al.*, 2003).

In a 12-day inhalation exposure study (where test animals were exposed 5 days/week, for 6 hours/day), a concentration of 10 mg nickel subsulphide/m^3 produced 100% mortality in mice and 20% mortality in rats (Benson *et al*., 1987). Lesions were observed in the nasal and lung epithelium and in bronchial lymph nodes of rats and mice. Pathological examination revealed the presence of emphysematous changes in the lungs of rats exposed to 5 or 10 mg/m^3, and fibrosis in mice exposed to 5 mg/m^3. There was also atrophy of lymphoid tissues, including spleen, thymus, and bronchial lymph nodes, was in both mice and rats exposed to either 5 or 10 mg/m^3.

A5-3.1.2 Sub-Chronic and Chronic Systemic Toxicity Studies

A number of subchronic and chronic studies have been conducted to investigate the effects of inhalation, oral and dermal exposure to various species of nickel on experimental animals. Some of the more relevant studies are described below.

Continuous exposure of rats to 0, 60, or 200 μg/m^3 nickel oxide for two years resulted in severe pulmonary damage and premature mortality (Glaser *et al.*, 1986). The pulmonary damage consisted of alveolar proteinosis and septal fibrosis.

ABC (1986) conducted a 90 day rat study with nickel chloride in water. A dose of 0, 5, 35 or 100 mg/kg/day was administered by gavage to male and female CD rats. Body weight and food consumption was found to be consistently reduced, relative to controls, for males in the 35 and 100 mg/kg/day dose groups. Female rats in these groups had lower body weights than controls, but food consumption was unaffected. Clinical signs of toxicity included lethargy, ataxia, irregular breathing, cool body temperature, salivation and discolored extremities, and were observed primarily in the 100 mg/kg/day group (and with lower severity in the 35 mg/kg/day group). The 5 mg/kg/day group did not show any significant clinical signs of toxicity. Mortality was 100% in the 100 mg/kg/day dose group, which dropped to 20% (males), and 27% (females), in the 35 mg/kg/day group. However, some deaths in the 35 mg/kg/day group were found to be related to gavage errors, rather than nickel toxicity. Upon necropsy, kidney, liver and spleen weights for 35 mg/kg/day males, and right kidney weights for 35 mg/kg/day treated females were significantly lower than in controls. Based on the results of this study, 5 mg/kg/day is a NOAEL, whereas 35 mg/kg/day can be considered a LOAEL for decreased body and organ weights (U.S. EPA, 1991a).

Dunnick *et al.* (1989) exposed rats and mice to nickel subsulphide for 90 days, by inhalation, for 6 hours/day, 5 days/week at concentrations between 0.1 and 1.8 mg/m^3. Alveolar macrophage hyperplasia was observed in rats at all tested concentrations and in mice at 0.2 mg/m^3 and above. Biochemical changes in the lungs were indicative of a cytotoxic and inflammatory response. Atrophy of the olfactory epithelium was observed at concentrations of 0.2 or 0.4 mg/m^3 in rats and mice, respectively. The LOEL was considered to be 0.1 mg/m^3 for rats, while this value was considered to be a NOEL in mice, based on the minimal respiratory effects that were observed. The same study authors also exposed male and female F344/N rats to nickel sulfate by inhalation for 6 hours/day, five days/week at concentrations between 0.02 and 0.4 mg/m^3 for 13 weeks. Lung and nasal lesions were observed at all doses tested. A dose-related inflammation of the lungs, and alveolar macrophage hyperplasia occurred in all animals. Atrophy of olfactory epithelium occurred at 0.2 mg/m^3. Lymphoid hyperplasia of the bronchial lymph nodes was reported at higher test concentrations (Dunnick *et al.*, 1989).

The NTP (1996 a,b,c) exposed rats to nickel sulfate, nickel subsulfide and nickel oxide for six hours/day, five days/week for 13 weeks. LOAELs for chronic active lung inflammation were reported to be 0.11 mg/m^3, 0.22 mg/m^3 and 3.9 mg/m^3 for nickel sulfate, nickel subsulfide and nickel oxide, respectively while NOAELs were 0.06 mg/m^3, 0.11 mg/m^3 and 2 mg/m^3, respectively. Sub-chronic NOAELs for respiratory effects in mice were respectively reported to be 0.22 mg/m^3, 0.22 mg/m^3 and 2.0 mg/m^3 for nickel sulfate, nickel subsulfide and nickel oxide (NTP, 1996a,b,c). The ATSDR (2005) reported these values to be the highest NOAELs for respiratory effects in mice.

Benson *et al.* (1995) exposed rats to nickel sulfate and nickel oxide for 6 hours / day for 5 days/week. At 0.11 mg/m^3 nickel sulfate and 1.96 mg/m^3 nickel oxide, alveolitis was reported. No effects were reported at 0.49 mg/m^3 nickel oxide in this study (Benson *et al.*, 1995). Similar sub-chronic effects have been reported in mice albeit at higher concentrations.

Adult male rats were fed 0, 0.02, 0.05 and 0.1% nickel sulfate (or 0, 44.7, 111.75, 223.5 mg Ni/L) for 13 weeks in their drinking water (Obone *et al.*, 1999). Significant increases in absolute and relative liver weights were observed in the 0.1% exposure group (converted to 28.8 mg/kg/day nickel as nickel sulfate). At this dose, a significant decrease was observed in total plasma proteins, plasma albumin and globulins, and plasma glutamic pyruvic transaminase activity. Lymphocyte subpopulations (T and B cells) were suppressed at 0.1% but induced at lower concentrations. No adverse gastrointestinal effects, no damage to testis or DNA in liver or kidneys, or no significant decrease in weight gain was observed at any dose tested (Obone *et al.*, 1999).

Significantly increased kidney weights were reported in male and female rats following 6 months exposure to 100 mg/L nickel as nickel sulfate in their drinking water (Vyskocil *et al.*, 1994). The authors reported that these results suggest oral exposure to soluble nickel at low doses may enhance the normal age-related glomerular nephritis lesions of ageing rats, or induce changes to glomerular permeability in female and possibly male rats (Vyskocil *et al.*, 1994).

Minor effects on the morphology and function of alveolar cells have been observed in rabbits exposed to concentrations of metallic nickel as low as 0.1 mg/m^3 for 6 hours/day, 5 days/week for up to 8 months (Camner and Johansson, 1992; Curstedt *et al.*, 1983; Johansson *et al.*, 1983; Lundborg and Camner, 1982).

There are a limited number of dermal studies with experimental animals. Of these, only a few have reported adverse effects following dermal exposure to nickel compounds. The following paragraphs summarize dermal animal studies where adverse effects were noted to occur.

Liver effects were observed in rats treated dermally (lateral abdominal area) with daily doses of 60 mg Ni/kg/day as nickel sulfate for 15 or 30 days (Mathur *et al.*, 1977). Observed effects included swollen hepatocytes and "feathery" degeneration after 15 days, and focal necrosis and vacuolization after 30 days. However, this study did not specify whether or not rats were prevented from licking the nickel from the skin; thus, the observed effects could have resulted from oral exposure.

Mathur and Gupta (1994) reported increased liver enzyme activity in guinea pigs treated with 100 mg Ni/kg/day as nickel sulfate (placed on skin of the back for 15 or 30 days). This study also noted that blood glucose levels were significantly increased in guinea pigs treated with 100 mg Ni/kg/day as nickel sulfate.

Nickel-induced skin sensitivity has been reported in guinea pigs following skin painting or intradermal injection with nickel sulfate (Turk and Parker, 1977; Wahlberg, 1976; Zissu *et al.*, 1987). Adverse skin effects were observed in rats treated dermally with ≥40 mg/kg/day of nickel sulfate for 15 or 30 days (Mathur *et al.*, 1977). Observed effects included distortion of the epidermis and dermis after 15 days, and hyperkeratinization, vacuolization, degeneration of the basal layer, and atrophy of the epidermis at 30 days. This study also reported tubular degeneration of the testes in rats treated dermally with nickel sulfate at 60 mg/kg/day for 30 days. No testicular effects were found at 40 mg/kg/day after 30 days, or at doses of up to 100 mg/kg/day after 15 days. ATSDR (2005) notes that this study gave no indication that

the rats were prevented from licking the nickel sulfate from the skin; thus, the observed effects could have resulted from oral, rather than dermal exposure.

A5-3.1.3 Animal Carcinogenicity Studies

Numerous carcinogenicity experiments have been conducted with nickel compounds, administered *via* injection, inhalation or ingestion. Chronic inhalation studies have clearly indicated that different nickel compounds have varying carcinogenic potentials, and that different animal species show varying carcinogenic responses to nickel compounds.

Ottolenghi *et al*. (1974) exposed Fischer 344 rats to 0.97 mg nickel sulphide/m^3 for 8 hours/day, 5 days/week for 78 weeks. Towards the end of treatment, an increase in mortality and a decrease in body weight was observed. An increased incidence of lung tumours was observed during treatment and during a 30-week observation period. Nickel sulphide-treated rats developed 29 tumours and 10 adenocarcinomas compared to control responses of two and one, respectively. The U.S. EPA (1986) chose the study by Ottolenghi *et al*. (1974) in the determination of a unit risk value representing the risk of lung carcinogenesis arising from inhalation of nickel sulphide. The unit risk values, calculated using 3 different models, ranged from 1.8×10^{-3} to 6.1×10^{-3} $(\mu g/m^3)^{-1}$ (U.S. EPA, 1986), with an average unit risk value of 3.88×10^{-3} $(\mu g/m^3)^{-1}$.

Nickel dust (obtained from roasting, and containing 31% nickel subsulfide and 33.4% nickel oxide + silicon oxide and oxides of iron and aluminum) was tested for carcinogenicity in rats *via* the inhalation route (Belobragina and Saknyn, 1964; Rigaut, 1983). The exposure conditions reported consisted of exposure to 80 to 100 mg/m^3, for five hours/day for 12 months. No tumours were reported in the study.

Three carcinogenicity studies (Schroeder and Mitchener, 1975; Schroeder *et al.*, 1964; 1974) using nickel acetate and an unspecified nickel salt at doses of 5 ppm in the drinking water of both Long-Evans rats and Swiss mice produced negative results for carcinogenicity.

The National Toxicology Program published three chronic inhalation studies (NTP, 1996a,b,c). This series of studies involved 16-day, 13-week and two-year inhalation exposures to nickel subsulphide, nickel sulphate hexahydrate and nickel oxide. A summary is provided in the following paragraphs for the chronic component (*i.e.*, two-year portion) of each study.

In the nickel subsulphide inhalation study (NTP, 1996a), rats were treated with 0, 0.11, or 0.73 mg Ni/m^3 and mice with 0, 0.44 or 0.88 mg Ni/m^3, 6 hours/day, five days/week for 104 weeks. The nickel subsulphide used in this study was in the form of high purity nickel subsulphide powder from which a fluid bed generated aerosol was produced. The survival rate of all treated animals was comparable to that of the controls. The mean body weights of high dose rats and all treated mice were lower than the controls. Rapid and shallow breathing was noted in treated rats as well as laboured respiration following exposure periods in mice. Some haematological changes including an elevated haematocrit were noted in treated animals. An increased incidence of non-neoplastic effects including chronic active inflammation and macrophage hyperplasia was noted in low dose rats and mice. There was an increased incidence of alveolar/bronchiolar adenoma or carcinoma or squamous cell carcinoma in male and female rats, benign or malignant pheochromocytoma in the adrenal medulla of males and benign pheochromocytoma in the adrenal medulla of female rats. There was no evidence of carcinogenic activity in mice.

It is important to note that pheochromocytoma and hyperplasia of chromaffin cells of the adrenal medulla that arise from inhalation exposure to nickel subsulphide in the rat is secondary to inflammation and fibrosis of the lung.

The NTP (National Toxicology Program) has reported similar pathology results from two year inhalation exposure studies for the particulate compounds nickel subsulphide, nickel oxide, talc, indium phosphide, and cobalt sulfate. Results from studies with these five substances indicated the occurrence of variably extensive pulmonary inflammatory lesions, alveolar-bronchiolar tumours and squamous cell carcinomas, and significantly increased incidences of adrenal medullary hyperplasia and pheochromocytomas in males and females (Ozaki *et al.*, 2002). With the exception of the nickel compounds, these as well as other substances that have been associated with a similar pattern of combined effects in the lung and the adrenal glands do not exhibit chemical similarity. Proliferative lesions of the adrenal that were reported to result from inhalation exposure to nickel subsulphide have been rarely observed in humans and other animal species (Ozaki *et al.*, 2002).

Hyperplasia and neoplasia occurs frequently in the rat adrenal medulla, either spontaneously or in response to a wide variety of xenobiotic agents (Ozaki *et al.*, 2002). Adrenal medullary hyperplasia produced in these studies consisted of irregular, small foci of small-to-normal-sized medullary cells arranged in packets or solid clusters slightly larger than normal. Benign pheochromocytomas arising in the adrenal medulla were identified as well-delineated masses that often exhibited altered architecture and variable compression of the surrounding parenchyma. Malignant pheochromocytomas were identified

when there was invasion into or beyond the adrenal capsule. Nickel subsulphide, nickel oxide, talc, and indium phosphide (NTP, 1996a; 1996b; 1993; 2001) showed a significant dose-related increase in the incidence of adrenal pheochromocytoma. Cobalt sulfate also showed an increased incidence of pheochromocytoma in the mid-dose group (NTP, 1998).

Multiple factors are known to affect the pathogenesis of pheochromocytoma in rats. These include genetic background, chronic high levels of growth hormone or prolactin associated with pituitary tumours, dietary factors, and stimulation of the autonomic nervous system (Rosol *et al.*, 2001). There is scant evidence to support a connection between DNA damage and the mechanism by which exogenous agents induce adrenal medullary neoplasia. It has been concluded that these agents influence the carcinogenic response observed in the adrenal medulla through an indirect mechanism related to hypoxemia (Ozaki *et al.*, 2002).

Chronic pulmonary lesions in the rat, especially fibrosis and inflammation, reduce the gas exchange area leading to hypoxemia followed by hyperventilation. Hypoxemia is one of the factors known to stimulate chromaffin cell proliferation ultimately leading to pheochromocytoma in rats (Ozaki *et al.*, 2002). Statistical analyses of the relationship between chronic lung lesions and adrenal pheochromocytoma or hyperplasia provided evidence that the incidence of pheochromocytoma was associated with the severity of lung fibrosis and inflammation produced by inhalation of the particulate compounds including nickel subsulphide, nickel oxide, talc, indium phosphide, and cobalt sulfate (Ozaki *et al.*, 2002).

Although the association between chronic pulmonary fibrosis and inflammation with the elevated incidence of adrenal pheochromocytoma in the male rat is not consistent over all NTP inhalation studies, it was concluded that conditions of prolonged hypoxemia related to chronic pulmonary inflammation and fibrosis was implicated in the development of pheochromocytoma in the rat (Ozaki *et al.*, 2002). In the nickel sulphate hexahydrate study (NTP, 1996b), rats were exposed by inhalation to 0, 0.03, 0.06 or 0.11 mg Ni/m^3, six hours/day, five days/week for 104 weeks, in the form of a nickel sulphate hexahydrate aerosol generated from aqueous solution. The survival rates of all exposed rats were similar to those of the controls. Final mean body weights of all exposed groups of male rats and 0.03 and 0.06 mg Ni/m^3 females were similar to those of control rats. No treatment related haematological differences were noted. The incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinases and fibrosis were significantly increased in the medium and high dose male and female rats. There was no evidence of carcinogenic activity. The NOAEL for rats was estimated to be 0.03 mg Ni/m^3 or 0.028 mg Ni/kg body weight/day (0.03 mg/m^3 x 0.36 m^3/day) 0.38 kg). In the same nickel sulphate hexahydrate study (NTP,

1996b), mice were treated with 0, 0.06, 0.11 or 0.22 mg Ni/m^3 according to the same protocol used for the rats. The survival rate of all exposed mice were similar to those of the controls. The mean body weights of high dose males and all treated female mice were lower than those of the controls. No treatment-related haematological differences were noted. The incidences of chronic active inflammation, bronchiolization, and macrophage hyperplasia were significantly increased in the medium and high dose males and all females. There was no evidence of carcinogenic activity. The NOAEL for male and female mice was determined to be 0.06 mg Ni/m^3 or 0.10 mg Ni/kg body weight/day (0.06 mg Ni/m^3 x 0.063 m^3/day) 0.0373 kg), the lowest dose tested.

In the nickel oxide inhalation study (NTP, 1996c), rats were treated with 0, 0.5, 1.0, or 2.0 mg Ni/m^3 and mice with 0, 1.0, 2.0, or 3.9 mg Ni/m^3, six hours/day, five days/week for 104 weeks. The nickel oxide exposure in this study was in the form of very pure high temperature green nickel oxide aerosol, generated from fluid bed generators. The survival rate of all treated animals was comparable to that of the controls. Mean body weights of high dose male rats and medium and high dose female rats were slightly lower than controls; high dose female mice body weights were also slightly lower than controls. No treatment related haematological differences were noted at the 15-month interim evaluation. An increase in the incidence of chronic inflammation and bronchial lymphoid hyperplasia was noted for low dose rats. An increase in the incidence of lung bronchiolization, proteinases, and chronic inflammation was noted in the low dose mice. There was some evidence of an increased incidence of alveolar/bronchiolar adenoma or carcinoma or squamous cell carcinoma, and benign or malignant pheochromocytoma in rats. There was no evidence of carcinogenic activity in male mice but equivocal evidence of alveolar/bronchiolar adenoma or carcinoma in female mice. The LOAEL for rats was determined to be 0.5 mg Ni/m^3 or 0.47 mg Ni/kg body weight/day (0.5 mg Ni/m^3 x 0.36 m^3/day) 0.38 kg). The authors of this study concluded that there was some evidence of carcinogenic activity in both male and female rats. The weight-of-evidence for pure NiO carcinogenicity indicates that it is not likely acting through a mutagenic process but rather as a threshold carcinogen. In light of this data it can be concluded that the observed rat lung tumours were the result of threshold mechanisms involving tissue damage secondary to an inflammatory response. In addition, the adrenal tumours were concluded to be an indirect effect of the chemical, possibly related to stress. It must be noted however, that there are substantial differences between rats and humans in the incidence of spontaneous and induced adrenal medullary lesions, and in the morphology and physiology of the lesions themselves. Adrenal medullary tumours, induced in rats, particularly for the F344 strain, may be species- and strain-specific and of little or no relevance to humans (Lynch *et al.*, 1996). The authors also concluded that there was equivocal

evidence of carcinogenic activity in the lungs of female mice. As outlined in the subcommittee comments section of this report (NTP, 1996c), there was some degree of disagreement surrounding this issue. While one reviewer (Dr. J. Reddy) contended that there was some evidence of carcinogenic activity in female mice, a second reviewer (Dr. J. Ward) pointed out that there was no dose response relationship and felt that the appropriate conclusion should have been no evidence of carcinogenicity.

Thus, there appears to be a threshold of exposure for NiO induced carcinogenicity. This conclusion is supported by the fact that there is little data to support differences in uptake and/or intracellular metabolism of nickel oxide between rats and mice. This suggests that patterns of carcinogenicity in response to a genotoxic, non-threshold carcinogen should be similar for both rodent species. The observation that exposure to nickel oxide demonstrates some evidence of carcinogenicity in rats and no evidence in mice at comparable doses is not consistent with what would be predicted for a non-threshold carcinogen. In addition, no tumours of the respiratory system were noted in treated rats at either of the interim evaluations, which is once again inconsistent with the profile of a non-threshold complete carcinogen. Furthermore, no lung tumours were observed in rats at the low dose of 0.5 mg Ni/m^3, which again is indicative of a threshold mechanism secondary to inflammation.

In contrast to the nickel oxide study, no tumours were observed in rats or mice in the nickel sulphate hexahydrate study, in spite of positive trends of an inflammatory response in the lungs of treated animals. The authors interpreted this finding by noting that the differences between severities of lung inflammation lesions in exposed and control rats in the nickel oxide and nickel subsulphide studies were greater than the differences between severities of exposed and control rats in the nickel sulphate hexahydrate study. In addition, nickel oxide and subsulphide treated rats had significant parenchymal damage secondary to inflammation, supportive of the conclusion that the lung tumours observed in the nickel oxide study represented a threshold response involving chronic inflammation and tissue damage. This conclusion is consistent with the interpretation of Oller *et al*. (1997) who conducted a detailed review of available *in vitro* and *in vivo* studies related to the carcinogenicity of nickel compounds.

Ambrose *et al*. (1976) reported data on rats and dogs exposed for two years to nickel sulphate hexahydrate in the diet at 100, 1,000, and 2,500 ppm (equal to 5,000, 50,000 and 125,000 µg/kg body weight/day for the rat, assuming a body weight of 0.350 kg and food consumption of 17.5 g/day). Non-neoplastic effects included decreased growth in dogs (mid and high doses) and rats (high dose), alterations in blood and urinary chemistry in high-dose dogs, and changes in relative organ weights for mid and high dose female rats (heart and liver) and high dose dogs (kidney and liver). No significant

histopathological effects were noted in rats. No significant histopathological findings were reported for the low and mid dose dogs, while high dose dogs had cholesterol granulomas of the lung. The NOAEL was estimated to be 5,000 µg Ni/kg body weight/day, based on the non-neoplastic changes in the rat. The authors acknowledged that the 2-year rat survival was poor, particularly in the control groups. However, the NOAEL of this chronic study (5,000 µg Ni/kg body weight/day) is consistent with the NOAEL of 5,000 µg Ni/kg body weight/day determined from a similar 90-day gavage study (ABC, 1986; U.S. EPA, 1998).

A5-3.1.4 Reproductive and Developmental Toxicity

A 3-generation study, carried out by Ambrose *et al*. (1976), noted a higher incidence of stillborns in the first generation of albino rats fed 250, 500, or 1,000 ppm nickel in their diet (nickel sulphate hexahydrate) and depressed body weights of weanlings on the 1,000 ppm diet in all generations. A higher incidence of stillborns was not observed in subsequent generations (Ambrose *et al*., 1976). The U.S. EPA concluded that this study had some statistical design limitations and concluded that a NOAEL could not be clearly defined (U.S. EPA, 1998).

In a reproductive toxicity study, mice were exposed *via* gavage to 5, 10 or 20 mg/kg/day nickel sulphate or nickel chloride dissolved in distilled water, five days/week for 35 days (Pandey and Srivastava, 2000). At a concentration of ≥2.2 mg Ni/kg as nickel sulfate or 2.5 mg Ni/kg as nickel chloride, mice exhibited significant decreases in sperm count and sperm mobility (Pandey and Srivastava, 2000; doses converted by ATSDR, 2005). Decreased sperm count was significant at 4.5 mg Ni/kg as nickel sulfate. No effects on sperm were reported at 1.1 mg Ni/kg as the sulfate or 1.2 mg Ni/kg as the chloride (Pandey and Srivastava, 2000; doses converted by ATSDR, 2005). In a similar previous study, mice were exposed to 5 and 10 mg Ni/kg body weight as the sulphate five days/ week for 35 days (Pandey *et al.*, 1999). Decreased sperm count and mobility, and an increase in sperm abnormalities were reported at 2.2 mg/kg and 1.1 mg/kg nickel as nickel sulfate, respectively (Pandey *et al.*, 1999; doses converted by ATSDR, 2005). In addition, a lower weight and smaller size of the seminal vesicles in addition to decreased normal (testosterone-dependant) proteinuria was reported in mice exposed to 20 mg Ni/kg body weight as the sulfate five days/week for six months (Pandey and Singh, 2001).

Dissolved nickel sulfate administered to male Wistar albino rats (2 mg/100 g body weight for 5 doses) as part of a protein or protein-restricted diet affected the steroidogenic enzymes, resulting in an alteration of the formation of testosterone in addition to decreased body and testes weights (Das and Dasgupta, 2002). These effects of nickel sulfate were reversible following a withdrawal period.

In an older study. Schroeder *et al*. (1974) administered 5 ppm nickel in drinking water (equivalent to 0.3 mg/kg body weight; based on parameters provided in Health Canada, 1994) to weanling rats for their remaining lifetime and observed no adverse effects on growth or survival.

A three-generation study in rats exposed to nickel in their food and drinking water at concentrations of 0.31 ppm and 5 ppm, respectively, displayed declining litter sizes with each successive generation (Schroeder and Mitchener, 1971). More treated young rats died in each generation relative to the control rats and significant numbers of runts occurred in the F1 and F3 generations. The total dose of nickel administered to these rats from food and drinking water is roughly equivalent to 0.32 mg/kg body weight/day. Criticisms of this study include the use of a single dose level, results are based on only five matings, which were not randomized nor were the males rotated, and limited exposure to other trace metals in the diet or drinking water may have contributed to the toxicity observed (U.S. EPA, 1991a).

Another multigenerational study involved the administration of 0, 10, 50, or 250 ppm of nickel chloride in the drinking water (equivalent to 0, 1.3, 6.8, or 31.6 mg/kg/day) for 11 weeks before mating and then through two successive periods of mating, gestation (G1,G2) and lactation (L1,L2) (Smith *et al*., 1993; Health Canada, 1994). A reduction in maternal weight gain during G1 in the mid- and high-exposure groups was observed. Also, the proportion of dead pups per litter was significantly elevated at the high dose in L1 and at 10 and 250 ppm, but not at 50 ppm in L2. Health Canada concluded that the LOAEL of this study was equal to 10 ppm or 1.3 mg/kg/day (Health Canada, 1994). However, the U.S. EPA concluded that neither a NOAEL nor a LOAEL could be established due to the lack of a clear dose response relationship (U.S. EPA, 1998). TERA (1999) also considers this LOAEL to be equivocal and without evidence of a dose response relationship, as well as the absence of reproductive effects in other reproductive toxicity studies at this dose level.

In a two-generation study conducted by RTI (1987), nickel chloride was administered in drinking water to male and female CD rats at dose levels of 0, 50, 250 and 500 ppm (roughly equivalent to 0, 7.3, 30.8 and 51.6 mg/kg/day), for 90 days prior to breeding. At 500 ppm, there was a significant decrease in the parental (P_o) generation maternal body weight, along with decreases in absolute and relative liver weights. Thus, 250 ppm (30.8 mg/kg/day) was considered a NOAEL for P_o breeders. The NOAEL was confirmed *via* histopathological examination of the liver, kidney, lungs, heart, pituitary, adrenals and reproductive organs. In the F1a generation (postnatal days one to four), the 500 ppm dose level was associated with a significant decrease in the number of live pups/litter, as well as significantly increased pup mortality, and significantly decreased average pup body weight, all relative to controls. Similar effects were reported

for the F1b litters of P_o dams exposed to 500 ppm nickel. In the 50 and 250 ppm dose groups, increased pup mortality and decreased live litter size was observed in the F1b litters. However, these effects were considered questionable because the room temperature tended to be 10 degrees F higher than normal at certain times during gestation and postnatal days, along with much lower levels of humidity. Temperatures that are 10 degrees F above normal during fetal development are known to cause adverse effects (Edwards, 1986). Therefore, the results seen at 50 and 250 ppm cannot be considered to be genuine nickel-related adverse effects (U.S. EPA, 1991a). F1b males and females were randomly mated on postnatal day 70 and their offspring (F2a and F2b) were then evaluated through postnatal day 21. This phase of the study included teratological evaluations of F2b fetuses. It was reported that the 500 ppm dose caused significant body weight depression of both mothers and pups, and increased neonatal mortality during the postnatal development period. At 250 ppm, there was a transient depression of maternal weight gain and water intake during gestation of the F2b litters. The 50 ppm nickel dose caused a significant increase in short ribs (11%). However, since this effect was not seen at all in the higher dose groups, the incidence of short ribs in the 50 ppm group was not considered to be biologically significant (U.S. EPA, 1991a).

Subchronic exposure of rats to up to 0.1% nickel sulfate in drinking water for 13 weeks did not result in toxic effects to the testes as measured using biochemical and morphological parameters (Obone *et al.*, 1999).

Exposure of males or males and females to nickel chloride before mating to 3.6 mg/kg/day for 28 days before copulation, resulted in decreased fertility and reduction in the number of live pups born (Kakela *et al.*, 1999). However, no significant reproductive or developmental effects were reported at this concentration when exposed males were mated with unexposed females. No effects on fertility were reported in females exposed to concentration up to 13 mg/kg/day. When females or both parents were exposed, a high incidence of pup mortality during lactation was reported (Kakela *et al.*, 1999).

Benson *et al*. (1987) reported that mice and rats exposed to 5 or 10 mg of nickel subsulfide/m^3 had degeneration of testicular germinal epithelium following 12 days exposure, for 6 hours/day, 5 days/week.

While in general, the available animal data indicates that nickel may result in reproductive and developmental toxicity, there is a substantial amount of inconsistency across the studies conducted to date. In a detailed review of the literature, TERA (1999) noted that although soluble nickel compounds can cause reproductive toxicity, the doses at which such effects occur tend to fall above those that cause kidney toxicity. Thus, it is suggested that the critical renal effect of increased albuminuria in rats, should

be protective of reproductive endpoints as well. Furthermore, while several multigenerational studies have been conducted with ingested nickel, and reported increased neonatal pup death, there has generally been no evidence of teratological effects. This has been confounded further by the fact that many studies could not establish a clear reproductive due to inconsistent dose-response data, incomplete reporting, and technical experimental difficulties and/or study design limitations (TERA, 1999).

Similarly, the Metals Subcommittee of the U.S. EPA Science Advisory Board reviewed the reproductive toxicity data in 1991, and had concluded that the available reproductive toxicity studies would not yield an RfD that was substantially different from that which the U.S. EPA derived from the Ambrose *et al.* (1976) study (SAB, 1991).

A5-3.2 Genotoxicity and Mutagenicity

Results of tests on a variety of nickel compounds *in vitro* and *in vivo* have demonstrated nickel to be genotoxic (ATSDR, 2005). Insoluble crystalline nickel compounds have been reported to be more active in genetic toxicity assays than soluble or amorphous forms of nickel (NTP, 1996a; ATSDR, 2005). Nickel has also been reported to interact with DNA (*e.g.*, crosslinks and strand breaks) (ATSDR, 2005). Mutagenicity test results in bacteria are equivocal which has been suggested to result from the different study conditions and sensitivity of bacterial strains tested (ATSDR, 2005). Although there are conflicting study results for some nickel compounds, the available genotoxicity and mutagenicity data strongly suggest that nickel compounds are genotoxic and mutagenic.

A5-3.2.1 Bacterial assays

In the *Salmonell*a gene mutation assay, nickel subsulphide yielded sporadic weakly positive results in strain TA100 with and without S9, whereas negative results were noted with strains TA97, TA98, TA102 and TA1535 with and without S9 (NTP, 1996c).

A5-3.2.2 *In vitro* Mammalian and Human Cell Line Studies

The genotoxicity and mutagenicity of nickel compounds *in vitro* mammal and human cell lines has been studied by a number of authors. Evidence of nickel mutagenicity includes DNA crosslinks (Lin *et al.*, 1992), chromosomal aberrations in Chinese hamster ovary cells (Sen and Costa, 1985), induction of genetic and chromosomal instability in V79 Chinese hamster cells by nickel sulfate (Ohshima, 2003), and DNA interaction in mammalian NRK cells (Chiocca *et al.*, 1991). Rossetto *et al*. (1994) found that soluble nickel compounds produced gene deletions after *in vitro* exposures of a transgenic Chinese

hamster ovary cell line (AS52) containing a bacterial gene substitute for the mammalian HGPRT gene. Mutagenesis of several insoluble nickel compounds including crystalline nickel sulfide, nickel subsulfide, and nickel oxides (black and green) has been demonstrated using the HGPRT gene of the well-defined V79 cell line, and in two transgenic derivative cell lines of V79 (Kargacin *et al*., 1993). A positive response for nickel sulphate hexahydrate was observed (in the absence of S9) for the induction of triflourothymidine resistance in L5178Y mouse lymphoma cells (NTP, 1996a). In a study in Chinese hamster ovary AS52 cells, it was concluded that nickel compounds are only weak or equivocal mutagens (Fletcher *et al.*, 1994). These authors reported that while NiO produced mutations in AS52 cells, neither black NiO nor green NiO showed a clear dose response relationship for mutation as has been demonstrated for several soluble nickel compounds. Analysis of the nickel content of the cytosol and nuclei of cells grown in tissue culture revealed significant concentrations of both forms of NiO in cell cytosol, but evidence for significant concentrations in the nuclei was less certain. The authors suggest that NiO may not reach the nucleus in sufficient concentrations to produce a mutagenic response in cultured AS52 cells.

Transformation by nickel oxide has been shown in Syrian hamster embryo cells (Costa *et al*., 1981; Sunderman *et al*., 1987), and rat tracheal epithelial cells (Patierno *et al*., 1993) and a number of other established mammalian cell lines (Hansen and Stern, 1983). Interestingly, the Sunderman *et al*. (1987) study showed that as the purity of the NiO species increased, the ability for metal particulate to induce cell transformation was lost. The increasing presence of traces of sulfur or copper resulted in an increase in the frequency of cell transformation. In addition, calcination of black NiO by treatment at temperatures from 735 to 1,045EC progressively decreased solubility and reduced the biological activity of the oxides both *in vitro* and *in vivo* (Sunderman *et al*., 1987). Nickel oxide did not induce chromosomal aberrations in human lymphocytes *in vitro* (Paton *et al*., 1972; Kanematsu *et al*., 1980). A recent study reported that nickel oxide (black and green) and nickel subsulfide induced morphological transformations in C3H/10T1/2 mouse embryo cells (Landolph *et al.,* 2002).

Nickel chloride was found to induce DNA damage and oxidative stress in human lymphocytes which was reported to be caused by oxygen radical intermediates (Chen *et al.*, 2003). Kawanishi *et al.* (2002) found that a significant increase in 8-hydrooxydeoxyguanosine formation in cultured HeLa cells was induced by Ni_3S_2 but not by NiO (black or green) or $NiSO_4$.

Chakrabarti *et al.* (1999; 2001) demonstrated that the speciation of the nickel compounds affects the extent of induction of DNA-protein crosslinks in isolated rat cortical cells or rat lymphocytes. In rat lymphocytes, no genotoxicity was reported for nickel chloride and nickel acetate, both of which are soluble nickel compounds (Chakrabarti *et al.*, 2001). Nickel subsulfide (soluble form) induced a significant increase in soluble nickel DNA-protein crosslinks (Chakrabarti *et al.*, 2001). In rat renal cortical cells, nickel subsulfide induced much higher levels of DNA-protein crosslinks than nickel sulphate at equimolar doses (Chakrabarti *et al.*, 1999).

A5-3.2.3 *In vivo* Studies

An *in vivo* study using Sprague-Dawley rats exposed to nickel chloride at a dose of 44 mg/kg body weight found evidence of DNA strand breaks in hepatic cells four hours post-treatment (Stinson *et al*., 1992). However, this dose was acutely toxic and resulted in mortality within 48 hours. At a lower dose of approximately 34 mg/kg body weight, no DNA strand breakage was observed in hepatic cells.

The nickel compounds, Ni_3S_2, NiO (black or green) and $NiSO_4$, when intratracheally administered to rats, significantly increased eight-hydroxydeoxyguanosine (Kawanishi, *et al.,* 2002).

No increase in the frequency of micronucleated normrochromatic erythrocytes was observed in peripheral blood samples from mice treated by inhalation exposure with either nickel oxide (NTP, 1996b), or with nickel subsulphide (NTP, 1996c). NTP (1996c) also found no evidence for micronucleus induction *in vivo*; however, this result is not unexpected given both the lack of solubility of NiO and the likelihood that the particulate material could not reach the hematopoietic system to any significant extent (NTP, 1996c). Saplakoglu *et al.* (1997) subcutaneously injected male albino rats with nickel chloride at a dose of 44 mg/kg body weight. They observed no DNA strand breaks in the liver. However single strand DNA breakage was observed in lung and kidney cells. Lung tissue was the most susceptible to DNA stand breakage. Male F344 rats administered a single intraperitoneal injection of 90 µmol nickel acetate/kg body weight were assayed for DNA base damage for up to 14 days post-treatment (Kasprzak *et al.,* 1997). Ten different damaged bases were quantified, with the DNA damage becoming significant from day one post-treatment. The magnitude and persistence of the base damage was organ and base-dependent. In the liver, levels of five damaged base products were significantly elevated over controls, while levels of three damaged base products were elevated in the kidney. As the levels of damaged base products persisted longer in the kidney than the liver for the duration of the study (14 days), the authors speculated that the nickel-induced oxidative DNA base damage may be associated with the susceptibility

of the rat kidney to nickel-initiated carcinogenesis. In a review article, Kawanishi *et al.* (2002) reported that through inflammation, certain nickel compounds induce oxidative DNA damage in rats lungs.

A significant increase in the incidence of chromosomal aberrations was observed in studies on refinery workers and welders (Waksvik and Boysen, 1982; Elias *et al.*, 1989). Due to concurrent exposures to other chemicals however, nickel could not be identified as the sole causal agent for this effect. While the data from these two occupational studies is limited, ATSDR (2005) reports that these studies indicate human exposure to nickel *via* inhalation can result in genotoxic effects.

Werfel *et al.* (1998) measured DNA damage (by alkaline filter elution) and SCE frequencies in the lymphocytes of 39 welders occupationally exposed to chromium and nickel and 39 control subjects. Welders showed a significantly higher rate of single strand DNA breaks and significantly elevated SCE frequency. The average concentration of nickel in the blood of these welders was 4.6 μg/L. The authors estimated that this blood concentration corresponded to an average nickel air concentration of 300 $\mu g/m^3$. The results of this study however, did not agree with previous studies conducted with lymphocytes from welders. Decreased SCE frequencies in lymphocytes of welders (relative to controls) were observed by Knudsen *et al.* (1992), Jelmert *et al.* (1995), and Popp *et al.* (1991). There is presently much uncertainty regarding the nature of the interaction between chromium and nickel in exposed welders, and whether there are dose-dependent effects of nickel on DNA strand breaks and SCE frequencies.

A5-3.2.4 Proposed Mechanism of Nickel Carcinogenicity

Whether or not nickel compounds are carcinogenic and the mechanisms by which carcinogenicity may occur are subjects of considerable controversy, and scientific understanding is limited at this time.

Salnikow, *et al*. (2003) states that nickel has been reported to be highly carcinogenic, but only weakly mutagenic. Both insoluble and soluble nickel compounds have been shown to have carcinogenic potential, but insoluble nickel is generally considered more carcinogenic than soluble nickel (Salnikow *et al.*, 2003; ATSDR, 2005). However, a recent study examining the changes of gene expression *in vitro*, found that both soluble and insoluble nickel compounds induced similar signaling pathways (Salnikow *et al*., 2003). The authors of this study reported that the results suggest both soluble and insoluble nickel compounds have similar carcinogenic potential *in vitro* (Salnikow *et al*., 2003). Grimsrud *et al.* (2002) suggested water-soluble nickel species play an important role in nickel-related cancer in other studies.

Nickel has been reported to bind maximally to RNA as compared to DNA, *in vivo* and *in vitro* studies using radiolabelled nickel in rats (Kaur and Dani, 2003). The authors suggest that this finding indicates nickel acts as a carcinogen by controlling gene expression post-transcriptionally (Kaur and Dani, 2003).

The carcinogenicity of insoluble nickel compounds has been reported to result from the ability of the nickel compound to deliver high concentrations of the Ni^{+2} ion intracellularly, and its ability to suppress or silence genes (such as tumour suppressor genes) (Kang *et al.,* 2003; Costa *et al*., 2003). In mammalian cells, gene silencing has been reported to result from DNA hypermethylation and its ability to inhibit histone acetylation (Costa, 2002). *In vivo*, bound-Ni^{+2} produces reactive oxygen species that are inversely related to the occurrence of mammalian cell resistance to Ni^{+2} (Kang *et al.,* 2003). Soluble nickel compounds have also been reported to exhibit carcinogenic potential by activating cell promotion (Costa *et al.,* 2003).

In a recent review article, Denkhaus and Salnikow (2002) reported that the mechanism of nickel carcinogenesis is still not fully understood; however, the following events are believed to play a role: cellular homeostasis is disturbed by exposure to high concentrations of nickel by changing intracellular calcium levels and producing oxidative stress; nickel-bound abnormal proteins appear or an oxygen sensor is poisoned which may lead to the activation of some signalling pathways and subsequent transcription factors; such effects may eventually alter gene expression and cellular metabolism. The authors also noted that inherent changes in gene expression by nickel result from induction of DNA damage, DNA methylation or suppression of histone actylation.

A5-3.3 Human Studies

Nickel is an essential element in all vertebrate animals and humans; however, nickel deficiency has never been reported in humans as nickel intake generally exceeds dietary requirements (Anke *et al.,* 1995). Nickel is widely considered to be a normal constituent of the diet, with daily intakes ranging from 100-300 µg/day (U.S. EPA, 1991a). However, ATSDR (2005) notes that the essentiality of nickel in humans has not been confirmed (as its functional importance is not yet clearly demonstrated), and nickel dietary recommendations have not been established for humans.

The primary target organs for toxicity *via* various exposure routes have been reported to be: the respiratory tract following inhalation exposure; the immune system following inhalation, oral, or dermal exposure; and the developing organism and reproductive system following oral and inhalation exposure (ATSDR, 2005). In industrial settings, and also in the general population, nickel is considered a prevalent

contact allergen (Hostynek and Maibach, 2002). ATSDR (2005) reports that approximately 10 to 15% of the general population is nickel-sensitive.

A5-3.3.1 Systemic Toxicity

Only limited data appears to exist on the systemic toxicity of ingested or inhaled nickel in humans. The majority of human studies with nickel compounds have focused on potential carcinogenic effects or dermal allergic responses.

Cirla *et al.* (1985) evaluated seven metal plating workers with occupational asthma for atopy, and conducted a pulmonary function challenge. Three asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were also noted in 3 asthmatic workers, all of which also had dermatitis. Six asthmatics exhibited significantly decreased forced expiratory volume FEV_1 (>15%) when exposed to 0.3 mg/m^3 nickel sulfate for 30 minutes. Control challenges with non-nickel-containing metal salts did not produce similar deficits in FEV_1.

Muir *et al.* (1993) examined the non-cancer effects of human inhalation exposure to soluble nickel. Exposure to soluble nickel in this study was estimated as either <8 mg Ni/m^3 or <4 mg Ni/m^3, depending on the time period (TERA, 1999). In this study, radiographs from nickel workers were examined and exhibited an increase in the prevalence of irregular opacities, that increases with the duration of exposure. However, this increase was largely attributable to ILO profusion scores of minimal severity, a relatively common effect in the general public. A LOAEL of 4 mg/m^3 was identified in this study. However, confidence in the results is greatly reduced by the wide variability in radiograph interpretation (TERA, 1999), and various other uncertainties and limitations (*e.g.*, there was no control group, exposure measurements were approximate, there was mixed exposure to soluble and insoluble forms of nickel, *etc*.).

A5-3.3.2 Carcinogenicity

There do not appear to be any studies in the available scientific literature that link ambient environmental exposure to nickel to increased cancer incidence in the general population. However, there are a number of studies which demonstrate that nickel refinery workers exposed by inhalation to various nickel compounds are at a significantly increased risk for cancer of the lung and the nasal cavity, relative to non-occupationally exposed individuals (EC, 2000; WHO, 2000; IARC, 1990; ICNCM; Doll *et al.*, 1990; CEPA, 1994).

Extensive reviews of prior-to-1990 studies of the toxicology of nickel and nickel compounds, including animal carcinogenicity and human epidemiological data, were published by the International Agency for Research on Cancer (IARC, 1990), and by the International Committee on Nickel Carcinogenesis in Man (ICNM, 1990; Doll *et al.,* 1990). These reviews examined ten cohorts (roughly 80,000 workers) involved in mining, smelting and refining nickel, as well as workers in nickel related industries (using metallic nickel powder and nickel alloy) (*e.g.*, Inco, Clydach, Wales, Falconbridge, Ontario, Huntington, W. Va., Inco, Copper Cliff and Coniston, Sudbury, Ontario, Inco, Port Colborne, Ontario, Falconbridge, Kristiansand, Norway, Oak Ridge, Tenn., Outokumpu Oy, Finland, Societe le Nickel, New Caledonia and Henry Wiggin Alloy Co., Hereford, England). In general, these reviews indicated increased risks for lung and nasal cancers associated with occupational inhalation exposures incurred during high temperature oxidation of nickel matte and nickel-copper matte (roasting, sintering, calcining), as well as with exposures in electrolytic refining and hydrometallurgy. The greatest risks were observed in workers involved in roasting, sintering and calcining operations, with much smaller risks observed for workers in refineries. However, these increased risks applied to only a small proportion of nickel workers. The reviews also indicated that different nickel compounds have different carcinogenic potencies, and more than one form of nickel may exhibit carcinogenic activity. From these reviews, it was evident that the increased respiratory cancer risk observed in nickel workers was largely attributable to mixtures of oxidic and sulphidic nickel at air concentrations in excess of 10 mg/m^3. There was also evidence that high concentrations of oxidic nickel, in the absence of sulphidic nickel, was associated with increased lung and nasal cancer risks in exposed workers. There was some evidence that soluble nickel species may enhance the risks associated with the less soluble forms of nickel. Respiratory cancer risks were associated with soluble nickel exposures, but only when there was co-exposure to less soluble forms of nickel, at air at concentrations in excess of 1 mg/m^3. There was no evidence that metallic nickel was associated with increased lung and/or nasal cancer risks.

It is important to recognize however, that many of the epidemiological studies reviewed by IARC (1990) and Doll *et al.* (1990) had several limitations. The principal limitation was the lack of data related to concentrations of nickel in air within the facilities that were evaluated. Both Doll *et al*. (1990) and IARC (1990) acknowledged that this is a critical limitation. Thus, it is not possible to determine exposure-response relationships from these studies. In addition, at the time these reviews were conducted, the mechanism underlying the carcinogenicity of the various nickel species was poorly understood. Furthermore, Doll *et al*. (1990) noted that the conclusions of many of the epidemiological studies (with respect to lung tumour incidence) were confounded by a lack of information about the smoking habits of

the workers. Finally, it must be recognized that nickel smelter and refinery workers have concomitant exposures to various other substances, including copper, cobalt, arsenic, lead, and sulphuric acid mist, depending on the type of nickel being processed, and the processing methods used. Some of these substances are strongly associated with increased respiratory cancer in workers (*i.e.*, arsenic, sulphuric acid mist). The potential influence of co-exposure to these substances has not been adequately accounted for in any occupational epidemiology study of nickel exposure and cancer incidence.

Since 1990, numerous studies, updates of previous cohort studies, and detailed interpretive reviews of the toxicological and epidemiological literature have been conducted. Key findings and conclusions from these most recent studies and reviews are summarized in the following paragraphs.

Update studies for the Clydach, Wales refinery cohorts have been reported by Easton *et al.* (1992) and Draper *et al.* (1994). Easton *et al.* (1992) confirmed the earlier observation that cancer risks are largely confined to workers employed in the 1930s and before. It is suggested that nasal cancer risk may be due to exposure to soluble nickel and at least one insoluble nickel species, possibly nickel oxide. Draper *et al.* (1994) suggest that the confinement of cancers to mostly pre-1930s workers likely reflects that leaching of nickel matte with an arsenic containing sulfuric acid ceased in the 1920s. Julian and Muir (1996) re-examined cancer incidence in male workers employed by Inco and Falconbridge. A small excess of laryngeal cancer was reported, but only in employees that were not exposed to the sintering process. Underground miners with more than 25 years exposure were shown to have a significant excess of lung and nasal cancers. It was speculated that the high prevalence of smoking among this cohort may have contributed to observed increased incidence rates for lung cancer. Andersen *et al.* (1992; 1996) conducted morbidity updates of the Kristiansand, Norway cohort. The updated analysis showed an excess risk of lung cancer in association with exposure to soluble forms of nickel. The study also suggested a multiplicative effect of smoking and nickel exposure. Arena *et al.* (1998) expanded upon and re-evaluated the cohort of U.S. high alloy nickel workers. The authors observed a statistically significant 13% increased risk for lung cancer, relative to the total U.S. population. However, no significant excess was found when smaller, local referent populations were used as the comparison.

Anttila *et al.* (1998) followed up on an earlier study of lung cancer incidence in workers at a nickel refinery in Harjavalta, Finland (Karjalainen *et al.*, 1992). In this first study, examination of cancer registry data for a cohort of workers followed from 1953 to 1987 revealed an elevated incidence of nasal cancer and a slight increase in lung cancer incidence. Anttila *et al.* (1998) followed up on this study by examining cancer registry data up to the end of 1995. A cohort of 1388 workers employed for at least

three months at the copper/nickel smelter and nickel refinery in Harjavalta was studied. Overall cancer incidence in this cohort was similar to the unexposed cohort; both were at expected levels for the Finnish population. A small increase in lung cancer incidence, and an increased risk of nasal cancer in the exposed cohort were observed. Refinery workers were primarily exposed to nickel sulfate at concentrations below 500 $\mu g/m^3$, but exposure to lower concentrations of other soluble nickel compounds also occurred. As the elevated lung and nasal cancer risks were confined to refinery workers, where the primary exposure was to nickel sulfate, the authors concluded that nickel sulfate is likely responsible for the excess cancer risk. An increased risk of stomach cancer in the nickel-exposed cohort was also observed in this study; however, as stomach cancer has not generally been associated with nickel exposure, the authors speculated that this was a chance finding.

Further evidence against an association between stomach cancer and nickel exposure was reported in the results of a negative two-year oral (gavage) carcinogenicity study dosing nickel sulphate hexahydrate (CRL, 2005). In this study, the daily oral administration of nickel sulphate hexahydrate did not produce a dose-related increase in any common tumour type (including stomach) or an increase in any rare tumours.

Pang *et al.* (1996) evaluated cancer mortality rates in a small cohort of English nickel platers with no known occupational exposure to chromium. There was no evidence of an increase in lung or nasal cancers in this cohort. However, there was weak evidence that nickel plating is associated with an excess risk for stomach cancer. TERA (1999) notes that these data are of interest in that this appears to be the only cohort that was exposed almost entirely to soluble nickel salts.

A clear dose-related effect was reported for cumulative exposure to water-soluble nickel and lung cancer in a case-control study of Norwegian nickel-refinery workers (Grimsrud *et al.*, 2002). Smoking was reported to be a low to moderate confounding factor. The authors indicated that although no other dose-dependant increases for other types of nickel were noted, a general increase in risks from other types of nickel could not be excluded. Soluble nickel compounds have also been reported to result in adverse effects on the kidney tubular function in humans albeit at high doses (Vyskocil *et al.*, 1994).

The results of an investigation conducted by Seilkop and Oller (2003, 2005) found that smoking and/or lifestyle factors are related to the marginal increase in lung cancer risks of nickel alloy workers, as opposed to low level exposure of nickel oxide and metallic nickel. Another recent study examined whether nickel content in inhaled smoke from cigarettes might be a supplementary source of nickel exposure to workers in a nickel refinery in Norway (Torjussen *et al.,* 2003). The authors of this study reported that blood plasma and urine concentrations were similar among randomly selected smoking and

non-smoking employees. In addition, after smoking the cigarettes ≤ 1.1% of nickel in the mainstream smoke was recovered with most of the tobacco nickel being recovered in the ash (Torjussen, *et al.*, 2003).

Seilkop and Oller (2003, 2005) also estimated safety limits for workers from fitted animal dose-response curves. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.5 to 1.1 mg inhalable nickel oxide/m^3. The authors report that although the animal data for nickel oxide suggest a threshold response for lung cancer, this cannot be concluded with certainty as sampling uncertainty in data make the non-threshold response equally as plausible. They report that the non-linearity of the observed dose-response of nickel oxide is well represented by benchmark dose models (excluding the high dose response).

In a review article, Oller (2002) suggested that in isolation, water soluble nickel compounds are not complete carcinogens. However, they may enhance the carcinogenic risks associated with other compounds when inhaled, if concentrations are large enough to induce chronic lung inflammation (Oller, 2002). Soluble and water-insoluble nickel compound exposures appear to increase respiratory cancer risks at concentrations in excess of 1 and 10 mg Ni/m^3 work place dust, respectively (ICNCM, 1990). By keeping exposure below levels resulting in chronic respiratory toxicity, Oller (2002) suggested that possible tumour-enhancing effects would be avoided.

The information from new studies and follow-up studies of existing cohorts generally confirms the conclusions previously made in the IARC (1990) and Doll *et al.* (1990) studies. That is, more than one form of nickel can contribute to an increased incidence of lung cancer. Also, it appears that the role of soluble nickel species as a contributor or enhancer of risks associated with exposure to insoluble nickel compounds is confirmed (*e.g.*, Easton *et al.*, 1992; Anttila *et al.*, 1998; Oller, 2002). It appears most likely that soluble nickel compounds enhance the potential carcinogenicity of insoluble nickel compounds. For example, Pang *et al.* (1996) found no increased incidence of lung or nasal cancer in nickel plating workers exposed almost entirely to soluble nickel compounds. The previous conclusion (Doll *et al.*, 1990) that respiratory cancer risk is primarily related to soluble nickel at concentrations in excess of 1 mg/m^3 and to exposure to insoluble forms at concentrations above 10 mg/m^3, do not appear to be confirmed; rather, the recent evidence suggests that lower concentrations of both soluble and insoluble nickel may contribute to an increased risk of respiratory tract cancer in exposed workers (EC, 2000).

Additional insights from the more recent suite of studies include two studies that found a small positive association between soluble nickel exposure and stomach cancer (*e.g.*, Pang *et al.*, 1996, Anttila *et al.*, 1998). The possible multiplicative effect of smoking in some studies is also of interest (*e.g.*, Andersen *et al.*, 1996; Julian and Muir, 1996).

A recent review by Sivulka (2005), shows that among occupational cohorts exposed to Ni compounds, the vast majority of affected human tissues were the lung or nasal sinus tissues. Meta-analysis of a large number of studies has shown that kidney, or stomach cancers that have been reported in some nickel cohorts are not related to nickel exposure. Generally metallic nickel has been identified as non-tumourigenic, especially at lower doses. This review does not focus on the subsulphide but on evidence of direct health effects attributable to metallic nickel. The review concludes that exposure to metallic nickel (nickel metal powder) does not appear to significantly increase the risk of respiratory cancer at concentrations that are as high or higher than those observed in current workplace environments.

A comprehensive review of the toxicology of soluble nickel salts (which included detailed evaluation of the available carcinogenicity evidence) has been prepared by TERA (1999). The key findings and conclusions from this review, with respect to the carcinogenic potential of soluble nickel compounds, are summarized below.

- The carcinogenic activity of insoluble nickel salts should not be used to predict the carcinogenic potential of soluble nickel salts, due to differences in tumorigenicity observed in animal studies (*e.g.,* NTP, 1996a,b,c).
- A non-linear dose-response relationship appears to exist for these compounds; although, data are presently insufficient to determine where non-linearities occur in the dose-response curves.
- Using the U.S. EPA's draft guidelines for carcinogen assessment, it was concluded that inhaled and ingested soluble nickel compounds should be classified as "cannot be determined" at this time. Using the current U.S. EPA (1986) guidelines, classification of the carcinogenic potential of soluble nickel compounds would be D - not classifiable as to human carcinogenicity.
- Multiple oral studies of soluble nickel compounds in different experimental animal species do not indicate a cancer-causing potential (however, all studies had design flaws that preclude a definitive conclusion, such as single doses, low dose ranges, high animal mortality, incomplete reporting, *etc*.). Despite these limitations, the oral exposure study results are consistent with those seen after inhalation and parenteral administration of soluble nickel compounds.

- The lack of definitive oral carcinogenicity data for soluble nickel, and absence of oral studies which investigate potential interactions between soluble and insoluble forms of nickel via the oral route, are key areas of scientific uncertainty.
- The evidence for genotoxicity of soluble nickel compounds is mixed and equivocal.
- As animal studies show clear differences in the ability to induce tumors by inhaled soluble and insoluble nickel (*e.g.,* NTP, 1996a,b,c), an animal carcinogenesis study involving co-exposure by inhalation to both soluble and insoluble forms of nickel would be useful in addressing the potential biological interactions between these forms.
- Extensive epidemiology data suggests inhalation exposure to mixed soluble and insoluble nickel salts can cause the development of lung and nasal cancer in humans. However, the possible contribution of soluble nickel exposure to the observed cancer deaths is controversial and poorly understood. Furthermore, interpretation of the data is confounded by co-exposure to other metals and other chemicals (some of which are known carcinogens such as arsenic and inorganic acid mists), limitations in exposure measurements, and smoking (possibly higher rates than in the general population in some cohorts).
- Overall, the available epidemiology data are insufficient to determine whether soluble nickel alone is a human carcinogen. The currently available evidence suggests soluble nickel is not carcinogenic by itself, but may act as an effect modifier, or promoter, which increases the lung and nasal cancer risk associated with exposure to other, less soluble forms of nickel.
- Potential means by which soluble nickel could increase the carcinogenicity of insoluble nickel species and possibly other chemicals, include: production of an inflammatory response and associated enhanced cell proliferation, impaired clearance, and local generation of oxygen radicals. However, it must be recognized that the available data are insufficient to determine which mechanism(s) operate and whether there is a dose-response relationships for this promotion effect of soluble nickel.
- The development of a cancer dose-response curve for soluble nickel for the inhalation route is not recommended at this time.
- It appears that toxcokinetic factors may predispose soluble nickel species to having a low carcinogenic potential. For example, soluble forms are inefficiently taken up by cells by passive

diffusion, and the soluble nickel that does enter cells binds preferentially to cellular proteins over DNA, thereby reducing the amount of soluble nickel that is available to react with DNA.

In its evaluation of nickel compounds, IARC (1990) concluded the following with respect to carcinogenicity of nickel compounds. This evaluation has not been updated since 1990.

- There is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry.
- There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys.
- There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulfides.
- There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides and nickel telluride.
- There is inadequate evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulfide and nickel titanate.

The overall IARC classification for nickel compounds is Group 1 – carcinogenic to humans. This was based on the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and other supporting data, as well as the underlying concept that nickel compounds can generate free nickel ions at critical sites in target cells. Metallic nickel was classified as Group 2B - possibly carcinogenic to humans. IARC has not conducted a more recent evaluation since 1990.

Other carcinogen classifications for nickel compounds are as follows. The U.S. EPA classifies nickel refinery dusts and nickel subsulfide as Class A – known human carcinogens. Health Canada (1994) classifies soluble nickel (primarily nickel sulfate and nickel chloride) as Group I, carcinogenic to humans. However, Health Canada only estimated the cancer potency of soluble nickel compounds *via* the inhalation route and did not estimate cancer potency *via* the oral route. Health Canada (1994) classifies metallic nickel as Group VI - unclassifiable with respect to carcinogenicity in humans. Health Canada classifies the groups of oxidic, sulphidic and soluble nickel compounds as Group I, carcinogenic to humans. The U.S. EPA has not evaluated soluble salts of nickel, as a class of compounds, for potential

human carcinogenicity, however nickel (various soluble and insoluble compounds) are presently being reassessed under the U.S. EPA IRIS Program.

It should be noted that the IARC, U.S. EPA and Health Canada classifications for nickel compounds do not take into account studies published since the early 1990's or the TERA (1999) review and recommendations.

A5-3.3.3 Nickel Contact Allergy and Dermatitis

It is well established that the nickel ion (*i.e.*, Ni^{2+}), when bound to proteins in the dermis, acts as an antigen that elicits a type IV (delayed type) hypersensitivity response. This response is mediated by T-lymphocytes, and results in dermal sensitivity (Menne and Maibach, 1989).

Nickel toxicity in humans is commonly exhibited as an allergic reaction (ATSDR, 2005). The percentage of individuals reported to be sensitized to nickel varies with each study, although ATSDR (2005) reports that approximately 10 to 15% of the population is sensitized to nickel. Andreassi *et al.* (1998) reported that approximately 10 to 15% of women and 1 to 3% of men living in industrialized countries are sensitized to nickel. Typically, nickel-sensitized individuals suffer from allergic contact dermatitis. Dermal contact with nickel leading to an antigenic response and dermatitis has been well documented in the literature (NAS, 1975; NCI, 1985; U.S. EPA, 1986).

Nickel can cause Type 1 and Type IV hypersensitivity following dermal or systemic exposure (Hostynek and Maibach, 2002; Hostynek, 2002). Although nickel is classified as an allergen of moderate potency (Kligman, 1966), there is a high risk of developing nickel allergic hypersensitivity occupationally and in the general public due to the ubiquitous occurrence of nickel in all aspects of daily life (Hostynek, 2002).

The prevalence of nickel allergic hypersensitivity has been reported to have increased substantially in some dermatological clinics (Hostynek, 2002). The increase was generally observed in women and mainly attributed to nickel in products such as jewelry (Hostynek, 2002). Similar reviews have reported a higher prevalence of contact dermatitis particularly in young females, believed to be related to nickel in consumer products such as jewelry rather than occupational exposure (ATSDR, 2005). In a study of 1,501 eighth grade Danish children, nickel allergy was most frequently found in girls and was associated with ear piercing, while in adolescents, nickel allergy was significantly associated with hand eczema (Mortz *et al.,* 2003). Interestingly, oral exposure to nickel *via* the application of dental braces prior to ear piercing was reported to significantly reduce the prevalence of nickel allergy (Mortz *et al*., 2003).

In a multifactorial analysis, 74,940 patients from German and Austrian contact dermatitis units between 1992 and 2000 were patch tested with nickel sulfate (Uter *et al.*, 2003). Age and sex were reported as being the most prominent risk factor for contact allergy to nickel, with female sex being the strongest risk factor. There was also a significant variation of risk factors between the occupations of the patients examined. Similar to other studies, it was felt that the strong predominance of nickel contact allergy in females (particularly young females) is related to wearing costume jewellery containing nickel (Uter *et al.*, 2003).

Recent studies have shown that acute oral exposure to nickel compounds can result in flare-ups of allergic contact dermatitis and eczema and in some cases, urticaria and respiratory symptoms in women that are sensitized to nickel (Andreassi *et al.,* 1998; Boscolo *et al.,* 1995). A recent study has suggested that long-term exposure to environmental nickel may induce immunologic tolerance resulting in a lower risk of developing contact allergy to nickel (Smith-Sivertsen *et al*., 2002).

Due to chemical and experimental variables in addition to individual variables (*e.g.*, differences in susceptibility to nickel, age, gender, integrity of skin), Hostynek (2002) reported that a threshold value for nickel inducing sensitization cannot be developed at this time. Similarly, Hostynek (2002) reported that identifying concentrations which will elicit a reaction in sensitized individuals is also not possible. While some investigators have attempted to determine a threshold value for nickel, conflicting results have been observed (Hostynek, 2002). A dose response relationship between nickel exposure and dermatitis was however, shown in a patch test study of 12 sensitive individuals (Emmett *et al.*, 1988). Dermatitis was reported at a nickel concentration of 316 ppm (0.0316%) in petroleum, while no effects were reported at 100 ppm (0.01%) or at 316 ppm in aqueous solution (Emmett *et al.*, 1988). Calabrese *et al.* (1997) reported that an acute human dose of 0.009 mg/kg was associated with contact dermatitis by 4 days. While no clear threshold value can be determined, based on a review of the data, Hostynek (2002) reported that the "best" estimate of the concentration of nickel necessary to elicit an allergic response is 0.6 ppm (based on Katz and Samitz, 1975). Hostynek (2002) also notes that some dermatologists consider an acceptable limit to be 10 times less than this value (*i.e.,* 0.06 ppm).

There is no evidence that airborne nickel causes allergic reactions in the general population (WHO, 2000).

A5-3.3.4 Reproductive and Developmental Toxicity

There is insufficient evidence demonstrating that nickel causes developmental or reproductive toxicity in humans (OEHHA, 1999; U.S. EPA, 1991a).

Few studies were identified that investigated reproductive/developmental outcomes in humans that were occupationally exposed to nickel compounds. Chashschin *et al.* (1994) studied pregnant female workers at a nickel refining plant in the Kola region in Russia. It was found that these workers had a 15.9% increase in spontaneous abortions relative to a spontaneous abortion rate of 8.5% in a control population of pregnant female construction workers (who had no known significant exposure to nickel). The refinery workers were exposed to nickel sulphate concentrations that were reported to range from roughly 0.08 to 0.196 mg nickel/m^3. Corresponding urinary nickel concentrations ranged from 3.2 to 22.6 µg/L. In contrast, nickel urinary concentrations in the control group ranged from <0.1 to 13.3 µg/L. Other findings included a 16.9% incidence of development effects (primarily cardiovascular and musculoskeletal defects) in refinery workers, relative to a 5.8% incidence in the control group. The authors expressed uncertainty over whether or not heavy lifting and heat stress could have contributed to the observed effects in this study. Although this report was not based on a scientifically rigorous study, it was suggestive enough to warrant further investigation. A note from the editors said:

> "This article is included in this special issue since it constitutes a first report on possible reproductive and developmental effects in humans due to occupational exposure to nickel. Although the results are incompletely documented and thus must be considered inconclusive, they identify a concern that requires more comprehensive and quantitative epidemiologic investigations."

As a direct consequence of the observations made by Chashschin *et al*. (1994), a detailed series of investigations of the same Russian cohort were begun in the late 1990s. While some of these studies are still being conducted to investigate reproductive outcomes in nickel-exposed communities in the Kola Peninsula region of Russia (MOE, 2004; Smith-Sivertsen *et al.*, 1997; Odland, 1999). To date, these studies show no indications of fetal toxicity in the general population within nickel smelter cities that can be related to parental exposure to nickel (Vaktskjold *et al*., 2006).

A5-4.0 REGULATORY EXPOSURE LIMITS FOR NICKEL COMPOUNDS

Recently collected Ontario data on nickel speciation in airborne PM indicates that the dominant nickel species in both urban and specific nickel source-affected areas (such as oil-fired power plants, petroleum refineries, nickel smelters (including those in the Greater Sudbury Area, and nickel refineries) is nickel sulphate (57 to 85%), followed by nickel oxide (6 to 31%), and nickel hydroxide (8 to 23%) (MOE, 2004). Very little or no sulphidic or metallic nickel species were detected in these areas. This is an important finding as the most toxic nickel species are the sulphidic compounds, particularly nickel subsulphide. This finding suggests that risk assessments investigating ambient environmental exposures to nickel should not use toxicity reference values that are based on nickel subsulphide, as this does not appear to be a relevant nickel species that people are exposed to in the ambient environment. Similarly, nickel refinery dust and metallic nickel are other form(s) of nickel for which there would be negligible ambient environmental exposure. That said, speciation analysis of dust samples and air filters from the Greater Sudbury area have indicated the presence of sulphidic and oxidic forms of nickel as well as the more prevalent soluble forms. Thus, the current review of regulatory agency exposure limits is focused on the forms of nickel found in the Sudbury area, including soluble nickel salts, nickel subsulphide and nickel oxide. Treatment of these different forms of nickel in the Sudbury environment is further discussed in Chapters 3 and 4 of this volume.

A number of regulatory agencies have derived health-based exposure limits for nickel compounds, as described below and summarized in the below Table. Exposure limits used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO), and the MOE. Limits derived by Seilkop and Oller (2003, 2005) and Seilkop (2004) have been considered as well.

It must be recognized that nickel soluble salts are currently being re-assessed by the U.S. EPA IRIS Program (Ambika Bathija, U.S. EPA, pers comm.). The revised IRIS profile was expected to be released in early 2004, however this has been delayed and it is not expected to be released until 2007 or 2008. The delay is a result of there being some new studies identified which the EPA wants to evaluate prior to releasing the revised profile including, a two year ingestion study being conducted by industry (Ambika Bathija, U.S. EPA, pers comm.).

A5-4.1 Inhalation Exposure Limits

Identified regulatory inhalation exposure limits for soluble nickel compounds, nickel sub-sulphide and nickel oxide are described below.

Soluble Nickel Compounds

The ATSDR (2005) chronic inhalation MRL (minimal risk level) is 0.00009 mg Ni/m^3. This MRL is considered most appropriate for soluble nickel salts. The MRL was derived from a NOAEL of 0.03 mg/m^3 in rats for respiratory effects (chronic active inflammation and lung fibrosis) (NTP, 1996b). This NOAEL was then adjusted for intermittent exposure (0.03 mg/m^3 x 6/24 x 5/7) and multiplied by the Regional Deposited Dose Ratio (RDDR) of 0.506 (to extrapolate from particle deposition in rats to deposition in humans), to yield a human-equivalent NOAEL value (NOAELHEC). The NOAELHEC was then divided by a cumulative uncertainty factor of 30 (3-fold for extrapolation from animals to humans and 10-fold for human variability) to yield the chronic MRL.

ATSDR (2005) also calculated an intermediate-duration inhalation MRL (Minimal Risk Level) for nickel of 0.0002 mg/m^3. This MRL is based on a nickel sulfate NOAEL of 0.06 mg/m^3 for lung inflammation (NTP, 1996b). This study NOAEL was adjusted for intermittent exposure (*i.e.*, 0.06 mg/m^3 x 6/24 x 5/7 = 0.01 mg/m^3), and then converted to a human-equivalent NOAEL (a NOAELHEC) by multiplying the adjusted NOAEL by a regional deposited dose ratio of 0.474. Following this, the MRL was obtained by dividing the NOAELHEC by a cumulative uncertainty factor of 30 (3 for inter -species extrapolation with dosimetric adjustments; 10 for intrahuman variability).

TERA (1999) and Haber *et al.* (2000b) suggest a reference concentration of 0.0002 mg/m^3 for inhaled nickel soluble salts, based on lung fibrosis in male rats, as reported in NTP (1996b). TERA reviewed the quantal data from the NTP (1996b) study on the incidence of respiratory tract lesions and then fit these data to a polynomial mean response regression model and a Weibull power mean response regression model, using the maximum likelihood method. This was followed by benchmark dose modelling. Based on the modelling results, the most sensitive endpoint was lung fibrosis in male rats. The $BMCL_{10}$(HEC) for lung fibrosis in male rats was 0.0017 mg Ni/m^3. An uncertainty factor of 10 was applied to this value to account for intrahuman variability, and yielded the RfC.

OEHHA (1999) derived an acute reference exposure level (REL) for nickel compounds (excluding nickel oxide) of 0.006 mg Ni/m^3. This value was derived using the LOAEL of 0.067 mg/m^3 for decreased forced expiratory volume (>15%) from the study by Cirla *et al.* (1985) involving seven volunteer metal plating workers with occupational asthma. The nickel species in this study was nickel sulphate hexahydrate, and the exposure duration was 30 minutes. OEHHA extrapolated the LOAEL to a 1 hour concentration, which was 0.033 mg/m^3. A cumulative uncertainty factor of six was applied to this value (based on use of a LOAEL) to yield the acute REL.

OEHHA (2003a) derived a chronic reference exposure level (REL) of 0.00005 mg/m^3 for nickel compounds (except nickel oxide). The principal study was NTP (1996b) and the critical effects were pathological changes in lung, lymph nodes, and nasal epithelium, which included active pulmonary inflammation, macrophage hyperplasia, alveolar proteinosis, fibrosis, lymph node hyperplasia, and olfactory epithelial atrophy. The study NOAEL was 0.03 mg/m^3. The nickel species tested was nickel sulfate hexahydrate. The study NOAEL was adjusted for continuous exposure (multiplied by 6/24 x 5/7) and then converted to a NOAELHEC by multiplying against an RDDR of 0.29. Following this, the NOAELHEC was divided by a cumulative uncertainty factor of 30 (three for interspecies uncertainty; 10 for intraspecies uncertainty) to yield the chronic REL.

Health Canada (1996) derived a tolerable concentration (TC) for nickel sulphate of 0.0000035 mg Ni/m^3. This TC was derived based on the LOEL in rats of 0.02 mg Ni/m^3 from the Dunnick *et al.* (1989) study. The LOEL was adjusted for continuous exposure by applying factors of 6/24 and 5/7, and an uncertainty factor of 1,000 was applied (*i.e.,* 10 for intraspecies variation, 10 for interspecies variation, and 10 for a less than chronic study). However, the Dunnick *et al.* (1989) study exposed rats to nickel oxide (a relatively insoluble form) rather than to a soluble nickel form, such as nickel sulphate. Thus, the appropriateness of using a nickel oxide study as the basis for a TC for nickel sulphate is questionable, especially given that nickel sulphate studies exist in the scientific literature. Given this, the TC was not considered for use in the human health risk assessment of the Greater Sudbury Area.

The only jurisdiction identified that classifies soluble nickel salts as human carcinogens is Health Canada. Health Canada (1996; 2003) reports an inhalation slope factor of 3.1 $(mg/kg/day)^{-1}$ and an inhalation unit risk of 0.71 $(mg/m3)^{-1}$ for soluble nickel salts. These values were calculated from the Health Canada (1996) tumorigenic concentration 5% (TC05) of 0.07 mg/m^3 for soluble nickel (primarily nickel sulfate and nickel chloride), which in turn, was based on Doll *et al.* (1990). It is important to note however, that the TERA (1999) review concluded that the available information on carcinogenicity of soluble nickel

salts is inadequate to derive a reliable TC05, slope factor or inhalation unit risk for any soluble nickel compound. Thus, these Health Canada values are not considered reliable estimates of carcinogenic potency for soluble nickel compounds and will not be used in the human health risk assessment of the Greater Sudbury Area. Health Canada (1996; 2003) also reports a TC05 of 0.040 mg/m^3 for combined oxidic, sulphidic and soluble nickel. Given the conclusions of the TERA (1999) review, this TC05 would appear to be equally flawed as the TC05 for soluble nickel compounds; thus, it is not considered a reliable estimate of carcinogenic potency for the combined nickel compounds, and will not be used in the human health risk assessment of the Greater Sudbury Area.

As noted previously, Oller (2002) suggests that in isolation, water soluble nickel compounds are not complete carcinogens. However, they may enhance the carcinogenic risks associated with other compounds when inhaled, if concentrations of soluble nickel compounds in air are large enough to induce chronic lung inflammation (Oller, 2002). By keeping airborne exposure below levels that result in chronic respiratory toxicity, Oller (2002) suggests that possible tumour-enhancing effects may be avoided. Seilkop (2004) has derived an inhalation unit risk of 1.9×10^{-4} $(\mu g/m^3)^{-1}$ for nickel sulphate when exposure is in the presence of a carcinogen. The basis of this unit risk is the incidence of an inflammatory response in the exposed animals.

Interestingly, when the IUR for nickel sulphate (Seilkop, 2004) is converted to a risk-specific concentration (RsC) assuming a target risk level of 1 in 100,000, the RsC is very similar to the OEHHA chronic REL for 'nickel compounds except nickel oxide'. The RsC that corresponds to the nickel sulphate IUR is 0.05 µg/m^3 (the OEHHA REL is also 0.05 µg/m^3). Thus, it can be extrapolated from this comparison that although the OEHHA chronic REL is not derived from a cancer endpoint, it would appear to be protective of both non-cancer and potential cancer effects of inhaled soluble nickel.

Nickel Subsulphide

Chronic exposure to nickel subsulphide has been found to result in inflammatory changes in the lungs The chronic MRL derived by ATSDR (0.00009 mg Ni/m^3) is based on the lowest of the LOAELs observed in a series of NTP studies which looked at various forms of nickel. The MRL was based on the most sensitive endpoint and form of nickel studied (sulphate) and as such is considered of protective similar effects following exposure to nickel subsulphide (ATSDR, 2005).

The U.S. EPA considered the following studies in the determination of unit risks for nickel compounds (refinery dust and subsulphide).

A cohort of employees of a nickel refinery in West Virginia who experienced a minimum one year exposure to nickel refinery dusts (containing nickel subsulphide, sulphate and oxide or only nickel oxide) did not show an increased incidence of lung cancer above expected rates (Enterline and Marsh, 1982). Chovil *et al.* (1981) studied a cohort of nickel refinery workers in Ontario, and observed a dose-related trend for the relationship between weighted exposure in years to the incidence of lung cancer. Similarly, a cohort of Welsh nickel refinery workers had elevated risks of cancer compared to the national average; increased rates of nasal cancer were observed in men employed prior to 1920, while this rate was less than the national average for those starting work between 1920 and 1925, and equalled the expected value for those employed after 1925 (Doll *et al.*, 1977). A significantly increased lung cancer-related mortality was observed in employees starting prior to 1925 but not in those starting between the years 1930 to 1944. Magnus *et al.* (1982) conducted a study of men employed at a nickel refinery in Norway, and reported an elevated occurrence of respiratory cancer for nickel- exposed workers compared to expected values, and for workers involved in nickel processing steps compared to non-processing employees.

Each of these epidemiology studies used in the U.S. EPA determination of the unit risk associated with nickel, had factors limiting their usefulness for a unit risk calculation. For example, none were able to account for exposures to other chemicals, metals or nickel species (such as nickel subsulphide), that were present in the occupational environment of a nickel refinery. Only one attempted to account for differences between smokers and non- smokers, an important consideration when examining the incidence of lung cancer. Three of the four studies did not provide measurements of airborne nickel concentrations or estimates of worker exposure. The U.S. EPA estimated exposures based on information provided in other reports in which concentrations of nickel in the work environment were projected on the basis of the operating procedures used. Other problems included poorly or heterogeneously defined cohorts, poor follow-up success, and no consideration of the role of the latency period for lung cancer. Of the four summarized above, Enterline and Marsh (1982) was the most relevant since estimated exposures were provided, the latent period could be examined, and the effects in refinery workers could be compared to non-refinery workers. However, the mixed exposure to other substances and to cigarette smoke were confounding factors that limit the interpretation of this study.

Based on these studies, the U.S. EPA derived slope factors for nickel refinery dust of 2.4×10^{-4} $(\mu g/m^3)^{-1}$ and 4.8×10^{-4} $(\mu g/m^3)^{-1}$ for nickel subsulphide (U.S. EPA, 1991b). The relevance of these slope factors to the Sudbury environment is questionable given the concomitant presence of multiple nickel species in proportions that will differ from those found within refineries. These slope factor were retained for

further consideration, most notably for consideration of the potential presence of nickel sulphide in the ambient environment.

Seilkop and Oller (2003, 2005) have estimated safety limits for workers from fitted animal dose-response curves after accounting for interspecies differences in deposition and clearance, differences in particle size distributions, and human work activity patterns. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.002 to 0.01 mg inhalable nickel subulphide/m^3. Subsequently, Seilkop (2004) has derived a non-linear inhalation unit risk of $6.3x10^{-4}$ $(\mu g/m^3)^{-1}$ for nickel subsulphide. This IUR is independent of any specific risk level and can be used to establish 10^{-5} or 10^{-6} acceptable risk levels as appropriate.

Nickel Oxide

OEHHA (2003b) derived a chronic REL specifically for nickel oxide of 0.0001 mg/m^3. The principal study was NTP (1996c), and the critical effects considered were pathological changes in lung and lymph nodes, including active pulmonary inflammation, lymph node hyperplasia, and adrenal medullary hyperplasia (females only). This study identified a LOAEL of 0.5 mg/m^3. The study NOAEL was adjusted for continuous exposure (multiplied by 6/24 x 5/7) and then converted to a NOAELHEC by multiplying against an RDDR of 0.29. Following this, the NOAELHEC was divided by a cumulative uncertainty factor of 300 (*i.e.,* 10 for use of a LOAEL, 3 for interspecies uncertainty, and 10 for intraspecies uncertainty) to yield the chronic REL.

Health Canada (1996; 2003) recommends a tolerable inhalation concentration (for non-cancer effects) of 0.00002 mg/m^3 for nickel oxide. This TC was derived based from the lowest identified LOEL of 0.02 mg/m^3 reported in Spiegelberg *et al.* (1984). In this study, rats exposed for four months to nickel oxide displayed a dose-related increase in lung granulocyte, lymphocyte, and multinucleated macrophage counts. An uncertainty factor of 1,000 was applied to this LOEL (*i.e.*, 10 for intraspecies variation, 10 for interspecies variation, and 10 for use of a less than chronic study, and minimal effects at the LOEL) to yield the TC.

Seilkop and Oller (2003, 2005) also estimated safety limits for workers from fitted animal dose-response curves. Using a 10^{-4} risk level (which they deemed an acceptable occupational lifetime cancer risk level), they derived an occupational exposure limit concentration of 0.5 to 1.1 mg inhalable nickel oxide/m^3. The authors report that although the animal data for nickel oxide suggest a threshold response for lung

cancer, this cannot be concluded with certainty as sampling uncertainty in data make the non-threshold response equally as plausible. They report that the non-linearity of the observed dose-response of nickel oxide is well represented by benchmark dose models (excluding the high dose response). Subsequently Seilkop (2004) has derived an inhalation unit risk of $2.3x10^{-5}$ $(\mu g/m^3)^{-1}$ for nickel oxide. This IUR is independent of any specific risk level and can be used to establish 10^{-5} or 10^{-6} acceptable risk levels as appropriate.

Interestingly, when the IUR for nickel oxide from Seilkop (2004) is converted to a risk-specific concentration (RsC) assuming a target risk level of 1 in 100,000, the RsC is very similar to the OEHHA chronic REL for nickel oxide. The RsC that corresponds to the nickel oxide IUR is 0.4 $\mu g/m^3$, while the OEHHA REL is 0.1 $\mu g/m^3$. Thus, it can be extrapolated from this comparison that although the OEHHA chronic REL is not derived from a cancer endpoint, it would appear to be protective of both non-cancer and potential cancer effects of inhaled nickel oxide.

Alternate Regulatory Approach

As part of a process to reshape their clean air policy through the generation of new ambient air quality directives, in the late 1990s the European Union established a series of Steering Groups and Working Groups to provide guidance back to the EU on development of appropriate ambient air quality target values. In the development of their proposed target value for nickel, the EU working group based its limit value on three distinct toxicological approaches – one non-cancer and two cancer approaches. The non-cancer approach was based upon non-cancer data on respiratory effects (specifically lung inflammation and fibrosis) of nickel sulphate hexahydrate in a 1996 NTP rodent study. The proposed limit was based upon a LOAEL of 0.06 mg Ni/m^3, a 1000-fold uncertainty factor (*i.e.*, 10-fold for extrapolation from LOAEL to NOAEL, 10-fold for interspecies extrapolation, and 10-fold for intrahuman variability), and a 6-fold correction factor to account for the fact that the animals were not continuously exposed to nickel sulfphate during the study. This yields a limit, based upon non-cancer endpoints, of 10 ng Ni/m^3 (European Commission DG Environment, 2001).

Under the EC classification system, nickel oxide, nickel monoxide, and nickel sulphide are considered "Group 1" (*i.e.*, known human carcinogens), whereas nickel, nickel carbonate, nickel hydroxide, nickel sulphate, and nickel tetracarbonyl are classified as "Group 3" (*i.e.*, possible carcinogens). As such, the EC working group also developed a nickel target value using two different cancer approaches. In the first approach, the EU working group based its first approach upon the International Committee on Nickel

Carcinogenesis in Man (ICNCM, 1990) report conclusion that more than one nickel species gives rise to concern with respect to lung and nasal cancer, and that soluble nickel is not carcinogenic in itself but acts so as to increase the overall risk of cancer associated with exposure to other forms of nickel (European Commission DG Environment, 2001).

In the first cancer approach, the EC working group selected three unit risk values (*i.e.*, the U.S. EPA, the Centre d'Etude sur l'Evaluation de la Protection dans le domaine Nucléaire [CEPN], and the WHO) to derive a range of unit risk values of 2.4×10^{-4} to 3.8×10^{-4} $(\mu g\ Ni/m^3)^{-1}$. Based upon the observations that nickel subsulphide is very low or absent in ambient air, and that these studies were based upon workplace exposures (*i.e.*, the possibility that nickel refinery workers are exposed to different nickel species than the general public), the EC working group concluded these estimates likely overestimate the true risk by an order of magnitude. This would result in an actual cancer unit risk range of 2.4×10^{-5} to 3.8×10^{-5} $(\mu g\ Ni/m^3)^{-1}$, or 0.026 to 0.042 $\mu g\ Ni/m^3$ assuming a 1-in-1,000,000 acceptable cancer risk level (European Commission DG Environment, 2001).

In the second cancer-based method to establishing a target value for nickel, the EU working group used a variety of threshold approaches, based upon three separate reports, which assumed a LOAEL for nickel refinery workers and applied various adjustments (*i.e.*, exposure time, work to lifetime, breathing rate) and uncertainty factors (*i.e.*, LOAEL to NOAEL and adjustments for sensitive subgroups). The three different applications of this threshold approach resulted in a calculated concentration range between 0.006 to 1.1 $\mu g\ Ni/m^3$ (European Commission DG Environment, 2001).

Following an evaluation of these three different approaches, the EU working group proposed a limit value range of 0.01 to 0.05 $\mu g\ Ni/m^3$ (as an annual mean), based upon non-cancer effects. The working group also believed that a limit value in this range can be judged compatible with the objective of limiting excess lifetime cancer risks to not more than one-in-a-million. The majority of the working group proposed a limit value at the lower end of this range, to represent an annual mean of total airborne nickel (European Commission DG Environment, 2001).

Based upon this work, in 2004 the European Parliament adopted a target value for airborne nickel of 20 $ng\ Ni/m^3$, or 0.02 $\mu g\ Ni/m^3$, considered protective of both cancer and non-cancer health endpoints (OJEU, 2005).

A5-4.2 Oral Exposure Limits

Identified regulatory oral exposure limits for soluble nickel compounds and nickel oxide are described below. No agency has derived oral exposure limits for nickel subsulphide.

ATSDR (2005) considers that there is insufficient data available to derive an acute, intermediate or chronic-duration oral MRL for any nickel compound.

The U.S. EPA (1991a) derived an oral RfD of 20 µg/kg body weight/day for soluble salts of nickel based on rat data from the Ambrose *et al.* (1976) study. This RfD was based on a NOAEL of 5000 µg/kg body weight/day, for decreased body and organ weights, which corresponded to a dietary concentration of 100 ppm nickel in the diet over the two-year study. The form of nickel administered was nickel sulphate hexahydrate. A cumulative uncertainty factor of 300 was applied to the NOAEL (*i.e.*, 10 for interspecies extrapolation, 10 to protect sensitive populations, and three to account for inadequacies in the available reproductive studies), to yield the oral RfD.

OEHHA (2003a) used the same principal study (*i.e.*, Ambrose *et al.*, 1976) as the U.S. EPA to derive a chronic oral REL of 50 µg/kg body weight/day. The only difference in derivation of the REL *versus* the U.S. EPA oral RfD is the magnitude of the cumulative uncertainty factor that was applied to the study NOAEL (5,000 µg/kg body weight/day). In both cases, the study NOAEL was equivalent to a NOAELHEC. OEHHA applied a cumulative uncertainty factor of 100 (10 for interspecies uncertainty and 10 for intraspecies uncertainty) to the NOAELHEC to yield the chronic REL.

Also using the Ambrose *et al.* (1976) study as the principal study, Health Canada (1996; 2003) recommends a TDI of 50 µg/kg body weight/day for nickel sulphate. Again, this TDI is based on the NOAEL from Ambrose *et al.* (1976). An overall uncertainty factor of 100 (10-fold for interspecies extrapolation and 10-fold for interspecies variation) was applied to the study NOAEL (considered the human-equivalent NOAEL) to yield the TDI. Unlike the U.S. EPA, Health Canada did not consider an additional uncertainty factor for design limitations in reproductive studies necessary.

Recently, in their review of the toxicology of soluble nickel compounds, TERA (1999) calculated an oral reference dose of 8 µg/kg/day nickel for ingested nickel-soluble salts. Several studies were considered as the basis for the RfD for soluble nickel salts including Vyskocil *et al.* (1994), Ambrose *et al.* (1976), American Biogenics Corp. (1988), Dieter *et al.* (1988), and Smith *et al.* (1993). The most sensitive endpoint was determined to be increased albuminuria (indicating renal glomerular dysfunction) in male

and female rats exposed to nickel in drinking water for 6 months (Vyskocil *et al.*, 1994). Increased albuminuria was observed at 6 months but not after three months of exposure. In this study, only a single dose was tested (*i.e.*, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day in females). These doses were the LOAEL values for this study, as albuminuria was considered a biologically significant effect. Despite this limitation of single doses, the LOAELs are supported by several other oral studies (TERA, 1999). Other limitations of Vyskocil *et al.* (1994) and the other available oral studies with soluble nickel compounds noted by TERA (1999) included subchronic durations, lack of comparison to baseline values, small numbers of test animals, considerable variability in responses in both control and exposed groups, uncertainty surrounding biological significance of renal effects, inadequate reporting, and high mortality rates in both treated and control groups. Upon consideration of these issues, TERA (1999) considered Vyskocil *et al.* (1994) to be the most appropriate choice for the principal study. As there was substantial variability for albuminuria in male rats, although not statistically significant, the LOAEL of 7.6 mg Ni/kg/day in females was considered the most appropriate study LOAEL for deriving an RfD from. An overall uncertainty factor of 1,000 was applied (*i.e.*, 10 for intrahuman variability, 10 for interspecies extrapolation, and a 10 for subchronic-to-chronic extrapolation, an insufficient toxicological database, and use of a minimal LOAEL) to the LOAEL of 7.6 mg/kg/day to yield the oral RfD of 8 µg/kg body weight/day.

TERA (1999) notes that the nickel doses used in the principal study did not include the nickel present in the diet. Therefore, the RfD represents the dose of nickel in addition to the amount received in food. TERA (1999) considers this oral RfD to agree well with the U.S. EPA oral RfD of 20 µg/kg body weight/day for total nickel exposure, and is within the expected inherent uncertainty surrounding an RfD. In addition, an independent peer review panel, through TERA's ITER Peer Review program has recently approved the oral RfD value. The TERA RfD was not used in this study as reference values derived for all other COC were expressed on a total exposure basis, whereas this value is considered an incremental value. For consistency purposes, the EPA value of 20 µg/kg/day was considered preferable.

Health Canada (1996; 2003) reports a TDI of 1.3 µg/kg body weight/day for nickel chloride. This TDI was obtained by applying a 1000-fold uncertainty factor (10-fold for use of a LOAEL, 10 fold for interspecies differences, and 10 fold for sensitive individuals in the population) to the LOAEL of 1.3 mg/kg/day based on reproductive toxicity in the rat study by Smith *et al.* (1993). It should be noted however, that this study was criticized by the U.S. EPA for lacking a clear dose-response relationship. Since the speciation analysis conducted as part of this study did not indicate the presence of any nickel chloride or related substances, this limit was deemed inappropriate. In addition, it is not expected that

nickel chloride would be very stable in the environment (*i.e.*, very soluble in water), nor is it anticipated to be a significant species in the facility emissions profiles.

No regulatory agencies were identified that developed chronic oral exposure limits (RfDs, TDIs, *etc*.) for nickel oxide, with the exception of RIVM in the Netherlands. This agency used the study by Ambrose *et al.* (1976), which used nickel sulphate, to derive a TDI of 50 µg/kg body weight/day for nickel oxide. An overall uncertainty factor of 100 was applied to the study NOAEL of 5,000 kg body weight/day to yield the TDI. The use of a nickel sulphate study to estimate a nickel oxide TDI appears scientifically questionable however, especially when nickel oxide studies are available in the scientific literature. As such, this TDI was not considered for use in the human health risk assessment of the Greater Sudbury Area.

Other credible regulatory agencies have recently published oral toxicity reference values for nickel. For example, the World Health Organization (WHO, 2007) published a nickel drinking water guidance (DWG) based on studies by Springborn (2000a,b) and Nielsen *et al*. (1999). In the Nielsen *et al*. study, soluble nickel was given to fasted (overnight + 4 hours post-exposure) human subjects at a single test concentration (12 µg/kg bw/day). There were 20 nickel-sensitized female (with hand eczema) and 20 age-matched controls. A LOAEL of 12 µg/kg/day was reported in pre-sensitized subjects (with nickel eczema). Nine of 20 women with pre-existing eczema had exacerbation of this condition. No effects were seen in the controls. WHO used this unbounded LOAEL as the basis for their drinking water guideline. No UF was applied as the LOAEL was from a sensitive human subpopulation.

There are numerous problems with using the Nielsen *et al.* (1999) study as the basis for a nickel oral TRV, including: i) the small numbers of subjects; ii) a single test concentration (an unbounded LOAEL was used with no information available as to whether similar or other effects occur at higher or lower doses); iii) use of fasted subjects which would greatly increase absorption of nickel beyond what would normally occur; and, iv) use of a very sensitive subpopulation that does not reflect the majority of people. Any one of these issues would often preclude a study from consideration as the basis for TRV development. In fact, OEHHA (2001) excluded this study from consideration in their derivation of a public health goal (PHG) for drinking water. Their concern appears to be that the results of this study do not represent the general population, due mainly to use of fasted subjects and a sensitive subgroup predisposed to nickel-induced eczema. Also, other studies seem to contradict Nielsen *et al*. (1999). OEHHA (2001) notes a few studies that demonstrated oral administration of soluble nickel at low doses

over time may reduce nickel contact dermatitis. The issues surrounding the use of the Springborn (2000a,b) data are discussed in detail below.

The State of California's Office of Environmental Health Hazard Assessment (OEHHA) published a public health goal (PHG) in 2005 based on consideration of three developmental toxicity studies in rats (*i.e.*, Smith *et al*., 1993 and Springborn Laboratory, 2000a,b), although the actual dose used to derive the PHG is from Springborn Laboratory (2000b). This dose is a reported NOAEL of 1120 µg/kg/day. The Smith *et al*. (1993) study used drinking water exposures while the Springborn studies used gavage exposures. OEHHA applied a total UF of 1000 (*i.e.*, 10 for interspecies, 10 for intraspecies and 10 to account for potential oral carcinogenicity of soluble nickel) to this NOAEL. The test compound was actually NiSO4 * 6H20. The resulting TRV used by OEHHA to derive the PHG for drinking water was 1.12 µg/kg bw/day. Interestingly, the same TRV could be derived from the Nielsen *et al*. (1999) study if one applied a 10-fold UF to the LOAEL. However, use of this TRV value is questionable given the following issues:

- While there are a number of studies demonstrating development and/or reproductive toxicity in experimental animals, there is no strong evidence in the scientific literature that nickel is a developmental toxicant in humans. One study (Chaschin *et al*., 1994) of workers at a Russian hydromet nickel refinery had increased rates of spontaneous abortions relative to controls. However, these exposures were inhalation only, and confounded by manual labour and heat stress. Interestingly, the estimated dose associated with the health outcome is 20 µg/kg bw/day (*i.e.*, the same value as the current U.S. EPA oral RfD).

- A number of the animal reproduction/developmental studies had design limitations which make the results questionable (*e.g.*, small sample sizes, inappropriate comparisons, non-random matings, other trace metals in diet, inadequate reporting of water intake rates, elevated temperature and humidity during experiments, questionable biological significance of some reported effects). However, the Springborn studies appear to have much fewer design limitations than previous studies.

- Interestingly, Springborn (2000b), which was a more detailed investigation, did not confirm the findings of Springborn (2000a). Springborn (2000a) was a preliminary study, and the dose identified as the LOAEL in the Springborn (2000a) study was identified as the NOAEL in the Springborn (2000b) study (*i.e.*, 10 mg/kg bw/day). This dose was the lowest test dose in the

Springborn (2000a) study, and the highest test dose in the Springborn (2000b) study (note that the NOAEL and LOAEL reported by OEHHA reflect adjusting on a relative molar mass basis; 10 mg/kg/day is the actual unadjusted test dose of the administered compound; see the footnote below for further information). OEHHA notes there is uncertainty around the actual effects threshold. To account for this, OEHHA selected the dose that is one dose level below the actual NOAEL in the Springborn (2000b) study as the "new" NOAEL (*i.e.*, 5 mg/kg/bw/day). OEHHA then adjusted this new NOAEL down to 1.12 mg/kg bw/day through a molar mass ratio procedure.

- Use of a 10-fold UF to protect against oral carcinogenic effects is not well supported by the scientific literature (*i.e.*, there is no strong evidence that nickel is a carcinogen by oral route for any chemical form). All oral animal studies in the literature are negative for carcinogenicity. Injection studies show tumours only in injection site tissues or in one highly susceptible mouse strain. The genotoxicity database on nickel compounds is mixed and equivocal. The basis for OEHHA using this 10-fold UF appear to be two ecological studies (*i.e.*, Isacson *et al*., 1985 and Ling-Wei *et al.*,1988) that reported an association between nickel in drinking water and bladder, nasopharyngeal and lung cancer rates. However, as is typical with ecological studies, these studies cannot show causation. For example, in the Isacson study, only males had the association (may be related to failure to account for confounding factors such as smoking rate or occupation). Even Isacson *et al.* (1985) suggest that nickel is really just an indicator of presence of other chemicals that might be related to the cancer rates. In the Ling-Wei study, there were only 15 subjects and 15 controls. It is the SARA Group's opinion that both studies are too weak to use as basis for this UF.

- While not all regulatory agencies follow this procedure, there was no use of a NOAEL-HEC value in the derivation of this TRV.

With respect to nickel subsulphide, no regulatory agency derived oral exposure limits were identified. Furthermore, there is no evidence to indicate that orally ingested nickel subsulphide would be carcinogenic. Nickel subsulphide would be expected to rapidly dissociate in the gut, and as such oral exposure to this compound would be negligible.

For consistency purposes, the U.S. EPA value of 20 µg/kg/day was selected for the purposes of this risk assessment.

A5-4.3 Dermal Exposure Limits

No regulatory dermal exposure limits for nickel compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAF_{dermal}). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

With respect to nickel dermal exposure, it is important to recognize that very little nickel penetrates the skin to enter the systemic blood circulation. Rather, nickel tends to penetrate only to outer layers of the skin (primarily the stratum corneum). A number of studies and reviews suggest that ionized nickel compounds do not penetrate intact skin, which results in dermal absorption of nickel being negligible in most circumstances (See Section A5-2.1.3 for further details).

A5-4.4 Criteria Summary

Current MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits) when health based exposure limits are available from major health agencies. Toxicological criteria used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO) and the MOE itself. However, given the uncertainty inherent in the available regulatory limits for inhalation exposures to nickel, the values derived by Seilkop (2004) have also been considered in the current assessment, in addition to a variety of other regulatory limits, as part of a weight of evidence approach.

The following table summarizes the exposure limits selected for the current study.

Table A5.1 Summary Table of Exposure Limits Selected for Nickel

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	20 µg/kg/day	RfD	Decreased body and organ weight (rats)	Ambrose *et al.*, 1976	U.S. EPA, 1991a
Inhalation	0.02 µg/m^3 (total nickel)	RfD	Respiratory effects (lung inflammation and lung fibrosis)	European Commission DG Environment, 2001	OJEU, 2005
Dermal [a]	NA		NA	NA	NA

Table A5.1 Summary Table of Exposure Limits Selected for Nickel

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	nickel oxide 2.3×10^{-5} $(\mu g/m^3)^{-1}$ nickel subsulphide 6.3×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Seilkop, 2004	none
	nickel refinery dusts 2.4×10^{-4} $(\mu g/m^3)^{-1}$ nickel subsulphide 4.8×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Extrapolations from epidemiologic datasets from Enterline and Marsh, 1982; Chovil *et al.*, 1981; Peto *et al.*, 1984; and Magnus *et al.*, 1982	U.S.EPA, 1991
	nickel compounds 3.8×10^{-4} $(\mu g/m^3)^{-1}$	IUR	Lung cancer	Andersen, 1992; Andersen *et al.*, 1996	WHO, 2000
	combined oxidic, sulphidic and soluble nickel 0.04 mg/m^3	TC05[b]	Lung cancer	Doll *et al.*, 1990	Health Canada, 1996
Dermal	NA		NA	NA	NA

NOTES:

NA = not available; RfD = reference dose; REL = reference exposure level ; IUR = inhalation unit risk.

[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

[b] Health Canada (2004) converts the TCO5 of 0.04 mg/m^3 to an IUR of 0.0013 $(\mu g/m^3)^{-1}$by dividing the 0.05/TC05.

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Sudbury Area Risk Assessment
Volume II

Appendix A6:

Detailed Toxicological Profile of Selenium

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SUDBURY AREA RISK ASSESSMENT
VOLUME II

APPENDIX A6: DETAILED TOXICOLOGICAL PROFILE OF SELENIUM

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APPENDIX A6 DETAILED TOXICOLOGICAL PROFILE OF SELENIUM

SUMMARY

The purpose of this toxicological profile of selenium is to: i) outline the most relevant toxicological and epidemiological information on this substance; ii) describe recent information that may challenge previous findings; and, iii) provide supporting rationale for the selenium exposure limits selected for use in the human health risk assessment of the Sudbury area. The toxicological review of selenium is based primarily on secondary sources, such as ATSDR toxicological profiles and other detailed regulatory agency reviews, and is supplemented with recent scientific literature.

The following is a summary of the toxicological profile for selenium. Detailed profiles for each of the Chemicals of Concern (COC) for the Sudbury Soils Study (*i.e.*, arsenic, cobalt, copper, lead, nickel and selenium) are provided on the attached CDs.

Environmental Forms of Selenium

Selenium has four valence states (I, II, IV, VI) and can exist in a variety of chemical forms in the environment including various salts, oxides, hydrides, sulfides, and metal selenides. The soluble oxyanions selenate [Se(VI)] and selenite [Se(IV)] are the primary forms of selenium in the ambient environment. In air, the most common selenium compounds are selenium dioxide, dimethyl selenide, dimethyl diselenide and hydrogen selenide. Hydrogen selenide is highly reactive, and is rapidly oxidized to elemental selenium and water (NAS 1976); thus, it does not persist.

In soils, sediments and water, the expected forms of selenium are mainly selenates and selenites. Selenates are among the most mobile selenium compounds, due to their high solubility and inability to adsorb onto particles (NAS, 1976; Kabatas-Pendias and Pendias, 1992). In acidic, organic-enriched soils, heavy metal selenides, selenium sulfides and selenites are the predominant species. In neutral, well-drained soils, sodium and potassium selenites dominate (ATSDR, 2003). In alkaline soils that are well-oxidized, selenates are the major selenium species present.

The majority of selenium in plant and animal material consumed by humans will be in the form of selenomethionine (Stahl *et al.,* 2002). Much of the selenium in the body also occurs as selenomethionine. Selenomethionine is biologically inert, relative to inorganic forms of selenium.

Absorption

Selenium compounds appear to be readily absorbed from the human gastrointestinal tract, with the degree of absorption dependent on the chemical form (*e.g.,* organic, inorganic), physical state (*e.g.,* solid, solution), and the dose (ATSDR, 2003). Inorganic forms of selenium are not absorbed as well as the organic forms (U.S. EPA, 1984). It also appears that with all forms of selenium, the degree of absorption is independent of the exposure level, and may be greater in individuals with a selenium deficiency (ATSDR, 2003). In humans, selenium absorption from the gastrointestinal tract has been reported to range from 20 to 97%, with the greatest absorption efficiencies observed for the organic forms of selenium (Thomson, 1974; Thomson and Stewart, 1974; Stewart *et al.,* 1978; Robinson *et al,* 1978; Thomson *et al.,* 1978; Griffiths *et al.,* 1976). In experimental animals, oral absorption efficiencies of 9.5 to 100% have been reported for various forms of selenium. A number of recent studies have examined oral bioavailability of selenium from milk-based products, infant formulae, as well as well as egg yolk and chicken meat. The absorption efficiencies were found to range from 61 to 97% (Chen *et al.,* 2004; Van Dael *et al.,* 2002). Fox *et al*. (2004) found that selenium intake from fish was 87.7 to 90.4 %. Most of the selenium compounds that occur in foods are organic. The retention of inorganic selenium is poor relative to organic selenium compounds (Fox *et al.,* 2004). Thus, the form (organic or inorganic) may play a significant role in the retention of selenium compounds in human tissues.

Selenium absorption in humans following inhalation exposure has only been studied in occupational settings. While such studies indicate humans absorb selenium compounds in the lungs, no quantitative data are available. Weissman *et al.* (1983) found that 16% of selenium inhaled as metal aerosols was absorbed in the lungs within two hours. The rate of selenium absorption from the lungs depends on the chemical form, with absorption of selenious acid aerosols occurring approximately twice as rapid as absorption of elemental selenium aerosols (Weissman *et al.,* 1983; Medinsky *et al.,* 1981).

The dermal absorption of selenium compounds is low in humans, as shown in studies with users of shampoos containing 1% selenium disulphide, or lotions containing seleniomethionine (ATSDR, 2003; NAS, 1980; Burke *et al.,* 1992). The Massachusetts Department of Environmental Protection (MDEP, 1994), the Ontario Ministry of the Environment (MOE, 1996) and the Canadian Council of Ministers of the Environment (CCME, 2002) assume a generic dermal absorption rate for selenium of 0.2% for exposure from contaminated soil on the skin. RAIS (2004) provides a human dermal absorption factor of 1% for selenious acid and selenite, and a factor of 0.1% for selenium and selenium sulfide.

Essentiality

There is scientific consensus that selenium is an essential trace element in both animal and human nutrition (NAS, 1976; Bennett, 1982; WHO, 1986; Foster and Sumar, 1997). The U.S. National Academy of Sciences (NAS, 2000) has recommended safe and adequate daily intakes ranging from 20 to 70 µg per person per day for adults. For children, the RDA is set at 0.87 µg/kg bw/day (NAS, 2000). Selenium dietary requirements for pregnant or lactating mothers are greater, with RDAs of 60 and 70 µg/day respectively (NAS, 2000). Whanger *et al.* (1996) suggest that an intake of less than 40 µg/day will likely result in deficiency. The minimum dose to cause toxicity in humans is not well defined, but the threshold appears to lie in the range of 400 to 900 µg/day (Yang *et al.,* 1989a,b; Longnecker *et al.,* 1991; Whanger *et al.,* 1996).

Various studies have indicated that supplementary selenium relieves a number of human health problems, including muscular discomfort, cardiomyopathy, arthritis, cataracts, cystic fibrosis, hemolytic anemia, multiple sclerosis, Kwashiorkor (a protein-calorie malnutrition), night blindness, and immunodeficiencies (Foster and Sumar, 1997; van Rij *et al.,* 1979; Johnson *et al.,* 1981). Selenium is also believed to have a protective function against certain types of cancers (Foster and Sumar, 1997; Levander,1987).

Toxicology of Selenium

It is generally believed that only the soluble forms of selenium are capable of causing toxicity, as they are absorbed most readily (ATSDR, 2003). It is also important to recognize that many manifestations of selenium toxicity are similar to those observed in cases of selenium deficiency. This complicates the association of selenium levels in various media with adverse health effects.

Signs of toxicity in humans orally exposed to elevated levels of selenium in foods and soils include: loss of hair; loss of, thickened or brittle nails; skin lesions, tooth decay, irregularities of the nervous system, garlicky breath; reduced hemoglobin; and altered thyroid hormone levels in blood (Yang *et al.,* 1983; Yang *et al.,* 1989a,b; Longnecker *et al.,* 1991; Brätter and Negretti De Brätter, 1996; Duffield *et al.,* 1999; Hagmar *et al.,* 1998; Contempre *et al.,*1991).

No reliable human studies were identified that investigated the toxicology of selenium compounds *via* the inhalation or dermal routes.

In experimental animals, oral exposure to elevated levels of various forms of selenium has been associated with a variety of effects, including: mortality, reduced body and organ weights, reduced food and water consumption, kidney effects, immunological effects, amyloidosis of lung, heart, and kidney, heart lesions, liver lesions and reduced liver weights, endocrine effects (thyroid hormone level changes), dermal effects; softening of bones, and increased red blood cell counts (NTP, 1994; ATSDR, 2003; U.S. EPA, 1991; Johnson *et al.,* 2000; Turan *et al.,* 1999a,b; Hotz *et al.,* 1997; Skowerski *et al.,*1997; Dausch and Fullerton, 1993). The available subchronic and chronic oral animal bioassays with selenium compounds are inadequate for determining reliable no-effect or lowest-effect threshold values, as many of the available studies used single doses, relatively short study durations, and had generally poor characterization of effects at various dose levels. In addition, some studies showed no effects at none or all of the dose levels tested, which makes it difficult to establish a dose-response relationship.

No studies investigating health effects in animals following subchronic or chronic inhalation or dermal exposure to selenium compounds were identified in the available scientific literature. There are few studies in the literature regarding reproductive or developmental effects in humans following exposure to selenium compounds. While the data are limited, there appears to be no documented human evidence of reproductive effects, teratogenesis, or developmental abnormalities following selenium exposure.

In experimental animals, some studies have found little or no reproductive or developmental effects (*e.g.,* NTP, 1996; Nobunga *et al.,* 1979; Tarantal *et al.,* 1991), while others have reported such effects following ingestion of various forms of selenium compounds in the diet or drinking water. No reproductive or developmental studies that used inhalation or dermal exposure routes were identified in the available scientific literature. Reported reproductive or developmental effects of selenium in experimental animals following oral exposure include: reduced fecundity and fertility, reduced juvenile growth and survival, sperm abnormalities, testicular atrophy, fetal malformations, and reduced fetal body weights (ATSDR, 2003; El-Zarkouny *et al.,* 1999; Turan *et al.,* 1999a,b; Kaur and Parshad, 1994; Ferm *et al.,* 1990).

Human and animal carcinogenicity studies with selenates, selenites, and organic selenium compounds have generally shown negative results. However, both the human and animal carcinogenicity databases are limited and inconclusive with a number of conflicting results that are difficult to interpret because of apparent anti-carcinogenic activity, high systemic toxicity of some selenium compounds, and flawed study designs. Furthermore, comparison of the available studies is difficult because several different selenium compounds with varying degrees of bioavailability were used in the cancer bioassays (U.S. EPA, 1991). Selenium sulfide is the only selenium compound that has been found to be carcinogenic in

animals. This compound is a pharmaceutical used primarily in anti-dandruff shampoos, and is not readily found in foods or the environment (ATSDR, 2003).

There are a number of studies that are suggestive of an anti-cancer activity for selenium compounds. This is based on various prospective, case-control, and nested case-control epidemiology studies that showed cancer patients had significantly lower blood selenium concentrations than healthy patients (U.S. EPA, 1991), findings that low selenium intake was positively associated with some forms of cancer, and findings of an inverse relationship between selenium intake and cancer incidence (ATSDR, 2003; U.S. EPA, 1991). However, other studies do not support an anticarcinogenic effect for selenium (*e.g.*, van den Drandt *et al.,* 1994).

In general, the weight of available evidence for selenium compounds is suggestive of either an inverse, or a lack of association between environmental selenium concentrations and human cancer incidence and/or cancer mortality rates. Only selenium sulfide appears to have sufficient evidence to suggest carcinogenic activity. Few regulatory agencies have classified selenium as to its carcinogenicity. The U.S. EPA currently classifies these substances as D - not classifiable as to human carcinogenicity, with the exception of selenium sulfide, which the U.S. EPA classifies as a probable human carcinogen (U.S. EPA, 1991). The International Agency for Research on Cancer (IARC, 1987) determined that selenium compounds should be classified as Group 3 - not classifiable as to its carcinogenicity to humans. It should be recognized that selenium sulfide is typically not present in soils, foods or other environmental media to any significant extent, thus human environmental exposure to selenium sulfide would likely be negligible.

Exposure Limits

The following organizations were consulted to select exposure limits for selenium: the U.S. EPA; ATSDR; HC; National Academy of Science; MOE; and, OEHHA.

Current MOE guidance discourages the development of de novo toxicological criteria (exposure limits). Toxicological criteria used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO) and the MOE itself.

As selenium compounds (with the exception of selenium sulfide) are widely considered non-carcinogenic, no regulatory agencies were identified that developed health-based exposure limits based on carcinogenic endpoints.

Oral Exposure Limits

In determining the oral RfD for selenium compounds, the U.S. EPA selected the epidemiology study by Yang *et al*. (1989a,b) as the principal and supporting study. The study NOAEL of 15 µg/kg bw/day was used to calculate an oral RfD of 5 µg/kg bw/day; a three-fold safety factor was applied to account for sensitive individuals (U.S. EPA, 1991). A 10-fold safety factor was not deemed necessary because of the high level of confidence in the Yang *et al.* (1989a,b) and additional supporting studies (U.S. EPA, 1991). The results of Longnecker *et al.* (1991) strongly corroborate the NOAEL identified by Yang *et al.* (1989a,b). In addition, numerous other epidemiological studies and animal studies also support the findings of Yang *et al.* (1989a,b) (U.S EPA, 1991). In addition, the ATSDR (2003) chronic MRL for selenium compounds is also 5 µg/kg bw/day, and is based on the same endpoint (selenosis) and utilizes the same magnitude of uncertainty factor as the U.S. EPA oral RfD. Furthermore, the Health Canada (1996) oral PTDI (assuming a 70 kg individual) of 7.14 µg/kg bw/day is in close agreement with the U.S. EPA oral RfD. The National Academy of Science (IOM, 2000) has derived an acceptable Upper Limit (UL) based on the NOAEL of 800 µg/day based on Yang and Zhou (1994). Application of two-fold uncertainty factor results in an upper limit of approximately 5 µg/kg/day for adults. There was no evidence of increased selenium toxicity for any age group (similar sensitivity for all ages) (IOM, 2000). IOM (2000) also derived an infant specific UL, based on a study by Brätter *et al*. (1996), of 47 µg or approximately, 7 µg/kg/day for two through six-month-old infants. Health Canada has indicated that in 2005/2006, the agency will officially be using adopt ULs as toxicity reference values for all essential elements (N. Roest, and S. Petrovic, Health Canada, Personal Communication) for contaminated sites human health risk assessments. In the derivation of the *Guideline for Use at Contaminated Sites in Ontario* (MOE, 1996), MOE utilized an oral RfD of 5 µg/kg/day for selenium.

Inhalation Exposure Limits

The U.S. EPA, ATSDR, and Health Canada have not developed inhalation exposure limits for selenium compounds. The Ontario Ministry of the Environment provides two health-based limits for selenium in air, a point-of-impingement limit and a 24-hour ambient air quality criterion of 20 and 10 µg/m^3, respectively (MOE, 2001). The California Environmental Protection Agency has developed a chronic

Reference Exposure Limit of 20 µg/m^3, for effects on the alimentary, cardiovascular and nervous systems, based on route-to-route extrapolation from the Yang *et al*. (1989a,b) study (OEHHA, 2001). The REL was derived by multiplying the U.S. EPA oral RfD of 5 µg/kg/day by an inhalation extrapolation factor of 3.5 µg/m^3 per mg/kg-day. Details of the origin of this factor were not provided in the supporting documentation from OEHHA (2001); however it can be obtained simply by dividing a default body weight of 70 kg by a default inhalation rate of 20 m^3/day. Route-to-route extrapolation assumes by default that a chemical is equally absorbed *via* both inhalation and oral routes and that the 'first pass' effect due to metabolism by the liver is not significant (OEHHA, 2001).

Dermal Exposure Limits

No regulatory dermal exposure limits for selenium compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can be expressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, is applied to dermal exposure estimates to adjust these exposures prior to comparison with oral exposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).
AF*dermal* = the fraction of the applied chemical absorbed through the skin.
AF*oral* = the fraction of the ingested chemical absorbed into the bloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

Criteria Summary

The following table summarizes the exposure limits selected for the current study.

Summary of Toxicological Criteria Selected For The HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	5.0 µg/kg/day	RfD/TRV	selenosis: hair loss and nail sloughing Clinical selenosis	Yang and Zhou (1994) Yang *et al.,* 1989a,b	IOM, 2000; Health Canada, 2005 U.S. EPA, 1991
Inhalation	20 µg/m^3	Chronic REL	Hepatic, cardiovascular, neurological	Yang *et al.,* 1989a,b	OEHHA, 2001
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

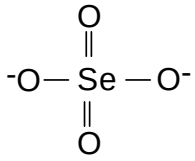
NOTES:
NA = not available; RfD = reference dose; REL = reference exposure level; TRV = toxicity reference value.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

References

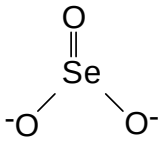
For a complete list of references, see Section A6-5.0 of the detailed toxicological profile on the accompanying CD.

A6-1.0 CHEMICAL CHARACTERIZATION AND GENERAL OVERVIEW

Selenium (CAS RN: 7782-49-2) is a Group 16 or *via* non-metal, and has four valence states (I, II, IV, VI). In nature, the valence states of selenium are responsible for a number of compounds in the environment, including bromides, fluorides, chlorides, oxides, hydrides, sulfides, and many metal compounds (*i.e.,* selenides). Selenium is naturally found in fossil fuels, shales, alkaline soils and as a constituent in over 40 minerals (*i.e.,* ferroselite, challomenite, schmeiderite). The soluble oxyanions selenate [Se(VI)] and selenite [Se(IV)] are the primary forms of selenium in oxic environments. Se(VI) does not readily undergo chemical reduction under physiological conditions of pH and temperature.

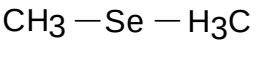


Selenate (VI)

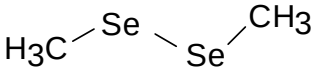


Selenite (IV)

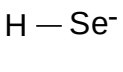
The most common selenium compounds in air include selenium dioxide, dimethyl selenide, dimethyl diselenide and hydrogen selenide. Hydrogen selenide is highly reactive, and is rapidly oxidized to elemental selenium and water (NAS 1976).



Dimethyl selenide



Dimethyl disenide



Hydrogen selenide

The toxicity of selenium can be attributed to the substitution of selenium for sulfur in thiol-containing proteins. In animals, selenium toxicity is conferred primarily by ingestion of food that is rich in seleniferous compounds (*e.g.,* selenomethionine). Selenium poisoning can result in conditions known as alkali disease and "the blind staggers" in domestic animals. Plants and microbes can incorporate inorganic selenium oxyanions into tissues by assimilatory reduction to the level of selenide, followed by their assembly into proteins (Raveendranathan, 2004).

In soil pore water, the expected forms of selenium are the salts of selenic and selenious acids (selenates and selenites, respectively). Selenates are among the most mobile selenium compounds, due to their high solubility and inability to adsorb onto soil particles (NAS, 1976; Kabatas-Pendias and Pendias, 1992). Selenites are less soluble than the selenates (NAS, 1980). Elemental selenium is essentially insoluble in water. In acidic, organic-enriched soils, heavy metal selenides, selenium sulfides and selenites are the predominant species. Selenides and selenium sulfide are also insoluble and tend to be immobile in soils (ATSDR, 2003). In neutral, well-drained soils, sodium and potassium selenites dominate, with soluble metal selenites occurring to a lesser extent (ATSDR, 2003). Selenites are typically complexed to iron oxides/hydroxides and clays in acidic and neutral soils and are of low solubility in this form (Mikkelsen *et al.,* 1989). In alkaline soils that are well-oxidized, selenates are the major selenium species present. Under highly reduced conditions, elemental selenium may dominate in soils but is of minimal bioavailability due to its low water solubility (ATSDR, 2003).

Selenium in foods derives from both vegetable and animal products. The majority of selenium in plant and animal material consumed by humans will be in the form of selenomethionine (Stahl *et al.,* 2002). Given that many inorganic forms of selenium are chemically reactive (*e.g.*, selenite can act as a strong oxidising agent), it is not surprising that much of the selenium in the body occurs as selenomethionine. Selenomethionine is comparatively chemically inert, and is metabolised to form the inorganic precursors of the essential selenoproteins. Mechanisms exist within the body to regulate the conversion of organic selenium in the diet into the inorganic precursors of the proteins (Arthur, 1999). Animals, that consume these forage crops grown in soils rich in selenium, have selenium status and selenium levels in tissues that closely reflect those in the forages. In extreme cases, the low selenium status results in clinical disease (Wichtel, 1998a,b). The main food sources are seafood, liver, meats and cereal products.

In the U.S. an intake for men and women of 55 µg per day with an upper safe level of 400 µg per day has been recommended (Standing Committee on the Scientific Evaluation of Dietary Reference, 2000). In the UK a recommended nutritional intake (RNI) of 60 µg per day for women and 75 µg per day for men has been set. The average UK intake is significantly less than the recommended nutritional intake (in the range 29 to 39 µg per day) but this figure includes children who have lower requirements (Stahl *et al.,* 2002).

The bioavailability of selenium is largely dependent on the mass of the element available through the diet. The form of selenium encountered in the diet influences absorption. In animal experiments, over 90% of both selenomethionine and selenite was absorbed from a variety of foodstuffs (Sunde, 1997; Vendeland *et*

al., 1992). In gut sac experiments, selenomethionine was absorbed against a concentration gradient by an energy-dependent process. This process is competitive with methionine, and clearly the seleno-amino acid uses the same pathway(s) as methionine. Selenate (Se VI) and sulphate have similar structures and are absorbed by an active process. On the other hand, selenite (Se IV) is absorbed *via* a passive process, which is not affected by sulphite. Very little is known about the uptake of selenium as selenocysteine and this does not seem to be influenced by cysteine (Sunde, 1997). The seleno-amino acids (selenomethionine and selenocysteine) originate from food sources, and must be released from the digestion of proteins prior to absorption (Stahl *et al.,* 2002).

There is no evidence that this digestive process limits bioactivity of selenium in any way. In the absence of other competitive factors in the diet, it is unlikely that the chemical form of selenium has a great effect on the absolute uptake of selenium into the body. The knowledge of distribution of selenium in human tissues, though limited, shows a distribution similar to that observed in experimental in animals. Thus, selenium concentrations are the highest in liver and kidney, followed by spleen, pancreas, blood, plasma, erythrocytes, skeleton, muscle and fat. Once selenium has been distributed between different tissues, the levels of expression of the specific selenoproteins within these tissues are subject to further regulation. An important point is that to define selenium bioactivity as total tissue concentrations it is often inadequate unless these can be guaranteed to provide optimal concentrations/activities of the essential selenoproteins (Stahl *et al.,* 2002).

The mechanism for specific selenocysteine incorporation into functional proteins has been investigated in greatest detail in bacterial systems. The mechanism in eukaryotes has been derived by analogy to procaryotic mechanisms (Low and Berry, 1996).

More scientific research effort has been expended on the production of selenocysteine in mammalian cells rather than on metabolism of inorganic and organic selenium (Stahl *et al.,* 2002). Selenate is probably converted to selenite, which is then metabolised by reductive process. Selenite reacts rapidly with glutathione to form selenodiglutathione. This compound may be further reduced, and the resulting selenide methylated to form dimethylselenide and the trimethylselenonium ion. The selenide formed by these pathways may be one source of selenium for the selenophosphate produced by selenophosphate synthetase in protein synthesis.

The excretion of selenium is dependent on several factors including: (i) the absolute level of intake; (ii) the type of diet from which the selenium is absorbed; and, (iii) the form in which the selenium is absorbed. With very high doses of selenium, which approach toxic levels, a lot of selenium may be

exhaled as volatile compounds (*e.g.*, dimethylselenide and dimethyldiselenide). In urine, one form of selenium has been identified as the trimethylselenonium ion (Combs and Combs, 1986; Sunde, 1997).

The focus of this report is on the direct intake, metabolism and effects associated with sources of inorganic selenium in the environment.

A6-2.0 FRACTION ABSORBED *VIA* DIFFERENT ROUTES

A6-2.1 Oral

Several selenium compounds appear to be readily absorbed from the human gastrointestinal tract, with the degree of absorption dependent on the chemical form (*e.g.*, organic, inorganic), physical state (*e.g.*, solid, solution), and the dosing regimen (ATSDR, 2003). Generally, inorganic forms of selenium are not absorbed as well as the organic forms (U.S. EPA, 1984). It also appears that with both inorganic and organic forms of selenium, the degree of absorption is independent of the exposure level, and may be greater in individuals with a selenium deficiency (ATSDR, 2003).

Bopp *et al.,* (1982) reported that selenium compounds are generally well absorbed by humans when ingested; further, exposure to selenium compounds from dietary sources is considered to be more significant than inhalation (Weissman *et al.,* 1983). In humans, selenium absorption from the gastrointestinal tract following administration of sodium selenite, ranged from 44 to 80% (Thomson, 1974; Thomson and Stewart, 1974; Stewart *et al.,* 1978). Absorption in human subjects of an aqueous solution of sodium selenite was reported to range from 46 to 95% (Thomson, 1974; Robinson *et al.,* 1978; Thomson *et al.,* 1978). Thomson (1974) observed 90 to 95% absorption of a low dose (0.01 mg) of aqueous sodium selenite. Absorption efficiency of a larger oral dose (1.0 mg) of either sodium selenite or selenomethionine was 90 to 95, and 97%, respectively (Thomson *et al.,* 1978). Griffiths *et al.,* (1976) reported 96 to 97% absorption of a single dose of 0.002 mg selenium administered as selenomethionine in solution. Robinson *et al.,* (1978) found that 75% of selenomethionine, but only 46% of sodium selenite, was absorbed during a 10 to 11 week administration of solutions (dose of 0.0013 to 0.0023 mg/kg bw/day) to New Zealand women. Thompson and Robinson (1986) reported an apparent absorption of selenate to be 95%, compared with 62% for selenite. RAIS (2004) provides human gastrointestinal absorption factors for selenious acid, selenite, selenium, and selenium sulphide of 87, 70, 44 and 20%, respectively.

A significant source of selenium in the diet is *via* milk and milk products (cow, goat, *etc*.) Absorption of selenium is much lower in ruminants than in nonruminants. Absorption of orally administered ^{75}Se was only 34% in sheep compared with 85% in swine. In ruminants, some dietary sources of selenium compounds are thought to be converted to insoluble selenides or elemental selenium in the rumen (Spears, 2003). On the other hand, most studies that have examined the bioavailability of selenium as inorganic selenite (IV) or selenate (VI) and as organic selenomethionine have shown similar results for absorption in ruminants. The predominant form of selenium in feed and forage is as organic selenomethionine. Seleno-methionine available from the diet is incorporated nonspecifically into proteins in place of

methionine. Organic selenium fed to ruminants as selenized yeast (rich in selanoamino acids) results in higher blood and milk concentrations of selenium than when similar doses of selenium are provided as inorganic selenite (IV).

Both the bioavailability of selenium as reflected in absorption and retention of inorganic and organic selenium compounds from dietary sources has been examined in human subjects by Fox *et al*. (2004). Selenium intake from fish was 87.7 to 90.4 %. The retention of absorbed selenium from fish test meals ranged from 85.1 to 86.2%. By contrast, only 53.5% of selenium in yeast preparations was absorbed, and only 59.3% of this dose was retained. The great majority of the selenium compounds found in foods are of organic origin (selenoamino acids including selenocysteine).

Experimental animals also appear to efficiently absorb selenium when administered by the oral route. Absorption efficiencies of 80 to 100% have been reported for rats administered selenium as sodium selenite, sodium selenate, selenomethionine, and selenocystine (Furchner *et al.,* 1975; Thomson and Stewart, 1973). Furchner *et al.* (1975) also reported that greater than 90% of an oral dose of selenious acid was absorbed in mice and dogs. However, an oral study with beagle dogs found that only 9.5% of administered selenium was absorbed in the gut (Weissmann *et al.,* 1983). Hodson and Hilton (1983) reported that uptake of selenium from food and water is more efficient at low water-borne and dietary concentrations than at high concentrations.

A number of recent studies have examined oral bioavailability of selenium from milk-based products, infant formulae, as well as well as egg yolk and chicken meat. The absorption efficiencies were found to range from 61 to 97% (Chen *et al.,* 2004; Van Dael *et al.,* 2002). Fox *et al.,* (2004) found that selenium intake from fish was 87.7 to 90.4 %. Most of the selenium compounds that occur in foods are organic. The retention of inorganic selenium is poor relative to organic selenium compounds (Fox *et al.,* 2004).

Thus, the form (organic or inorganic) may play a significant role in the retention of selenium compounds in human tissues.

A6-2.2 Inhalation

Selenium absorption in humans following inhalation exposure has only been studied in occupational settings. While these studies indicate that humans absorb selenium compounds in the lungs, no quantitative data are available. Weissman *et al.* (1983) reported that 20% of selenium inhaled as metal aerosols was deposited in the lungs and upper respiratory tract of beagle dogs. Of the deposited selenium, 80% was absorbed in the lungs within two hours. Thus, 16% of the administered dose was absorbed in the lungs. These authors also reported that 97% of selenious acid retained in the lungs was absorbed within two hours.

The rate of selenium absorption from the inhalation route depends on the chemical form, with absorption of selenious acid aerosols occurring approximately twice as rapid as absorption of elemental selenium aerosols (Weissman *et al.,* 1983; Medinsky *et al.,* 1981).

A6-2.3 Dermal

Users of shampoos containing 1% selenium disulphide have shown no dermal uptake (ATSDR, 2003; NAS, 1980). Dermal absorption was not observed in women tested with a 0.05% L-selenomethionine lotion, though the authors concluded that absorption might occur at higher doses (maximum dose in this study was 0.0029 mg/kg) (Burke *et al.,* 1992). Mice treated with 0.02% selenomethionine lotion three times a week for 30 weeks (0.29 mg/kg/day) showed significantly higher concentrations of selenium in the liver and in skin away from the application site than controls (Burke *et al.,* 1992). In rats, 9 to 27% of dermally applied ^{75}Se-selenious acid was absorbed (Medinsky *et al.,* 1981). The Massachusetts Department of Environmental Protection (MDEP, 1994), the Ontario Ministry of the Environment (MOE, 1996) and the Canadian Council of Ministers of the Environment (CCME, 2002) assume a generic dermal absorption rate for metals of 0.2% for exposure from contaminated soil on the skin. RAIS (2004) provides a human dermal absorption factor of 1% for selenious acid and selenite, and a factor of 0.1% for selenium and selenium sulfide.

A6-3.0 TOXICOLOGY

It is generally believed that only the soluble forms of selenium are capable of causing toxicity, as they are absorbed most readily (ATSDR, 2003). A number of studies have shown that soluble selenium compounds are the most toxic and that these compounds tend to exert a cumulative toxicity over time (ATSDR, 2003). There is general agreement that sodium selenite, sodium selenate, selenomethionine and selenoglutathione are among the more toxic species (Combs and Combs, 1986). The specific mechanisms by which selenium compounds produce toxicity are not well understood. Evidence that selenium can replace sulfur in biological macromolecules (especially when the Se: S ratio is high), is thought to be a possible mechanism for chronic selenium toxicity (Stadtman 1983; Tarantal *et al.,* 1991).

It should be noted however, that many manifestations of selenium toxicity are remarkably similar to those observed in cases of selenium deficiency. This complicates the association of selenium levels in various media with adverse health effects.

A6-3.1 Essentiality of Selenium

There is widespread scientific consensus that selenium is an essential trace element in both animal and human nutrition (NAS, 1976; Bennett, 1982; WHO, 1986; Levander, 1982; Robinson, 1982; Foster and Sumar, 1997). Selenium has even been reported to be an essential trace element in all vertebrates (Bowen, 1979).

Selenium deficiency in isolation seldom causes overt illness; however it leads to biochemical changes that predispose selenium-deficient individuals to illness associated with other stresses. (NAS, 2000). A deficiency of selenium in the human diet is associated with Keshan disease (juvenile cardiomyopathy endemic to certain areas of China) and Kashin-Beck disease (osteoarthropathy endemic to Eastern Siberia) as well as numerous other diseases, conditions and effects (Chen *et al.,* 1980; Sokoloff, 1985; Wu *et al.,* 1987).

Selenium is a rather unique element in that there is a small margin of safety (ranging from a factor of approximately 5 to 18 between levels of selenium compounds that constitute dietary deficiency and those that result in toxicity (Lemly, 1997). The U.S. National Academy of Sciences (NAS, 2000) has recommended a safe and adequate daily intakes ranging from 20 to 70 µg per person per day for adults (Table A.1). The estimated average requirements for selenium were extrapolated from human balance studies and laboratory animal studies (NAS, 2000). The RDAs for selenium of 70 µg/day for adult men

and 70 µg/day for adult women were based on a daily dose 0.87 µg/kg bw/day derived from a series of depletion studies carried out in Chinese males (Yang *et al.,* 1989a,b; Yang *et al.,* 1988; Levander, 1991). RDAs for children and infants were extrapolated from the adult RDAs on the basis of body weight. For children, the RDA is set at 0.87 µg/kg bw/day (NAS, 2000). Selenium dietary requirements for pregnant or lactating mothers are greater, with RDAs of 60 and 70 µg/day respectively (NAS, 2000). The dietary requirement below which adverse human health effects resulting from deficiency may occur has been tentatively estimated to range from 2 to 120 µg/day (Stewart *et al.,* 1978). Whanger *et al.,* (1996) suggest that an intake of less than 40 µg/day will likely result in deficiency. The minimum dose to cause toxicity in humans is not well defined, but the threshold appears to lie in the range of 400 to 900 µg/day (Allegrini *et al.,* 1985; Yang *et al.,* 1989a; 1989b; Longnecker *et al.,* 1991; Whanger *et al.,* 1996).

Table A6.1 Recommended Allowable Intakes for Selenium[a]

Age	Intake/day AI or EAR[b]	RDA[b]
Selenium AI: Summary, Ages 0 through 12 months		
0 to 6 months	15 µg (0.19 µmol)/day	≈ 2.1 µg/kg
7 to 12 months	20 µg (0.25 µmol)/day	≈ 2.2 µg/kg
Selenium EAR/RDA: Children and Adolescents ages 1 through 18 years		
Children 1 to 3 years	17 µg (0.22 µmol)/day	RDA = 20 µg/day
Children 4 to 8 yrs	23 µg (0.29 µmol)/day	RDA = 30 µg/day
Boys and Girls 9 to 13 yrs	35 µg (0.45 µmol)/day	RDA = 40 µg/day
Boys and Girls 14 to 18 yrs	45 µg (0.57 µmol)/day	RDA = 55 µg/day
Selenium EAR/RDA: Adults 19 through 50 yrs		
Men and women 19 to 30 yrs	45 µg (0.57 µmol)/day	RDA = 55 µg/day
Men and Women 31 to 50 yrs	45 µg (0.57 µmol)/day	RDA = 55 µg/day
Selenium EAR/RDA: Adults 19 through 50 yrs		
Men and Women 51 to 70	45 µg (0.57 µmol)/day	RDA = 55 µg/day
Men and women >70 yrs	45 µg (0.57 µmol)/day	RDA = 55 µg/day
Selenium EAR/RDA: Women during and after pregnancy		
Pregnant women 14 to 18 yrs	49 µg (0.62 µmol)/day	RDA = 60 µg/day
Pregnant women 19 to 30 yrs	49 µg (0.62 µmol)/day	RDA = 60 µg/day
Pregnant women 31 to 50 yrs	49 µg (0.62µmol)/day	RDA = 60 µg/day
Lactating women 14 to 18 yrs	59 µg (0.75µmol)/day	RDA = 70 µg/day
Lactating women 19 to 30 yrs	59 µg (0.75µmol)/day	RDA = 70 µg/day
Lactating women 31 to 50 yrs	59 µg (0.75µmol)/day	RDA = 70 µg/day

[a] Adapted from NAS, 2000.
[b] EAR = estimated average requirement; RDA = recommended dietary allowance.

Various studies have indicated that supplementary selenium relieves a number of human health problems. These include muscular discomfort, cardiomyopathy, arthritis, cataracts, cystic fibrosis, hemolytic anemia, multiple sclerosis, Kwashiorkor (a protein-calorie malnutrition), night blindness, and immunodeficiencies (Foster and Sumar, 1997; van Rij *et al.,* 1979; Johnson *et al.,* 1981). Furthermore, selenium is an essential component of glutathione peroxidase (GSHPx), an enzyme which protects cell membranes from oxidative damage, and type 1 iodothyronine 5`-deiodinase, an enzyme which interacts with iodine to prevent abnormal thyroid hormone metabolism (Foster and Sumar, 1997).

Selenium is also believed to have a protective function against certain types of cancers (Foster and Sumar, 1997). Levander (1987) hypothesized that the "anti-cancer" protective effects of selenium are due to its roles in alleviating oxidative damage, altering carcinogen metabolism, and selective toxicity against rapidly dividing tumour cells. It should be noted that there is conflicting evidence with respect to this function of selenium. Nonetheless, relatively high levels of selenium have been used successfully to protect against both chemically-induced and spontaneously occurring tumours in laboratory animals (Combs and Combs, 1986; Ip and Ganther, 1992; Whanger, 1983). Selenium supplementation has also been shown to significantly inhibit tumours induced by viruses, or ultraviolet radiation (ATSDR, 2003). Methylated forms of selenium appear to be the most important with respect to cancer prevention.

A6-3.2 Systemic Toxicity

A6-3.2.1 Human Studies

An epidemiological study by Yang *et al.* (1983) reported selenium intoxication and deleterious effects in residents in China. The symptoms of selenosis were characterized by loss of hair and nails, skin lesions, tooth decay and some irregularities of the nervous system. Significant correlations were produced between the incidence of selenosis and concentrations of selenium in the hair, blood and urine of exposed persons and in the vegetable crops grown in the area and consumed by the people. By comparing the estimated daily intake of selenium for those in selenium-deficient areas who exhibited deficiency symptoms with those in high selenium areas exhibiting selenosis, a range representing the minimum required intake versus the toxic intake could be determined. The minimum required level was determined to be 0.003 or 0.04 µg/kg/day in a 70 kg individual. The dose resulting in selenosis was 3.2 or 46 µg/kg/day in a 70 kg individual.

In a follow-up study, Yang *et al*. (1989a,b) studied approximately 400 individuals, living in areas of China with high environmental concentrations of selenium. These individuals were compared with individuals living in areas with low to medium selenium soil and food levels. A positive correlation was observed between the concentrations of selenium in soil and food, and those in blood and tissues. The daily average intake of selenium, based on lifetime exposures, were 70, 195 and 1,438 µg for adult males and 62, 198 and 1,238 µg for adult females in the low-, medium- and high-selenium areas, respectively. Selenosis was observed in only 5/349 adults who had blood selenium concentrations ranging from 1.054 to 1.854 with a mean of 1.346 mg/L. Clinical signs of selenosis included garlic odour breath, thickened and brittle nails, hair and nail loss, reduced hemoglobin, mottled teeth, skin lesions, and central nervous system abnormalities (*e.g.,* limb pain, peripheral anaesthesia). No clinical signs of selenosis were found in the next lowest blood selenium concentration of 1.0 mg/L, which corresponds to a selenium intake of 853 µg Se/day (Yang *et al.,* 1989a,b). Based on the blood selenium concentrations that were shown to reflect clinical signs of selenosis, the authors concluded that a whole blood selenium concentration of 1.35 mg/L, which corresponds to a daily intake of 1,261 µg/day, is indicative of the lowest selenium intake causing overt signs of selenium toxicity. The authors also concluded that a whole blood selenium concentration of 1.0 mg/L, corresponding to a daily intake of 853 µg/day, produced no clinical signs of selenosis. Therefore, the NOAEL and LOAEL values from this study were estimated to be 15 and 23 µg/kg bw/day, respectively, assuming an average adult body weight of 55 kg (U.S. EPA, 1991).

Longnecker *et al*. (1991) observed results similar to those of Yang *et al*. (1989a,b). Volunteers (142) from regions of Wyoming and South Dakota known to be elevated in selenium responded to questionnaires, were given physical examinations, and submitted blood, hair and urine samples for analysis. The average daily selenium intake was estimated at 239 µg/day. High correlations were observed between selenium concentrations in blood, urine, toenails and diet. Blood selenium concentrations correlated highly with daily intake, which is consistent with what Yang *et al.* (1989a,b) reported. No signs of selenium toxicity were observed in this population, including individuals for which the daily selenium intake was as high as 724 µg/day. The results of this study corroborate those of Yang *et al.* (1989a,b), who reported a NOAEL of 853 µg/day.

A number of studies of the effects of selenium compounds on thyroid hormone status have been conducted. Brätter and Negretti De Brätter (1996) examined the thyroid hormone levels in lactating women. Among women who resided in areas of Venezuela with high soil selenium concentrations (estimated selenium intake 250 to 980 µg/day) these authors found a significant decrease (but still within the normal range) in serum 3,5,3'-triiodothyronine (T3) levels as compared with women having normal

selenium intakes (90 to 350 µg/day). There was no correlation between selenium intake and levels of thyroxine (T4) or thyroid stimulating hormone (TSH).

New Zealanders given 10, 20, 30 or 40 µg/day selenium supplements for 20 weeks had reduced serum T4 concentrations, but thyroglobulin concentration did not change significantly (Duffield *et al.,* 1999). Hagmar *et al*. (1998) found a significant inverse correlation between serum selenium concentration and TSH but not T3 or T4 in a study of male Latvian fish consumers. Oral administration of selenomethionine to normal schoolchildren, and to children suffering from thyroid deficiency (cretin) for a period of two months resulted in decreased levels of T4 thyroid hormone for both groups of children (Contempre *et al.,* 1991). The authors concluded that selenomethionine increases sensitivity to iodine deficiency.

Vinceti *et al*. (1996) reported an increased incidence of amyotrophic lateral sclerosis (standardized incidence ratio = 4.14), among a cohort exposed to drinking water with high selenium content. Amyotrophic lateral sclerosis is a human motor neuron disease that occurred with increased frequency among residents from the surrounding area with a lower drinking water content of selenium (<1 µg/L).

No reliable human studies were identified that investigated the toxicology of selenium compounds *via* the inhalation route, and no human studies were identified that investigated the toxicology of selenium compounds *via* the dermal route of exposure.

A6-3.2.2 Animal Studies

A table summarizing the toxicology of selenium in a large number of studies is presented in Table A.2. These studies are described in more detail below.

Table A6.2 Animal Studies for Adverse Effects of Selenium Intake

ANIMAL Selenium (IV or VI)	Route	Duration	Dose Range	Immunological	Hormonal	Cardio-vascular	Liver	Lung	Renal	Weight	NOAEL/ EFFECT mg/kg-bw/day	Reference
Rat (VI)	dw	13 wk	0-60 mg/L	-	-	-			+ >7.5g/L	↓ 30 mg/L	0.4	NTP 1994
Rat (VI)	dw	144 wk	0.5 mg/kg/d				+	-				Nelson *et al.*, 1943
Rat (VI) Na_2SeO_4	dw	6 wk	2 ppm							+ (↓wt gain)		Palmer and Olson, 1974
Rat (VI) Na_2Se or Na_2SeO_4	Diet	5 wk	10 ppm				+ (↓wt)			+ (↓wt gain)		Dausch and Fullerton, 1993
Rat (IV)	dw	13 wk	0-60 mg/L	-	-	-			+ all	↓ 32 mg/L	0.4	NTP 1994
Rat (IV)	Diet						+ > 8mg/kg				0.04	Halverson *et al.*, 1966
Rat (IV)	dw	10 wk		↓ Ab	↓ prostaglandin						0.75 (LOAEL)	Koller *et al.*, 1986
Rat (IV)	dw	8 wk	0.28 mg/kg/d	-	-	-	-	-	-		0.28 mortality LOAEL	Schroeder and Mitchener, 1971
Rat (IV) Na_2SeO_3	dw	6 wk	2 ppm							+ (↓wt gain)		Palmer and Olson, 1974
Rat (IV) SeS_2	gavage	13 wk	31.6 mg/kg/d				+	-	-		17.6	NTP 1980c
Rat (IV) Na_2SeO_3 or SeS_2	Diet	5	10 ppm				+ (↓wt)					Dausch and Fullerton, 1993
Rat (IV) Na_2SeO_3	Diet	12 to 14 wk	0.324 mg/kg/d			+	+					Turan *et al.*, 1999a
Rat (IV) Na_2SeO_3	Diet	12 wk	0.002 or 0.005 mg/kg/d				+					Kolodziejczyk *et al.*, 2000
Rat (IV) Na_2SeO_3	Diet	6	0.009 mg/kg/d		↑ TSH							Hotz *et al.*, 1997
Mouse (VI)	dw	13 wk	0-32 mg/L	-	-	-			-	↓ 32 mg/L	0.9	NTP 1994
Mouse (VI)	w	144 wk	0.57 mg/kg/d			+		+	+			Schroeder and Mitchener, 1972
Mouse Selenomethionine	dw		1.36 mg/kg/d				-		-			Johnson *et al.*, 2000

Table A6.2 Animal Studies for Adverse Effects of Selenium Intake

ANIMAL Selenium (IV or VI)	Route	Duration	Dose Range	Immunological	Hormonal	Cardio-vascular	Liver	Lung	Renal	Weight	NOAEL/ EFFECT mg/kg-bw/day	Reference
Mouse (IV) Na_2SeO_3	dw	2 wk	0.82 mg/kg/d	+			+ (↓ wt)		+ (↑ wt)			Johnson *et al.*, 2000
Mouse (IV)	dw	13 wk	0-32 mg/L	-	-	-			-	↓ 30 mg/L	0.8	NTP 1994
Mouse (IV) Na_2SeO_3	dw	50 wk	0-8 mg/L								↓ growth at 8 mg/L	Jocob and Forst, 1981
Mouse (IVor VI)	dw	8 wk	0.57 mg/kg/d	+	+	+	+	+	+	-	0.57 (LOAEL)	Schroeder and Mitchener, 1971
Mouse (IV) Na_2SeO_3	Diet	12 wk	0.2 mg/kg				+					Skowerski *et al*, 1997
Mouse (IV) Na_2SeO_3	Diet	12 wk	0.2 mg/kg			+						Kolodziejczyk *et al.*, 2000
Mouse (IV) SeS_2	gavage	13 wk	464 mg/kg/d				+	-	-		216	NTP 1980c
Hamster (IV)	Diet	144 wk	0.42 mg/kg/d				-		+		NOAEL 0.42	Birt, 1986
Hamster (IV) Na_2SeO_3	Diet	6 wk	1-20 ppm				+					Beems and van Beek, 1985
Rabbit (IV) Na_2SeO_3	Diet	12 wk	0.137 mg/kg/d			+						Turan *et al.*, 1999b
Pig *via* Se in plant material	Diet	5 day	1.25 mg/kg/d								paralysis	Panter *et al.*, 1996
Pig (VI) Na_2SeO_3 or selenomethionine	Diet	5 day	1.25 mg/kg/d								Dermal, no neurological	Panter *et al.*, 1996

A6-3.2.2.1 Drinking Water Exposure Studies

Male and female F344/N rats and B6C3F1 mice received oral doses sodium selenite and sodium selenate in drinking water for 13 weeks (NTP, 1994). In the selenate group, 10 male and 10 female rats and mice received 0, 3.75, 7.5, 15, 30, or 60 mg/L. The selenite group of 10 male and female rats and mice received 0, 2, 4, 8, 16, or 32 mg/L. All rats exposed to 60 mg/L selenate died, while two female rats exposed to 32 mg/L selenite died. Rats and mice exposed to 30 mg/L selenate and 32 mg/L selenite had decreased body weights. Rat and mouse water consumption decreased as the dose levels increased. Selenate concentrations greater than 7.5 mg/L, and all tested selenite concentrations were associated with an increased incidence of renal papillary degeneration in rats. No lesions were observed in mice as a result of selenate or selenite administration. Increases in estrous cycle length were also observed in female rats and mice during this study. A NOAEL of 0.4 mg/kg bw/day in rats for both selenate and selenite (based on 15 and 16 mg/L, respectively), was estimated, and based upon a consideration of all observed effects. In mice, a NOAEL of 0.8 and 0.9 mg/kg bw/day was estimated for selenate and selenite, respectively, based on body weight depression and decreased water consumption.

Koller *et al.* (1986) reported that rats administered sodium selenite in drinking water at a dose of 0.75 mg/kg bw/day, for 10 weeks, displayed a reduction in humoral antibody production in response to an administered antigen. Reduced prostaglandin synthesis was also observed. At lower doses (0.075 and 0.28 mg/kg bw/day), natural killer cell (NKC) cytotoxicity was enhanced, while prostaglandin activity and delayed-type hypersensitivity were reduced. The authors reported a LOAEL of 0.75 mg/kg bw/day for reduced humoral antibody production. The authors did not identify a NOAEL due to the conflicting effects of enhanced NKC activity occurring at the same dose level as reduced prostaglandin activity and delayed-type hypersensitivity.

Administration of sodium selenite in drinking water at a dose of 0.28 mg/kg bw/day for 58 days resulted in the death of 25 to 50 male rats (Schroeder and Mitchener, 1971). There was no increase in mortality for female rats receiving the same dose over the same duration. These authors also noted that the te-travalent form of selenium (*i.e.,* selenite), was more toxic in male rats than the hexavalent selenate. However, this observation did not hold true in female rats (Schroeder and Mitchener, 1971). Schroeder and Mitchener (1972) reported a LOAEL for respiratory, cardiovascular, hepatic, renal, endocrine and dermal effects in mice of 0.57 mg/kg bw/day for both selenate and selenite.

Rosenfeld and Beath (1954) found that in rats receiving a dose of 1.05 mg/kg bw/day in drinking water as potassium selenate for eight months, no mortalities occurred. In contrast, decreased survival was reported in rats fed sodium selenate or selenite at a dose of 0.4 mg/kg bw/day for two years (Harr *et al.,* 1967;

Tinsley *et al.,* 1967). At a dose of 0.25 mg/kg bw/day, hepatitis was commonly observed, with liver lesions occurring at doses as low as 0.10 mg/kg bw/day. In addition, liver weights showed a dose-dependent decrease with increasing levels of selenate or selenite in the diet (Harr *et al.,* 1967; Tinsley *et al.,* 1967). Harr *et al.* (1967) also reported that sodium selenate or selenite caused softening of bones in rats at doses as low as 0.2 mg/kg bw/day.

Lifetime exposure of mice to sodium selenate in drinking water at 0.57 mg/kg bw/day resulted in amyloidosis of the lung, heart, and kidney in some animals (Schroeder and Mitchener, 1972). In an early study, Nelson *et al.* (1943) had previously reported that no effects on lungs occurred in rats administered 0.50 mg/kg bw/day in drinking water for two years, although doses ranging from 0.25 to 0.5 mg/kg bw/day were noted to produce cirrhosis of the liver in rats.

Mice given drinking water that contained 9 ppm selenium (0.82 mg selenium/kg/day) as sodium selenite for 14 days had significantly increased red blood cell counts, significantly decreased relative liver weight, and signs suggestive of reduced immunological responses (Johnson *et al.,* 2000). Effects at the 9 ppm level may have resulted from a severe reduction in food and water consumption. No effects on haematology, drinking water consumption or liver weight were observed at the 3 ppm dose level; however, relative kidney weights were increased at this dose level. No effects on hematology, drinking water consumption, kidney or liver weight were observed at doses of up to 1.36 mg selenium/kg bw/day as selenomethionine.

Mice treated with 0, 1, 4, or 8 mg/L Na_2SeO_3 in drinking water over 50 weeks showed decreased growth rates at 8 ppm (Jacobs and Forst, 1981).

A6-3.2.2.2 Dietary Exposure Studies

Post-weaning Sprague-Dawley rats were fed selenite or seleniferous wheat *ad libitum* at dietary dose levels of 1.6, 3.2, 4.8, 6.4, 8.0, 9.6, and 11.2 mg/kg in a subchronic study (Halverson *et al.,* 1966). A NOAEL of 4.8 mg/kg (40 µg/kg bw/day) was observed. At selenium dietary concentrations greater than 8.0 mg/kg, effects such as decreased liver and spleen weights, and reduced hemoglobin were observed. Mortality was observed in the three highest dose groups fed both selenite and seleniferous wheat.

Mortality was 100% in the group fed wheat with a selenium concentration of 11.2 mg/kg. In addition, significant growth reduction was observed for animals fed both selenite and wheat containing 6.4 mg/kg selenium or higher.

Rats fed 0.324 mg selenium/kg/day as sodium selenite in food for 12 to14 weeks showed several diffuse degenerative cardiovascular changes and degenerative changes to the liver (Turan *et al.,* 1999c). Degenerative changes indicating disintegration of the internal structure of the myocytes were observed in the hearts of rabbits fed 0.137 mg selenium/kg/day as sodium selenite in food for 3 months (Turan *et al.,* 1999c).

Salbe and Levander (1990) administered selenium to rats either in the organic form as selenomethionine or in the inorganic form as sodium selenate. Rats given a diet deficient in methionine had a decrease in final body weight, with the most significant decrease observed in rats fed inorganic sodium selenate. A NOAEL for reduced body weight of 0.125 mg/kg bw/day of selenium as selenate was identified.

Hotz *et al*. (1997) exposed weanling rats to 0.009 mg selenium/kg bw/day as sodium selenite in food for 6 weeks and observed a significant increase in serum concentrations of the thyroid hormone TSH.

Skowerski *et al.* (1997) observed hepatic damage in mice exposed to 0.2 mg selenium/kg bw/day as sodium selenite in food for 12 weeks. Observed liver damage that increased with dose in rats fed 0.002 or 0.005 mg selenium/kg bw/day as sodium selenite in food for three months. Mice exposed to 0.2 mg selenium/kg/day as sodium selenite in food for 12 weeks also exhibited cardiac damage.

A study with hamsters found no mortality following the dietary administration of sodium selenite at a dose of 0.42 mg/kg bw/day for 122 to 144 weeks (Birt, 1986).

Hamsters treated with 0.1 (unsupplemented), 1, 5, 10, or 20 ppm Na_2SeO_3 in the diet for 42 days showed histopathological changes to the liver (Beems and van Beek, 1985).Rats treated in the diet with SeS_2, Na_2Se, Na_2SeO_3, or Na_2SeO_4 showed increased relative liver weights and/or decreased body weight gain at 10 ppm (for each compound tested) over a 5 week exposure period (Dausch and Fullerton, 1993). Decreased body weights were observed in rats treated for 6 weeks in drinking water with 2 ppm Na_2SeO_3 or Na_2SeO_4 (Palmer and Olson, 1974).

A commonly observed finding in livestock that graze in selenium-enriched areas is the development of "blind staggers". This condition is characterized by impaired vision, aimless wandering behaviour, reduced consumption of food and water, paralysis and death (Rosenfeld and Beath, 1964 and Shamberger,

1986). Panter *et al*. (1996) found that pigs fed 1.25 mg selenium/kg bw/day in the selenium accumulator plant, *A. bisulcatus,* experienced severe paralysis within five days. In pigs fed similar doses of sodium selenate or D,L-selenomethionine, some dermal but no neurological effects were observed. Interestingly, studies in which cattle were treated with known amounts of selenium have not produced similar neurological effects, nor have studies with laboratory animals produced symptoms similar to blind staggers. However, there is a neurological syndrome associated with chronic selenosis in human studies (ATSDR, 2003). In the report prepared by the ATSDR (2003), it was speculated that: 1) small laboratory mammals may not be an appropriate model for selenium toxicity in humans; 2) some unidentified organic form of selenium contributes to the neurological effects associated with chronic selenosis in humans and grazing livestock; or, three) unrecognized confounding factors have contributed to the observed neurological effects.

A study by NTP (1980c) found no respiratory or musculoskeletal effects in mice and rats administered selenium sulfide at doses of 464 and 31.6 mg/kg bw/day, by gavage, once daily for 13 weeks. There were also no gastrointestinal and renal effects in rats at the 31.6 mg/kg bw/day dose level; however, rats displayed liver necrosis at this dose. Liver effects in rats were not observed at a lower dose of 17.6 mg/kg bw/day. No liver effects were observed in mice at 464 mg/kg bw/day but an increased incidence of interstitial nephritis was observed at this dose level. A dose of 216 mg/kg bw/day produced no renal effects in mice.

No studies investigating health effects in animals following subchronic or chronic inhalation or dermal exposure to selenium or selenium compounds were identified in the available scientific literature. In general, the available subchronic and chronic oral animal bioassays with selenium compounds are inadequate for determining reliable NOAEL or LOAEL values, as the available studies used single doses, relatively short study durations, and had generally poor characterization of effects at various dose levels. In addition, some studies showed no effects at none or all of the dose levels tested, which makes it difficult to establish a dose-response relationship.

A6-3.3 Reproductive/Developmental

A6-3.3.1 Human Reproductive Studies

Few studies were identified in the literature that relate to either reproductive or developmental effects in humans from exposure to selenium compounds. Roy *et al.* (1990) studied 211 men but found no correlation between the concentration of Se in seminal plasma and lowered sperm counts or decreased

mobility in sperm samples. Robertson (1970) and Zierler *et al.* (1988) found no association between prenatal exposure to selenium compounds and birth defects or developmental abnormalities.

Vinceti *et al*. (2000) reported a non-significant increase in spontaneous abortions (RR=1.75) among births within a cohort exposed to high selenium levels in drinking water (7 µg/L). Similar body weights and lengths were recorded for infants of exposed and unexposed women, and there was no significant increase in congenital abnormalities in the exposed group.

Overall, while data are limited, there appears to be no human evidence of reproductive effects, teratogenesis, or developmental abnormalities following selenium exposure.

Animal Reproductive Studies

Rosenfeld and Beath (1954) exposed rats for two generations to 1.5, 2.5 or 7.5 mg/L of potassium selenate in drinking water. No adverse effects on reproduction were observed in rats exposed to 1.5 m/L. In the 2.5 mg/L group, there was a 50% reduction in the fecundity of females, and fertility. Juvenile growth and survival were adversely affected in the 7.5 mg/L group (Rosenfeld and Beath 1954). A NOAEL of 0.20 mg/kg bw/day and a LOAEL of 0.33 mg/kg bw/day is identified from this study by Sample *et al.* (1996) using a water consumption rate of 0.046 L/day and a body weight of 0.35 kg.

A short-term reproductive study in female rats reported some reproductive toxicity following exposure to sodium selenate in drinking water, but only at doses which also produced signs of severe maternal toxicity (*i.e.,* 0.418 mg selenium/kg bw/day) (NTP, 1996).

In a study by Lijinsky *et al.* (1989), young rats (7 weeks of age) were exposed for 28 weeks to 0, 1.4 and 2.1 mg/kg sodium selenite in the diet. At the 1.4 mg/kg dose, rats displayed slower growth rates and reached a smaller maximum weight; however, the magnitude and statistical significance of this effect was not documented in this study.

A three-generation study of mice exposed to a single dose of selenate in both their food and drinking water (0.056 mg/kg and 3 mg/L, respectively), demonstrated an inhibition of reproductive success and a failure to breed (Schroeder and Mitchener, 1971). A significant increase in deaths of young was noted in the F1 generation, and the number of runts increased in generations F1 through F3. The number of breeding events decreased by the F3 generation.

Mice exposed to concentrations of 0.9, 3 or 6 mg/L sodium selenite in drinking water for 30 days prior to reproduction, and through day 18 of gestation, showed no adverse effects on reproduction. No significant differences between treated and control animals were observed with respect to fertility, number of litters, total implants, number of implants per dam, foetal and embryonic mortality, resorptions, litter size, and gross or skeletal malformations (Nobunga *et al.,* 1979). The only significant effect observed was reduced body weight in surviving fetuses of mice administered 6 mg/L.

Tarantal *et al.* (1991) reported no adverse effects in 40 long-tailed macaques exposed to 0.003 or 0.025 mg/kg bw/day organic selenium (L-selenomethionine) by nasogastric intubation for 30 days during gestational days 20 to 50. Foetal mortality and maternal toxicity were observed in macaques exposed to

0.15 and 0.3 mg/kg bw/day for the same duration. However, these effects occurred within the range observed among the macaque colony at large; thus, they could not be attributed to the selenium treatment. Overall, there were no significant differences in pregnancy loss between treated and control animals. In addition, no statistically significant treatment-related effects were observed upon necropsy at gestational day 100. The authors concluded there was no evidence of any significant foetal developmental, teratogenic, or maternal effects at any of the doses tested.

El-Zarkouny *et al*. (1999) observed a significant reduction in serum testosterone levels, as well as a non-significant increase in spermatozoa without an acrosome in male rabbits that received 0.3 mg selenium/kg bw/day as sodium selenite by gavage once a week for 6 weeks.

Testicular atrophy was experienced in male rats given 0.234 mg selenium/kg bw/day as sodium selenite in water (Turan *et al.,* 1999c). A decreased percentage of live spermatozoa, altered sperm morphology, and decreased body weight-gain were observed in rats treated for five weeks with 2 ppm $Na_2 SeO_3$ in the diet (Kaur and Parshad, 1994). Nebbia *et al.* (1987) found that rats exposed to 0, 4, 8, or 16 ppm $Na_2 SeO_3$ in drinking water for 240 days showed alterations in testicular LDH and β-glucuronidase activity at the 4 ppm level.

In a study to examine the teratogenic potential of selenium in Syrian hamsters, embryotoxicity was reported after oral gavage during gestation with several selenium compounds including sodium selenite, sodium selenate and selenomethionine (Ferm *et al.,* 1990). The oral LOAELs for developmental effects were 7.9, 7.1 and 5.9 mg/kg bw/day for selenite, selenate and selenomethionine, respectively. Foetal malformations and decreased foetal body weights and lengths were observed in the treatment groups. A strong association was reported between the effects observed in offspring and maternal toxicity. Weight

loss and severely reduced nutrient intake in the dames during gestation were confounders for clear attribution of embryotoxic effects to selenium.

A6-3.4 Carcinogenicity

Generally, carcinogenicity studies with selenates, selenites, and organic selenium compounds have shown negative results. The animal carcinogenicity database is poor and equivocal with a number of conflicting results that are difficult to interpret because of apparent anti-carcinogenic activity, high toxicity of some selenium compounds, and flawed study designs. Furthermore, comparison of the available studies is difficult because several different selenium compounds with varying degrees of bioavailability were used in the cancer bioassays (U.S. EPA, 1991).

A6-3.4.1 Human Cancer Studies

Studies investigating the relationship between selenium exposure and human cancer are conflicting and inconclusive. Few selenium-related instances of cancer have been reported in epidemiological studies, despite its widespread use in industry, livestock husbandry and some therapeutic agents (U.S. EPA, 1984).

Vinceti *et al*. (1998) observed an excess incidence of melanoma in a cohort that received an excess exposure to selenate *via* a municipal water supply. The strength of this association was limited by the fact that individual exposure levels were not measured, and individuals were classed as exposed or unexposed based on their place of residence (ATSDR, 2003). Two other epidemiology studies indicated that there is no statistically significant increase in breast cancer in women regardless of selenium dietary intake or tissue concentrations (Hunter *et al.,* 1990; van't Veer *et al.,* 1990).

Several investigators have studied the association between serum selenium and cancer risk through prospective, case-control, and nested case-control epidemiology studies. In general, patients with gastrointestinal cancer, pancreatic cancer, or Hodgkins lymphoma had significantly lower blood selenium concentrations than healthy patients (U.S. EPA, 1991). In a case-control study of lung cancer patients, Menkes *et al.,* (1986) found that the risk of lung cancer was not associated with levels of selenium in serum.

Some prospective studies have reported an association between low serum selenium levels and an increased incidence of cancer (Salonen *et al.,* 1984; 1985; Willett *et al.,* 1983). A review of breast cancer

epidemiological studies (Garland *et al.,* 1993) found reports of inverse correlations, positive correlations, or no correlation between tissue selenium concentrations and breast cancer incidence.

Epidemiological data do not support a causal association between the inhalation of elemental selenium dusts or selenium compounds and the induction of cancer in humans (Gerhardsson *et al.,* 1986; Wester *et al.,* 1981). Postmortem tissue samples in these studies showed lower concentrations of selenium compounds in lung and kidney tissues than those from controls or workers who died from non-cancer causes. It was difficult to draw a firm conclusion from this study as chronically ill and/or older individuals naturally tend to have lower organ and tissue selenium concentrations than younger, healthier individuals (Martin *et al.,* 1991).

Some studies have noted a relationship between selenium exposure and a reduction in the incidence of cancer (Shamberger and Frost, 1969; Willet *et al.,* 1983; Salonen *et al.,* 1984; 1985; Fex *et al.,* 1987; Willet and Stampfer, 1988). Moreover, low serum concentrations of selenium in men have been associated with an increase in the incidence of cancers of the stomach and lung (Knekt *et al.,* 1990). These studies support the anti-carcinogenic effect of selenium intake. A recent cohort study by Vinceti *et al.* (1995) reported that a strong inverse relationship exists between dietary intake of selenium and cancer mortality.

Shamberger and Frost (1969) reported an inverse relationship between cancer mortality rates and selenium concentrations in the food crops of several Canadian provinces. Cancer mortality rates (per 10^{-5} risk level) were found to be greatest in the provinces with the lowest amounts of selenium in food crops. In a similar study conducted in California, Shamberger and Willis (1971) reported a correlation between decreased cancer death rates and an increase in the selenium content of forage crops. The authors also investigated the ratio of observed to expected cancer mortality rates by anatomic site for men in 17 paired cities which included both seleniferous and non-seleniferous areas. Gastrointestinal cancers and bladder cancer showed substantially lower rate ratios in the "high-selenium" cities versus the "low-selenium" cities.

Clark et al. (1997; 1998) were able to demonstrate a significant reduction in overall cancer mortality with selenium exposure. The incidences of lung, colorectal and prostate cancers among patients with a history of skin carcinoma were reduced for individuals who received selenium supplements of 200 µg/day for an average of 4.5 years. Supplementation with selenium had no effect on the incidence of skin cancers. In a nested case-control study Yoshizawa *et al*. (1998) found that higher prediagnostic selenium levels were

associated with reduced prostate cancer incidence (OR = 0.35 after controlling for various confounding factors).

Other studies have not supported an anticarcinogenic effect for selenium (van den Drandt *et al.,* 1994). A study investigating the levels of selenium in toenails and susceptibility to breast cancer found no evidence for an inverse relationship. The etiology of breast cancer development may be unrelated to selenium exposure and more a matter of genetics. Establishing a mechanism to support a relationship between reductions in the frequency of breast cancers and selenium levels is improbable.

Overall, conflicting results reported for the available human epidemiological studies that correlate selenium exposure with cancer risk are difficult to interpret. This is partly due to the lack of toxicological assessment of many specific selenium compounds. Additional factors confounding epidemiological results include the flawed study designs and poor statistical support for effects reported in many studies (U.S. EPA, 1991; ATSDR, 2003). Only selenium sulfide appears to have sufficient evidence to support a carcinogenic effect. In general, the weight of available evidence for selenium compounds is suggestive of either an inverse, or a lack of association between environmental selenium concentrations and human cancer incidence and/or cancer mortality rates.

The U.S. EPA has evaluated the carcinogenicity of selenium compounds, and currently classifies these substances as D - not classifiable as to human carcinogenicity. An exception is selenium sulfide, which the U.S. EPA classifies as B2 - *probable human carcinogen* (U.S. EPA, 1991). The Tenth Annual Report on Carcinogens (NTP) classed selenium sulphide as "reasonably anticipated to be a human carcinogen" (ROC, 2002). The International Agency for Research on Cancer (IARC) last reviewed the carcinogenicity data for selenium compounds in 1987. IARC has determined that selenium compounds should be classified as Group 3 - *not classifiable as to its carcinogenicity to humans*. According to IARC (1987), the available data are insufficient to allow an evaluation of the carcinogenicity of selenium compounds in animals, and available data provide no suggestion that selenium is carcinogenic in humans (IARC, 1987). Selenium sulfide is typically not present in soils, foods or other environmental media to any significant extent, human environmental exposure to selenium sulfide would likely be negligible.

A6-3.4.2 Animal Cancer Studies

In general, there is no clear evidence for selenium carcinogenicity in experimental animals. Selenium (as selenite, selenate, or organic selenium in forage) was first reported to be a possible carcinogen in rat studies conducted by Nelson *et al.* (1943), Seifter *et al.* (1946), Tsuzuki *et al.* (1960), and Volgarev and

Tscherkes (1967). However, all these studies suffered from a number flaws including study design, short exposure durations, incomplete quantification of results, and other inconsistencies. For example, Nelson *et al.* (1943) reported tumours only in animals with cirrhotic livers, the investigators had problems discerning malignant tumours from non-malignant tumours, and a large number of animals had died of liver cirrhosis prior to the appearance of liver tumours. Volgarev and Tscherkes (1967) and Tsuzuki *et al.* (1960) used no control groups. A follow-up experiment by Volgarev and Tscherkes (1967) that utilized a control group, found no increase in tumour incidence in 100 rats administered sodium selenate in the diet at a dose of 0.22 mg/kg bw/day for 25 months.

Wistar rats fed sodium selenite or selenate at dietary concentrations ranging from 0.5 to 16 mg/kg, over their lifetime, developed no tumours (Harr *et al.,* 1967; Tinsley *et al.,* 1967). However, there were non-neoplastic liver effects, such as hepatocyte hyperplasia that were observed at dietary concentrations greater than 4 mg/kg selenium. As this study employed a large number of animals (1,437 were used in this study and 1,126 of them were necropsied), and both positive and negative controls, the negative result for carcinogenicity is considered to be a robust finding (ATSDR, 2003).

A study in Long-Evans rats administered 2 mg/L sodium selenite or selenate in drinking water for the period of a year, followed by a 3 mg/L dose rate for the remainder of the animals' lifespan (Schroeder and Mitchener, 1971). The incidence of all tumours and of malignant tumours was significantly increased in the selenate-treated rats versus controls. Selenite-treated rats showed a small number of tumours; however, this may have been due to the greater toxicity of selenite versus selenate which shortened the survival time of the selenite-treated group (U.S. EPA, 1991). This study is considered inadequate as only heart, lung, liver, kidney, and spleen tissues were examined histologically, and an increase in longevity was observed in the selenate-treated female rats (U.S. EPA, 1991). Furthermore, this study was confounded by a virulent pneumonia epidemic among the test animals (ATSDR, 2003).

A subsequent study (Schroeder and Mitchener, 1972), in which Swiss mice were administered 3 mg/L sodium selenate or selenite in drinking water showed no significant increase in total tumour, or malignant tumour incidence in treated versus control animals. Similar to what was observed in the previous experiment, longevity was increased in male and female mice administered selenate, while the selenite-treated females showed a decrease in longevity relative to control animals.

Selenium sulfide is the only selenium compound that has been found to be carcinogenic in animals. This compound is a pharmaceutical used primarily in anti-dandruff shampoos, not readily found in foods or the environment (ATSDR, 2003).

NTP (1980a) conducted a bioassay of Selsun® (selenium sulfide) for possible carcinogenicity by applying this substance dermally to ICR Swiss mice. Selsun® is an antidandruff shampoo that contains 2.5% selenium sulfide. Groups of 50 mice of each sex were exposed to 0.05 ml of 25 or 50% Selsun® in distilled water three times a week on a 2 x 3 cm clipped dorsal surface. Vehicle controls consisted of 50 mice of each sex that were clipped and treated with distilled water. Untreated controls consisted of 50 mice of each sex that were only clipped. Mean body weights of mice in all groups were comparable throughout the experiment. Amyloidosis was a factor in the deaths of most animals after one year. In males, alveolar/bronchiolar carcinomas or adenomas occurred with a significant dose-related trend. However, the incidence of the high-dose group, when compared with that of the untreated controls, was not significant. Under the conditions of this bioassay, dermal application of Selsun® was not carcinogenic for ICR Swiss mice. It was noted that this study was limited by the relatively short lifespan of this particular strain of mouse. In addition, ATSDR (2003) noted that since the dermal application sites were not covered, there may have been some ingestion of the selenium sulfide through licking and preening.

NTP (1980b) conducted a study to investigate the carcinogenicity of selenium sulfide in which a suspension of this substance was applied to the skin of ICR Swiss mice. Groups of 50 mice of each sex were treated by applying 0.5 mg or 1.0 mg selenium sulfide three times a week for 86 weeks to a clipped 2 x 3 cm dorsal surface. The selenium sulfide was suspended in 0.05 ml saline solution containing 0.5% carboxymethylcellulose. Mean body weights in all groups were comparable throughout the study. Amyloidosis was a factor in the deaths of most treated and control mice after one year, and the study was terminated after 88 weeks when the majority of animals in all dosed and control groups had died. Under the conditions of this bioassay, dermal application of selenium sulfide did not produce a carcinogenic effect in ICR Swiss mice, but as noted in the NTP (1980a) study, the relatively short lifespan of this strain of mouse limits interpretation of the study findings.

NTP (1980c) conducted a bioassay of selenium sulfide for possible carcinogenicity by administering this substance by gavage to F344 rats and B6C3F_1 mice. Groups of 50 rats and 50 mice of each sex were administered selenium sulfide suspended in 0.5% aqueous carboxymethylcellulose for seven days per week for 103 weeks at a dose of either 3 or 5 mg/kg/day for rats, and 20 or 100 mg/kg/day for mice. Vehicle controls consisted of groups of 50 rats and 50 mice of each sex administered only the 0.5% aqueous carboxymethylcellulose. Similar groups of untreated controls were also used. The significant effects that could be related to administration of selenium sulfide at the doses tested were decreased body weight and increased tumor formation in female mice, and in rats of both sexes. The rats and female mice

had an increased incidence of hepatocellular carcinomas and adenomas. Treated female mice also had an increased incidence of alveolar/bronchiolar carcinomas and adenomas. Under the conditions of this bioassay, selenium sulfide was considered carcinogenic for F344 rats and female $B6C3F_1$ mice, based on hepatocellular carcinomas in male and female rats and female mice, and alveolar/bronchiolar carcinomas and adenomas in female mice. Selenium sulfide was not found to be carcinogenic for male mice.

Lijinsky *et al*. (1989) examined the effects of 28 weeks exposure to 0, 1.4 or 2.1 mg/kg selenium as sodium selenite in the diet on tumour induction in rats. Decreased growth rates, smaller maximum weights and slightly accelerated death rates were reported in mid-dose rats. No increase in tumour incidence was observed in the selenium-exposed groups.

A6-4.0 EXPOSURE LIMITS

The following organizations were consulted to select exposure limits for selenium: the U.S. EPA; ATSDR; HC; National Academy of Science; MOE; and, OEHHA.

As selenium compounds (with the exception of selenium sulfide) are widely considered non-carcinogenic, no regulatory agencies were identified that developed health-based exposure limits based on carcinogenic endpoints.

Oral Exposure Limits

In determining the oral RfD for selenium compounds, the U.S. EPA selected the epidemiology study by Yang *et al.* (1989a,b) as the principal and supporting study. The study NOAEL of 15 µg/kg bw/day was used to calculate an oral RfD of 5 µg/kg bw/day; a three-fold safety factor was applied to account for sensitive individuals (U.S. EPA, 1991). A 10-fold safety factor was not deemed necessary because of the high level of confidence in the Yang *et al.* (1989a,b) and additional supporting studies (U.S. EPA, 1991). The results of Longnecker *et al.* (1991) strongly corroborate the NOAEL identified by Yang *et al.* (1989a,b). In addition, numerous other epidemiological studies and animal studies also support the findings of Yang *et al.* (1989a,b) (U.S EPA, 1991). In addition, the ATSDR (2003) chronic MRL for selenium compounds is also 5 µg/kg bw/day, and is based on the same endpoint (selenosis) and utilizes the same magnitude of uncertainty factor as the U.S. EPA oral RfD. Furthermore, the Health Canada (1996) oral PTDI (assuming a 70 kg individual) of 7.14 µg/kg bw/day is in close agreement with the U.S. EPA oral RfD. The National Academy of Science (IOM, 2000) has derived an acceptable Upper Limit (UL) based on the NOAEL of 800 µg/day based on Yang and Zhou (1994). Application of a 2-fold uncertainty factor results in an upper limit of approximately 5 µg/kg/day for adults. There was no evidence of increased selenium toxicity for any age group (similar sensitivity for all ages) (IOM, 2000). IOM (2000) also derived an infant specific UL, based on a study by Brätter *et al*. (1996), of 47 µg or approximately 7 µg/kg/day for two through six-month-old infants. Health Canada has indicated that in 2005/2006, the agency will officially be using adopt ULs as toxicity reference values for all essential elements (N. Roest, and S. Petrovic, Health Canada, Personal Communication) for contaminated sites human health risk assessments. In the derivation of the Guideline for Use at Contaminated Sites in Ontario (MOE, 1996), MOE utilized an oral RfD of 5 µg/kg/day for selenium.

For the purposes of this risk assessment an oral RfD/TRV of 5.00 µg/kg/day was selected for selenium (IOM, 2000; Health Canada, 2005).

Inhalation Exposure Limits

The U.S. EPA, ATSDR, and Health Canada have not developed inhalation exposure limits for selenium compounds. The Ontario Ministry of the Environment provides two health-based limits for selenium in air, a point-of-impingement limit and a 24-hour ambient air quality criterion of 20 and 10 µg/m^3, respectively (MOE, 2001). The California Environmental Protection Agency has developed a chronic Reference Exposure Level of 20 µg/m^3, for effects on the alimentary, cardiovascular and nervous systems, based on route-to-route extrapolation from the Yang *et al*. (1989a,b) study (OEHHA, 2001). The REL was derived by multiplying the U.S. EPA oral RfD of 5 µg/kg/day by an inhalation extrapolation factor of 3.5 µg/m^3 per mg/kg-day. Details of the origin of this factor were not provided in the supporting documentation from OEHHA (2001); however it can be obtained simply by dividing a default body weight of 70 kg by a default inhalation rate of 20 m^3/day. Route-to-route extrapolation assumes by default that a chemical is equally absorbed *via* both inhalation and oral routes and that the 'first pass' effect due to metabolism by the liver is not significant (OEHHA, 2001).

For the purposes of this risk assessment a chronic REL RfC of 20 µg/m^3 was selected (OEHHA, 2001).

Dermal Exposure Limits

No regulatory dermal exposure limits for selenium compounds were identified in the literature reviewed for the current assessment. In fact, for a number of chemicals, exposure limits are not always available for all exposure routes of concern. In these circumstances, exposure limits may be extrapolated from other routes. For example, it is common in human health risk assessment to assess the risks posed by dermal absorption of a chemical based on the exposure limit established for oral exposure. The systemic dose absorbed dermally is scaled to the 'equivalent' oral dose by correcting for the bioavailability of the dermally-applied chemical relative to an orally-administered dose.

The relative absorption difference between the oral and dermal routes of exposure can beexpressed as a relative absorption factor (RAFdermal). This factor, calculated as follows, isapplied to dermal exposure estimates to adjust these exposures prior to comparison with oralexposure limits when route-to-route extrapolation is necessary.

$$RAF_{dermal} = \frac{AF_{dermal}}{AF_{oral}} x\,100$$

Where:

RAF*dermal* = relative absorption factor for dermal exposure (%).

AF*dermal* = the fraction of the applied chemical absorbed through the skin.

AF*oral* = the fraction of the ingested chemical absorbed into thebloodstream.

It must be recognized however that route extrapolation is only appropriate where effects are systemic in nature, and not closely associated with the point of exposure. Further discussion of bioavailability considerations and route extrapolation is provided in Chapter 4, Section 4.2.2.

No regulatory agencies were identified that have developed health-based exposure limits for the carcinogenic selenium compound, selenium sulfide.

Toxicological Criteria Summary

Current MOE guidance discourages the development of *de novo* toxicological criteria (exposure limits). Toxicological criteria used in human health risk assessments are generally values recommended by regulatory agencies such as Health Canada, the U.S. EPA, U.S. Agency for Toxic Substances and Disease Registry (ATSDR), World Health Organization (WHO) and the MOE itself.

Table A6.3 summarizes the exposure limits selected for the current study.

Table A6.3 Summary of Toxicological Criteria Selected For The HHRA

Route of Exposure	Exposure Limit	Type of Limit	Toxicological Basis	Reference	
				Study	Regulatory
Non-cancer (Threshold) Effects					
Oral	5.0 µg/kg/day	RfD/TRV	selenosis: hair loss and nail sloughing Clinical selenosis	Yang and Zhou (1994) Yang *et al.*, 1989a,b	IOM, 2000; Health Canada, 2005 U.S. EPA, 1991
Inhalation	20 µg/m^3	Chronic REL	Hepatic, cardiovascular, neurological	Yang *et al.*, 1989a,b	OEHHA, 2001
Dermal[a]	NA		NA	NA	NA
Cancer (Non-threshold) Effects					
Oral	NA		NA	NA	NA
Inhalation	NA		NA	NA	NA
Dermal	NA		NA	NA	NA

NOTES:
NA = not available; RfD = reference dose; REL = reference exposure level; TRV = toxicity reference value.
[a] No regulatory dermal exposure limits were identified in the literature reviewed for the current assessment.

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