Health Council of the Netherlands

Arsenic and inorganic arsenic compounds

Health-based calculated occupational cancer risk values

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Arsenic and inorganic arsenic compounds*
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Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan arseen en anorganische arseenverbindingen.

Dit advies maakt deel uit van een uitgebreide reeks waarin concentratieniveaus in lucht worden afgeleid die samenhangen met een extra kans op overlijden aan kanker van 4 per 1.000 en 4 per 100.000 door beroepsmatige blootstelling. De conclusies van het genoemde advies zijn opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Dutch Expert Committee on Occupational Safety (DECOS)
a Committee of the Health Council of The Netherlands

to:

the Minister of Social Affairs and Employment

No. 2012/32, The Hague, December 11, 2012

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden op de werkplek. Deze advieswaarden vormen vervolgens de basis voor grenswaarden – vast te stellen door de minister – waarmee de gezondheid van werknemers beschermd kan worden.

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan arseen en anorganische arseenverbindingen en presenteert zij concentratieniveaus in de lucht (HBC-OCRV) die samenhangen met een extra kans op overlijden aan kanker van 4 per 1.000 en 4 per 100.000 door beroepsmatige blootstelling. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór 3 september 2012 zijn verschenen.

Fysische en chemische eigenschappen

Arseen (As; CAS nr. 7440-38-2) is een in de natuur voorkomend, grijs vast metalloïde met een molecuulgewicht van 74,92. Elementair arseen sublimeert bij 613°C, heeft een erg lage dampspanning en een log $P_{octanol/water}$ van 0,680.

Arseenverbindingen kunnen voorkomen als drie- en vijfwaardige verbindingen. Arseen trioxide (CAS nr. 1327-53-3), de belangrijkste arseenverbinding met betrekking tot blootstelling voor werkers, heeft een molecuulgewicht van 197,84, een smelttemperatuur van 312°C, een kooktemperatuur van 465°C, een erg lage dampspanning en een log $P_{octanol/water}$ van -0,310.

Gebruik

Arseen en anorganische arseenverbindingen worden gebruikt als houtconserveringsmiddelen, als bestrijdingsmiddel in de landbouw (vooral in het verleden), als droogmiddel van katoen, in halfgeleiders, bij de fabricage van glas, bij de productie van niet-ijzerhoudende metaalmengsels en als medicatie voor mensen en dieren.

Monitoring

Voor monitoring van arseen en arseenverbindingen in omgevingslucht zijn gevalideerde meetmethoden beschikbaar (NIOSH methoden 5022, 7300, 7900 en 7901 en OSHA methode ID-105). Er bestaan verschillende methodes voor biologische monitoring van arseen en arseenverbindingen, deze methoden zijn echter niet door NIOSH of OSHA gevalideerd.

Grenswaarden

Voor blootstelling aan de combinatie van alle anorganische arseenverbindingen gedurende gemiddeld een achturige werkdag (tgg 8 uur) geldt in Nederland een wettelijke grenswaarde van 0,05 mg As/m^3. Bovendien geldt in Nederland een grenswaarde van 0,1 mg As/m^3 voor kortdurende blootstelling (tgg 15 minuten) aan de combinatie van alle anorganische arseenverbindingen.

De huidige wettelijke grenswaarden voor wateroplosbare anorganische arseenverbindingen in Nederland zijn 0,025 (gemiddeld over een achturige werkdag; tgg 8 uur) en 0,05 mg As/m^3 (tgg 15 minuten). Er is momenteel geen Europese norm vastgesteld door de SCOEL. In Duitsland is een TRK (Technische Richtkonzentration) voor 8 uur en 15 minuten vastgesteld (0.1 respectievelijk 0.4 mg/m^3). In het Verenigd Koninkrijk, Denemarken en Zweden zijn grenswaarden (tgg 8 uur) vastgesteld van respectievelijk 0,1 mg/m^3, 0,01 mg/m^3 en 0,03 mg/m^3. De grenswaarde (tgg 8 uur) in de Verenigde Staten is 0,01 mg/m^3. NIOSH beveelt een 15 minuten waarde aan van 0,002 mg/m^3 voor anorganische arseenverbindingen.

Kinetiek en toxisch werkingsmechanisme

Arseen en arseenverbindingen worden snel opgenomen na orale blootstelling. Opname na inhalatie van arseen partikels is afhankelijk van de oplosbaarheid en omvang van de partikels. De dermale opname verloopt veel trager. Na opname vindt een verdeling plaats over alle organen. Arseen passeert gemakkelijk de placenta. Het metabolisme wordt gekarakteriseerd door twee afwisselende reacties: reductie van vijfwaardige naar driewaardige arseenverbindingen en een oxidatieve reactie waarbij driewaardige verbindingen overgaan in vijfwaardige en (een) methylgroep(en) krijgen. Arseen en arseen metabolieten worden voornamelijk uitgescheiden via de urine. Verschillende studies tonen aan dat arseen uitgescheiden kan worden via de moedermelk.

Driewaardig arseen reageert met sulfhydryl groepen in eiwitten en inactiveert verschillende enzymen. De mitochondriën in het bijzonder zijn gevoelig voor arseen. Vijfwaardig arseen kan werken als een fosfaat analoog en mogelijk verschillende biologische processen beïnvloeden, zoals ATP-productie, botvorming en DNA-synthese (ontkoppeling van de oxidatieve fosforylering).

Effecten bij mensen

Er is relatief weinig informatie beschikbaar betreffende de lokale effecten van arseen. Arseen trioxide is een corrosieve stof en kan schade veroorzaken aan de huid, ogen en luchtwegen.

Er zijn geen gevallen van sterfte bij mensen beschreven na acute inhalatoire blootstelling aan anorganische arseenverbindingen, zelfs niet bij hoge blootstellingniveau's (1-100 mg As/m^3) die voorheen gevonden werden op werkplekken.

Acute orale inname van grote hoeveelheden arseen kan effecten veroorzaken in het maagdarmkanaal en in het cardiovasculaire- en zenuwstelsel met uiteindelijk de dood als gevolg. Verder zijn beenmergdepressie, haemolyse, leververgroting en melanose waargenomen.

Arseen en arseenverbindingen worden als niet-stochastisch genotoxische verbindingen beschouwd. Er worden voornamelijk chromosoomafwijkingen waargenomen. Remming van DNA repair enzymen, hypo- en hypermethylering van DNA en oxidatieve schade door reactieve zuurstofradicalen zijn de belangrijkste genotoxische werkingsmechanismen van arseen.

Chronische blootstelling aan arseen in drinkwater kan huid-, lever-, long-, blaas-, en nierkanker en overmatige verhoorning en pigmentatie van de huid veroorzaken. Verhoogde risico's voor long- en blaaskanker en voor huideffecten

zijn geassocieerd met een arseen blootstellingcategorie van ≤ 50 µg/l in drinkwater. Arseen blootstelling via drinkwater kan verschillende perifere vasculaire aandoeningen veroorzaken. Of enkel arseenblootstelling de extreme vasculaire aandoening, 'blackfoot disease', kan veroorzaken, is niet bekend. Of arseen andere nadelige gezondheidseffecten (hypertension, diabetes, cerebrovascular disease) kan veroorzaken is minder duidelijk.

Beroepsmatige inhalatoire blootstelling aan arseen kan longkanker veroorzaken. Er zijn meerdere studies waar verhoogde risico's zijn waargenomen bij relatief lage cumulatieve blootstellingen in smelterij cohorten in Zweden (Zweden, Rönnskär; arseen blootstellingcategorie < 250 µg/m^3·jaar) en de Verenigde Staten (Tacoma; arseen blootstellingcategorie < 750 µg/m^3·jaar). Studies tonen aan dat roken een synergistisch effect heeft op het optreden van longkanker door arseen blootstelling.

Verschillende studies zijn uitgevoerd om te onderzoeken of arseen reproductietoxische eigenschappen heeft. Er werd geen duidelijk verband tussen inhalatoire blootstelling aan arseen en reprotoxische effecten waargenomen. Echter, studies naar orale blootstelling aan arseen via drinkwater laten zien dat arseen niet uitgesloten kan worden als een causale factor voor reprotoxische effecten (spontane abortus, neonatale en postnatale sterfte, vroeggeboorten en verlaagde geboortegewichten) (zie Annex K).

Effecten bij dieren

Natrium arseniet and natrium arsenaat zijn niet sensibiliserend. Verder is er relatief weinig informatie aanwezig met betrekking tot lokale effecten van arseen en arseenverbindingen.

In een ontwikkelingstudie trad 100% sterfte op in groepen van 10 zwangere ratten na 1 dag blootstelling aan arseen trioxide ≥ 100 mg/m^3 (76 mg As/m^3). De acute dermale dosering van calcium arsenaat en lood arsenaat die 50% sterfte veroorzaakte in ratten is ≥ 2.400 mg/kg bw (≥ 400 mg As/kg bw). Orale letale doseringen variëren van 15 tot 960 mg As/kg lichaamsgewicht/dag.

Arseen veroorzaakt chromosoomafwijkingen in vivo en in vitro.

In een recente studie werd een verhoogde incidentie waargenomen van long, lever, maagdarm en huid tumoren in vrouwelijke muizen die via drinkwater blootgesteld waren aan 500 µg As^V/l* gedurende twee jaar.

* vijfwaardig arseen (As^v) itt driewaardig arseen (As^{III})

In studies met muizen, ratten en hamsters werden reprotoxische effecten waargenomen (o.a. verlaagde geboortegewichten, foetale misvormingen en foetale sterfte) na inhalatoire, orale en parenterale toediening van relatief hoge arseen doseringen die gewoonlijk toxisch (en vaak bijna fataal) waren voor de moederdieren. Er zijn geen reprotoxische effecten waargenomen in ratten na inhalatoire blootstelling (20 mg As/m^3) en orale blootstelling (8 mg As/kg lichaamsgwicht/dag).

Er zijn weinig gegevens beschikbaar over immunologische effecten van arseen en arseenverbindingen in dieren.

Evaluatie en advies

Carcinogeniteit van arseen is het kritische effect. Omdat er voldoende adequate humane gegevens beschikbaar zijn betreffende arseen en arseenverbindingen zal voor het afleiden van de gezondheidskundige advieswaarde uitgegaan worden van de humane data.

Arseen en arseenverbindingen worden als kankerverwekkend beschouwd voor mensen (classificatie categorie 1A, zie Annex I en J). Longkanker is het kritische effect na inhalatoire blootstelling. Er is voldoende kwantitatieve informatie beschikbaar over blootstelling aan arseen afkomstig van een drietal cohorten van werknemers in kopersmelterijen (Tacoma, Washington (USA), Anaconda, Montana (USA) and Rönskär (Zweden)) om de blootstellings-respons relatie kwantitatief te kunnen evalueren.

Arseen en anorganische arseenverbindingen hebben een non-stochastisch genotoxisch werkingsmechanisme (zie Annex I). De commissie besluit om voor arseen concentratieniveaus in de lucht af te leiden die samenhangen met een kans op 4 extra sterfgevallen door kanker per 1.000 en 4 per 100.000 (HBC-OCRV).

De commissie beoordeelt de kwaliteit van een viertal epidemiologische studies over long- en respiratoire kanker in werknemers van bovengenoemde kopersmelterijen (Lubin et al. 2000[1], 2008[2], Järup et al. 1989[3] en Enterline et al. 1995[4]) en de geschiktheid voor kwantitatieve risicobeoordeling. Uiteindelijk selecteert de commissie de studie van Lubin et al. 2000[1] voor de afleiding van risicogetallen (HBC-OCRV).

De commissie berekent op grond van de gegevens uit deze studie dat de concentratie van arseen in lucht , die samenhangt met een extra kans op overlijden aan kanker van

- 4 per 1.000 ($4x10^{-3}$), bij 40 jaar beroepsmatige blootstelling, gelijk is aan 28 µg arseen/m^3

- 4 per 100.000 ($4x10^{-5}$), bij 40 jaar beroepsmatige blootstelling, gelijk is aan 0,28 µg arseen/m^3.

Het afleiden van een ‘Short Term Exposure Limit’ (STEL) of ‘ceiling value’ wordt niet nodig geacht op basis van de beschikbare informatie.

Een huidnotatie wordt niet nodig geacht.

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands sets Health-Based Calculated Occupational Cancer Risk Values (HBC-OCRVs) for chemical substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Safety (DECOS). These recommendations serve as a basis in setting legally binding occupational exposure limits by the Minister .

In this report, the Committee discusses the consequences of occupational exposure to arsenic and arsenic compounds and presents HBC-OCRVs associated with excess mortality levels of 4 per 1,000 and 4 per 100,000 as a result of working life exposure. The Committee's conclusions are based on scientific papers published prior to September 3, 2012.

Physical and chemical properties

Arsenic (As; CAS no. 7440-38-2) is a naturally occurring grey, crystalline solid with metallic luster and has a molecular weight of 74.92. Elemental arsenic sublimes at 613°C, has a very low vapour pressure and a log $P_{octanol/water}$ of 0.680. Arsenic compounds, crystalline, amorphous or hygroscopic substances, occur in trivalent and pentavalent forms. Arsenic trioxide (CAS no. 1327-53-3), the major arsenic compound with regard to occupational exposure, has a molecular weight

of 197.84, melts at 312°C, boils at 465°C, has also a very low vapour pressure and a log $P_{octanol/water}$ of -0.130.

Use

Arsenic and/or arsenic compounds are used as wood preservative, in agriculture (mainly in the past), as a cotton desiccant/defoliant, in a variety of semiconductor applications, as a decolouriser and fining agent in the production of bottle glass and other glassware, in the production of non-ferrous alloys and as a medication.

Monitoring

Arsenic and arsenic compounds in air are usually associated with particulate matter and therefore standard methods of the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) involve collection of air samples on glass fibre or membrane filters, acid extraction of the filters (digestion with nitric, sulphuric and/or perchloric acids) and arsine generation. Hydride generation atomic absorption spectrometry and graphite furnace atomic absorption spectrometry are the major analysis techniques (NIOSH method 5022 (particulate organoarsenicals), NIOSH method 7900 (arsenic and compounds as As, except AsH_3 and As_2O_3), NIOSH method 7901 (arsenic trioxide, as As) and OSHA method ID-105 (arsenic)). Furthermore, inductively-coupled argon plasma, atomic emission spectroscopy is used to analyse arsenic (NIOSH method 7300 (arsenic)).

Although different methods for biological monitoring are available, none of these methods is validated.

Guidelines

Currently, the legal time weighted average (TWA) (8 hr) and short-term (15 min) occupational exposure limits for the combination of all inorganic arsenic compounds in the Netherlands are 0.05 and 0.1 mg As/m^3, respectively. The legal TWA (8 hr) and short-term (15 min) occupational exposure limits for the watersoluble inorganic arsenic compounds are 0.025 and 0.05 mg As/m^3, respectively. There is currently no limit value for exposure to arsenic and arsenic compounds at the European level. In Germany, an 8 hr TRK (Technische Richtkonzentration) and a short time (15 min) TRK of 0.1 mg/m^3 and 0.4 mg/m^3 are established, respectively. The United Kingdom, Denmark and Sweden have

set an occupational exposure limit (8 hr TWA value) for arsenic and compounds (as As) of 0.1 mg/m^3, 0.01 mg/m^3 and 0.03 mg/m^3, respectively. The American Conference of Governmental Industrial Hygienists (ACGIH) has specified a threshold limit value (TLV) of 0.01 mg/m^3 (as As) (8 hr TWA value). The permissible exposure limit (PEL) for inorganic arsenic of the Occupational Safety and Health Administration (OSHA) is 10 µg/m^3. Furthermore, the recommended standard of NIOSH amounts to 0.002 mg/m^3 as determined by a 15-minute sampling period (inorganic compounds, as As).

Kinetics and mechanism of action

Both pentavalent and trivalent soluble arsenic compounds are rapidly and extensively absorbed from the gastrointestinal tract. Absorption of arsenic from inhaled airborne particles is highly dependent on the solubility and the size of particles. Dermal absorption appears to be much less than absorption by the oral or inhalation routes. Arsenic and its metabolites distribute to all organs in the body; preferential distribution has not been observed. Arsenic readily crosses the placenta.

Arsenic metabolism is characterised by alternation of two main types of reactions: (1) two-electron reduction reactions of pentavalent to trivalent arsenic and (2) oxidative methylation reactions in which trivalent forms of arsenic are converted to (mono-, di- or tri-) methylated pentavalent products. Arsenic and its metabolites are largely excreted via the renal route. Excretion can also occur via faeces; minor excretion pathway are nails and hair. Different studies indicated that arsenic can be excreted in human milk.

Trivalent (in)organic arsenic reacts strongly with sulfhydryl groups in proteins and inactivates many enzymes. A particular target in the cell is the mitochondria. Pentavalent inorganic arsenic may exert effects by acting as a phosphate analogue and could potentially affect a number of biological processes, including ATP production, bone formation, and DNA synthesis (uncoupling of oxidative phosphorylation).

Effects – Human toxicity data

Relatively little information is available on the local effects of arsenic and arsenic compounds. Arsenic trioxide is a corrosive compound and may cause local damage to the skin, eyes and respiratory tract.

No cases were located regarding death in humans from inhalation exposure to inorganic arsenicals following acute exposure, even at the very high exposure levels (1-100 mg As/m^3) found previously in the workplace.

Acute ingestion of large doses of arsenic leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.

Arsenic is considered to be a non-stochastic genotoxic compound. Clastogenic damage was observed in different cell types of exposed humans and in mammalian cells in vitro. For point mutations, the results are largely negative. With regard to the mechanism which caused the genotoxic effects, there is evidence that DNA repair enzymes are inhibited by arsenicals, that DNA is hypo- or hypermethylated and that oxidative damage by reactive oxygen species plays a role in arsenic genotoxicity.

Long-term exposure to arsenic in drinking-water is causally related to increased risks of cancer in the skin, liver, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. The effects have been most thoroughly studied in Taiwan but there is considerable evidence from studies on populations in other countries as well. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been reported to be associated with arsenic exposure categories of $\leq$ 50 µg/L. Chronic oral (drinking water) arsenic exposure in Taiwan may-be associated with blackfoot disease, a severe form of peripheral vascular disease which leads to gangrenous changes. There is good evidence from studies in several countries that arsenic exposure causes other forms of peripheral vascular disease.

Conclusions on the causality of the relationship between oral arsenic exposure and other health effects (hypertension, cardiovascular disease, diabetes, cerebrovascular disease, long-term neurological effects) are less clear-cut.

Occupational exposure to arsenic by inhalation is causally associated with lung cancer. Exposure-response relationships and high risks have been observed. Increased risks have been observed at relatively low cumulative exposure levels in smelter cohorts in Sweden (Rönnskär; arsenic exposure category of < 250 µg/m^3·year) and in the USA (Tacoma; arsenic exposure category of < 750 µg/m^3·year). Studies indicated that smoking had a synergistic effect on the lung cancer effects of arsenic exposure.

Several studies have examined a number of reproductive end-points in relation to arsenic exposure. Occupational exposure studies are not conclusive on a causal relationship between arsenic and reprotoxic effects. Studies on oral exposure to arsenic in drinking water show that arsenic can not be excluded as a

causal factor for reproduction toxicity (spontaneous abortion, neonatal and postnatal mortality, preterm delivery, reduced birth weight) (see Annex K).

Effects – Animal toxicity data

Relatively little information is available on the local effects of arsenic and arsenic compounds in animals. Sodium arsenite and sodium arsenate were not allergenic in the guinea-pig maximisation test.

In a developmental toxicity study, 100% mortality in groups of 10 pregnant rats after 1 day of inhalation exposure to arsenic trioxide concentrations ≥100 mg/m^3 was observed (76 mg As/m^3). The acute dermal LD_{50} for the pentavalent arsenicals calcium arsenate and lead arsenate in the rat is ≥ 2400 mg/kg bw (≥ 400 mg As/kg bw). Oral and parenteral lethal doses range from 15 to 960 mg As/kg bw/day, depending on the compound and the animal species.

Arsenic produced chromosomal aberrations in vivo and in vitro

Several animal carcinogenicity studies on arsenic have been carried out, but limitations such as limited time of exposure and limited number of animals make these inconclusive. In a recent study, female C57B1/6J mice had an increased incidence in tumours involving mainly lung, liver, gastrointestinal tract and skin after exposure to 500 µg As^V/L^* drinking water for 2 years. One study has indicated that dimethylarsinic acid may cause cancer of the urinary bladder in male rats at high doses.

Studies with mice, rats and hamsters revealed that inorganic arsenic caused reprotoxic effects (reduced birth weight, foetal malformations, increased foetal mortality) upon inhalatory, oral and parenteral administration of relatively high arsenic doses which were usually maternally toxic (and often nearly fatal). Reproductive performance was not affected in female rats that received inhalation exposures to concentrations as high as 20 mg As/m^3 or gavage doses as high as 8 mg As/kg bw/day from 14 days prior to mating through gestation day 19.

Little information is available with regard to immunological effects of arsenic and arsenic compounds in animals.

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* pentavalent arsenic (As^v) versus trivalent arsenic (As^{III})

Evaluation and advice

Arsenic and arsenic compounds are considered to be carcinogenic in humans (classification category 1A, see Annex I and J). Because sufficient adequate human data on arsenic and arsenic compounds are available the available human data are used for derivation of the occupational limit value.

Lung cancer is the critical effect after inhalation exposure to arsenic and arsenic compounds. Sufficient quantitative information from human studies on the levels of arsenic exposure to ensure reliable assessment of the exposure-response relationship was available for three copper smelter cohorts: Tacoma, Washington (USA), Anaconda, Montana (USA) and Rönnskär (Sweden).

Arsenic and inorganic arsenic compounds have non-stochastic genotoxic mechanisms (see Annex I). For quantitative hazard assessment the Committee decided not to pursue a threshold approach but to calculate excess lifetime cancer mortality risks (health-based calculated occupational cancer risk values (HBC-OCRV)), using mathematical modeling and extrapolation.

The Committee compared the quality and suitability for quantitative hazard assessment of four epidemiological studies on lung and respiratory cancer mortality among workers in these smelters (Lubin et al. 2000[1], 2008[2], Järup et al. 1989[3] and Enterline et al. 1995[4]) and selects the study of Lubin et al. (2000)[1] for the derivation of HBC-OCRV using a linear model.

The Committee calculates that the concentration of arsenic in the air, which corresponds to an excess cancer mortality of

- 4 per 1,000 ($4x10^{-3}$), for 40 years of occupational exposure, equals to 28 µg/m^3
- 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 0.28 µg/m^3.

The available data do not warrant the setting of a Short Term Exposure Limit (STEL) or ceiling value.

The rate of absorption of arsenic and arsenic compounds through the skin does not warrant a skin notation.

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, at the request of the Minister of Social Affairs and Employment (Annex A). The purpose of the Committee's evaluation is to set a health-based recommended occupational exposure limit for the atmospheric concentration of the substance, provided the database allows the derivation of such a value. Such an exposure limit cannot be derived if the toxic action cannot be evaluated using a threshold model, as is the case for substances with genotoxic carcinogenic properties. In that case, an exposure-response relationship is recommended for use in regulatory standard setting, i.e., the calculation of so-called health-based calculated occupational cancer risk values (HBC-OCRVs). The Committee calculates HBC-OCRVs for compounds, which are classified as genotoxic carcinogens by the European Union or by the Committee.

For the establishment of the HBC-OCRVs, the Committee generally uses a linear extrapolation method, as described in the Committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens'.[5]

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister on the feasibility of using the HBC-OCRVs as

regulatory occupational exposure limits. In the final step of the procedure, the Minister of Social Affairs and Employment sets the legally binding occupational exposure limits.

1.2 Committee and procedure

This document contains the assessment of DECOS, hereafter called the Committee, of the health hazard of arsenic and arsenic compounds (as specified in WGD document 84-103-16 concerning inorganic arsenic (excluding arsine)). The members of the Committee are listed in Annex B. The submission letter to the Minister can be found in Annex C.

Because WGD document 84-103-16 is rather outdated (1984) and recent toxicological profiles of WHO/IPCS Environmental Health Criteria (EHC 224, 2001)[6] and the Agency for Toxic Substances and Disease Registry (ATSDR, 2007)[7] were available, these were used as starting documents (see section 1.3). In sections 2.4-7.2, first the WHO and ATSDR data, which describe the data available before 2007, are mentioned. Subsequently, additional data are discussed (up to September 3, 2012) which were obtained from several online databases (see section 1.3).

In July 2012, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The Committee's recommendations on the health-based occupational exposure limit of arsenic and arsenic compounds have been based on scientific data, which are publicly available. For evaluation of the available data before 2007, the toxicological profiles of WHO/IPCS Environmental Health Criteria (EHC 224, 2001)[6] and the Agency for Toxic Substances and Disease Registry (ATSDR, 2007)[7] were used as starting documents. Additional scientific publicly available data up to September 3, 2012 were obtained from the following online databases: Toxline, Medline, Chemical Abstracts and TSCATS. The CAS numbers of arsenic (7440-38-2); arsenic trioxide (1327-53-3); arsenic pentoxide (1303-28-2); arsenic acid (7778-39-4); arsenic trisulphide (1303-33-9); arsenic trichloride (7784-34-1); sodium arsenite (7784-46-5); arsenic acid, trisodium salt (13464-

38-5); arsenenous acid, potassium salt (13464-35-2); potassium arsenate (7784-41-0); arsenic acid, calcium salt (10103-62-5); calcium arsenite (52740-16-6); lead arsenate (7784-40-9); cupric acetoarsenite (12002-03-8); copper(II) arsenite (10290-12-7); magnesium arsenate (10103-50-1) were used in combination with the following key words: expos*, kinetic*, toxic, animal, human, adverse effects. Literature references containing one of the following key terms were excluded: environmental, soil, marine, pollution, pharmacology, drug effect, therapeutic. The literature from this search was selected based on titles and abstracts. The last search was performed on September 3, 2012.

Chapter 2

Identity, properties and monitoring

2.1 Chemical identity

In this section, the chemical identity of arsenic and different arsenic compounds are described.[7-9]

Chemical name: Arsenic

Synonyms: Arsenic Black; Arsenic, elemental; Arsenic - 75; Arsenicals; Colloidal arsenic; Gray arsenic
Molecular formula: As
CAS-number: 7440-38-2
EINECS-number: 231-148-6

Chemical name: Arsenic trioxide

Synonyms: AI3-01163; Arseni trioxydum; Arsenic (III) oxide; Arsenic oxide; Arsenic oxide (As_2O_3); Arsenic sesquioxide
Molecular formula: As_2O_3
CAS-number: 1327-53-3
EINECS-number: 215-418-4

Chemical name: Arsenic pentoxide

Synonyms: Arsenic acid anhydride; Arsenic anhydride; Arsenic oxide; Arsenic oxide (As_2O_5); Arsenic pentaoxide

Molecular formula: As_2O_5
CAS-number: 1303-38-2
EINECS-number: 215-116-9

Chemical name: Arsenic acid

Synonyms: Arsenate; Arsenic acid; Arsenic acid (H_3AsO_4); Arsenic acid, liquid; Crab grass killer
Molecular formula: H_3AsO_4
CAS-number: 7778-39-4
EINECS-number: 231-901-9

Chemical name: Arsenic trichloride

Synonyms: Arsenic butter; Arsenic chloride; Arsenic chloride ($AsCl_3$); Arsenic trichloride; Arsenic(III) chloride
Molecular formula: $AsCl_3$
CAS-number: 7784-34-1
EINECS-number: 232-059-5

Chemical name: Arsenic trisulphide

Synonyms: AI3-01006; Arsenic Red; Arsenic Sulphide Yellow; Arsenic Yellow; Arsenic sesquisulphide; Arsenic sesquisulphide
Molecular formula: As_2S_3
CAS-number: 1303-33-9
EINECS-number: 215-117-4

Chemical name: Sodium arsenite

Synonyms: Arsenenous acid, sodium salt; Arsenious acid, monosodium salt; Arsenious acid, sodium salt; Arsenite de sodium (French); Atlas “A”
Molecular formula: $NaAsO_2$
CAS-number: 7784-46-5
EINECS-number: 232-070-5

Chemical name: Arsenic acid, trisodium salt

Synonyms: Sodium arsenate; Sodium orthoarsenate; Trisodium arsenate
Molecular formula: Na_3AsO_4
CAS-number: 13464-38-5
EINECS-number: 236-682-3

Chemical name: Arsenenous acid, potassium salt
Synonyms: Arsenenous acid, potassium salt; Arsenic acid ($HAsO_2$), potassium salt; Arsenious acid, ($HAsO_2$), potassium salt (8CI); Potassium arsenite
Molecular formula: $KH(AsO_2)_2$
CAS-number: 13464-35-2
EINECS-number: -

Chemical name: Potassium arsenate
Synonyms: Arsenic acid (H_3AsO_4), monopotassium salt; Arsenic acid, monopotassium salt; Macquer's salt; Monopotassium arsenate; Monopotassium dihydrogen arsenate Molecular formula: KH_2AsO_4
CAS-number: 7784-41-0
EINECS-number: 232-065-8

Chemical name: Arsenic acid, calcium salt
Synonyms: Calcium arsenate
Molecular formula: $Ca_3(AsO_4)_2$
CAS-number: 7778-44-1
EINECS-number: 231-904-5

Chemical name: Calcium arsenite
Synonyms: Arsenous acid, calcium salt; Arsonic acid, calcium salt (1:1); Calcium arsonate (1:1); Calcium meta-arsenite; Mono-calcium arsenite
Molecular formula: $CaAsO_3H$
CAS-number: 52740-16-6
EINECS-number: 258-147-3

Chemical name: Lead arsenate
Synonyms: Acid lead arsenate; Acid lead orthoarsenate; Arsenate of lead; Arsenic acid (H_3AsO_4), lead(2+) salt (1:1); Arsenic acid, lead(2+) salt(1:1)
Molecular formula: $Pb_3(AsO_4)_2$
CAS-number: 7784-40-9
EINECS-number: 232-064-2

Chemical name: Copper(II) arsenite
Synonyms: Acid copper arsenite; Air-flo Green; Arsenious acid (H_3AsO_3), copper(2+) salt (1:1); Arsonic acid, copper(2+) salt (1:1); Copper arsenite
Molecular formula: $Cu(AsO_2)_2$
CAS-number: 10290-12-7

EINECS-number: 233-644-8

Chemical name: Cupric acetoarsenite
Synonyms: (Acetato)trimetaarsenitodicopper; (Acetato-O)(trimetaarsenito)dicopper; Basle Green; CI Pigment Green 21; Copper acetate arsenite
Molecular formula: $C_4H_6As_6Cu_4O_{16}$
CAS-number: 12002-03-8
EINECS-number: -

Chemical name: Magnesium arsenate
Synonyms: Arsenic acid, magnesium salt; Magnesium O-arsenate; Magnesium arsenate phosphor
Molecular formula: $Mg_3(AsO_4)_2$
CAS-number: 10103-50-1
EINECS-number: 233-285-7

2.2 Physical and chemical properties

In Table 1, the physical and chemical properties of arsenic and different arsenic compounds are presented.[7,9,10] No data on physical and chemical properties of the following arsenic compounds were available: arsenic acid, trisodium salt; arsenenous acid, potassium salt; arsenic acid, calcium salt and calcium arsenite.

2.3 EU Classification and labeling

The classification of arsenic and arsenic compounds based on EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures is presented in Table 2.[11] No concentration limits are specified for the different arsenic compounds.

Table 1 Physical and chemical properties of arsenic and arsenic compounds.

	Arsenic	Arsenic trioxide	Arsenic pentoxide	Arsenic acid	Arsenic trichloride	Arsenic trisulphide	Sodium arsenite	Potassium arsenate	Lead arsenate	Copper (II) arsenite	Cupric acetoarsenite	Magnesium arsenate
Physical description	grey, crystlline solid with metallic luster	white, amorphous or crystalline powder	white hygroscopic powder	white solid substance	oily, colourless liquid with acrid smell	yellow-red powder	grey-whitish powder	white, crystalline powder	white, crystalline solid	n.d.	emerald green, crystalline powder	n.d.
Molar mass (g/mol)	74.9	197.8	229.8	141.9	181.3	246.0	129.9	180.0	347.1	277.4	1013.8	350.8
Melting point (°C)	sublimation at 613	312	decomposition at 315	35.5	-16	300-325	n.d.	288	decomposition at 720	decomposition	n.d.	86.3
Boiling point (°C)	-	465	-	loses H_2O at 160	130	707	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Density kg/m^3	5,727	3,738	4,320	2,000-2,500	2,100	n.d.	1,870	2,900	n.d.	n.d.	n.d.	n.d.
Solubility	insoluble in water; soluble in nitric acid	soluble in water (37 g/L at 20°C and 115 g/L at 100°C); slightly soluble in alcohol; soluble in hydrogen chloride	soluble in water (1500 g/L at 16°C and 767 g/L at 100°C); soluble in alcohol; soluble in acid	soluble in alcohol	decomposed by water; soluble in ethanol, ether and concentrated mineral acids	insoluble in cold water, slightly soluble in hot water; soluble in alkali, acids and ethanol	very soluble in water (1*10^6 mg/L at 25°C); slightly soluble in ethanol	soluble in cold water (190 g/L at 6°C), very soluble in hot water; soluble in acid, glycerol and ammonia	soluble in water (8.5*10^5 mg/L at 25°C); soluble in nitric acid and in caustic alkalis	n.d.	soluble in acids	soluble in water (2.7*10^5 mg/L at 17°C)
Log $P_{octanol/water}$	0.680	-0.130	n.d.	3.140	1.610	n.d.	-3.280	n.d.	-2.490	n.d.	n.d.	-7.290
Vapour pressure (kPa; 25°C)	3.3*10^{-10}	3.7*10^{-11}	n.d.	7.6*10^{-20}	1.3 (23.5°C)	n.d.	8*10^{-19}	n.d.	1.9*10^{-19}	n.d.	n.d.	n.d.

Table 1 Continued.

	Arsenic	Arsenic trioxide	Arsenic pentoxide	Arsenic acid	Arsenic trichloride	Arsenic trisulphide	Sodium arsenite	Potassium arsenate	Lead arsenate	Copper (II) arsenite	Cupric acetoarsenite	Magnesium arsenate
Relative density (air=1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.79	n.d.	n.d.	n.d.
Flash point	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Odor threshold	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: no data

Table 2 Classification of arsenic and arsenic compounds.

Arsenic compound	CAS number	Classification
Arsenic	7440-38-2	Acute Tox. 3; H331 (Toxic if inhaled) Acute Tox. 3; H301 (Toxic if swallowed)
Arsenic trioxide	1327-53-3	Carc. 1A; H350 (May cause cancer) Acute Tox. 2; H300 (Fatal if swallowed) Skin Corr. 1B; H314 (causes severe skin burns and eye damage)
Arsenic pentoxide	1303-28-2	Carc. 1A ; H350 (May cause cancer) Acute Tox. 3; H331 (Toxic if inhaled) Acute Tox. 3; H301 (Toxic if swallowed)
Arsenic acid	7778-39-4	Carc. 1A ; H350 (May cause cancer) Acute Tox. 3; H331 (Toxic if inhaled) Acute Tox. 3; H301 (Toxic if swallowed)
Arsenic trichloride	7784-34-1	-
Arsenic trisulphide	1303-33-9	-
Sodium arsenite	7784-46-5	-
Arsenic acid, trisodium salt	13464-38-5	-
Arsenenous acid, potassium salt	13464-35-2	-
Potassium arsenate	7784-41-0	-
Arsenic acid, calcium salt	10103-62-5	-
Calcium arsenite	52740-16-6	-
Lead arsenate	7784-40-9	Carc. 1A ; H350 (May cause cancer) Repr. 1A ; H360df (May damage fertility or the unborn child) Acute Tox. 3; H331 (Toxic if inhaled) Acute Tox. 3; H301 (Toxic if swallowed) STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure)
Cupric acetoarsenite	12002-03-8	-
Copper(II) arsenite	10290-12-7	-
Magnesium arsenate	10103-50-1	-

2.4 Validated analytical methods

In this chapter the analytical methods which are available for detecting and/or measuring and monitoring arsenic and arsenic compounds in air and in biological samples are described. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify arsenic and arsenic compounds. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis.

2.4.1 *Environmental monitoring*

WHO/ATSDR data

Arsenic in air is usually associated with particulate matter and therefore standard methods involve collection of air samples on glass fibre or membrane filters, acid extraction of the filters (digestion with nitric, sulphuric, perchloric acids, hydrogen peroxide and/or hydrochloric acid) and arsine generation. Atomic absorption spectrophotometry (AAS) is as yet the major technique used to analyse arsenic and arsenic compounds in air.

Table 3 summarizes methods reported in the literature for detecting arsenic and arsenic compounds in air samples.

Table 3 Analytical methods for determining arsenic and arsenic compounds in air samples.

Sample matrix	Sampler	Sample preparation	Assay procedure	Limit of detection	References
Air (arsenic and compounds, as As (except AsH_3 and As_2O_3) (NIOSH method 7900)	Filter (0.8 µm cellulose ester membrane)	Collection filter; digestion with nitric acid, sulphuric acid, and perchloric acid	Hydride generation atomic absorption spectrometry (HGAAS)	0.02 µg/sample	NIOSH, 1994a[12]
Air (arsenic trioxide, as As) (NIOSH method 7901)	Filter (Na_2CO_3-impregnated, 0.8 µm cellulose ester membrane + backup pad)	Collection on filter and H_2O_2	Graphite furnace atomic absorption spectrometry (GFAAS)	0.06 µg/sample	NIOSH, 1994b[13]
Air (arsenic) (NIOSH method 7300)	Filter (0.8-½ m cellulose ester membrane, or 5-½ m, polyvinyl chloride membrane)	Collection on filter, digestion with nitric acid, sulphuric acid, and perchloric acid	Inductively-coupled plasma, atomic emission spectroscopy (ICP-AES)	5.6 ng/mL	NIOSH, 2003[14]
Air (particulate organoarsenal) (NIOSH method 5022)	Filter (1 µm polytetrafluoroethylene (PFTE) membrane + backup pad)	Collection on filter	Ion chromatography, hydride generation atomic absorption spectrometry	0.02 µg As/sample	NIOSH, 1994[15]
Air, wipes (smear tabs) or bulks (OSHA method ID-105)	Filter (0.8 µm mixed-cellulose ester filter and backup pad)	Collection on filter, digestion with nitric acid and stabilisation by addition of nickel; thereafter, hydrochloric acid is added; arsine collected on charcoal is extracted using a dilute nitric acid/nickel solution	Graphite furnace atomic absorption spectrometry (GFAAS)	0.003 µg/mL (qualitative); 0.01 µg/mL (quantitative)	OSHA, 1991[16]

Air (except volatile arsenic compounds) (NEN 2951)	Filter (0.8 µm cellulose-ester membrane)	Collection on filter; digestion with nitric acid and hydrochloric acid	Graphite furnace atomic absorption spectrometry (GFAAS)	200 ng	Nederlands-Normalisatie-instituut, 1999[17]
Air (arsenic and inorganic compounds except AsH_3) (MDHS 41/2)	Cellulose ester membrane filter and sodium carbonate impregnated back-up paper pad, mounted in an inhalable dust sampler	Particulate arsenic and inorganic compounds of arsenic collected on cellulose ester membrane filter; arsenic trioxide vapour collected by reaction with sodium carbonate on the paper pad. Digestion using nitric acid, sulphuric acid and hydrogen peroxide	Continuous flow or flow injection analysis hydride generation atomic absorption spectrometry	0.3 ng/mL (qualitative); 1 ng/mL (quantitative)	Health and Safety Executive (HSE), 1994[18]

2.4.2 *Biological monitoring*

WHO/ATSDR data

Atomic absorption spectrophotometry (AAS) is the most common analytical procedure for measuring total arsenic in biological materials. In AAS analysis, the sample is heated in a flame or in a graphite furnace until the element atomises. The ground-state atomic vapour absorbs monochromatic radiation from a source and a photoelectric detector measures the intensity of transmitted radiation. Inductively-coupled plasma atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS) are used techniques for the analysis of arsenic; both methods can generally provide lower detection limits than absorbance detection methods. Samples may be prepared for AAS in a variety of ways. Most often, the gaseous hydride procedure is employed. In this procedure, arsenic in the sample is reduced to arsine (AsH_3), a gas which is then trapped and introduced into the flame. This approach measures total inorganic arsenic, but may not detect all organic forms unless preceded by a digestion step. Digestion or wet-ashing with nitric, sulphuric and/or perchloric acids degrades the organic arsenic species to inorganic arsenic so that recovery of total arsenic from biological materials can be achieved. For accurate results, it is important to check the completeness of the oxidation, however, this is seldom done.
The arsenic concentration in biological fluids and tissues may also be determined by neutron activation analysis (NAA). In this approach, the sample is irradiated with a source of neutrons which converts a portion of the arsenic atoms to radioactive isotopes which can be quantified after separation from radioisotopes of other chemicals. Neutron activation has limited use because it depends on the

presence of the number of nuclear reactors providing this service and the need to dispose of radioactive waste.
X-ray fluorescence (XRF) is also capable of measuring arsenic in biological materials. This method has the advantage that no sample digestion or separation steps are required. Hydride generation combined with atomic fluorescence spectroscopy (HG/AFS) is a relatively new technique that provides freedom from interference offered by hydride generation with sensitivity better than to 20 parts per trillion and linearity up to 10 ppm.

The ATSDR in 2007[7] summarizes details of a number of methods for the analysis of total arsenic in biological samples e.g. total arsenic in blood using HG AAS (Foa et al., 1984; Valentine et al. 1979), total arsenic in serum using NAA (Versieck et al.,1983), total arsenic in urine using colorimetry (Pinto et al. 1975), NAA (Landsberger and Simsons,1987), HG AAS (Guo et al., 1997) or XRF (Clyne et al.,1989), total arsenic in hair using HG AAS (Valentine et al.,1979; Curatola et al., 1978), total arsenic in nails (Agahian et al.,1990), total arsenic in soft tissue using GF AAS (Mushak et al.,1977).

Speciation of arsenic (i.e., analysis of organo-arsenicals or different inorganic species, rather than total arsenic) is usually accomplished by employing separation procedures prior to introduction of the sample material into a detection system. Various types of chromatography or chelation-extraction techniques are most commonly used in combination with AAS, ICP-AES, or ICP-MS detection methods. In one method, HPLC is combined with HG/AFS to quantify arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid and dimethylarsinic acid. Another approach involves selective reduction of arsenate and arsenite (permitting quantification of individual inorganic arsenic species), and selective distillation of methyl arsines to quantify monomethylarsonic acid and dimethylarsinic acid. Most methods for measuring arsenic in biological samples, are unable to measure arsenobetaine with any accuracy because it does not form a hydride and it gives a different response from inorganic arsenic in electrothermal AAS.

The ATSDR in 2007[7] summarizes details of a number of methods for the speciation analysis of arsenic metabolites in urine. Ion exchange chromatography (IEC) was combined with HG-AAS (Johnson and Farmer, 1989) or ICP-MS (Inoue et al., 1994). HPLC was combined with HG-AAS (Norin and Vahter, 1981) or ICP-MS (Ebdon et al., 1999). Selective distillation was combined with ICP-AES (Braman et al., 1977).

Detection limits for arsenic in blood and urine are about 0.1-1 ppb for most techniques; limits for hair and tissues are usually somewhat higher.

Additional data

Especially the recognition of the role of methylated arsenicals that contain As^{III} in the toxicity and metabolism of arsenic (see Chapter 5, 6 and 7) emphasised the need for hyphenated analytical methods to detect and quantify these species in biological samples. Hence, a method was developed by Del Razo et al.(2001)[19] to exploit pH-dependent differences in the generation of arsines from inorganic and methylated arsenicals that contain either As^{V} or As^{III} followed by gaschromatographic separation and atomic absorption spectrophotomery. Le et al. (2000)[20] also developed a method for determination of methylated arsenicals: ion-pair chromatographic separation with hydride generation atomic absorption spectrometry detection. Verdon et al. (2009)[21] developed a hyphenated method combining HPLC with ICP-MS and Dynamic Reaction Cell (DRC) technology allowing the separation of 7 As species in human urine.

Table 4 summarises details of a variety of methods for measuring total arsenic and individual arsenic species in biological materials.

Table 4 Analytical methods for determining arsenic compounds in biological samples.

Sample matrix	Sample preparation	Assay procedure	Limit of detection	References
Urine	Filtering of urine specimens through a 0.45 µm Millipore membrane filter; microwave digestion of a mixture of sample and nitric and hydrochloric acids	Graphite furnace atomic absorption spectrometry (GFAAS)	0.03 µg/L	Horng et al., 2002[22]
Urine (inorganic acid plus monomethylarsonic acid and dimethylarsinic acid)	Reduction with sodium borohydride to arsine	Hydride generation atomic absorption spectrometry (HGAAS)	1 µg/L	ACGIH, 2001[23]
Urine	Reduction with borohydride at pH 6: generation of arsines from inorganic As^{III}, methyl As^{III}, and dimethyl As^{III}, but not from inorganic As^{V}, methyl As^{V}, and dimethyl As^{V}; reduction with borohydride at pH 2 or lower: generation of arsines from arsenicals that contained either As^{V} or As^{III}. Arsines are trapped in a liquid nitrogen-cooled gas chromatographic trap, which is subsequently warmed to allow separation of the hydrides by their boiling points.	Hydride generation atomic absorption spectrometry (HGAAS)	inorganic As^{III}: 1.1 µg As/L methyl As^{III}: 1.2 µg As/L dimethyl As^{III}: 6.5 µg As/L	Del Razo et al., 2001[19]

Urine (determination of As^{III}, As^{V}, monomethylarsonic acid, dimethylarsinic acid and monomethyl-arsonous acid)		Ion-pair chromatographic separation/Hydride generation atomic absorption spectrometry (HGAAS)	4 µg/L	Le et al., 2000[20]
Urine (As^{III}, As^{V}, monomethyl-arsonate, dimethylarsinate)	Dilution with ammonium acetate, pH 5, centrifugation and injection of supernatant into HPLC	High-performance liquid chromatography (HPLC) with anion-exchange column/ Inductively-coupled plasma mass spectrometry (ICP-MS) with dynamic reaction cell (DRC)	0.4-1.7 µg As/L for various species	Verdon et al., 2009[21] (CDC method)
Urine (As^{III}, As^{V}, MMA, DMA	Dilution with water, filtration and injection of filtrate into HPLC	High-performance liquid chromatography (HPLC) with anion and cation-exchange column/ Inductively-coupled plasma mass spectrometry (ICP-MS)	(LODs for anion- resp. cation mode) As^{III} 0.3/0.3 µg As/L As^{V} 0.2/0.4 µg As/L MMA 0.2/0.3 µg As/L DMA 0.3/0.2 µg As/L	Suzuki et al., 2009[24]
Urine (As^{III}, As^{V}, MMA^{V}, DMA^{V}, MMA^{III}, DMA^{III}	Dilution with water, filtration and injection of the filtrate into HPLC	High-performance liquid chromatography (HPLC) with anion and cation-exchange column/ Inductively-coupled plasma mass spectrometry (ICP-MS)	As^{III} 0.11 µg As/L As^{V} 0.25 µg As/L MMA^{V} 0.18 µg As/L DMA^{V} 0.17 µg As/L MMA^{III} 0.61 µg As/L DMA^{III} 0.44 µg As/L	Xie et al., 2006[25]

Chapter 3

Sources

3.1 Natural occurrence

WHO/ATSDR data

Arsenic is the main constituent of more than 200 mineral species, of which about 60% are arsenate, 20% sulphide and sulphosalts and the remaining 20% include arsenides, arsenites, oxides and elemental arsenic (Onishi, 1969). The most common of the arsenic minerals is arsenopyrite, FeAsS, and arsenic is found associated with many types of mineral deposits, especially those including sulphide mineralisation (Boyle and Jonasson, 1973). It has been estimated that about one-third of the atmospheric flux of arsenic is of natural origin. Volcanic action is the most important natural source of arsenic, followed by low-temperature volatilisation.

The ability of arsenic to bind to sulphur ligands means that it tends to be found associated with sulphide-bearing mineral deposits, either as separate arsenic minerals or as a trace of a minor constituent of the other sulphide minerals. This leads to elevated levels in soils in many mineralised areas where the concentrations of associated arsenic can range from a few milligrams to > 100 mg/kg. Concentrations of various types of igneous rocks range from < 1 to 15 mg As/kg, with a mean value of 2 mg As/kg. Similar concentrations (< 1-20 mg As/kg) are found in sandstone and limestone. Significantly higher concentrations of

up to 900 mg As/kg are found in argillaceous sedimentary rocks including shales, mudstone and slates. Up to 200 mg As/kg can be present in phosphate rocks (O'Neill, 1990). Concentrations of arsenic in open ocean water are typically 1-2 µg/L. The concentrations of arsenic in unpolluted surface water and groundwater are typically in the range of 1-10 µg/L. Elevated concentrations in surface water and groundwater of up to 100-5,000 µg/L can be found in areas of sulphide mineralisation (Welch et al., 1988; Fordyce et al., 1995). Elevated concentrations (> 1 mg As/L) in groundwater of geochemical origins have also been found in Taiwan (Chen et al., 1994), West Bengal, India (Chatterjee et al., 1995; Das et al., 1995, 1996; Mandal et al., 1996) and more recently in most districts of Bangladesh (Dhar et al.,1997; Biswas et al., 1998). Elevated arsenic concentrations were also found in the drinking water in Chile (Borgono et al. 1977); North Mexico (Cebrian et al., 1983); and several areas of Argentina (Astolfi et al.,1981; Nicolli et al., 1989; De Sastre et al.,1992). Arsenic-contaminated groundwater was also found in parts of PR China (Xinjiang and Inner Mongolia) and the USA (California, Utah, Nevada, Washington and Alaska) (Valentine, 1994). More recently, arsenic concentrations of < 0.98 mg/L have been found in wells in south-western Finland (Kurttio et al.,1998). Levels as high as 35 mg As/L and 25.7 mg As/L have been reported in areas associated with hydrothermal activity (Kipling, 1977; Tanaka, 1990).
In nature, arsenic-bearing minerals undergo oxidation and release arsenic to water. This could be one explanation for the problems of arsenic in the groundwater of West Bengal and Bangladesh. In these areas the groundwater usage is very high. It has been estimated that there are about 4-10 million tube wells in Bangladesh alone. The excessive withdrawal and lowering of the water table for rice irrigation and other requirements lead to the exposure and subsequent oxidation of arsenic-containing pyrite in the sediment. As the water table recharges after rainfall, arsenic leaches out of the sediment into the aquifer.

3.2 Man-made sources

3.2.1 Production

WHO/ATSDR data

Arsenic is presently obtained as a byproduct of the smelting of copper, lead, cobalt, and gold ores. Arsenic trioxide is volatilised during smelting and accumulates in the flue dust from the roasting of ores, which may contain up to 30% arsenic trioxide. The crude flue dust is further refined by mixing with small

amounts of galena or pyrite and roasting to yield a arsenic trioxide of 90-95% purity. By successive sublimations, a purity of 99% can be obtained. Subsequently, arsenic and arsenic compounds can be prepared by the reduction of arsenic trioxide with charcoal. Demand for metallic arsenic is limited and thus most arsenic is marketed and consumed in combined form, principally as arsenic trioxide which is subsequently converted to arsenic acid.

3.2.2 *Use*

WHO/ATSDR data

Arsenic and arsenic compounds have different uses[6,7,26-28], e.g.:

- as wood preservative, primarily chromium copper arsenate (CCA) (CrO_3-$CuO•As_2O_5$). Chrome copper arsenate is a water-based product that protects several commercially-available species of western lumber from decay and insect attack. It is widely used in treating utility poles, building lumber, and wood foundations. 10,10'-Oxybisphenoxarsine is an antimicrobial used primarily in the plastics industry
- as a cotton desiccant/defoliant
- in the manufacture of galium arsenide and other intermetallic compounds that are used in a variety of semiconductor applications including solar cells, light-emitting diodes, lasers, and integrated circuits (high purity arsenic)
- as a decolouriser (elimination of the green colour) and fining agent in the production of bottle glass and other glassware (arsenic, arsenic trioxide (e.g., manufacturing of low-melting glasses, lightening glass and removing of air bubbles) and arsenic acid)
- in the production of non-ferrous alloys, principally lead alloys used in lead-acid batteries (used in automobiles) and copper alloys (arsenic pentoxide and As_2O_3 are used as additives in alloys). Arsenic may be added to alloys used for bearing, type metal, lead ammunition, automotive body solder. It is also added to some brasses to improve corrosion resistance
- in agriculture (mainly in the past). Organic arsenicals, namely cacodylic acid, disodium methylarsenate, monosodium methylarsenate, and arsenic acid are still used as herbicides. From the mid-nineteenth century to the introduction of organic pesticides in the 1940s, inorganic arsenic compounds were the dominant pesticides available to farmers and fruit growers. Calcium arsenate was formerly used to control the boll weevil and cotton worm and used as a herbicide. Lead arsenate was used on apple and other fruit orchards as well as on potato fields. Sodium arsenite was used to control weeds on railroad right-

of-ways, potato fields, and in industrial areas, as well as in baits and to debark trees. Sodium arsenate had some application in ant traps. The use of inorganic arsenic compounds in agriculture has virtually disappeared beginning around the 1960s. Inorganic arsenic's remaining allowable uses are in ant baits and wood preservatives (see above). All agricultural uses of arsenic were banned because of concerns about human health risk during production and application or accidental poisoning at the point of use
- as a medication. Inorganic arsenic was used as a therapeutic agent through the mid twentieth century, primarily for the treatment of psoriasis, and chronic bronchial asthma; organic arsenic antibiotics were extensively used in the treatment of spirochetal and protozoal disease. Organic arsenical drugs continue to be used in treating the meningoencephalitic stage of African trypanosomiasis and amoebic dysentery
- in veterinary medicine to treat parasitic diseases, including filariasis in dogs and black head in turkeys and chickens
- in dental surgery (until the early eighties)
- as a feed additive for poultry and swine (arsenilic acid (*p*-aminophenyl-arsonic acid) until the late nineties, and for cattle and sheep dips (sodium arsenite).

Additional data

- Inorganic arsenic may be a part of a wide variety of traditional herbal medicines, often from asian origin and therefore the development and production of such herbal praparations requires strict control.[29]

With regard to agriculture and/or (veterinary) medical purposes of arsenic and inorganic compounds in the Netherlands:
- 'Superwolmanzout-CO' (CCA), a biocide contaning arsenic pentoxide as an active substance [30] was allowed in The Netherlands until 2006
- different drugs for usage in humans are registered by the Netherlands' Medicines Evaluation Board which contain arsenic compounds as the active substance [31] e.g. arsenic trioxide is recently allowed for the treatment of acute promyelocytic anemia
- according to the Netherlands' Medicines Evaluation Board, which is also responsible for the authorisation of veterinary medicines, arsenic and arsenic compounds are not used as (active substances in) veterinary medicines [32].

Chapter 4

Exposure

4.1 General population

WHO/ATSDR data

Arsenic is found naturally in the environment and therefore exposure to arsenic may occur by eating food, drinking water, or breathing air. 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^-) occur in water, soil, or food. Food is usually the largest source of arsenic (also in the Netherlands). Fish and seafood contain the greatest amounts of arsenic, mostly the organic form of arsenic. Children may also be exposed to arsenic by eating dirt.[7] In some areas arsenic in drinking water is a significant source of exposure to inorganic arsenic. In these cases, arsenic in drinking water often constitutes the principal contributor to the daily arsenic intake. Contaminated soils such as mine tailings are also a potential source of arsenic exposure.[6] Exposure may also occur by skin contact with water that contains arsenic or by contact with contaminated soil. Furthermore, arsenic compounds are used as a desiccant for cotton. If arsenic is retained in cotton, the general public may be exposed.[7]

The concentration of arsenic in natural surface and groundwater is generally about 1 µg/L but may exceed 1,000 µg/L in mining areas or where arsenic levels in soil are high. Groundwater is far more likely to contain high levels of arsenic

than surface water. Levels of arsenic in food range from about 20 to 140 µg/kg.[7] Limited data indicate that approximately 25% of the arsenic present in food is inorganic, but this depends highly on the type of food ingested. Inorganic arsenic levels in fish and shellfish are low (< 1%). Foodstuffs such as meat, poultry, dairy products and cereals have higher levels of inorganic arsenic. The daily intake of total arsenic from food and beverages is generally between 20 and 300 µg/day.[6]

Mean total arsenic concentrations in air from remote and rural areas range from 0.02 to 4 ng/m^3. Mean total arsenic concentrations in urban areas range from 3 to about 200 ng/m^3; much higher concentrations (> 1,000 ng/m^3) have been measured in the vicinity of industrial sources. Pulmonary exposure may contribute up to approximately 10 µg/day in a smoker and about 1 µg/day in a non-smoker, and more in polluted areas.[6]

In addition to the normal levels of arsenic in air, water, soil, and food, exposure to higher levels of arsenic may occur:

- some hazardous waste sites contain large quantities of arsenic. If the material is not properly disposed of, it can get into surrounding water, air, or soil. For people living near such a site, exposure to elevated levels of arsenic from these media may occur
- when sawing or sanding arsenic-treated wood, inhalation of some of the sawdust may occur. Similarly, if burning arsenic-treated wood, inhalation of arsenic from the smoke may occur
- in a formerly agricultural area where arsenic was used on crops, the soil could contain high levels of arsenic
- in the past, several kinds of products used in the home (rat poison, ant poison, weed killer, some types of medicines) had arsenic in them. However, most of these uses of arsenic have ended, so exposure from home products is not likely any longer.[7]

Additional data

Data on Dutch emission registrations showed a total emission of arsenic and its compounds into air of 0.425 tonnes.[33]

4.2 Working population

WHO/ATSDR data

Occupational exposure to arsenic may be significant in several industries, mainly nonferrous smelting, arsenic production, electronics, wood preservation, wood joinery, glass manufacturing, arsenical pesticide production and application, and pharmaceutical [6]. Since arsenic compounds are used as a desiccant for cotton, workers involved in harvesting and ginning cotton may be exposed to arsenic. If any arsenic is retained in the cotton, workers in the fabric may be exposed. The electronics industry is expanding the use of gallium arsenide in the production of electro-optical devices and integrated circuits, and workers in the industry where gallium arsenide is used may be exposed to arsenic.[7]

Exposure is primarily through inhalation of arsenic-containing particulates, but ingestion and dermal exposure may be significant in particular situations (e.g. chromium copper arsenate (CCA)-treated timber). It is extremely rare for workers to be exposed to arsenic alone; the exposure is usually to arsenic in combination with other elements.[6]

The WHO stated that data on typical exposure levels of arsenic in the workplace are difficult to obtain and may vary considerably between different locations of the same industry because of the level of occupational hygiene in place and the chemical properties of the materials processed. Also, they are often out of date with regard to the current level of industrial hygiene. In workplaces with up-to-date occupational hygiene practices, exposure generally does not exceed 10 µg/m^3 (8-h time-weighted average (TWA)). However, in some places workroom atmospheric arsenic concentrations as high as several milligrams per cubic meter have been reported.[6]

The following data illustrate levels found in specific industries in various locations worldwide and provide some information on present and past exposures of workers to arsenic. They should not be considered as representative of all similar industrial sites.

Exposure investigations indicated that the arsenic exposure concentrations (8-hour TWA) in copper smelters ranged from 0.8-746 µg/m^3 (Vahter et al., 1986; Hakala and Pyy, 1995; Jakubowski et al., 1998; Ferreccio et al., 1996; Offergelt et al., 1992; Liu and Chen, 1996). Much higher arsenic exposure concentrations were reported in older exposure investigations.

Workers in certain glass-manufacturing industries may be exposed to airborne arsenic through the use of As_2O_3 (IARC, 1993). A study in the USA of

35 crystal glassworkers within the mix-and-melt and batch-house areas indicated the potential for arsenic exposure. Personal air monitoring of 8 workers found airborne arsenic concentrations of 2-11 mg/m^3 (Chrostek et al., 1980).

In a study of six wood joinery shops in Sweden (Nygren et al., 1992), airborne arsenic concentrations between 0.54 and 3.1 µg/m^3 were reported. In two workshops machining wood impregnated with CCA preservatives, levels of arsenic in personal air samples were reported to be 30-67 µg/m^3 in one plant (8 workers) and 10-62 µg/m^3 in another plant (8 workers) (Subra et al., 1999).[6] In a study performed in Denmark to evaluate arsenic exposure in workers impregnating wood with CCA solutions (Jensen and Olsen, 1995), the maximum exposure concentration was 17.3 µg/m^3, found for a single worker who was filling an impregnation container with CCA paste.[7]

Workers in coal-powered power plants may also be exposed to arsenic found in the coal, or more likely that found in the fly ash during cleaning. Yager et al. (1997) reported arsenic concentrations (8-h TWA) between 0.17 and 375.2 µg/m^3 (mean 48.3) in the breathing zone of maintenance workers in a coal-fired power plant in Slovakia.

Concentrations of arsenic in the breathing zone of underground gold-miners in Ontario (Canada) were reported to range between 2.4 and 5.6 µg/m^3 (geometric mean) (Kabir and Bilgi, 1993). In a study relating arsenic exposures to lung cancer among tin-miners in Yunnan province (China), Taylor et al. (1989) reported mean concentrations of airborne arsenic to range from 0.42 mg/m^3 in 1951 to 0.01 mg/m^3 in 1980.[6]

NIOSH researchers conducted a study of arsenic exposures and control systems for gallium arsenide operations at three microelectronics facilities during 1986-1987 (Sheehy and Jones, 1993). Results at one plant showed that in all processes evaluated but one, the average arsenic exposures were at or above 5 µg/m^3, with a maximum exposure of 8.2 µg/m^3. While cleaning the liquid encapsulated Czochralski (LEC) pullers, the average potential arsenic exposure of the cleaning operators was about 500 µg/m^3. Area arsenic samples collected at the plant in break-rooms and offices, 6-20 meters from the process rooms, had average arsenic concentrations of 1.4 µg/m^3. At the other two plants, personal exposures to arsenic were well controlled for all processes evaluated. In another study (de Peyster and Silvers, 1995), airborne arsenic was found in areas where equipment was cleaned but not in administrative areas in a semiconductor fabrication facility. The highest airborne arsenic level found in the study, 15 µg/m^3, was collected from the breathing zone of a maintenance employee who was cleaning a source housing over a period of 2 hours in an area with local exhaust ventilation.[7]

Additional data

In a study performed at three semiconductor manufacturing facilities[34], twenty of 31 personal airborne arsenic exposure samples were below the detection limit of 0.01 µg/m^3. The geometric mean arsenic level of the remaining 11 samples was 1.66 µg/m^3 (GSD = 2.2). The highest personal arsenic level was 7 µg/m^3 and was experienced by an engineer who was dismounting and scrubbing major parts of the ion implanter for 4 hours.

Smith and Coulehan (2002) reported that exposure to arsenic may also occur when handling museum artifacts (e.g. skins, furs, baskets, feathers).[35] In previous centuries, various toxic pest control treatments and preservatives including arsenic compounds were used to maintain the integrity of artifacts. However, no quantitative information was reported.

Baptiste (2000) reported exposure to arsenic in a battery manufacturing facility. No quantitative information was reported.[36]

Grillet et al. (2004) reported exposure to arsenic in the wine growing industry (use of arsenic as a fungicide) without reporting quantitative information.[37]

Chapter 5

Kinetics

5.1 Absorption

WHO/ATSDR data

Arsenic absorption depends on its chemical form. The rate of absorption of arsenic in highly insoluble forms (e.g., arsenic sulphide, lead arsenate) is much lower than that of more soluble forms via both oral and inhalation routes. In humans, As^{III}, As^{V}, monomethylarsonic acid, and dimethylarsinic acid are orally absorbed $\geq$ 80%.

Arsenic is also absorbed via inhalation. In lung cancer patients exposed to arsenic in cigarette smoke, deposition was estimated to be about 40% and absorption was 75-85% (Holland et al., 1959). Thus, overall absorption (expressed as a percentage of inhaled arsenic) was about 30-34%. In workers exposed to arsenic trioxide dusts in smelters, the amount of arsenic excreted in the urine was about 40-60% of the estimated inhaled dose (Pinto et al., 1976; Vahter et al., 1986). Absorption appears to be by passive diffusion in humans and mice, although there is evidence for a saturable carrier-mediated transport process for arsenate in rats.

Absorption by the dermal route has not been well characterised, but is low compared to the other routes.

Additional data

Because human occupational exposure to high levels of arsenic in air has been associated with lung cancer, but generally not other types of cancer, Beck et al. (2002) investigated the relationship between airborne arsenic exposures and systemic uptake in rabbits.[38] New Zealand white rabbits were chosen as the test animal because metabolism of arsenic in rabbits and humans is fairly similar, with the exception that humans excrete somewhat more monomethylarsonic acid than rabbits. The animals (n=6/sex/concentration) were exposed to one of four levels of arsenic trioxide in air for 8 hr/day, 7 days/week, for 8 weeks (0.05, 0.1, 0.22, or 1.1 mg/m^3; Mass Median Aerodynamic Diameter (MMAD) ranged from 3.2 to 4.1 µm; GSD not specified). The airborne arsenic within each exposure chamber was reasonably well mixed, and variations in exposure concentrations within an exposure group are likely to have been minimal. Plasma levels of inorganic arsenic, monomethylarsonic acid, and dimethylarsinic acid were measured following the last exposure. Total arsenic calculated as the sum of inorganic arsenic, monomethylarsonic acid, and dimethylarsinic acid showed a concentration-related increase. Although there was also a concentration-related increase in plasma levels of methylated arsenic metabolites, statistically significant increases in mean inorganic arsenic levels in plasma were observed only in male rabbits exposed to 0.22 mg/m^3, and in both males and females exposed to 1.1 mg/m^3. Mean inorganic arsenic levels in plasma in males and females exposed to 0.05 and 0.1 mg/m^3, and females exposed to 0.22 mg/m^3, were not significantly elevated compared to controls. According to the authors these results suggest that low level arsenic inhalation has a negligible impact on body burden of inorganic arsenic until air levels are above ± 0.15 mg/m^3. Based on plasma measurements of inorganic arsenic, the two lowest exposure levels in this study (0.05 and 0.1 mg/m^3) are indistinguishable from background.

In contrast to the others, Hazelton et al. (2001) found indications for accumulation of arsenic in the lung.[39] In this study Hazelton et al. used the two-stage clonal expansion model to analyse lung cancer mortality in a cohort of Yunnan tin miners. As part of this study, models were tested with variable arsenic clearance rates. Analysis suggested that particles containing arsenic accumulate in the lung with very slow clearance. The best estimate was for a half-life of about 6 years, but the difference in likelihood for a 6-year half-life compared with no decay was not significant.

5.2 Distribution

5.2.1 Distribution through the body

WHO/ATSDR data

Data on distribution after inhalation exposure are limited, but it appears that arsenic is transported to nearly all tissues. Arsenic and its metabolites distribute to all organs in the body; preferential distribution has not been observed in human tissues at autopsy or in experiments with animal species other than rat (in which arsenic is concentrated in red blood cells). Since the liver is a major site for the methylation of inorganic arsenic, a first-pass effect after gastrointestinal absorptionis possible; however this has not been investigated in animal models. Furthermore, arsenic accumulates in keratin-rich tissues such as skin, hair and nails.

Additional data

After the administration of arsenicals (route not specified) to animals, elevated levels were found especially in liver, kidney, spleen, and lung; several weeks later, arsenic is translocated to ectodermal tissues (hair, nails) because of the high concentration of sulphur-containing proteins in these tissues.[40]

Hughes et al. examined the disposition of arsenic after repeated oral administration of arsenate in mice.[41] Adult female B6C3F1 mice (n=10) were administered nine repeated oral daily doses of 0.5 mg As/kg ([^{73}As]arsenate) (estimated dose regimen for attaining steady-state levels of whole-body arsenic). Accumulation of radioactivity (ng As/g tissue) was highest in bladder, kidney, and skin. Loss of radioactivity was most rapid in the lung and slowest in the skin.

Monomethylarsonic acid was detected in all tissues except the bladder. Bladder and lung had the highest percentage of dimethylarsinic acid after a single exposure to arsenate, and it increased with repeated exposure. In kidney, inorganic arsenic was predominant. There was a higher percentage of dimethylarsinic acid in the liver than the other arsenicals after a single exposure to arsenate. The percentage of hepatic dimethylarsinic acid decreased and that of inorganic arsenic increased with repeated exposure. A trimethylated metabolite was also detected in the liver.

5.2.2 *Placental transfer*

WHO/ATSDR data

Human studies

Case reports of arsenic poisoning in pregnant women resulting in death of the foetus; by toxic levels of arsenic in foetal organs and tissues demonstrate that arsenite readily passes through the placenta (Lugo et al., 1969; Bollinger et al., 1992). In a more recent study, Concha et al. (1998) reported that arsenic concentrations were similar in cord blood and maternal blood (~9 µg/L) of mother - infant pairs exposed to drinking water containing high levels of arsenic (~200 µg/L). Another study of an 'unexposed' population in the southern USA found that concentrations of arsenic in cord blood and maternal blood (about 2 µg/L) were also similar, and suggests that arsenic readily crosses the placenta (Kagey et al., 1977).

Animal studies

Both older and more recent studies have documented the ability of trivalent and pentavalent inorganic arsenic to cross the placenta in laboratory animals. Lindgren et al., (1984) reported that in pregnant mice given a single intravenous injection (4 mg As/kg) of sodium arsenate or sodium arsenite, both forms passed through the placenta easily and to approximately the same extent. These investigators also reported that the rate of placental transfer was lower in a marmoset monkey (non-methylating species) injected intravenously with arsenite than in mice, and suggested that this was a consequence of stronger binding in maternal tissues.

Hood et al. (1987) compared the foetal uptake of sodium arsenate after oral (40 mg/kg) or intraperitoneal (20 mg/kg) administration to pregnant CD-1 mice on day 18 of gestation. Arsenic levels peaked later and over 5-fold lower in foetuses of mice dosed orally, most likely reflecting both slower uptake from the gastrointestinal tract and greater opportunity for methylation in the liver before the arsenic reached the systemic circulation. The quantity of dimethylated metabolite present in the foetuses rose over time (to ~80% of total metabolites present for both routes of administration) and remained relatively constant from ~10 h after dosing until the study ended, 24 h after dosing.

Hood et al. (1988) also compared the foetal uptake of sodium arsenite after oral (25 mg/kg) or intraperitoneal (8 mg/kg) administration to mice that were 18 days pregnant. As was the case with arsenate, injected mice achieved both higher

foetal and placental levels of arsenic more quickly than did mice dosed orally. Both valence forms followed similar time-course trends after oral administration. However, levels of arsenic in foetuses of dams injected with arsenite reached a plateau 12-24 h after dosing, whereas levels of arsenic in foetuses of dams injected with arsenate peaked at 2-4 h after dosing and then declined quickly. The proportion of arsenic present in foetuses as methylated metabolite increased over time to 88% and 79% after oral and intraperitoneal administration, respectively. A higher fraction of monomethylated arsenic was present in foetuses of dams dosed with arsenite than with arsenate.

Older studies have demonstrated that dimethylarsenic acid is capable of crossing the placenta of rats (Stevens et al., 1977).

Additional data

Human studies

The abovementioned study of Concha et al. (1998) also showed that arsenic metabolites originating from inorganic As in the blood of both the newborns and their mothers was in the form of DMA, which indicated that DMA is the major form of arsenic transferred from mothers to their foetuses.

In a recent study in Bangladesh (Hall et al., 2007) where people are exposed to waterborne arsenic a study was conducted in 101 pregnant women who gave birth.[42] Maternal and cord blood pairs were collected and concentrations of total As were analyzed for 101 pairs and As metabolites for 30 pairs. Strong associations between maternal and cord blood concentrations were observed for total As ($r=0.93$, $p<0.0001$), but also for DMA (0.94, $p < 0.0001$), MMA ($r=0.80$, $p<0.0001$), arsenite ($r=0.8$, $p< 0.0001$) and arsenate ($r=0.89$, $p < 0.0001$). This implies that exposure to all metabolites of inorganic As occurs in the prenatal period.

Animal studies

Jin et al. (2006) investigated in an experimental study in mice the transfer of arsenic species from the mother through the placenta in newborn pups, and the speciated arsenic distribution in the liver and brain of newborn mice after gestational maternal exposure to inorganic arsenic.[43] The mother mice were exposed to 10 and 30 ppm inorganic AsIII and 10 and 30 pm AsV in drinking water during gestation. The livers and brains of the mother mice and their newborn pups were collected and As, monomethylarsonic acid (MMA^V), dimethylarsinic acid (DMA^V), and trimethylarsenic (TMA) were analysed.

Contents of inorganic arsenic As, MMA, and DMA in the liver of mother mice increased with the concentration of arsenite or arsenate in their drinking water. However, only DMA increased with the concentration of arsenate or arsenite in the drinking water in the brain of the mother mice. On the other hand, the contents of both inorganic As and DMA in the liver and brain of newborn mice increased with the concentration of arsenate or arsenite administered to their mother orally. Contents of arsenic species in the liver and brain of both mother mice and their newborn pups were significantly lower in the 10 ppm As^{V} group than in the 10 ppm inorganic As^{III} group. Ratios of As or DMA levels between the brain and the liver of newborn mice were larger than 1, whereas those in mother mice were much smaller than 1. Arsenic taken from drinking water was distributed and metabolized mainly in the liver of mother mice. As^{III} in low levels may be taken up and metabolized easily in the liver compared to iAs^{V}. Both inorganic As and DMA are transferred from the mother through the placenta and cross the immature blood-brain barrier easily. Compared to that in the liver of newborn mice, DMA as an organic metabolite is prevalent in brain, a lipidic organ, if the blood-brain-barrier is not matured enough to prevent it from entering the brain.

5.3 Biotransformation

WHO/ATSDR data

In many species arsenic metabolism is characterised by two main types of reactions: (1) two-electron reduction reactions 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 glutathione as an essential co-factor. Methylation of inorganic arsenic facilitates the excretion of inorganic arsenic from the body, as the endproducts monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) are readily excreted in urine. There are major qualitative and quantitative interspecies differences in methylation, to the extent that some species exhibit minimal or no arsenic methylation (e.g. marmoset monkey, guinea-pig, chimpanzee). However, in humans and most common laboratory animals, inorganic arsenic is extensively methylated. Factors such as dose, age, gender and smoking contribute only minimally to the large inter-individual variation in arsenic methylation observed in humans. Studies in humans suggest the existence of a wide difference in the activity of methyltransferases, and the existence of polymorphism has been hypothesised.

Animal and human studies suggest that arsenic methylation may be inhibited at high acute exposures. The metabolism of inorganic arsenic may be influenced by its valence state, particularly at high dose levels. Studies in laboratory animals indicate that administration of trivalent inorganic arsenic such as As_2O_3 and arsenite initially results in higher levels in most tissues than does the administration of pentavalent arsenic. However, the trivalent form is more extensively methylated.

Additional data

Monomethylarsonic acid (MMA^V) may be methylated to dimethylarsinic acid (DMA^V), but neither monomethylarsonic acid nor dimethylarsinic acid are demethylated to yield inorganic arsenic. Recent data showed that both monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) are persistent metabolites that can be identified in the urine of individuals chronically exposed to arsenic in drinking water.[20,19] A simplified scheme of overall arsenic metabolism in many mammals including humans is shown in Figure 1.

There is a variation in susceptibility to arsenic and arsenic compounds among individuals, which is related to variation in metabolism. On average, the urine of people exposed to inorganic arsenic occupationally, experimentally, or in the general environment, contains 10-30% inorganic, 10-20% monomethylarsonic acid, and 60-70% dimethylarsinic acid, but there is a considerable inter-individual variation. Also, recent studies have identified groups with unusually low or high urinary excretion of monomethylarsonic acid. Thus, there appears to be a genetic polymorphism in the biomethylation of arsenic. Most likely, there is a genetic polymorphism in the regulation of arsenic methyltransferases (Schläwicke et al., 2009).[44] However, the methyltransferases involved in arsenic methylation have not been characterised. Possibly, arsenic metabolism is also affected by the polymorphism in enzymes involved in the remethylation of homocysteine.[45]

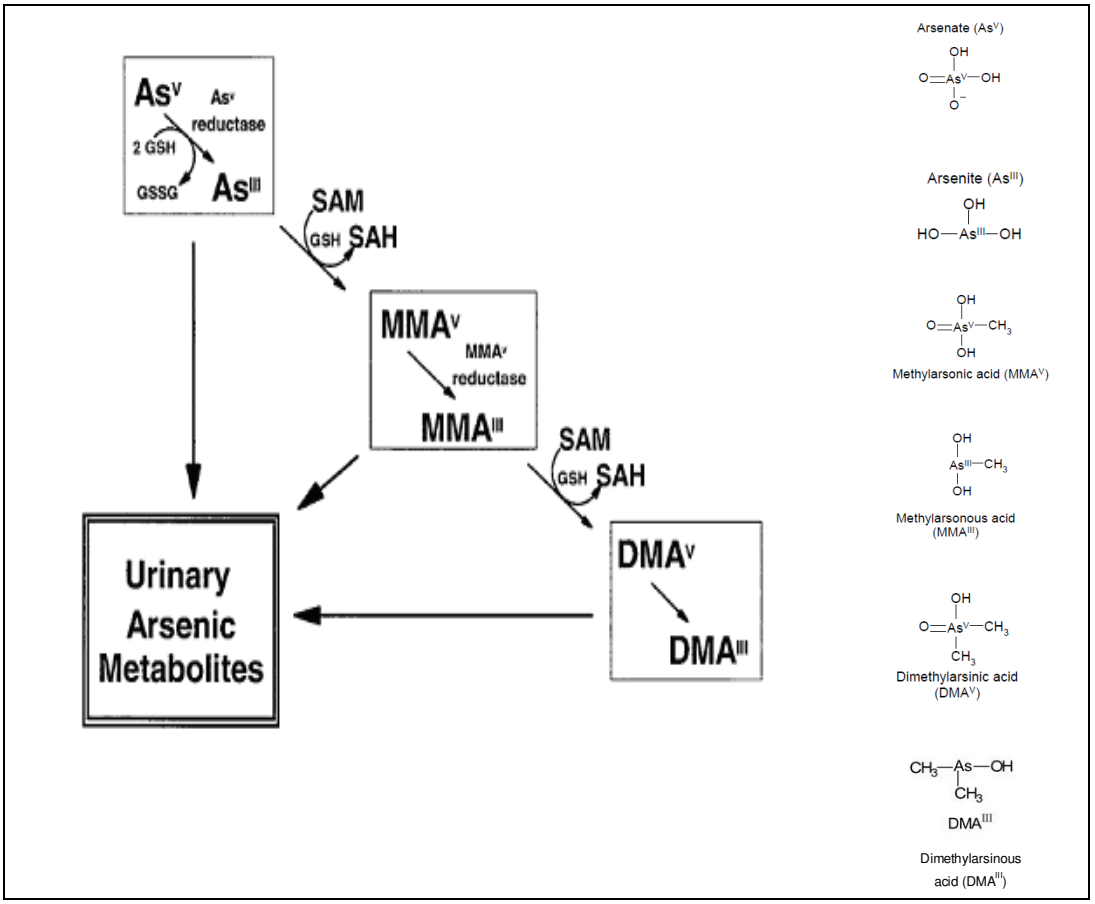

Figure 1 Metabolism of arsenic in the liver: reduction from pentavalent to trivalent arsenic states may occur nonenzymatically via glutathione or enzymatically. Oxidation and methylation are coupled in arsenic metabolism with the trivalent arsenic form as substrate and a methylated pentavalent form as the product. As^V, As^{III}, monomethylarsonic acid (MMA^V) (humans excrete a relatively high amount of monomethylarsonic acid in their urine), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^V) (the major form in many mammals; 60-80% in humans), and dimethylarsinous acid (DMA^{III}) are found in human urine. In rats, some arsenic is further metabolised to a form with three methyl groups, TMAO. Some forms of arsenic can reversibly change valence state from pentavalent to trivalent and back again (e.g. arsenate ↔ arsenite). SAM (S-adenosyl methionine): serves as the methyl donor; SAH (S-adenosylhomocysteine); GSH (glutathione reduced); GSSG (glutathione oxidised) (sources: Kitchin[46] and Tchounwou et al.[28]). There is a considerable variation in the methylation of inorganic arsenic among mammalian species. Compared to human subjects most experimental animals (mouse, rat, rabbit, hamster, dog) excrete very little MMA, while the methylation to DMA is more efficient than in humans. There is an overall higher excretion of arsenic in urine of experimental animals than in humans except for the rat since most of the produced DMA is retained in the erythrocytes. The chimpanzee and the marmoset monkey however lack the ability to methylate arsenic.[33]

5.4 Elimination

5.4.1 Elimination from the body

WHO/ATSDR data

In humans arsenic is largely excreted via the renal route as a mixture of As^{V}, As^{III}, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid and dimethylarsinous acid. This excretion mechanism is not likely to be saturated within the dose range expected from human exposure. The proportion of metabolites recovered in urine are roughly consistent in humans regardless of the exposure scenario. Smaller amounts are excreted in faeces. Some arsenic may remain bound to tissues (especially skin, hair, and nails), depending inversely on the rate and extent of methylation.

Additional data

Mice (n=5) were administered a single oral dose of 0.5 mg As/kg ([^{73}As]arsenate) to estimate the half-life of the terminal elimination phase of arsenic-derived radioactivity (Hughes et al.).[41] The half-life of the phase describing the rapid elimination of radioactivity following its absorption from the gut and entry into the plasma amounted to 2.2 h. The phase describing elimination of radioactivity which had first distributed throughout the whole animal, some of it perhaps retained, and then was released and finally eliminated had a half-life of ± 44 h.

Furthermore, Hughes et al. examined the elimination of arsenic after repeated oral administration of arsenate.[41] Adult female B6C3F1 mice (n=10) were administered nine repeated oral daily doses of 0.5 mg As/kg ([^{73}As]arsenate) (estimated dose regimen for attaining steady-state levels of whole-body arsenic). Radioactivity was eliminated from mice after repeated [^{73}As]arsenate exposure, primarily by urinary excretion (the urinary vs. fecal elimination of arsenic amounted to 9.8 ± 0.4 and 1.9 ± 0.3 µg As/day, respectively). There was no significant difference in the amount of arsenic eliminated in urine or faeces each day after administration of [^{73}As]arsenate. After repeated dosing ceased, the amount of arsenic excreted decreased rapidly. Dimethylarsinic acid was the predominant arsenic metabolite detected (approximately 9-10 µg As/day) in urine during each 24 h period after administration of [^{73}As]arsenate. Smaller amounts of arsenate, arsenite, and monomethylarsonic acid were detected (all

<0.5/µg As/day). The amount of each metabolite excreted in urine each day did not vary significantly over the 9-day dosing period.

5.4.2 *Elimination in human milk*

Various studies indicated that arsenic can be excreted in human milk.

WHO/ATSDR data

In the Bombay area (India) Dang et al. (1983) reported arsenic levels ranging from 0.2 to 1.1 ng/g in breast milk of nursing mothers 1-3 months postpartum. Arsenic was detected in human breast milk at concentrations of 0.13-0.82 ng/g (Somogyi and Beck, 1993). In human milk sampled from 88 mothers on the Faroer Islands whose diets were predominantly seafood, arsenic concentrations were 0.1-4.4 ng/g (Grandjean et al., 1995). Exposure to arsenic from the seafood diet in this population was most likely to organic "fish arsenic." In a population of Andean women exposed to about 200 ng/g of inorganic arsenic in drinking water, concentrations of arsenic in breast milk ranged from about 0.8 to 8 ng/g (median 2.3 ng/g) (n=10) (Concha et al., 1998). The arsenic concentration in the breast milk of 35 women in Ismir, Turkey, a volcanic area with high thermal activity ranged from 3.24 to 5.41 ng/g, with a median of 4.22 ng/g (Ulman et al., 1998).

Additional data

Two hundred and twenty-six breast milk samples were collected from lactating women in arsenic-affected districts of west Bengal (Samanta et al., 2009).[47] In only 39 (17%) samples arsenic was detected. The maximum arsenic concentration in breast milk was 48 µg/L. Hair and nail arsenic was highly correlated with drinking water arsenic concentrations. Women who had both high arsenic body burden and arsenical skin lesions also had elevated levels of arsenic in their breast milk.

5.5 Possibilities for biological monitoring

WHO/ATSDR data

The three most commonly employed biomarkers used to identify or quantify arsenic exposure are total arsenic in hair or nails, blood arsenic, and total or speciated metabolites of arsenic in urine.

Arsenic is rapidly cleared from blood. It is for this reason that blood arsenic is typically used only as an indicator of very recent or relatively high-level exposure (e.g. in cases of poisoning), or chronic stable exposure (e.g. to drinking water). The limitation of blood arsenic levels as indicators of low-level exposure or drinking water is that it is difficult to distinguish the contributions of inorganic arsenic from water and organic arsenic from food (speciation of chemical forms in blood is difficult).

Because arsenic accumulates in keratin-rich tissues such as skin, hair and nails as a consequence of its affinity for sulfhydryl groups, arsenic levels in hair and nails may be used as an indicator of past arsenic exposure (exposure that occurred 1-10 months earlier). Hair and nails have the advantage of being readily and non-invasively sampled, but a major issue of concern is whether external contamination can be removed (e.g., when exposed to water containing high arsenic levels, hair can bind arsenic externally which may not be removed readily by washing procedures). Arsenic levels in both hair and nails are elevated within one to a few weeks after acute poisoning, and return to background levels within a few months (Choucair and Ajox, 1988). Fingernail arsenic has been reported to be significantly correlated with hair arsenic content (Lin et al., 1998). Since the rate of hair growth is about 1 cm/month, the segmental distribution of arsenic along the hair shaft has been used to distinguish between acute and chronic poisoning, as well as to estimate length of time since a poisoning incident (Koons and Peters, 1994). The use of toenails rather than fingernails has been recommended in some studies because of the larger amount of sample that can generally be obtained (Garland et al., 1993; Karagas et al., 1996).

Since arsenic is rapidly metabolised and excreted into the urine, total arsenic, inorganic arsenic and the sum of arsenic metabolites (inorganic arsenic + monomethylarsonic acid + dimethylarsinic acid) in urine have all been used as biomarkers of recent arsenic exposure. Urinary levels are generally considered to

be the most reliable indication of recent exposures. In common with other biomarkers of arsenic exposure, levels of arsenicals in urine may be a consequence of inhalation exposure or ingestion of arsenic from drinking water, beverages, soil or foodstuffs (NRC, 1999). In many older studies, total urinary arsenic was used as a biomarker of recent arsenic exposure. However, this is increasingly uncommon because organoarsenicals present in substantial amounts in certain foodstuffs are also excreted in urine. Therefore, assessment of inorganic arsenic exposure using total urinary arsenic would result in overestimation of inorganic arsenic exposure. To avoid the potential for overestimation of inorganic arsenic exposure inherent in using total urinary arsenic, most studies now measure specific metabolites in urine and use either inorganic arsenic or the sum of arsenic metabolites (inorganic arsenic + monomethylarsonic acid + dimethylarsinic acid) as an index of arsenic exposure. Relatively recently it has been found that adding all arsenic metabolites together can give misleading results unless a careful diet history is taken and/or seafood consumption is prohibited for 2-3 days before urine collection (Buchet et al., 1996). There are two reasons for this. First, some seafoods contain arsenic metabolites MMA and DMA, particularly DMA, in fairly high amounts. Secondly, arsenosugars present in seaweeds and some bivalves are extensively metabolised to DMA (either by the body itself or the gut microbiota, which is than excreted in urine (Le et al., 1994, Ma and Le, 1998, WHO 2001[6]).

Additional data

Hwang et al. (2002)[48] analysed urinary inorganic arsenic metabolites in the urine of 12 office-based engineers (control group) and 30 maintenance engineers (exposure group) from six wafer fabrication facilities (semiconductor industry). No personal airborne arsenic exposure samples were taken. First morning-voided urine samples of each study subject were collected for 7 consecutive days. The levels of the various arsenic species for the exposed group were 1.7±1.4, 1.4±1.1, 6.2±6.7, 20.2±14.1, and 29.5±17.2 µg/L for As^{III}, As^{V}, monomethylarsonic acid, dimethylarsinic acid, and total inorganic arsenic, respectively. Although there was no significant difference in total urinary arsenic concentrations of the control (27.4 ± 17.7 µg/L) and the exposed group, monomethylarsonic acid content was significantly higher in the exposed group than in the control group (4.0± 5.5 µg/L; $P<0.05$). The data also suggested that, at low-level occupational arsenic exposure, the concentration of total urinary inorganic arsenic metabolites might be misleading due to the confounding effect resulting from intake of seafood, such as arsenosuger. Nevertheless, the authors concluded that using the

percentage change of monomethylarsonic acid in total urinary inorganic arsenic metabolites as an indicator for the verification of arsenic exposure is appropriate for monitoring of urinary arsenic species.

5.6 Possibilities for biological effect monitoring

WHO/ATSDR data

The effects of arsenic are mainly nonspecific, but the combined presence of several of the most characteristic clinical signs (e.g., nausea, diarrhoea, peripheral neuropathy, anaemia, vascular lesions, hyperkeratinisation, hyperpigmentation) is usually adequate to suggest arsenic intoxication. Although there are standard clinical methods for detecting and evaluating each of these effects, there are no recognised methods for identifying early (preclinical) effects in exposed persons. Neurophysiological measurements of nerve conduction velocity or amplitude have been investigated (Goebel et al., 1990; Jenkins, 1966; Le Quesne and McLeod, 1977; Morton and Caron, 1989; Murphy et al., 1981), but at present, this approach does not seem to offer much advantage over a standard neurological examination. Arsenic is known to affect the activity of a number of enzymes, and some of these may have potential as biomarkers of effect. Most promising is the spectrum of effects caused by arsenic on the group of enzymes responsible for heme synthesis and degradation, including inhibition of coproporphyrinogen oxidase and heme synthetase and activation of heme oxygenase. Changes in urinary excretion levels of several heme-related metabolites appear to be a good indication of preclinical effects of arsenic toxicity in animals (Albores et al., 1989; Sardana et al., 1981; Woods and Fowler, 1978; Woods and Southern, 1989), but this has not been established in humans and is not specific for arsenic-induced effects.

Additional data

Apostoli et al. (2002) evaluated the possible effect of inorganic arsenic and of its species on the urinary excretion of porphyrin homologues.[49] Total porphyrins and their homologues (copro, penta, hexa, hepta, uroporphyrins) and arsenic species (trivalent and pentavalent As; monomethyl arsonic acid; dimethyl arsenic acid; arsenobetaine) were measured respectively by HPLC and HPLC-ICP-MS in urine from 86 art glass workers exposed to As_2O_3 and from 54 controls (workers from tool makers without exposure to inorganic arsenic). Individuals with liver or kidney diseases were excluded. A significant increase in the

excretion of penta and uroporphyrins was demonstrated for workers exposed to As; As^{III} was the species best correlated with urinary porphyrin excretion. The increase of urinary excretion for some porphyrin homologues appears to be consistent with the inhibition by arsenic of uro-decarboxylase in the heme biosynthesis pathway.

Wu et al. (2004) administered young female C57Bl/6J mice drinking water containing 0, 100, 250 and 500 µg As^{V}/L as sodium arsenate ad libitum for 12 months.[50] Urine was collected bimonthly for urinary arsenic methylation assay and porphyrin analysis. All detectable arsenic species showed strong linear correlation with administered dosage and the arsenic methylation patterns were similar in all three treatment groups. No significant changes of methylation patterns were observed over time for either the control or test groups. Urinary coproporphyrin III was significantly increased in the 8th month in 250 and 500 µg/L groups and remained significantly dose-related after 10 and 12 months. Coproporphyrin I also showed a significant dose-response relationship after 12 months.

5.7 Summary

Absorption via both oral and inhalation routes is dependent on the solubility and the size of particles. Both pentavalent and trivalent soluble arsenic compounds are rapidly and extensively (>70%) absorbed from both gastrointestinal tract and the lung. Dermal absorption appears to be much less than by the oral or inhalation routes. Arsenic and its metabolites distribute to all organs in the body; preferential distribution has not been observed. Arsenic readily crosses the placenta.

Arsenic metabolism is characterised by two main types of reactions: (1) two-electron reduction reactions of pentavalent to trivalent arsenic, which may occur nonenzymatically via glutathione or enzymatically, 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 reduced glutathione (GSH) as an essential co-factor. Some forms of arsenic can reversibly change valence state from pentavalent to trivalent and back again.

Arsenic and its metabolites are largely excreted via the renal route. Excretion can also occur via faeces; a minor excretion pathway is nails and hair. Different studies indicated that arsenic can be excreted in human milk.

Blood arsenic is a useful biomarker in the case of acute arsenic poisoning or stable chronic high-level exposure. Arsenic in hair and nails can be indicators of past arsenic exposure. Arsenic in hair may also be used to estimate relative length of time since an acute exposure. Speciated metabolites in urine expressed either as inorganic arsenic or as the sum of metabolites provide the best quantitative estimate of recently absorbed dose of arsenic.

Arsenic is known to affect the activity of a number of enzymes, and some of these may have potential as biomarkers of effect. Most promising is the spectrum of effects caused by arsenic on the group of enzymes responsible for heme synthesis and degradation.

Chapter 6

Mechanisms of action

6.1 Mechanisms of toxicity and carcinogenicity

WHO/ATSDR data

Mechanisms of arsenic-induced toxicity and carcinogenicity have not been clearly identified. Due to the extremely large amount of mechanistic data for arsenic, it is not feasible to include all primary studies that address issues concerning proposed mechanisms. Therefore, the discussion of mechanisms is based on information from several review articles (Chen et al., 2004, 2005; Florea et al., 2005; Hughes, 2002; Kitchin, 2001; Lantz and Hays, 2006; Navas-Acien et al., 2005; Rossman, 2003; Roy and Saha, 2002; Thomas et al., 2007; Vahter, 2002).

It is becoming increasingly evident that the toxicity and carcinogenicity of arsenic is likely to be closely associated with metabolic processes. Absorbed pentavalent arsenic (As^{V}) is rapidly reduced to trivalent arsenic (As^{III}), at least partially in the blood. Much of the formed AsIII is distributed to tissues and taken up by cells (particularly hepatocytes). Many cell types appear to accumulate As^{III} more rapidly than As^{V}. Because As^{III} (as arsenite) is known to be more toxic than As^{V} (as arsenate), the reduction step may be considered bioactivation rather than detoxification. Glutathione appears to play a role in the reduction of As^{V} to As^{III}, which is required prior to methylation. Methylation of

arsenic ultimately forms relatively less toxic MMA and DMA; this process is accomplished by alternating between the reduction of As^{V} to As^{III} and the addition of a methylgroup; S-adenosylmethionine (SAM) is considered to be the source of the methylgroup. The methylation process appears to include multiple intermediates, some of which are more reactive than inorganic arsenic. For example, reactive trivalent metabolites, MMA^{III} and DMA^{III}, have been detected in the urine of human subjects chronically exposed to arsenic in drinking water, and in vitro studies have demonstrated MMA^{III} to be more toxic than arsenite or arsenate to human hepatocytes, epidermal keratinocytes, and broncial epithelial cells. Additional in vitro studies have demonstrated genotoxic and DNA damaging properties of both MMA^{III} and DMA^{III}.

Molecular action – Trivalent inorganic arsenic

Trivalent inorganic arsenicals, such as arsenite, readily react with sulfhydryl groups in proteins and inactivate many enzymes, thereby inhibiting critical functions such as gluconeogenesis and DNA repair (Scott et al., 1993; Delnomdedieu et al., 1994a). The complex between arsenic and vicinal sulfhydryl reagent is particularly strong. The activity of enzymes or receptors essential to cellular metabolism is due in part to the functional groups on amino acids such as the sulfhydryl group on cysteine or coenzymes such as lipoic acid, which has vicinal thiol groups. Thus, if arsenite binds to a critical thiol or dithiol, the enzyme may be inhibited (Aposhian, 1989). Arsenite inhibits pyruvate dehydrogenase (Peters, 1955; Szinicz and Forth, 1988), a lipoic-acid-dependent enzyme involved in gluconeogenesis. The acute toxicity of inorganic arsenic may result in part from inhibition of gluconeogenesis and ultimately depletion of carbohydrates from the organism (Reichl et al., 1988; Szinicz and Forth, 1988). However, binding of arsenite to protein at non-essential sites may be a detoxication mechanism (Aposhian, 1989). Arsenite inhibits the binding of steroids to the glucocorticoid receptor, but not other steroid receptors (Lopez et al., 1990; Simons et al., 1990). The glucocorticoid receptor has vicinal thiols that are involved with steroid binding (Simons et al., 1990).

A particular target in the cell for trivalent inorganic arsenic is the mitochondrion, which accumulates arsenic (Goier, 1991). A major mechanism by which arsenic exerts its toxic effect is through impairment of cellular respiration by the inhibition of various mitochondrial enzymes.

Molecular action – Pentavalent inorganic arsenic

A mechanism of toxicity of pentavalent inorganic arsenic, such as arsenate, is its reduction to a trivalent form, such as arsenite. The reduction of arsenate to

arsenite occurs in vivo. Arsenite is more toxic than arsenate, as evidenced by the lower amount of it needed to elicit a toxic response. Another potential mechanism is the replacement of phosphate with arsenate. Kenney and Kaplan (1988) have reported that in the human erythrocyte, arsenate can replace phosphate in the sodium pump and the anion exchange transport system. In KB oral epidermoid carcinoma cells, arsenate accumulates at a greater rate when the cells are grown in phosphate-free media (Huang and Lee, 1996). Arsenate uptake by these cells is inhibited by phosphate in a concentration-dependent manner. Arsenate can form esters with glucose and gluconate (Lagunas, 1980; Gresser, 1981), forming glucose-6-arsenate and 6-arsenogluconate, respectively. These compounds resemble glucose-6-phosphate and 6-phosphogluconate. Glucose-6-phosphate and glucose-6-arsenate have similar K_M and V_{max} values as substrates for glucose-6-phosphate dehydrogenase and each can inhibit hexokinase.

As a phosphate analogue, pentavalent arsenic could potentially affect a number of biological processes, including ATP production (uncoupling of in vitro oxidative phosphorylation), bone formation, and DNA synthesis. During glycolysis, arsenate can substitute for phosphate to form 1-arsenato-3-phospho-d-glycerate, instead of 1,3-biphospho-d-glycerate, from d-glyceraldehyde-3-phosphate. The arsenic anhydride is unstable and hydrolyses to arsenate and 3-phosphoglycerate. Normally adenosine-5'-triphosphate (ATP) is generated in this reaction, but with arsenate present instead of phosphate, ATP is not formed (Crane and Lipmann, 1953; Aposhian, 1989). Adenosine-5'-diphosphate-arsenate is synthesised by submitochondrial particles from adenosine-5'-diphosphate (ADP) and arsenate in the presence of succinate (Gresser, 1981). ADP-arsenate hydrolyses more easily than ATP. The formation and hydrolysis of ADP-arsenate results in arsenolysis. The depletion of ATP in rabbit erythrocytes exposed in vitro to arsenate has been reported (Delnomdedieu et al., 1994b).

Molecular action – Organic arsenic (metabolites)

Trivalent organic arsenicals react with sulfhydryl groups, as observed with trivalent inorganic arsenicals. In vitro binding of monomethylarsonous acid (MMAIII) and dimethylarsinous acid (DMA^{III}) to protein occurs to a greater extent than with the pentavalent organic forms (Styblo and Thomas, 1997). Monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) have been found to be bound to protein of rat liver cytosol incubated with arsenite (Styblo et al., 1995) and in liver and kidney of mice administered arsenite (Styblo et al., 1996). These compounds would be in the trivalent oxidation state when bound to protein.

Methylated trivalent arsenicals are potent inhibitors of GSH reductase (Styblo et al., 1997). The activity of these chemicals is greater than that of inorganic trivalent arsenic and the pentavalent organic arsenicals. GSH reductase contains five cysteine residues in each dimeric unit (Collinson and Dawes, 1995), which may provide a binding site for trivalent arsenic to inactivate the enzyme.

Pentavalent organic arsenicals are reduced in vitro by thiols to trivalent organic arsenicals which then bind other thiols (Cullen et al., 1984; Delnomdedieu et al., 1994a). The reduction of organic pentavalent arsenicals to their trivalent forms, as observed with inorganic pentavalent arsenicals, is a potential mechanism of action of the pentavalent organic arsenicals.

Carcinogenesis – Inorganic arsenic

Because trivalent inorganic arsenic has greater reactivity and toxicity than pentavalent inorganic arsenic, it is generally believed that the trivalent form is the carcinogen. Arsenic is not a point mutagen but does induce chromosomal abnormalities including changes in structure and number of chromosomes, endoreduplication and sister chromatid exchanges. DNA repair is inhibited by arsenic, and this inhibition can result in a co-mutagenic effect with x-rays, UV radiation and several chemicals. However, concentrations of arsenite that are required to inhibit DNA ligase activity in vitro are higher than that needed to inhibit repair within cells. This suggests that arsenite does not directly inhibit DNA ligase, but affects repair processes controlled by the cell (Li and Rossmann, 1989; US EPA, 1997; Hu et al., 1998). Arsenic may cause hypermethylation of DNA, particularly the promoter region, which can result in inactivation of tumour suppressor genes or genes involved in DNA repair (US EPA, 1997).

Rossman and Wang (1999) isolated two cDNAs from arsenite-resistant Chinese hamster V79 cells. One of these cDNAs is almost homologous with the rat tumour suppressor gene fau. This tumour suppressor gene contains a ubiquitin-like region fused to ribosomal protein (Michiels et al., 1993). Klemperer and Pickart (1989) have shown that arsenite inhibits the ubiquitin-dependent proteolytic pathway. Rossman and Wang (1999) suggest that the gene product, or a component within the ubiquitin system, is targeted by arsenic, resulting in alterations that may result in genotoxicity and carcinogenicity. Mass and Wang (1997) showed that arsenite increased the methylation of the tumour suppressor gene p53 and induced hypermethylation of DNA. Zhao et al. (1997) have shown that exposure of arsenite to rat liver cells results in DNA hypomethylation. The rat liver cells are transformed by the exposure to arsenite,

which may have resulted from aberrant gene expression. With respect to oxidative stress, arsenite induces metallothionein and heat shock protein. Catalase and superoxide dismutase reduce arsenite-induced micronuclei in CHO cells (Wang and Huang, 1994) and sister chromatid exchanges in human lymphocytes (Nordenson and Beckman, 1991). Antioxidants such as vitamin E, methylamine and benzyl alcohol reduce the killing of human fibroblasts by arsenite (Lee and Ho, 1994).

Induction of ornithine decarboxylase, an indicator of cellular proliferation, was observed in rat liver after administration of arsenite (Brown and Kitchin, 1996). The co-carcinogenic effect of inorganic arsenic was proposed from the observed co-genotoxic effect of arsenite and the inhibition of DNA repair. It is concluded that each of the modes of action could operate, that more than one may act at the same or different concentration-levels, and there is little evidence of favouring one over any other mode of action.

The cell-specificity of arsenic carcinogenicity in humans has been studied in primary human epidermal keratinocytes (Germolec et al., 1997). Low micromolar concentrations of sodium arsenite resulted in increased mRNA transcripts and secretion of growth factors including granulocyte macrophage-colony stimulating factor (GM-CSF), transforming growth factor alpha (TGF-α), and the cytokine tumour necrosis factor alpha (TNF-α). Total cell numbers were also elevated. Arsenic in drinking water also increased the number of skin papillomas in transgenic mice in which dermal application of phorbol esters induces papillomas (genetically initiated mice). These results support a hypothesis that chronic low-level exposure to arsenic stimulates keratinocyte secretion of growth factors, the resulting increased cellular division (and concomitant DNA replication) allows greater opportunities for genetic damage to occur.

Carcinogenesis – Organic arsenic (metabolites)

Dimethylarsinic acid has been suggested to be an initiator, on the basis of the DNA damage it induced in rat lung (Brown et al., 1997). Wei et al. (1999) have reported that dimethylarsinic acid is a rat bladder carcinogen after a 2 year drinking water exposure. Most studies have focused on the tumour-promoting activity of dimethylarsinic acid. Dimethylarsinic acid promotes tumour development in several different organs (Yamamoto et al., 1995; Wanibuchi et al., 1996; Yamanaka et al., 1996). Yamanaka et al. (1996) have suggested that dimethylarsinic acid may be a tumour progressor. The nitrosamine-initiated tumours were primarily benign, but after exposure to dimethylarsinic acid the tumours progressed to adenocarcinomas.

One potential mechanism of tumour promotion by dimethylarsinic acid is increased cell proliferation, as observed in the bladder (Wanibuchi et al, 1996), kidney (Murai et al., 1993) and liver (Wanibuchi et al., 1997) of uninitiated rats. A second mechanism could be dimethylarsinic acid-induced oxidative stress. A dimethylarsinic acid peroxyl radical has been detected in vitro (Yamanaka et al., 1990) and dimethylarsinic acid induces oxidative damage in the lung of mice (Yamanaka et al., 1991), in the liver of rats (Wanibuchi et al., 1997) and heat shock and other stress-related proteins such as metallothionein. Trivalent organic arsenicals inhibit GSH reductase, which might result in a decreased ability of cells to protect against oxidants.

Additional data

Molecular action

The biomethylation of arsenic, particularly the conversion to methylated metabolites that contain trivalent arsenic, monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), should be considered as a pathway for activation of arsenic, rather than a mode of detoxification.[51-55] The trivalent metabolites MMA^{III} and DMA^{III} exceed inorganic arsenic in the trivalent oxidation state (As^{III}) in potency as cytotoxins[56], enzyme inhibitors[57] and genotoxins[52,58,59].

According to Kitchin and Wallace (2008) there are three main ways in which arsenic species can interact with biologically important molecules.[60] First, trivalent arsenicals (a.o. arsenite, MMA^{III}, DMA^{III}, TMA^{III}, can bind to the sulfhydryls of peptides and proteins. For example, this may give rise to inhibition of DNA repair enzymes. Other arsenite binding sites such as selenocysteines, selenium atoms and molybdenum atoms are known. Second, arsenical exposures may generate free radicals and other reactive species in biological systems. Arsenic does not generate reactive oxygen by itself but it inhibits the scavenging systems of reactive oxygen species. Third, arsenic exposures can result in changes in the methylation state of cellular DNA. Following arsenic exposures, both hypomethylation and hypermethylation of DNA have been demonstrated in several different experimental systems. Altered DNA methylation could be caused by prior changes in the amount and activities of the DNA methylation enzymes involved in both de novo and maintenance of DNA methylation. Alternatively DNA methylation changes could be due to a shortage of cellular S-adenosylmethionine (SAM) concentration due to As.

Genotoxicity/Carcinogenesis

In a study with Chinese hamster ovary cells involving the biomethylation, genotoxic effects and uptake of different arsenic compounds is described by Dopp et al. (2004).[61] Biomethylation covered the subsequent conversion via reduction (pentavalent to trivalent state) and oxidative methylation (trivalent to pentavalent state) from arsenate (As^{V}) via arsenite (As^{III}), monomethylarsonic acid (MMA^{V}), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^{V}), dimethylarsinous acid (DMA^{III}), trimethylarsenic oxide (TMAO) to trimethylarsine. The potency of the DNA damage decreased in the order dimethylarsinous acid (DMA^{III}) > monomethylarsonous acid (MMA^{III}) > arsenate and arsenite > monomethylarsonic acid (MMA^{V}) > dimethylarsinic acid (DMA^{V})> trimethylarsenic oxide (TMAO). The cellular uptake of the compounds was measured by ICP-MS analysis, demonstrating an uptake of 0.03% for monomethylarsonic acid and dimethylarsinic acid, 2% for monomethylarsonous acid, As^{III} and As^{V}, and 10% uptake for dimethylarsinous acid. It was postulated that the induction of genotoxic effects caused by the different arsenic species is primarily dependent upon their ability to penetrate cell membranes.[61]

Kitchin (2001)[46] discussed in a review 9 different possible modes of action of arsenic carcinogenesis: induced chromosomal abnormalities, oxidative stress and formation of reactive oxygen species (ROS), altered DNA repair, altered DNA methylation patterns, altered growth factors, enhanced cell proliferation (induction of cell signaling pathways that lead to expression of genes involved in cell growth and proliferation[62]), promotion of carcinogenesis and progression to malignancy, suppression of p53 and gene amplification. As yet (in 2012), four of these modes of action, chromosomal abnormality, oxidative stress, inhibition of DNA repair, and a continuum of altered growth factors → cell proliferation → promotion of carcinogenesis for arsenic carcinogenesis have a degree of positive evidence, both in experimental systems (animal and human cells) and in human tissues, while the remaining possible modes of carcinogenic action for arsenic (progression to malignancy, p53 suppression, altered DNA methylation patterns and gene amplification) do not have as much evidence (Kitchin, 2001[46]; Kitchin and Ahmad, 2003[54], Beyersmann and Hartwig; 2008[63]; Hughes and Kitchin, 2006[64]).

- *Chromosomal abnormality.*[7,46] Collectively, in vitro and in vivo genotoxicity assays have demonstrated that arsenic cause single strand breaks, formation of apurinic/apyrimidinic sites, DNA base and oxidative base damage, DNA-protein crosslinks, chromosomal aberrations, aneuploidy, sister chromatid exchanges, and micronuclei.[65-70] Chromosomal aberrations, characterized by

chromatid gaps, breaks and fragmentation, endoreduplication, and chromosomal breaks, are dose-dependent and arsenite is more potent than arsenate. Both MMA^{III} and DMA^{III} are directly genotoxic and are many times more potent than arsenite at inducing DNA damage. Inorganic arsenic can potentiate the mutagenicity observed with other chemicals, although arsenic itself does not appear to induce point mutations. Arsenic-induced genotoxicity may involve oxidants or free radical species.

- *Oxidative stress.*[7,46,64,71] Mechanistic studies of arsenic toxicity have suggested a role for the generation of reactive oxygen species in the toxicity of inorganic arsenic. Results of both in vivo and in vitro studies of arsenic-exposed humans and animals suggest the possible involvement of increased lipid peroxidation, superoxide production, hydroxyl radical formation, blood non-protein sulfhydryls, and/or oxidant-induced DNA damage. Reduction of cellular oxidant defense by treatment with glutathione-depleting agents results in an increased sensitivity of cells to arsenic toxicity. Support for mechanisms of toxicity that involve arsenic-induced oxidative stress includes findings that inhaled arsenic can predispose the lung to oxidative damage, chronic low-dose arsenic alters genes and proteins that are associated with oxidative stress and inflammation, and major transcriptional regulators of altered genes are redox sensitive.
- *Altered DNA repair.*[7,46] Arsenite is known to inhibit more than 200 enzymes. Early work on DNA repair enzymes showed that DNA ligases I and II were both inhibited by arsenite (Li and Rossman, 1989[72]; Lee-Chen et al. 1993[73]). Later work with purified human DNA repair enzymes showed that arsenite actually increased the activities of DNA polymerase beta, O6-methylguanine-DNA methyltransferase and DNA ligases I, II, and III (Hu et al., 1998[74]). Human poly(ADP-ribose) polymerase (PARP) activity is also inhibited by arsenite (Yager and Wiencke, 1997[75]). Especially zinc-finger DNA repair proteins may be sensitive tartgets for arsenicals. The theory that altered DNA repair is the cause of arsenic carcinogenesis is particularly attractive because trivalent arsenic species, such as arsenite, can bind strongly to dithiols as well as free sulfhydryl groups in proteins. Such protein binding could induce inhibition of DNA repair, mutation in key genetic sites, or increased cell proliferation (Kitchin, 2001[46]; Beyersmann and Hartwig, 2008[63]). An association between arsenic exposure and a decreased expression of genes involved in nucleotide excision repair, provided a plausible mechanism for the inhibition of DNA-repair mechanisms.[76]
- *Altered growth factors → Cell proliferation → Promotion of carcinogenesis.*[7,46] Increased concentrations of growth factors can lead to cell

proliferation and eventual promotion of carcinogenesis. Arsenic-induced cell death can also lead to compensatory cell regeneration and carcinogenesis. Altered growth factors, cell proliferation, and promotion of carcinogenesis have all been demonstrated in one or more systems exposed to arsenics. Altered growth factors and mitogenesis were noted in human keratinocytes. Cell death was observed in human hepatocytes and rat bladder epithelium. Cell proliferation was demonstrated in human keratinocytes and intact human skin and rodent bladder cells. Promotion of carcinogenesis was noted in rat bladder, kidney, liver, and thyroid, and mouse skin and lung.

To date a uniform mechanism of toxicity and carcinogenicity for inorganic arsenic and its compounds has not been put forward. Identification and understanding of the mode of action of arsenic genotoxicity is helpful in estimating cancer risk. The overall mechanistic evidence supports the view that genotoxicity is not caused by a direct effect of inorganic arsenic or its metabolites on the DNA, but via other processes which are triggered by arsenic and its trivalent metabolites MMA^{III} and DMA^{III}. This implies that the genotoxic mechanism should be considered as non-stochastic.

6.2 Summary

Trivalent inorganic arsenicals readily react with sulfhydryl groups in proteins and inactivate many enzymes, thereby inhibiting critical functions such as gluconeogenesis and DNA repair. A mechanism of toxicity of pentavalent inorganic arsenic is its reduction to a trivalent form. In addition, as a phosphate analogue, pentavalent arsenic could potentially affect a number of biological processes (uncoupling of in vitro oxidative phosphorylation).

The trivalent organic compounds, monomethylarsonous acid and dimethylarsinous acid, are metabolites of inorganic arsenic and exceed inorganic arsenic in the trivalent oxidation state in potency as cytotoxins, enzyme inhibitors and genotoxins. Therefore, the biomethylation of arsenic, particularly the conversion to methylated metabolites that contain trivalent arsenic, should be considered as a pathway for activation of arsenic.

As yet (in 2012), four of modes of action underlying carcinogenesis, i.e. chromosomal abnormality, oxidative stress, inhibition of DNA repair, and a continuum of altered growth factors → cell proliferation → promotion of carcinogenesis, have a degree of positive evidence, both in experimental systems (animal and human cells) and in human tissues, while the remaining possible

modes of carcinogenic action for arsenic (progression of carcinogenesis, p53 suppression, altered DNA methylation patterns and gene amplification) do not have as much evidence.

Identification and understanding of the mode of action of arsenic genotoxicity and arsenic compounds is helpful in the assessment of the cancer risk. The overall mechanistic evidence supports the view that genotoxicity should be considered as non stochastic since is not caused by a direct effect of inorganic arsenic or its metabolites on the DNA, but via indirect processes which are triggered by arsenic and its trivalent metabolites.

Chapter 7

Effects

WHO/ATSDR references are summarized in Annex F.

7.1 Observations in humans

Human data are summarized in Tables 10-15 (Annex G).

7.1.1 Irritation and sensitisation

WHO/ATSDR data

Arsenite can induce irritative contact dermatitis after occupational exposure (Goncalo et al., 1980), but dermal sensitisation to inorganic arsenic appears to be rare. Barbaud et al. (1995) reported on the contact hypersensitivity of arsenic in a crystal factory employee. A patch test was done with various compounds from the workplace, and arsenate was the only chemical that tested positive.

Local effects on the respiratory tract

Inhalation of inorganic arsenic dusts (usually containing mainly arsenic trioxide) is irritating to the nose, throat, and lungs, and can lead to laryngitis, bronchitis, and rhinitis (without an indication of an allergic response) (Dunlap, 1921; Lundgren, 1954; Morton and Caron, 1989; Pinto and McGill, 1953). However,

chronic functional impairment of respiration is not usually observed in workers even exposed to high levels of arsenic trioxide in air (Perry et al., 1948).

Local effects on the skin

Relatively little information is available on effects due to direct dermal contact with inorganic arsenicals, but several studies indicate the main effect is local irritation and dermatitis, with little risk of other adverse effects. Usually the effects are mild (erythema and swelling) but may progress to papules, vesicles, or necrotic lesions in extreme cases (Holmqvist, 1951). These conditions tend to heal without treatment if exposure ceases. Effects of this type have only been observed in workplace environments where there are high levels of arsenic dusts (Holmqvist, 1951; Pinto and McGill, 1953), and have not been noted in people exposed to arsenic in water or soil (presumably because the concentrations of arsenic that contact the skin from water or soil are too low to cause significant irritation).

Local effect on the eyes

Chemical conjunctivitis, characterised by redness, swelling, and pain, usually in combination with facial dermatitis, has been observed in workers exposed to arsenic dusts in air (Dunlap, 1921; Pinto and McGill, 1953). No information was located regarding air levels of arsenic that produce this effect.

Additional data

No additional data on irritation and sensitisation of arsenic and arsenic compounds was found in literature for the period up to September 3, 2012.

According to EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures[11], arsenic trioxide is labelled Skin Corr. 1B; H314 (causes severe skin burns and eye damage).

7.1.2 Acute toxicity

WHO/ATSDR data

Inorganic arsenic is acutely toxic and ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and central nervous system functions, multiorgan failure and eventually death. In survivors, bone

marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.

No cases were located regarding death in humans from inhalation exposure to inorganic arsenicals following acute exposure, even at the very high exposure levels (1-100 mg As/m^3) found previously in the workplace (e.g., Enterline and Marsh, 1982; Jarup et al., 1989; Lee-Feldstein, 1986). Dermal exposure to inorganic arsenicals has not caused lethality in humans. Acute lethality caused by ingestion of inorganic arsenic is usually attributable to cardiopulmonary collapse (Levin-Scherz et al., 1987; Saady et al., 1989), while delayed lethality results from failure of one or more of the many tissues injured by arsenic (Campbell and Alvarez, 1989). Estimates of the minimum lethal oral dose in humans range from 1 to 3 mg As/kg bw/day (Armstrong et al., 1984; Holland, 1904; Vallee et al., 1960), although there may be considerable variation between individuals. Facial oedema, generally involving the eyelids, was a prominent feature of inorganic arsenic poisoning among 220 cases associated with an episode of ingestion of soy sauce contaminated with arsenic in Japan (Mizuta et al., 1956) and has also been reported in poisoning cases in the United States (Armstrong et al., 1984). The oedema developed soon after the initial exposure and then subsided.

Additional data

No case reports were found on inhalation exposure. Several human case reports on arsenic ingestion were found in literature for the period up to September 3, 2012 (see Annex G, Table 11).[77-85] The acute oral toxicity of arsenic was summarised as follows: fatal consequences have been reported with acute doses of inorganic arsenic between 70-300 mg, equivalent to 1-4 mg/kg bw.

Symptoms of acute intoxication usually occur within 30 min of ingestion but may be delayed if arsenic is taken with the food. Initially, a patient may have a metallic taste or notice a slight garlicky odor to the breath associated with a dry mouth and difficulty in swallowing. Early clinical symptoms at acute arsenic intoxication may be muscular pain, weakness, with flusking skin. Severe nausea and vomiting, colicky abdominal pain, and profuse diarrhea with rice-water stools abruptly follow. Capillary damage leads to generalized vasodilation, transudation of plasma, and vasogenic shock. Arsenic's effect on the mucosal vascular supply, not a direct corrosive action, leads to transudation of fluid in the bowel lumen, mucosal vesical formation, and sloughing of tissue fragments. The patient may complain of muscle cramps, numbness in hands and feet, reddish rashes in the body, and intense thirst.

In severe poisoning, the skin becomes cold and clammy, and some degree of circulatory collapse usually occurs along with kidney damage and decreased urine output. Drowsiness and confusion are often seen along with the development of a psychosis associated with paranoid delusions, hallucinations, and delirium. Finally, seizures, coma, and death, usually due to shock, may follow. Following the gastrointestinal phase, multisystem organ damage may occur. If death does not occur in the first 24 h from irreversible circulatory insufficiency, it may result from hepatic or renal failure over the next several days. Cardiac manifestations include acute cardiomyopathy, subendocardial hemorrhages, and electrocardiographic changes. The most common changes on an electrocardiogram are prolonged QT intervals and nonspecific ST-segment changes. [86,87,88]

According to EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures[11], arsenic, arsenic pentoxide and lead arsenate are labelled Acute Tox. 3; H331 (Toxic if inhaled) and H301 (Toxic if swallowed). Furthermore, arsenic trioxide is labelled Acute Tox. 2; H300 (Fatal if swallowed).

7.1.3 Short-term toxicity

WHO/ATSDR/additonal data

No data were available on inhalation exposure. The major effects of subacute oral exposure are gastrointestinal, haematological and dermal.

7.1.4 Long-term toxicity

Genotoxicity

WHO/ATSDR data

Genotoxicity studies in relation to oral arsenic exposure have included exposed and unexposed individuals from several populations and analyses have been based on various tissues, including blood, buccal and bladder cells as well as sections from tumour biopsies.

Even with some negative findings, the overall weight of evidence indicates that arsenic can cause clastogenic damage in different cell types, with different

end-points, in exposed individuals. Clastogenic effects have also been observed in cells from cancer patients. Arsenic is thus clastogenic in humans in vivo.

No HPRT gene mutation was seen in the single study in lymphocytes or increases in ras or p53 gene expression in cells from cancer patients with long-term exposure to arsenic, except for one study with increased p53 expression in Bowen's disease patients with such exposure compared to patients without exposure.

Studies of humans have detected higher-than-average incidence of chromosomal aberrations in peripheral lymphocytes, both after inhalation exposure and oral exposure. These studies must be interpreted with caution, since in most cases there was only a small number of subjects and a number of other chemical exposures was possible.

Additional data

In Annex G, Table 13a (in vivo) and 13b (in vitro), the available human data with regard to genotoxicity up to 2012 are summarised. These studies showed chromosome aberrations and sister chromatid exchanges in different cell types of people exposed to relatively high arsenic concentrations in drinking water.[65-70,89-92]

In vitro studies with human lymphocytes and fibroblasts also showed genotoxic effects of arsenic: nicking (unwinding) of DNA, double-stranded DNA breaks, induction of alkaline labile sites, sister chromatid exchanges, oxidative damage and interference with the formation and repair of DNA adducts. Methylated trivalent arsenicals were more potent DNA damaging compounds than the other arsenicals.[58,59,91-96]

Carcinogenicity

Human data on carcinogenicity of arsenic are summarised in Table 14 of Annex G. The Table includes the key studies reported by the WHO (2001)[6] and ATSDR (2007)[7] and additional material up to September 2012 (IARC 2012).[97]

Because sufficient epidemiological data involving inhalation exposure to arsenic (the most relevant route of occupational exposure) is available, the oral studies concerning carcinogenicity, mainly epidemiological drinking water studies, are only briefly mentioned in this section (based on the summaries from WHO[6] and ATSDR[7]).

WHO/ATSDR data

Studies of populations occupationally exposed (primarily by inhalation) to arsenic, such as smelter workers, pesticide manufacturers and miners in many countries, consistently demonstrate an excess lung cancer risk among the arsenic-exposed. Although all these groups are exposed to other chemicals in addition to arsenic, it is unlikely that some other common factor could explain the findings.

There are three occupational cohorts (non-ferrous smelters) in which quantitative exposure assessments allow an evaluation of the relation between exposure to arsenic (arsenic trioxide) and lung cancer, those of the copper smelters in Tacoma, Washington (USA), Anaconda, Montana (USA), and Rönnskär (Sweden).

Tacoma copper smelter

Results from the Tacoma copper smelter have been published in a series of papers (Pinto and Bennett, 1963; Pinto et al., 1977 and 1978; Enterline and Marsh, 1980 and 1982; Enterline et al., 1987[98], Enterline et al., 1995[4]). In the 1995 update[4], the vital status of 2802 men who worked at the smelter for a year or more during the period 1940-1964 was followed for the period 1941-1986; exposure assessment was extended to 1984, the time the smelter closed. The vital status was determined for 98.5% of the cohort, and of the 1583 known deaths, death certificates were obtained for 96.6%. The expected numbers of deaths for various diseases were calculated for white males in the state of Washington (all studied workers were males and nearly all were white).

Exposure to arsenic was estimated from departmental measurements of arsenic in air from the annual company reports, available since 1938 (the factory began operation in 1913), and from measurements of urinary arsenic since 1948. Before 1971, the airborne arsenic concentrations came from “spot” samples and “tape” samples (apparently surface sampling), thereafter from personal air sampling. These data were combined to allow for an analysis of the relation between the concentrations of arsenic in air and various cancers. The conversion of data of urinary arsenic to airborne arsenic was made by the identification of departments and years for which data from both air and urinary arsenic were available and by the determination of the mathematical relation between the two. Twenty-eight pairs of data represented 11 of the 33 departments at the smelter.

The relationship between arithmetic mean arsenic concentrations in air and geometric mean concentrations of arsenic in the urine was described as follows:

$$Air\ arsenic\ (\mu g/m^3) = 0.0064 \times (urine\ arsenic\ (\mu g/L))^{1.942}$$

Using this equation, urinary arsenic concentrations were transformed into airborne data for departments for which no air data were available. From the data an exposure matrix of arsenic in air was developed by department and year from 1938 up to the time the smelter closed in 1984. For each worker, cumulative exposure in ($\mu g/m^3$) · year was then calculated on the basis of individual history of work in different departments. For years before 1938 the exposure data for 1938 were used.[98]

An increase in lung cancer risk related to cumulative arsenic exposure was observed. The lung cancer standard mortality ratio (SMR)* was 188 in the group with < 20 years after the first exposure, and 217 among those with > 20 years since first exposure, indicating a rather short latency period. The SMRs for respiratory cancer by cumulative airborne arsenic and date of hire are presented in Table 5.

Table 5 Standard mortality ratios (SMRs) for respiratory cancer by cumulative airborne arsenic and date of hire.

Cumulative exposure (mean exposure) ($\mu g/m^3$·years)	Total cohort	Hired < 1940	Hired ≥ 1940
<750 (405)	154	65	178*
750-1,999 (1,305)	176**	68	256**
2,000-3,999 (2,925)	210**	246*	170
4,000-7,999 (5,708)	212**	150	300**
8,000-19,999 (12,334)	252**	255**	244*
20,000-44,999 (28,356)	284**	252**	406**
45,000+ (58,957)	316**	339*	-

*: P<0.05; **: P<0.01

The reason for the stratification is that for the cohort hired before 1940 only the person-years accumulated from 1941 were followed up for deaths, whereas for the cohort hired in 1940 and later all the person-years, to the end of the follow-up, period, were assessed. The stratification to some extent also separated workers before 1940 with relatively high exposure and with poor respiratory protection from workers with lower exposure, but with better quality exposure data and perhaps better respiratory protection. According to the authors, smoking could be an important confounder in respiratory cancer and data on the histories of the study population for smoking were collected.

* Standard mortality ratio: Ratio between the observed number of deaths in a study population and the number of deaths that would be expected in a standard population.

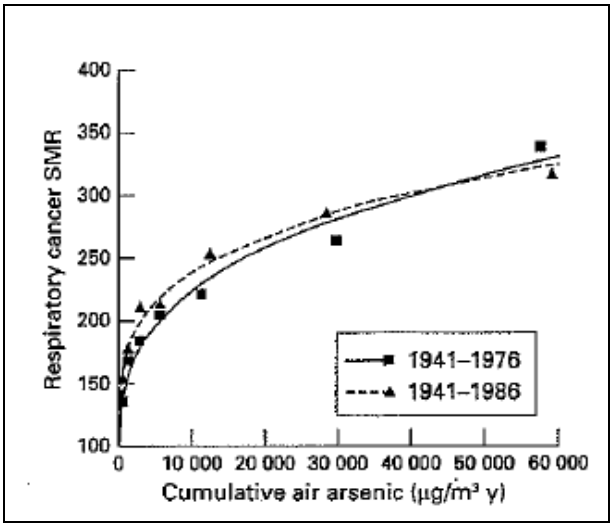

Figure 2 The SMRs for respiratory cancer for cumulative airborne arsenic. Copper smelter workers from Tacoma 1941-1976 and 1941-1986. Data are plotted at the means of exposure intervals. Fitted lines are from corresponding power functions. Source: Enterline et al., 1995[4].

When the SMR is plotted against cumulative arsenic exposure on an arithmetic exposure scale (Figure 2), relatively larger increments in respiratory cancer risk are observed at low exposure levels, i.e. the exposure-response curve is concave downward. This had already been observed in the previous report on the same cohort, where the follow-up time was 10 years shorter.[98] However, when lung cancer SMR was plotted against measured urinary arsenic concentrations, a linear relationship was observed.[98]

An earlier publication of the Tacoma copper smelter[98] contained data on actual daily exposure concentrations, duration of exposure and the risk on lung cancer. In this study, an arsenic exposure category of < 400 µg/m^3 (mean 213 µg/m^3) was associated with a statistically significant SMR of 238.7 for copper smelter workers who were exposed to arsenic for 30 or more years.

Anaconda copper smelter

An elevated risk of lung cancer among workers in the Anaconda copper smelter in Montana was originally reported by Lee and Fraumeni (1969). Updates and further cohort and nested case-referent analyses were published later (Lubin et al., 1981; Welch et al., 1982; Brown and Chu, 1983a,b; Lee-Feldstein, 1983,1986 and 1989[99]; Lubin et al., 2000[1]).

The study population of the latest cohort update[1] consisted of 8,014 white males, who were employed for ≥ 12 months before 1957. Their vital status was followed from 1 January 1938 to 31 December 1987; a total of 4,930 (63%) were deceased, including 446 from respiratory cancer. The vital status at the end of the follow-up period was not known for 1175 workers (15%), and they were assumed to be alive at the end of the study period (except the 81 workers born before 1900, who were assumed to have died).

Industrial hygiene data (702 measurements), collected between 1943 and 1958, were used to categorise each work site to an exposure category on a scale 1-10, and work areas were then grouped as representing “light”, “medium” or “heavy” exposure. Based in addition on estimates of workers' daily exposure time, time-weighted average (TWA) exposures for each category were created, and were considered to be 0.29, 0.58 and 11.3 mg/m^3 arsenic for the “light”, “medium”, and “heavy” exposure category. It should be noted that in earlier reports (Lee-Feldstein 1986) on this cohort the TWA exposure estimates used were different, notably for the "heavy" exposure category (0.38, 7.03, and 61.99 mg/m^3, respectively). These earlier estimates were not weighted by workers’ exposure time. For each worker, the cumulative exposure was estimated from the time of working in different work areas. The authors note that industrial hygiene measurements were actually available for less than half of the 29 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. The locations for sampling were not randomly selected.

Altogether 446 deaths from respiratory cancer (SMR 155, 95% CI 141-170) were observed. A trend of increasing risk with increasing estimated exposure was seen; the risk increased linearly with time of employment in each exposure category.

Furthermore, it was found that estimated relative risk for respiratory cancer declined with calendar year of follow-up. Measurements of arsenic in air were available only for the years 1943-1958, and the exposure assessment implicitly assumed that arsenic levels were constant over time. Available monitoring data and anecdotal information indicated that airborne arsenic levels declined over time in work areas with heavy and medium exposures with lesser reductions of airborne arsenic in work areas with light exposure. These variations in exposure probably accounted at least partly for the observed significant downward trend in the relative risk for respiratory cancer by year of follow-up. In support of this, it was found that the trend in the relative risks with duration of exposure declined with follow-up for medium and heavy, but not for light, arsenic exposures.[1]

Although information on smoking was not available, according to the authors it is noteworthy that mortality from smoking-related cancers, except for chronic obstructive pulmonary disease, was not excessive. In a sample of 1469 workers from the original cohort, there was a higher proportion of smokers compared with US white males. However, the proportion of cigarette smokers did not vary significantly by extent of exposure to airborne arsenic, indicating that it was unlikely that smoking confounded the assessment of lung cancer risk with arsenic exposure according to the authors.[1]

Rönnskär copper smelter

The elevated lung cancer incidence among workers of the Rönnskär smelter in northern Sweden was originally reported in a population-based case-referent study in St Örjan parish in 1978 (Axelson et al., 1978). Since then, studies using both cohort and case-referent approaches have been published (Wall, 1980; Pershagen et al., 1981 and 1987; Järup et al., 1989[3]; Sandström et al., 1989; Järup and Pershagen, 1991[100]; Sandström and Wall, 1993). The cohort consisted of 3916 male smelter workers, who had worked for at least 3 months at the smelter between 1928 and 1967. The vital status of all but 15 (0.4%) of them was verified. Mortality of different causes, as defined on death certificates, was compared to local rates. Reference rates were not available for the period before 1951, but the contribution of deaths during this period (89 out of a total of 1275, i.e. 7%) was minor.

Air concentrations of arsenic were estimated by the factory industrial hygienists. The first measurements were carried out in 1945, and from 1951 exposure data were more generally available; production figures were used to extrapolate exposures before 1951. Each work site was characterised by an exposure level during three consecutive time periods, and the workers' cumulative exposure was assessed on the basis of their working history in these different work sites.

The SMRs were very similar whether they were calculated with no latency, 10 years minimum latency or 10 years minimum latency with exposure lagged 5 years. A positive dose-response relationship was found between cumulative arsenic exposure and lung cancer mortality with an overall SMR of 372 (95% CI 304-450), and a statistically significantly increased risk was observed even in the lowest exposure category, < 0.25 (mg/m^3) $\cdot$ year. In Table 6 the SMRs for lung cancer by cumulative airborne arsenic are presented.

Table 6 Standard mortality ratios (SMRs) for lung cancer by cumulative airborne arsenic.

Cumulative exposure (mg/m^3·years)	SMR (mean (95% confidence interval))
< 0.25	271 (148-454)
0.25-<1	360 (192-615)
1-<5	238 (139-382)
5-<15	338 (189-558)
15-<50	461 (309-662)
50-<100	728 (267-1.585)
100+	1.137 (588-1.986)

A sensitivity analysis showed that the SMRs were fairly robust, particularly among the workers with low and medium exposure (Järup, 1992). Even when the exposure estimates before 1940 were reduced dramatically (assuming there was a large overestimation of the early exposures), these SMRs changed only marginally. As expected, the SMRs in the highest exposure group increased as the early exposures were reduced. An overestimation of the early exposures would thus tend to decrease the strength of the exposure-response association.

Little difference was observed in the SMRs for workers hired before 1940, in 1940-1949, or after 1949, when the estimated level of exposure was similar, meaning that a longer follow-up did not increase the apparent risk.[3] In most subcohorts, and in the total cohort, the mortality increased with increasing average intensity of exposure, but no clear-cut trend was observed for the duration of exposure. Exposure to sulphur dioxide was also assessed. The lung cancer risk was elevated in all groups exposed to sulphur dioxide, but there was no exposure-response with the estimated cumulative sulphur dioxide exposure.

In a nested case-referent study on the interaction between smoking and arsenic exposure as lung cancer-causing agent in the cohort as described above[100], lung cancer risks were positively related to cumulative arsenic exposure with smoking standardised relative risks ranging from 0.7 to 8.7 in different exposure groups. A negative confounding by smoking was suggested in the highest exposure category. The interaction between arsenic and smoking for the risk of developing lung cancer appeared less pronounced among heavy smokers.

In a cancer incidence study (Sandström et al., 1989), partly overlapping with the above described study, the cancer risk of the smelter workers over a moving 5-year period was observed to decrease steadily from 1976-1979 to 1980-1984, showing that the later the date of first employment the lower the incidence of cancer, especially for lung cancer. This trend may be explained by decreasing

exposure levels to arsenic. Further follow-up of an expanded Rönnskär cohort (n = 6,334) by Sandström and Wall (1992) showed also a decreasing trend in lung cancer incidence and mortality, but there was still an elevated lung cancer incidence among the workers when compared with Swedish men.

Other studies

A very high excess of lung cancer (SMR 2500; 10 observed and 0.40 expected cases in the heavy exposure category), which was related to duration and level of exposure, was observed in the copper smelter of a Japanese metal refinery (Tokudome and Kuratsune, 1976); the study was prompted by an earlier case-referent study that demonstrated an excess lung cancer rate among copper-smelter workers (Kuratsune et al., 1974). There was an approximately 3-fold increase in the relative death rate from lung cancer among employees of a copper smelter in Utah, in comparison to workers of the same company not employed in the smelter (mainly mine and concentrator workers), and also in comparison to Utah state figures (Rencher et al., 1977). The risk was related to all estimated exposure parameters (cumulative exposure to arsenic, sulphuric acid, lead and copper), and was similar for smokers and non-smokers. This refinery was a part of a cohort study in eight copper smelters (Enterline et al., 1987b), the SMR for respiratory cancer < 20 years since first exposure was 170 (11 deaths), and ≥ 20 years 108 (39 deaths). In this study, the only smelter with an appreciable exposure to arsenic was the Utah one, and this was the only one with a statistically significant excess in lung cancer.

- Pesticide manufacture and application

Ott et al. (1974) conducted a proportionate mortality study of decedents who had worked at a factory producing arsenical pesticides, mainly lead arsenate, calcium arsenate, copper acetoarsenite and magnesium arsenate. The cause of death of 173 workers who had worked at least 1 day in jobs with presumed arsenic exposure was compared to that of 1809 decedents (age- and calendar-year-adjusted) from the same factory, with no exposure to arsenic or asbestos. The exposure of the workers was analysed from a job exposure matrix covering the working history. The proportionate mortality ratio (PMR)* for lung cancer increased with estimated exposure, from a PMR of 200 at an exposure level of 1-1.9 (mg/m^3) · month to a PMR of 700 at the highest cumulative exposure group ≥ 96 (mg/m^3) · month.

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* Proportionate mortality ratio: Number of deaths from a specific cause in specific period of time per 100 deaths from all causes in the same time period.

Ott et al. (1974) also conducted a cohort study at the same pesticide plant. The cohort was expanded and updated through December 1982 (Sobel et al., 1988) to include 611 workers altogether; the mortality was compared to age- and calendar-time standardised data on US white males. A significant excess of lung cancer mortality was observed (35 observed vs. 15.6 expected cases; SMR 225, 95% CI 156-312). The small number of deaths made analyses by duration and latency difficult; analysis by exposure level or cumulative exposure was not reported.

In a cohort study of pesticide manufacturing workers in Baltimore, the vital status of 1050 men and 343 women was followed from 1946 through 1977 (Mabuchi et al., 1979 and 1980). The vital status was determined for 86.9% of men and 66.8% of women; the non-traced subjects were counted as being alive at the time of ending the follow-up. Cause-specific mortality was compared to that of Baltimore city whites, age- and calendar time adjusted, and 23 lung cancer deaths were identified, which represents an excess lung cancer mortality (SMR 168 based on Baltimore City whites, or 265 based on US whites; $p < 0.05$ for both). There was an exposure-response relationship between presumed cumulative exposure (no relevant measurement data on exposure were available) and the SMR. The SMR reached 2750 in the highest exposure category (3 lung cancer deaths). No exposure-response relationship was observed between presumed cumulative exposure to non-arsenical pesticides and SMR.

In an autopsy series of 163 winegrowers from the Moselle area (Lüchtrath, 1983), 130 cases of cancer in internal organs were observed. Of these, 108 were lung cancers. In an age- and sex-adjusted control group of 163 people, there were 23 malignant tumours, out of which 14 were lung tumours. Exposure to arsenic was considered to be by inhalation of arsenic-containing insecticide, but to a much larger extent, by drinking arsenic-contaminated "Haustrunk" (a wine substitute made from already pressed grapes), which was estimated to lead to a daily intake of about 3-30 mg arsenic.

In 1938 a cohort of 1231 people living in the Wenatchee area in Washington, where lead arsenate was extensively used in orchards, was studied. The mortality of this cohort was reported by Nelson et al. (1973), Wicklund et al. (1988) and Tollestrup et al. (1995). No difference in lung cancer mortality was observed between orchardists exposed to arsenical insecticides and consumers who were not significantly exposed to arsenicals (hazard ratio 0.59, 95% CI 0.19-1.85) (Tollestrup et al., 1995). It is likely that the overall exposure to arsenic for orchardists was low. A case-control study included all white male orchardists (n = 155) who died in Washington state between 1968 and 1980 from respiratory cancer, using orchardists who died of other causes as controls (n = 155)

(Wicklund et al., 1988). Lead arsenate exposure did not differ between cases and controls, and smoking habits were similar.

- Miners and other

In a cohort study on tin-miners in the UK (Hodgson and Jones, 1990), 13 workers had worked in arsenic calcining. Three of them had died of cancer of the trachea, bronchus, lung or pleura (0.55 expected, SMR 550, $p < 0.05$), and 2 of stomach cancer (0.2 expected, SMR 890, $p < 0.05$). A very high lung cancer mortality has been demonstrated among tin-mine workers exposed to arsenic and radon in Yunnan, China (Taylor et al., 1989; Qiao et al., 1997). The lung cancer risk increased with estimated cumulative exposure to arsenic (Qiao et al., 1997). A 2-fold excess (SMR 213; 95% CI 148-296) in lung cancer mortality was observed among workers in a gold-mine and refinery in France, mainly among workers with a history of exposure to arsenic, diesel exhaust, radon and silica. There was little change in the relative risk with length of employment, and the risk was similar among refinery workers and miners (Simonato et al., 1994). An exposure-related increase in the lung cancer mortality was also observed among gold-miners in Ontario, exposed to arsenic and radon daughters (Kusiak et al., 1991 and 1993). Similarly, lung cancer mortality among Australian gold-miners was higher than that expected from the experience of all Western Australian men (SMR 140, 59 observed and 40.8 expected cases, $p < 0.01$). The gold-miners were exposed to arsenic, radon daughters and silica, and apparently smoked more than the referent population (Armstrong et al., 1979).

Female hat-makers, probably exposed to arsenic while making felt hats, had an elevated risk of lung cancer (6 cases versus 0 in controls) in a case-referent study (376 cases with 892 controls) on occupational risk factors of lung cancer in Italy (Buiatti et al., 1985).

A cohort mortality study of workers in a Russian fertiliser plant, including 2039 men and 2957 women, showed an excess mortality from all cancers combined (SMR 143) and lung cancer (SMR 186) for the male production workers (Bulbulyan et al., 1996). Excess mortality from all cancers and stomach cancer was found for the workers with the highest average exposure to arsenic, and excess lung cancer mortality was attributed to exposure to arsenic.

- Interactions of arsenic exposure and tobacco smoking

Hertz-Picciotto et al. assembled data from numerous published case-control and cohort studies with regard to lung cancer due to smoking and to occupational arsenic exposure to examine whether active smoking and occupational exposure to arsenic act synergistically to increase the risk of lung cancer.[101] There were six

studies on two overlapping smelter populations (Tacoma and Rönnskär), where a direct evaluation of the interaction could be assessed (Rencher et al., 1977; Pershagen et al., 1981; Enterline, 1983; Pershagen, 1985; Enterline et al., 1987b; Järup and Pershagen, 1991[100]). The joint effect from both exposures consistently exceeded the sum of the separate effects by about 70 to 130%. The calculated excess fractions for the synergism showed that a minimum of between 30% and 54% of lung cancer cases among those with both exposures could not be attributed to either one or the other exposure alone. Taken as a whole, the evidence is compelling that arsenic and smoking act in a synergistic manner to produce lung cancer. The mechanism for the synergism is however unclear.[101]

- Lung cancer in the vicinity of arsenic-emitting industries

Mortality rates for lung cancer for white men and women in 1950-1960 were significantly higher in several counties of the United States with copper, lead, or zinc smelting and refining industries (Blot and Fraumeni, 1975), and a 2-fold increased mortality of lung cancer was observed among people with residence near a zinc smelter, and in areas with high topsoil concentrations of arsenic, cadmium, copper, lead and manganese (Brown et al., 1984). A slightly higher mortality of lung cancer was observed among male residents of Rouyn-Noranda, a community with a copper smelter, than among male residents of a referent community (SMR 150) or Quebec (Canada) as a whole (SMR 120); no such difference was observed among women (only 7 exposed cases) (Cordier et al., 1983). Although the lung cancer mortality between 1935 and 1969 in women living in three geographically defined areas in the vicinity of an arsenic-emitting smelter was not different from that expected from nationwide expected figures, there was a positive trend following predicted exposure levels (Frost et al., 1987). No difference was observed between the frequency of lung cancer and that of other cancers in the vicinity of non-ferrous smelters (a lead-zinc smelter and 10 copper smelters) in the USA (Greaves et al., 1981).

The lung cancer mortality among people living in the vicinity of a copper smelter in Rönnskär (Sweden) was studied in a cohort and a case-referent study (Pershagen et al., 1977; Pershagen, 1985). In the cohort study, a significantly higher mortality from lung cancer was observed among men living close to the smelter than among men in a reference area (Pershagen et al., 1977). The difference disappeared, however, when men working in the smelter were excluded. In the case-referent study, the odds ratio (OR)* for residence in the

* Odds ratio: Ratio between the proportions of cancer deaths in the exposed cohort and the non-exposed cohort.

exposed area was 2.0 (95% CI 1.2-3.4), and it was not explained by occupation in the smelter, or by differences in smoking habits (Pershagen, 1985). Lung cancer mortality was higher in men living in the vicinity of a factory producing arsenical pesticides in Baltimore (Maryland, USA) (Matanoski et al.,1981). No association between the distance from a smelter and lung cancer risk was observed in a case-referent study where 575 lung cancer cases were compared with 1490 breast and prostate cases collected from 1944 to 1973 in El Paso, Texas (USA), where a smelter had been operating since 1887 (Rom et al., 1982).

In a study of lung cancer mortality in 6 Arizona (USA) copper smelter towns, using 185 lung cancer cases and 2 matched controls per case from deceased residents during 1979-1990, information on lifetime residential, occupational, and smoking history was obtained (Marsh et al., 1997 and 1998). Historical environmental exposures to smelter emissions were linked with residential histories to derive individual profiles of residential exposure. Occupational histories were characterised by potential exposure to smelter emissions, asbestos and ionising radiation. No statistically significant associations were observed between lung cancer risk and residential exposure to smelter emissions, when adjustment for potential confounding factors (gender, Hispanic ethnicity, and smoking) were made. The authors concluded that the study provided little evidence of a positive association between lung cancer mortality and residential exposure to smelter emissions.

A Chinese case-control study including 1249 lung cancer patients and 1345 population-based controls showed 3-fold elevated risks among smelter workers (Xu et al., 1989 and 1991). Soil levels of arsenic rose with increasing proximity to the Shenyang copper smelter, and, after controlling for smoking and work experience in the smelter, elevated risks of lung cancer were found among men, but not women, living within 1 km of its central stacks.

It has been noted that epidemiological studies designed to detect lung cancer risk and other health effects in communities surrounding arsenic-producing copper smelters usually have insufficient statistical power to detect the small increases in risk that may occur (Hughes et al., 1988).

- Exposure-response relationships

Sufficient information on the levels of exposure to ensure reliable assessment of the exposure-response relationships can be found only in the three copper smelter cohorts: Tacoma, Anaconda and Rönnskär. Figure 3 shows the dose-response relation reported for the three copper smelter cohorts. The horizontal scale is logarithmic. Although the figure shows three lines, the dose-response relations are not modeled, but plotted by connecting the SMRs of the different

exposure categories. In all, there was an increase in lung cancer risk with increasing exposure (Figures 2 and 3). The risk seems to increase more rapidly with dose at low cumulative dose levels than at higher exposures (which is clear from Figure 2, but not from Figure 3 due to the logarithmic scale), and the general form of the exposure-response is rather similar in the three studies (Figures 2 and 3).

The shape of the exposure-response curve has been further analysed and discussed by Hertz-Picciotto and Smith, 1993[102], who noted that all of the studies with quantitative data are consistent with a nonlinear, i.e. supralinear (decreasing slope), exposure-response relationship. Two of these studies (Lee-Feldstein, 1986 and Järup, 1989[3]) are also consistent with a linear relationship over an elevated background risk of lung cancer among arsenic-exposed workers. Neither toxicokinetic mechanisms nor confounding from age, smoking, or other workplace carcinogens that differ by exposure level appears likely to explain this curvilinearity. The authors argue that a plausible explanation may be synergism

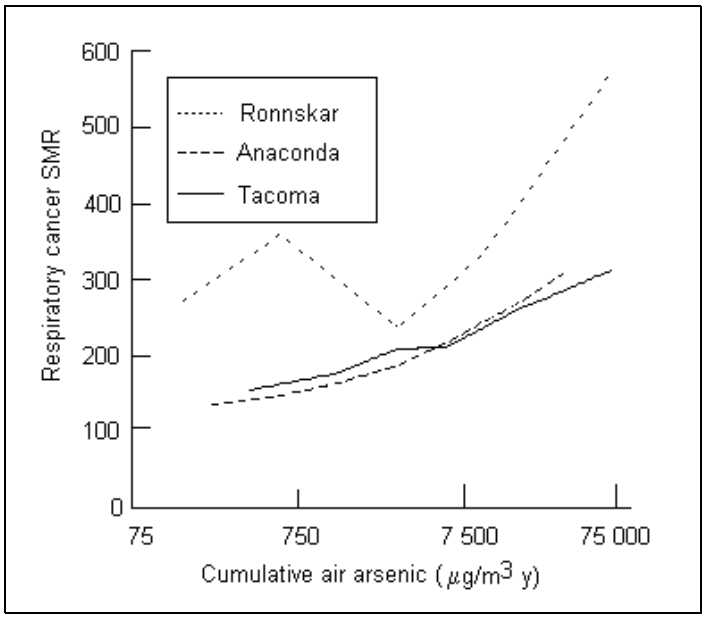

Figure 3 The SMRs for respiratory cancer (Anaconda and Tacoma) and lung cancer (Rönnskär) for cumulative airborne arsenic. To facilitate presentation the upper three exposure categories for Rönnskär have been combined. Anaconda and Rönnskär are plotted at the midpoint of exposure intervals except for upper intervals 30,000 and 57,000, which were based in part on the exposure distributions in the data from Tacoma. Source: Enterline et al., 1995[4].

(with smoking) which varies in magnitude according to the level of arsenic exposure. Another possible explanation may be a long-term survivorship in higher-exposure jobs among the healthier, less susceptible individuals (Hertz-Picciotto and Smith, 1993[102]; Arrighi and Hertz-Picciotto, 1996). It is also plausible that exposure estimate errors are more prominent at higher exposure levels as a result of past industrial hygiene sampling or worker protection practices[102], which is consistent with the findings of the sensitivity analysis of the Rönnskär data (Järup, 1992), and the update of the Anaconda cohort[1].

Using the updated data on the Tacoma smelter cohort of Enterline et al., 1995[4], Viren and Silvers (1999)[103] explored the (non)linearity in the lung cancer dose-response in this cohort. Lung cancer risk was expressed in terms of both the SMR and the excess mortality rate (EMR). Analyses were undertaken by subcohort as there was strong evidence of confounding by year of initial hire. Subcohort analyses based on initial employment, prior to 1940 or thereafter, showed that the nonlinearity in the dose-response was strongly influenced by date of initial hire. Whether the cohort risk was measured by either the SMR or EMR, a nonlinear dose-response was evident only among workers hired prior to 1940. This, however, was strongly related to the artifactually low lung cancer mortality seen among workers hired between 1930 and 1939. Among workers hired after 1940, analyses showed that a linear dose-response provided a clearly superior fit.

Re-analysis of the Anaconda cohort[1] is also in favour of a linear exposure-risk relationship, and ascribes the apparent non-linearity observed in other studies to overestimation of the high exposures. According to Lubin *et al.* (2000) the conclusion of Hertz-Picciotto and Smith[102] that a concave relation existed between respiratory cancer and cumulative airborne arsenic exposure was strongly influenced by the previous analysis of the Anaconda data and analysis of the Tacoma data for which the exposure-response relations for the individual studies varied substantially in magnitude and shape.

- Other types of cancer

There have been occasional reports of other types of cancer (i.e., non-respiratory cancer) potentially associated with inhalation exposure to inorganic arsenic, but there is no strong evidence for any of them. Enterline et al., 1995[4] found a statistically significant increase in cancer of the large intestine and bone, and SMRs > 150 for cancer of the buccal cavity and pharynx, rectal cancer, and kidney cancer. However, neither cancer showed any relation to cumulative arsenic exposure, and the purported increase in bone cancer risk was based on a very small number of observations. Bulbulyan et al., 1996 reported an increase in

risk of stomach cancer among workers exposed to the highest average arsenic concentrations at a Russian fertiliser plant, but this finding, which was based on a small number of observations and was only marginally statistically significant, was confounded by exposure to nitrogen oxides. Wingren and Axelson, 1993 reported an association between arsenic exposure and stomach and colon cancer in Swedish glass workers, but this result was confounded by concomitant exposure to other metals. Lee-Feldstein, 1983 observed a small, marginally significant increase in digestive tract cancer (SMR=125) in one study of the Anaconda cohort, but this was not found in other studies of this cohort (Lee and Fraumeni, 1969; Lee-Feldstein, 1986; Welch et al., 1982). Wulff et al., 1996 observed an apparent increase in the risk of childhood cancer (all types combined) in the population living within 20 km of the Rönnskär smelter, but the apparent increase was based on a small number of cases (13 observed vs. 6.7 expected) and was not statistically significant, and exposure to arsenic was confounded by exposure to lead, copper, cadmium, sulphur dioxide, and possibly other emissions such as nickel and selenium. Various case reports have implicated occupational arsenic exposure as a potential contributing factor in workers who developed sinonasal cancer (Battista et al., 1996), hepatic angiosarcoma (Tsai et al., 1998), and skin cancer (Col et al., 1999; Tsuruta et al., 1998), but provide no proof that inhaled arsenic was involved in the etiology of the observed tumours. Wong et al., (1992) found no evidence that environmental exposure to airborne arsenic produced skin cancer in residents living near the Anaconda smelter.

Additional data

A small number of studies (Binks et al., 2005[104]; Jones et al. 2005[105]; Sorahan 2009[106]) have been published in support of the view that recent (late) exposures can be more important than exposures received in the distant past (i.e. that arsenic is a late stage carcinogen) and that analyses of lifetime cumulative exposure can fail to identify a potent occupational carcinogen. Binks et al. (2005)[104] studied lung cancer mortality in employees from a tin smelter operation (Capper Pass, North Humberside in UK). These employees were *a.o.* exposed to lead, arsenic and cadmium. A cohort consisting of 1462 males who had been employed for at least 12 months between 1/11/1967 and 28/7/1995, followed up through 31/12/2001. Lung cancer mortality was significantly elevated (62 death, SMR 161, 95% CI 124-206, P<0.001). Lung cancer mortality had been enhanced by occupational exposure to one or more carcinogens. However, this effect diminished with time since leaving exposure. Using

available data from the Capper Pass tin smelter cohort Jones et al. (2007)[105] tried to explain this excess lung cancer mortality by attributing this to specific exposure (lead, arsenic, cadmium *a.o.*). They conclude that a substantial proportion of the excess lung cancer mortality can be attributed to the effects of arsenic exposure, but only if it is assumed that the resulting relative risk of lung cancer declines with time since exposue.

Sorahan (2009)[106] reexamined lung cancer mortality data in a cohort from a cadmium recovery factory facility located in the state of Colorado.[107,108] These workers were employed for at least 6 months between 1 January 1940 and 31 December 1969 and exposed to cadmium and arsenic. Sorahan categorized this cohort according to the period from leaving arsenic exposed employment (years) (< 9; 10-19; 20-29; 30-39; ≥40) and observed that there was a statistically significant ($P < 0.05$) negative trend in lung cancer SMR's in relation to the period from ceasing arsenic exposure (SMR 300; 113; 140; 113; 75 respectively). These findings are consistent with the hypothesis that arsenic is a late stage carcinogen.

These three studies (Binks et al., 2005[104]; Jones et al., 2007[105]; Sorahan 2009[106]) suggest that simple cumulative inhalation exposure may not be a good predictor of excess relative risk and that when arsenic is a late stage carcinogen this may have consequences for the risk estimation.

Lubin et al. (2008)[2] reanalyzed the Anaconda copper smelter cohort. The original cohort study enrolled 8,014 workers employed for ≥ 1 year before 1957, with follow up starting 1 year after initial employment or 1 january 1938 whichever was later. It should be noted that in the reanalysis, Lubin et al.[2] used an exposure reduction factor in the higher exposure categories to account for the use of personal protection equipment. They showed that RR's for respiratory cancer increased linearly with cumulative arsenic exposure when analyzed for specific concentrations and concentration ranges of arsenic (0.29 mg/m^3; 0.30-0.39 mg/m^3; 0.40-0.49 mg/m^3; ≥ 50 mg/m^3). In addition, they showed that the slope of the linear exposure-response relationship increased with increasing arsenic concentration. This pattern implied that for equal cumulative arsenic exposure, the RR of respiratory cancer mortality was greater for cumulative arsenic exposure delivered at higher concentration for shorter duration compared with cumulative exposure delivered at lower concentration for longer duration. As an explanation for this effect they suggest that the concentration dependent mechanisms involved in methylation and excretion of arsenic play an important role and can become rate-limited at higher concentrations.

Carcinogenicity – dermal exposure

WHO/ATSDR/additional data

No studies were found that have associated cancer in humans with dermal exposure to arsenic.

Carcinogenicity – oral exposure

WHO/ATSDR data

Studies in Taiwan, Chile and Argentina show consistently high mortality risks from lung, bladder and kidney cancer, as well as other skin changes such as hyperkeratosis and pigmentation changes among populations exposed to arsenic via drinking water. Where exposure-response relations have been studied, the risk of cancer for these sites increases with increasing exposure. Even when tobacco smoking has been considered, the exposure-response relationship remains.

Not all studies of populations exposed to arsenic have reported positive findings for increased lung, bladder and kidney cancer. Exposure in these studies have not been as high as those in Taiwan, Chile or Argentina, and the sample sizes of the study populations may not have provided the statistical power to detect increased risks.

Most of the studies where these effects have been observed were conducted in Taiwan. The exposure categories of studies conducted in the blackfoot disease (BFD)-endemic area in Taiwan have historically been rather broad (e.g. < 300 µg/L, 300-600 µg/L, and > 600 µg/L). A recent paper on the BFD-endemic area in Taiwan, however, reported increased risks of bladder and lung cancer mortality in persons consuming drinking water with arsenic concentrations < 50 µg/L.

In Argentina, significantly elevated bladder, lung and kidney cancer mortality were found in the high-exposure group where over 75% of the measurements of arsenic in drinking water were higher than the detection limit of 40 µg/L. For the measurements above the detection limit, the average concentration was 178 µg/L. Bladder, lung and kidney cancer mortality were also significantly elevated for men, and lung cancer mortality was significantly elevated for women in the < 40 µg/L-exposure groups. Thus the lowest exposure where elevated kidney cancer risk could be observed would have to be considerably lower than 178 µg/L.

In a case-control study conducted in Chile, there was an exposure-response relationship for the risk of lung cancer over all exposure categories, and the increased risk was statistically significant at exposure strata of 30-50 µg/L and above. In a case-control study in Finland, a statistically significantly elevated risk of bladder cancer was observed at ≥ 0.5-64 µg As/L drinking water concentration but only when exposure was 3-9 years before diagnosis.

Arsenic ingestion in drinking water has also been shown to be associated with a high risk of skin cancer. Well-documented studies on skin cancer after arsenic ingestion from drinking water have been conducted in several populations in different countries, the largest of which were in Taiwan. Association of exposure to arsenic with skin cancer has also been observed in studies on patients treated with arsenicals.

The lowest arsenic drinking water concentration where an increased risk of skin cancer could be observed is in the lowest exposure group in the exposed Taiwan population (i.e. < 300 µg/L). It should be noted that this is a very broad exposure category and the lowest concentration associated with skin cancer could have been considerably lower.

The lowest arsenic drinking water concentration where an elevated risk of arsenic-associated skin lesions (hyperpigmentation and/or keratosis) has been found can be estimated from a study in West Bengal (India) to be less than 50 µg/L.

In two partly overlapping studies in Taiwan, an elevated mortality from liver cancer was observed in relation to arsenic exposure from drinking water. In one of the two studies in Chile, but not in the study in Argentina, such a relationship was observed.

Cancer at other sites in relation to arsenic exposure has been little studied outside Taiwan. The sites that have exhibited an elevated risk include oesophagus, stomach, small intestine, colon, nose, larynx, bone and prostate, as well as lymphoma and leukaemia. A study in the USA and another in Australia, neither of which showed a clear-cut increase in the risk of lung, bladder, or kidney cancer, showed moderately elevated mortality from cancer of the prostate.

Additional data

Long-term exposure (years) to drinking water at levels as low as 0.001 mg As/kg/day have been recently associated with skin diseases and skin, bladder, kidney, liver, and also lung cancer. [109-121] The available oral studies (on arsenic in food and drinking water) were recently (2009) reviewed and evaluated by the

European Food Safety Authority (EFSA) and will not be further discussed here.[122]

According to EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures[11], arsenic acid, arsenic trioxide, arsenic pentoxide and lead arsenate are labelled Carc. 1A ; H350 (May cause cancer).

The National Toxicology Program (NTP) classified arsenic compounds as a known human carcinogen, i.e., human studies (epidemiology studies and/or experimental studies) provide 'sufficient evidence' of carcinogenicity in humans[123].

The International Agency for Research on Cancer (IARC) rates arsenic and arsenic compounds as carcinogenic to humans (Group 1: exposures 'known to be carcinogenic to humans based on sufficient human evidence, and limited evidence in experimental animals). This evaluation applies to the group of chemicals (i.e. arsenic and arsenic compounds) as a whole and not necessarily to all individual chemicals within the group.[124-126]

The U.S. Environmental Protection Agency (EPA), through its Integrated Risk Information System (IRIS)[127], classifies arsenic as a human carcinogen (Group A).

Reproduction toxicity

Effects on fertility

WHO/ATSDR/additional data

No effects of arsenic on fertility in humans were retrieved from the literature.

Developmental effects-inhalation exposure

WHO/ATSDR/additional data

Several older epidemiological studies (see WHO 2000[128], ATSDR 2007[7]) have reported an association between exposure to inorganic arsenic and increased risk of adverse developmental effects (congenital malformations, low birth weight, spontaneous abortion)(Nordström et al., 1978a[129], 1978b[130], 1979a[131], 1979b[132]).

The Rönnskär copper smelter in northern Sweden emitted a number of potentially toxic substances, of which arsenic, lead and sulphur dioxide have

caused most public concern. Birth weights were studied in the offspring of women working at the Rönnskär smelter and in four populations (A-D) at different distances from the smelter (Nordström 1978a[129]). Records for all women living in any of these four areas were studied, the distance from the smelter increasing from A to D. In the offspring of employees and of women living in areas A and B close to the smelter statistically significantly decreased birth weights were found. This decrease showed a consistent parity dependence, affecting mainly later pregnancies. The same authors studied frequencies of spontaneous abortion in the abovementioned populations. In the population located close to the smelter (A) a statistically significant increase of the abortion frequency was found, compared to more distantly located populations (B, C, D) (Nordström 1978b[130]).

In another report, Nordström et al. (1979a[131]) focussed more on the population of female employees working in the smelter. An increased frequency of spontaneous abortion was found in pregnancies where the mother was employed during pregnancy or had been employed before pregnancy and was still living close to the smelter. Women occupied in close connection with the smelting processes had a significantly higher abortion frequency than other employees. In the offspring of mothers working at the smelter, birth weight was decreased mainly in later pregnancies. The lowest birth weights were found in the offspring of women working in close contact with the smelting processes. Also the frequencies of congenital malformations were studied in the offspring of female employees at the Rönnskär smelter and in the population near the smelter. In the offspring of women who had worked at the smelter during pregnancy the frequency of congenital malformations was increased (Nordström 1979b[132]).

Inhalatory studies of more recent date are scarce in the literature. Ihrig et al. (1998)[133] investigated the association between chronic inhalation exposure to arsenic and stillbirth. A case control study was conducted in the vicinity of a Texas arsenic pesticide factory. Data were collected on 119 cases of stillbirth and 267 controls randomly selected from healthy live-births at the same hospital and matched for year of birth. Arsenic exposure levels were estimated from airborne emission estimates and an atmospheric dispersion model. A conditional logistic regression model was fitted including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. There was a statistically significant increase in the risk of stillbirth in the highest exposure category (> 100 ng As/m^3, midpoint=682 ng/m^3) for Hispanics only with an odds ratio (OR) of 8.4 (95%CI 1.4-50.1).

Developmental effects-oral exposure

WHO/ATSDR/additional data

The association between trace element levels in community drinking water and spontaneous abortion was studied in 286 women having a spontaneous abortion through 27 weeks of gestation with that of 1,391 women having live birth (Aschengrau et al.,1989[134]). Trace element levels were gathered from routine analyses of public tap water supplies from the communities where the women resided during pregnancy. After adjustment for potential confounders, the authors conclude that an increase in the frequency of spontaneous abortions was dose-dependently associated with levels of mercury above the detection limit and with high levels of arsenic, potassium and silica. [The Committee observed that the data in the original publication do not support the association with arsenic].

In a case-control study (270 affected children and 665 healthy children) the association was investigated between chemicals (arsenic, lead, mercury, selenium) in maternal drinking water consumed during pregnancy and congenital heart disease in the offspring (Zierler et al.,1988[135]). Contaminant levels in maternal drinking water was available from the records of routine water analysis of the samples taken from public taps in the communities where the mothers resided during pregnancy. Mothers provided information on their health, pregnancy management and demographic characteristics during telephone interviews. None of the chemicals was associated with an increase in frequency of congenital heart disease overall but arsenic exposure was associated with an increase in the occurrence of coarctartion of the aorta (OR 3.4, 95% CI 1.3-8.9) whereas selenium was associated with a lower frequency of any congenital heart diseases (OR 0.82, 95% CI 0.4-0.97).

Associations between developmental effects and chronic exposure of women to arsenic in the drinking water has been reported in more recent studies in populations in different areas of the world with elevated levels of arsenic in drinking water e.g., Taiwan (Yang *et al.*, 2003[136]), Chile (Hopenhayn-Rich, 2000[137]; Hopenhayn et al., 2003[138]; Smith et al., 2006[139]) and Bangladesh/ Bengal (Ahmad et al., 2001[140]; Milton et al., 2005[141]; von Ehrenstein et al., 2006[142]; Tofail et al., 2009[143]; Rahman et al., 2009[144]; Rahman et al., 2010[145]).

Taiwan. The well water in Lanyang Basin, which is located in the northeastern part of Taiwan, was found to have high levels of arsenic ranging from undetectable levels (< 0.15 ppb) to 3.59 ppm. A study was performed to compare the risk of adverse pregnancy outcomes (preterm delivery and birthweight) between an area with historic high well water arsenic levels

(arsenic-exposed area (AE)) and a comparison area with no historic evidence of arsenic water contamination (non-arsenic-exposed area (NAE)) (Yang et al., 2003[136]). Data from 3,872 first parity singleton live births for AEs and 14,387 for NAEs were included in the analysis. Babies born in AEs were on average 30 g lighter than those born in NAEs after adjustment for confounders. AEs had a higher rate of preterm delivery than NAEs (3.74% vs 3.43%). The results of this study suggest that, after adjustment for potential confounders, arsenic exposure from drinking well water was associated, although not statistically significant, with the risk of preterm delivery, with an odds ratio of 1.10 (0.91-1.33). The estimated reduction in birth weight was 29.05 g (95% CI 13.55-44.55).

Chile. Associations were found between late foetal mortality, neonatal mortality, and postneonatal mortality and exposure to high levels of arsenic in the drinking water (up to 0.86 mg/L during more than a decade), based on comparisons between subjects in low- and high-arsenic areas of Chile (Hopenhayn-Rich et al., 2000[137]). The Antofagasta area has a well-documented history of arsenic exposure from naturally contaminated water, and Valparaíso, a comparable low-exposure city. Antofagasta is the second largest city in Chile and had a distinct period (1958 until 1971) of very high arsenic exposure that began when an arsenic removal plant was installed. A retrospective study design was used to examine time and location patterns in infant mortality showing the general declines in late foetal and infant mortality over the study period in both locations. The data also indicate an elevation of the late fetal, neonatal, and postneonatal mortality rates for Antofagasta, relative to Valparaíso, for specific time periods, which generally coincide with the period of highest arsenic concentration in the drinking water of Antofagasta. Poisson regression analysis yielded associations between arsenic exposure and late foetal mortality (rate ratio (RR) 1.7, 95% CI 1.5-1.9), neonatal mortality (RR 1.53, 95% CI 1.4-1.7), and postneonatal mortality (RR = 1.3, 95% CI 1.2-1.3) after adjustment for location and calendar time.

Hopenhayn et al. (2003)[138] also investigated the association between drinking water arsenic exposure and fetal growth, reflected in birth weight in a prospective cohort study in these two Chilean cities with contrasting drinking water arsenic levels: Antofagasta (40 µg/L) and Valparaiso (<1 µg/L). Study subjects completed in-depth interviews and provided urine samples for exposure analysis. Pregnancy and obstetric information was obtained from medical records. The final study group consisted of 424 infants from Antofagasta and 420 from Valparaiso. After controlling for confounders, results of the multivariable analysis indicated that Antofagasta infants had lower mean birth weight (-57 g, 95% CI: -123 to 9). This study suggests that moderate arsenic exposures from

drinking water (<50 µg/L) during pregnancy are associated with reduction in birth weight.

Increased standardized mortality ratios (SMRs) were reported for lung cancer and bronchiectasis among subjects in Antofagasta in Chile who had probable exposure in utero or during childhood to high levels of arsenic (near 0.9 mg/L) in the drinking water (Smith et al., 2006[139]). In this study, mortality rates in Antofagasta in the period 1989-2000 were compared with those of the rest of Chile, focusing on subjects who were born during or just before the peak exposure period and who were 30-49 years of age at the time of death. For the birth cohort born just before the high-exposure period (1950-1957) and exposed in early childhood, the SMR for lung cancer was 7.0 [95% CI 5.4-8.9; $p < 0.001$] and the SMR for bronchiectasis was 12.4 (95% CI 3.3-31.7; $p < 0.001$). For those born during the high-exposure period (1958-1970) with probable exposure *in utero* and early childhood, the corresponding SMRs were 6.1 (95% CI 3.5-9.9; $p < 0.001$) for lung cancer and 46.2 (95% CI 21.1-87.7; $p < 0.001$) for bronchiectasis. These findings suggest that exposure to arsenic in drinking water during early childhood or in utero has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults from both malignant and nonmalignant lung disease.

Bangladesh/Bengal. In a cross-sectional study a group of 96 women in the reproductive age (15-49 years) chronically exposed to arsenic through drinking water in Bangladesh was studied to identify the pregnancy outcomes in terms of live birth, stillbirth, spontaneous abortion, and preterm birth (Ahmad et al., 2001[140]). In a cross-sectional study, pregnancy outcomes of exposed women were compared with pregnancy outcomes of 96 women of reproductive age (15-49 years) who were not exposed to arsenic-contaminated water matched for age, socioeconomic status, education, and age at marriage. Of the women in the exposed group, 98% had been drinking water containing ≥ 0.10 mg/L arsenic (approx. 0.08 mg/As/kg/day) and 43.8% had been drinking arsenic-contaminated water for 5-10 years. Frequency of the adverse pregnancy outcomes of spontaneous abortion, stillbirth, and preterm birth rates were higher in the exposed group than in the nonexposed group ($p = 0.008$, $p = 0.046$, and $p = 0.018$, respectively).

In a mixed-design study in 533 women in Bangladesh the association was assessed between arsenic in drinking water and spontaneous abortion, stillbirth, and neonatal death (Milton et al., 2005)[141]. Information on sociodemographic characteristics, drinking water use, and adverse pregnancy outcomes (spontaneous abortion, stillbirth and neonatal death) was obtained through a structured interviewer-administered questionnaire. The range of arsenic

concentrations in tube well water ranged from non-detectable to 1710 µg/L. Excess risks of spontaneous abortion and stillbirth were observed among the chronically exposed participants after adjusting for participant's height, history of hypertension and diabetes, and (for neonatal death only) age at first pregnancy. Comparing exposure to arsenic concentration of greater than 50 µg/L with 50 µg/L or less, the ORs were 2.5 (95% CI 1.5-4.3) for spontaneous abortion, 2.5 (95% CI 1.3-4.9) for stillbirth, and 1.8 (95% CI 0.9-3.6) for neonatal death.

Pregnancy outcomes and infant mortality were studied among 202 married women in West Bengal, India (von Ehrenstein et al., 2006[142]). Reproductive histories were ascertained using structured interviews and arsenic exposure during each pregnancy, including all water sources used, was assessed; this involved measurements from 409 wells. Exposure to high concentrations of arsenic (≤ 200 µg/liter) during pregnancy was associated with a sixfold increased risk of stillbirth after adjustment for potential confounders (odds ratio (OR)=6.07; 95% CI 1.54-24.0; p = 0.01). The odds ratio for neonatal death was 2.81 (95% CI 0.73-10.8). No association was found between arsenic exposure and spontaneous abortion (OR 1.01, 95% CI 0.38- 2.70) or overall infant mortality (OR 1.33, 95% CI 0.43-4.04).

Rahman et al. (2009)[144] conducted a prospective cohort study, based on 1,578 mother-infant pairs, in Matlab, Bangladesh. Arsenic exposure was assessed by analysis of arsenic in urine collected at around gestational weeks 8 and 30. In analysis over the full range of exposure (6-978 µg/L), no dose-effect association was found with birth size. However, statistically significant negative dose effects were found for birth weight and head and chest circumferences at a low level of arsenic exposure (<100 µg/L in urine). In this range of exposure, birth weight decreased by 1.68 (standard error (SE), 0.62) g for each 1-µg/L increase of arsenic in urine. For head and chest circumferences, the corresponding reductions were 0.05 (SE, 0.03) mm and 0.14 (SE, 0.03) mm per 1 µg/L, respectively. No further negative effects were shown at higher levels of arsenic exposure.

Tofail et al. (2009)[143] performed a population-based cohort study with 4,436 pregnant women in Matlab, Bangladesh (an area with high-arsenic contaminated tube wells). A subsample of 1,799 infants born to these mothers was assessed on two problem solving tests (PST), the motor scale of the Baley Scales of Infant Development, and behaviour ratings at 7 month of age. Arsenic concentrations in spot urine specimens at 8 and 30 weeks of pregnancy were 81 µg/L (range 37-207) and 84 µg/L (range 42-230) respectively. No significant effect of arsenic exposure during pregnancy on infant development (motor, PST score and

behaviour rating) was detected. However, it is possible that other effects are as yet unmeasured or that effects will become apparent at a later age.

Associations of arsenic exposure with adverse pregnancy outcomes and infant mortality were assessed in a prospective cohort study of pregnant women (n=2924) in Matlab, Bangladesh (Rahman et al., 2010[145]). Spontaneous abortion was evaluated in relation to urinary arsenic concentrations at gestational week 8. Stillbirth and infant mortality were evaluated in relation to the average of urinary arsenic concentrations measured at gestational weeks 8 and 30. The odds ratio of spontaneous abortion was 1.4 (95% CI 0.96-2.2) among women with urine arsenic concentrations in the fifth quintile (249-1253 µg/L; median = 382 µg/L), compared with women in the first quintile (<33 µg/L). There was no clear evidence for increased rates of stillbirth. The rate of infant mortality increased with increasing arsenic exposure: the hazard ratio was 5.0 (95% CI 1.4-17.8) in the fifth quintile of maternal urinary arsenic concentrations (268-2019 µg/L; median = 390 µg/L), compared with the first quintile (<38 µg/L).

The ATSDR (2007)[7] reports that chronic oral exposure of humans to arsenic concentrations of approximately 10 µg/kg/day (LOAEL) or more, can lead to reproduction toxicity. For human cancer however, LOAELS are reported of approximately 1 µg/kg/day indicating that carcinogenicity in humans may be expected at far lower concentrations than reproduction toxicity. Although a LOAEL after inhalation exposure to arsenic is not reported by th ATSDR (2007) the Committee is of the opinion that effects of arsenic and inorganic arsenic compounds on reproduction may be expected at exposure levels exceeding the exposure levels for carcinogenicity.

Lactation

WHO/ATSDR/additional data

Different studies indicated that arsenic can be excreted in human milk. In the Bombay area (India) Dang et al. (1983)[146] reported arsenic levels ranging from 0.2 to 1.1 ng/g in breast milk (25) samples of nursing mothers 1-3 months postpartum. Arsenic was detected in human breast milk at concentrations of 0.13-0.82 ng/g (Somogyi and Beck, 1993[147]). In human milk sampled from 88 mothers on the Faroer Islands whose diets included predominantly seafood, arsenic concentrations were 0.1-4.4 ng/g (Grandjean et al., 1995[148]). Exposure to arsenic from the seafood diet in this population was most likely to organic arsenic (arsenobetaine).” In a population of Andean women exposed to approximately 200 ng/g of inorganic arsenic in drinking water, concentrations of

arsenic in breast milk ranged from about 0.8 to 8 ng/g (median 2.3 ng/g) (n=10) (Concha et al., 1998[149]). The arsenic concentration in the breast milk of 35 women in Ismir, Turkey, a volcanic area with high thermal activity ranged from 3.24 to 5.4 ng/g, with a median of 4.2 ng/g (Ulman et al., 1998[150]).

Samanta et al. (2009)[47] collected 226 breast milk samples from lactating women in arsenic-affected districts of west Bengal. In only 39 (17%) samples arsenic was detected. The maximum arsenic concentration in breast milk was 48 µg/L. Hair and nail arsenic was highly correlated with drinking water arsenic concentrations. Women who had both high arsenic body burden and arsenical skin lesions also had elevated levels of arsenic in their breast milk.

Recently EFSA (2009) calculated for the European population that the average exposure of inorganic arsenic from breast milk for infants (up to 6 months) amounted 0.0275 µg/kg/day.[122]

No data on the toxic effects from arsenic in breast milk on the development of breastfed babies could be retrieved from the literature.

Immunological effects

WHO/ATSDR data

Workers exposed to arsenic in air from burning coal (not further specified) did not have altered levels of antibodies in their blood (Bencko et al., 1988). Depression of white blood cells has not been reported in workers exposed by the inhalation route (e.g., Beckett et al., 1986; Bolla-Wilson and Bleecker, 1987; Ide and Bullough, 1988; Morton and Caron, 1989). Leukopenia is observed in cases of oral exposure to inorganic arsenicals (e.g., Armstrong et al., 1984; Franzblau and Lilis, 1989; Kyle and Pease, 1965).

Additional data

No additional human data on immunological effects of arsenic and arsenic compounds was found in literature.

Neurological effects

WHO/ATSDR data

Signs of peripheral and/or central neuropathy are common in humans exposed to inorganic arsenicals by the inhalation and oral route. Acute, high-dose exposure

can lead to encephalopathy, with clinical signs such as confusion, hallucinations, impaired memory, and emotional lability (Beckett et al., 1986; Danan et al., 1984; Morton and Caron, 1989). In fatal or near-fatal cases, this may progress to seizures and coma (Armstrong et al., 1984; Fincher and Koerker, 1987), while lower-level exposure can lead to significant peripheral neuropathy (e.g., Feldman et al., 1979; Huang et al., 1985; Landau et al., 1977; Mizuta et al., 1956; Silver and Wainman, 1952). This neuropathy is usually first detected as a numbness in the hands and feet, but may progress to a painful "pins and needles" sensation (Franzblau and Lilis, 1989; Jenkins, 1966; Le Quesne and McLeod, 1977). Both sensory and motor neurons are affected, with distal axon degeneration and demyelination (Goebel et al., 1990; Hindmarsh and McCurdy, 1986). More advanced symptoms include weakness, loss of reflexes, and wrist-drop or ankle-drop (Chhuttani et al., 1967; Heyman et al., 1956). These effects may diminish after exposure ceases, but recovery is slow and usually not complete (Beckett et al., 1986; Fincher and Koerker, 1987; Le Quesne and McLeod, 1977; Morton and Caron, 1989; Murphy et al., 1981).

Inhaled inorganic arsenic can produce neurological effects. A study by Gerr et al. (2000) reported an elevated incidence of peripheral neuropathy in subjects who lived near an arsenic-using pesticide plant (13/85=15.3%; odds ratio [OR]=5.1, p=0.004), relative to subjects who lived farther from the plant (4/118=3.4%). Studies of copper smelter workers at the smelter in Tacoma, Washington (Feldman et al.,1979), a power station in Slovakia (Buchancová et al.,1998), and the Rőnnskär smelter in Sweden (Blom et al. 1985; Lagerkvist and Zetterlund, 1994) have demonstrated peripheral neurological effects in workers associated with arsenic trioxide exposure. At the Tacoma smelter, the prevalence of clinically diagnosed peripheral neuropathy was markedly higher in arsenic-exposed workers (26/61=43%) than controls (4/33=12%), and although not statistically significant, mean peroneal motor NCV (nerve conduction velocity) was lower in arsenic-exposed workers than controls and all 12 cases of abnormally low NCV occurred in the arsenic group (Feldman et al.,1979). In the study of 70 workers in Slovakia, the investigators described 16 cases of arsenic intoxication. Among these, 13 had signs and symptoms of sensory and motor polyneuropathy on both upper and lower extremities, 10 were diagnosed with pseudoneurasthenic syndrome, and 6 suffered from toxic encephalopathy (Buchancová et al., 1998). The average length of exposure was 22.3 years (SD ±8.4 years) and the average arsenic exposure in inhaled air ranged from 4.6 to 142.7 μg/m^3. Similar results were observed at the Rőnnskär smelter, where Blom et al. (1985) reported significantly increased prevalence of workers with abnormally low NCV in the exposed group, and lower, but not statistically

significant, mean NCV in five peripheral nerves. A follow-up study on the Rőnnskär workers 5 years later found that the prevalence of abnormally low NCV 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). The follow-up Rőnnskär study provided enough information to estimate that mean arsenic exposure was 0.31 mg As/m^3 and lasted an average of 28 years in the exposed group.

Chronic oral exposure to arsenic may be associated with intellectual deficits in children. Wasserman et al. (2004) conducted a cross-sectional evaluation of intellectual function in 201 children 10 years of age whose parents were part of a larger cohort in Bangladesh. Intellectual function was measured using tests drawn from the Wechsler Intelligence Scale for Children. The mean arsenic concentration in the water was 0.118 mg/L. The children were divided into four exposure groups, representing <5.5, 5.6-50, 50-176, or 177-790 µg As/L drinking water. A dose-related inverse effect of arsenic exposure was seen on both Performance and Full-Scale subset scores; for both end points, exposure to ≥50 µg/L resulted in statistically significant differences (p<0.05) relative to the lowest exposure group (<5.5 µg/L).

The same group of investigators examined 301 6-year-old children from the same area (Wasserman et al., 2007). The children were categorized into quartiles based on water arsenic concentration: 0.1-20.9, 21-77.9, 78-184.9, and 185-864 µg/L. Water arsenic was significantly negatively associated with both Performance and Processing speed raw scores. Analyses of the dose-response showed that compared to the first quartile, those in the second and third categories had significantly lower Performance raw scores (p<0.03 and p=0.05, respectively). Those in the fourth category had marginally significantly lower Full-Scale and Processing Speed raw scores.

Additional data

A cross-sectional study examined the effects of chronic inhalatory exposure to lead (Pb), arsenic (As) and undernutrition on the neuropsychological development of children living in the vicinity of a smelter complex (San Luis Potosi, Mexico) (Calderon et al., 2001).[151] Two populations chronically exposed to either high (41 children) or low (39 children) levels of As and Pb were analyzed using the Wechsler Intelligence Scale for Children(WISC). Geometric means of urinary arsenic (AsU) and lead in blood (PbB) were 62.9 ± 0.03 (µgAs/g creatinine) and 8.9 ± 0.03 (µg/dL) for the exposed group and 40.2 ± 0.03

(μgAs/g creatinine)and 9.7 ± 0.02 (μg/dL) for the reference group. The height for age index (HAI) was used as an indicator of chronic malnutrition and sociodemographic information was obtained with a questionnaire. Lead and arsenic were measured by atomic absorption spectrophotometry. Data on full, verbal, and performance intelligence quotients (IQ) scores, long-term memory, linguistic abstraction, attention span, and visuospatial organization were obtained through the WISC (Wechsler Intelligence Score for Children) . Verbal IQ ($p < 0.01$) decreased with increasing concentrations of AsU. The HAI correlated positively with full-scale and performance IQ ($p < 0.01$). Higher levels of AsU were significantly related to poorer performance on WISC factors examining long-term memory and linguistic abstraction, while lower scores in WISC factors measuring attention were obtained at increasing values of PbB.

Other effects

Vascular effects

WHO/ATSDR data

Several studies in Taiwan have demonstrated an association between arsenic ingestion and blackfoot disease (BFD), with clear exposure-response effects related to both the well-water arsenic levels and duration of use of arsenic-contaminated drinking water (Chen et al.,1988b; Ch'i and Blackwell, 1968; Tseng, 1977, 1989; Tseng et al.,1968. 1995, 1996). Several other studies and case reports of subjects exposed to arsenic from many sources, in countries other than Taiwan, document an association with peripheral vascular alterations. However, the extreme form and high prevalence of BFD found in Taiwan has not been reported in other parts of the world.

Hypertension is associated with long-term oral exposure to arsenic, but this evidence is limited to cross-sectional studies, one occupational and two environmental (Taiwan and Bangladesh), all three of which found elevations in blood pressure with arsenic exposure (Rahman et al.,1999; Wang et al., 2003; Chen et al.,1995). The two environmental studies demonstrated exposure-response relationships. It should be noted that although hypertension is not a very important cause of death itself, it is a major risk factor for other vascular diseases.

Several studies in Taiwan show a relationship between oral arsenic exposure and mortality from cardiovascular diseases (CVD), including exposure-response relationships (Chiou et al.,1977; Wang et al., 2002, 2003; Chang et al., 2004;

Chen et al., 1996; Hsueh et al.,1998; Tsai et al., 1999; Tseng et al., 2003). Similar results have generally not been observed in other arsenic drinking water studies or in a medicinal study, in all of which the exposure levels have been lower. In the occupational studies, mortality from arteriosclerosis and coronary heart disease was elevated in the report from the Tacoma cohort (USA), but no statistically significant increases for these effects have been found in the Rönnskär (Sweden) or Anaconda (USA) smelter cohorts (Enterline et al.,1995; Lee-Feldstein, 1983; Welch et al., 1982; Wall, 1980). The study in Utah found an excess of mortality from hypertensive heart disease but there were only a small number of deaths.

Only very limited evidence exists for an association between oral arsenic exposure and cerebrovascular disease. Some of the Taiwanese studies have shown an elevated risk of death from cerebrovascular disease, but the data are inconsistent across studies and the elevations, where present, are small compared with those for CVD. Studies from other countries provide only very limited support for the Taiwanese findings, but exposure levels were considerably lower.

Additional data

Wang et al. (2010) reported a 17-year follow-up of a cohort consisting of 280 men and 355 women living in area in southwestern coast in Taiwan for 17 years.[152] Cumulative arsenic exposure was significantly associated with QT-dispersion (QTD) showing a dose-response relationship ($p < 0.001$). Significant associations of the QTD with coronary artery disease and carotid atherosclerosis existed after adjustment for potential confounders in the multiple linear regression analysis (all p values < 0.05). In the multivariate Cox regression analyses, the hazard ratios (95% confidence interval, p value) of cumulative cardiovascular and all-cause mortality were 3.9 (2.1-6.2, P = 0.002) and 1.4 (0.9-2.3, p = 0.10), respectively, for QTD > or = 65 ms compared with QTD < 65.

Diabetes mellitus

WHO/ATSDR data

Two occupational studies found an association of borderline statistical significance between diabetes mellitus and inhalation exposure to arsenic. In Taiwan, the prevalence and mortality rates of diabetes mellitus were higher among the population of the BFD-endemic area. There was also an exposure-response relationship between cumulative oral arsenic exposure and the

prevalence of diabetes mellitus. A similar exposure-response pattern was observed in a study in Bangladesh, where prevalence of keratosis was used as a surrogate for arsenic exposure.

Additional data

Navas-Acien et al. (2008) conducted a cross-sectional study in 788 adults aged 20 years or older who participated in the 2003-2004 National Health and Nutrition Examination Survey (NHANES).[153] The median urine levels of total arsenic, dimethylarsinate, and arsenobetaine were 7.1, 3.0, and 0.9 µg/L, respectively. The prevalence of type 2 diabetes was 7.7%. After adjustment for diabetes risk factors and markers of seafood intake, participants with type 2 diabetes had a 26% higher level of total arsenic (95% CI 2.0%-56.0%) and a nonsignificant 10% higher level of dimethylarsinate (95% CI -8.0% to 33.0%) than participants without type 2 diabetes, and levels of arsenobetaine were similar to those of participants without type 2 diabetes. After similar adjustment, the odds ratios for type 2 diabetes comparing participants at the 80th vs the 20th percentiles were 3.58 for the level of total arsenic (95% CI 1.18-10.83), 1.57 for dimethylarsinate (95% CI 0.89-2.76), and 0.69 for arsenobetaine (95% CI 0.33-1.48).

The same cross-sectional data on urinary arsenic and type 2 diabetes mellitus in 795 adults from the 2003-2004 National Health and Nutrition Examination Survey were analyzed by Steinmaus et al. (2009) to assess this evidence.[154] They found an odds ratio (OR) near 1.0 for diabetes, comparing the 80th versus 20th percentiles of urinary total arsenic (OR 0.88, 95% CI 0.39-1.97). This OR increased to above 3.0 when urinary arsenobetaine was added to the logistic risk model. The authors claim that this high OR was a statistical artifact. because arsenobetaine, which is ingested from fish and is essentially nontoxic, is a part of measured total urinary arsenic. Upon correction an OR of 1.15 (0.53-2.50) was calculated. These findings show no evidence of increased risk of diabetes with arsenic exposure in this dataset.

Anaemia

WHO/ATSDR data

Anaemia is often observed in humans exposed to arsenic by the oral route (e.g., Armstrong et al., 1984; Glazener et al., 1968; Mizuta et al., 1956; Westhoff et al., 1975). This is probably due mainly to a toxic effect on the erythropoietic cells of

bone marrow (Franzblau and Lilis, 1989; Lerman et al., 1980; Westhoff et al., 1975), although increased haemolysis may also contribute (Goldsmith and From, 1986; Kyle and Pease, 1965).

Additional data

The association between arsenic exposure and anemia, based on blood haemoglobin concentration was studied by Heck et al. (2008).[155] Haemoglobin measures, skin lesions, arsenic exposure, and nutritional and demographic information were collected from 1954 Bangladeshi participants in the Health Effects of Arsenic Longitudinal Study. Arsenic exposure (urinary arsenic >200 µg/L) was negatively associated with haemoglobin among all men and among women with haemoglobin <10 g/dL.

7.2 Animal experiments

Animal data are summarized in Tables 16-19 (Annex H).

7.2.1 Irritation and sensitisation

WHO/ATSDR data

Sodium arsenite and sodium arsenate were not allergenic in the guinea-pig maximisation test (Wahlberg and Boman, 1986).

No animal data on local effects on the respiratory tract was reported for arsenic and inorganic arsenic compounds.

No studies were located on ocular effects in animals after inhalation exposure to inorganic arsenicals.

Additional data

Animal data with regard to irritation and sensitisation of arsenic and arsenic compounds are very limited.

Fukuyama et al. (2008) used the local lymph node assay to evaluate the ability of chromated copper arsenate (CCA), a commonly used wood preservative, and its components to cause sensitizing reactions.[156] After CBA/J mice were treated topically with 0.3 to 10% CCA, 0.3-3% chromium oxide, 0.3-

3% arsenic oxide, or 0.3-3% copper oxide, their auricular lymph nodes (LN) were weighed and used in lymphocyte proliferation assays. In addition, total levels of chromium and arsenic in blood samples were measured. In all groups treated with CCA, all parameters, including LN weight and lymphocyte proliferation, increased in a dose-dependent manner. The stimulation index (SI; the mean [3H]-TdR incorporation of the treatment group divided by that of the control group) showed a positive response (SI >3) in all treatment groups. In addition, it was confirmed that the three components of CCA – chromium oxide, arsenic oxide and copper oxide – each individually exerted sensitizing ability.

7.2.2 Acute toxicity

WHO/ATSDR/additional data

The acute dermal LD_{50} for the pentavalent arsenicals calcium arsenate and lead arsenate in the rat is ≥ 2400 mg/kg bw (≥ 400 mg As/kg bw) (Gaines, 1960).

In a developmental study, 100% mortality in groups of 10 pregnant rats after 1 day of inhalation exposure to arsenic trioxide concentrations of ≥100 mg/m^3 was observed (76 mg As/m^3) (Holson et al., 1999).

Oral and parenteral lethal doses range from 15 to 960 mg As/kg bw/day, depending on the compound and the animal species (Jaghabir et al., 1988; Kaise et al., 1989; NTP, 1989; Rogers et al., 1981; Stevens et al., 1979).

7.2.3 Short-term toxicity

WHO/ATSDR/additional data

In a developmental toxicology study, four of nine pregnant rats died and one rat was euthanised in extremis between days 12 and 19 of gestation after 30-35 days of exposure to an aerosol of arsenic trioxide at an exposure concentration of 20 mg As/m^3 (Holson et al., 1999). These animals exhibited severe hyperemia and plasma discharge into the intestinal lumen at autopsy.

The lowest oral arsenic lethal level in an animal study was 1.5 mg As/kg bw/day in pregnant rabbits dosed repeatedly throughout gestation.

7.2.4 *Long-term toxicity*

Genotoxicity

WHO/ATSDR data

Inorganic arsenic did not induce point mutations in bacteria or in mammalian cells. However, arsenic can produce chromosomal aberrations in vitro, affect methylation and repair of DNA, induce cell proliferation, transform cells, and promote tumours.

There have been a large number of in vitro studies of the genotoxic effects of arsenic. The results are mixed, but in general, it appears that the inorganic arsenicals are either inactive or weak mutagens, but are able to produce chromosomal effects (aberrations, sister chromatid exchange) in most systems.[7]

Additional data

Additional data on genotoxicity in animals is presented in Table 19a and 19b (Annex H). Ahmad et al. (2000, 2002)[157,158] demonstrated that dimethylarsinous acid and dimethylarsinic acid are capable of releasing iron from horse spleen ferritin, inducing DNA damage via formation of reactive oxygen species (ROS). Furthermore, DNA-damaging activity by dimethylarsinous acid and monomethylarsonous acid mediated by ROS towards supercoiled ΦX174 RFI DNA, using a DNA nicking assay[58,59,159] and towards isolated PM2 DNA was demonstrated[94]. In the DNA nicking assay, nicking and/or degradation of ΦX174 DNA depended on concentration; monomethylarsonous acid was effective at nicking ΦX174 DNA at 30 mM; however, at 150 µM dimethylarsinous acid, nicking could be observed.

Dopp et al. observed a significant increase in the number of micronuclei, chromosome aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells after exposure to dimethylarsinous acid and monomethylarsonous acid.[61] As^{III} and As^{V} induced chromosome aberrations and sister chromatid exchanges. Trimethylarsenic oxide, monomethylarsonic acid and dimethylarsinic acid were not genotoxic in the concentration range tested (up to 5 mM).

Akram et al. (2009) examined the genotoxic effects of arsenite in ovarian tissue of rats at 56 days of age. Immature (28 days old) female rats were exposed to different doses (50, 100, and 200 ppm) of sodium arsenite in drinking water

for 28 days.[160] DNA damage in ovarian tissue was measured by the comet assay. All doses induced significant decrease in ovarian weight in a dose-dependent manner compared to control, more prominently at ($p < 0.001$) 100 and 200 ppm. All the comet assay parameters showed significant difference with arsenite treatment compared to control group. In treatment groups, mean number of cells with intact DNA decreased while, mean comet number increased ($p < 0.001$) in a dose-dependent manner compared to control. Significant decrease ($p < 0.05$) was observed in mean comet length, height, comet head diameter and %DNA in comet head of high dose groups compared to control group. Dose dependent increase was found in mean comet tail length, %DNA in tail, tail moment and olive tail moment in high dose groups compared to control group. The study indicates that arsenic caused DNA damage to ovarian cells particularly at high doses.

In an in vivo study, mice (Swiss albino) (n=6 females/dose) were exposed orally to 0 and 2.5 mg/kg bw sodium arsenite (exposure for 24 h). Sodium arsenite produced significantly high frequencies of chromosome aberrations in bone marrow cells.[161]

Yamanaka et al. observed that the amount of 8-oxodG (a biomarker of DNA oxidation) was significantly increased not only in lung and liver, but also, though not significantly, in urinary bladder in male ddY mice which were exposed for 4 weeks to 400 mg/L dimethylarsinic acid in drinking water (n=5/dose).[162] No increase in 8-oxodG was observed in spleen or kidney. The amount of 8-oxodG in epidermis of the dorsal skin was also significantly increased (in female HR-1 hairless mice which were exposed for 2 weeks to 400 mg/L dimethylarsinic acid in drinking water (n=5/dose)). Furthermore, when the same dose of As as arsenite or dimethylarsinic acid (a single gavage dose of 15.2 mg (11.5 mg As)/kg arsenite or 21.1 mg (11.5 mg As)/kg dimethylarsinic acid) was administered to male ddY mice, the amount of 8-oxodG was significantly higher in the urine after 9 hour of mice exposed to dimethylarsinic acid.

Noda et al. (2002) conducted a study to evaluate whether arsenite or its metabolite, DMA, could initiate carcinogenesis via mutagenic DNA lesions *in vivo* that can be attributed to oxidative damage.[163] A transgenic mouse model, MutaMouse, was used in this study and mutations in the lacZ transgene and in the endogenous cII gene were assessed. When DMA was intraperitoneally injected into MutaMice at a dose of 10.6 mg/kg per day for 5 consecutive days, it caused only a weak increase in the mutant frequency (MF) of the lacZ gene in the lung, which was at most 1.3-fold higher than in the untreated control animals. DMA did not appreciably raise the MF in the bladder or bone marrow. Further analysis of the cII gene in the lung, the organ in which DMA induced the DNA

damage, revealed only a marginal increase in the MF. Following DMA administration, no change in the cII mutation spectra was observed, except for a slight increase in the G:C to T:A transversion. Administration of arsenic trioxide (arsenite) at a dose of 7.6 mg/kg per day did not result in any increase in the MF of the lacZ gene in the lung, kidney, bone marrow, or bladder. Micronucleus formation was also evaluated in peripheral blood reticulocytes (RETs). The assay for micronuclei gave marginally positive results with arsenite, but not with DMA. These results suggest that the mutagenicity of DMA and arsenite might be too low to be detected in the MutaMouse.

Carcinogenicity

WHO/ATSDR data

Several animal carcinogenicity studies on arsenic have been carried out, but limitations such as limited time of exposure and limited number of animals make these inconclusive. However, in a recently reported animal study, female C57B1/6J mice exposed to arsenic in drinking water containing 500 µg As^V/L over 2 years was associated with increased incidence in tumours involving mainly lung, liver, gastrointestinal tract and skin.[7] One study has indicated that dimethylarsinic acid may cause cancer of the urinary bladder in male rats at high doses.[7]

No studies were located regarding cancer in animals after inhalation exposure to inorganic arsenicals, although several intratracheal instillation studies in hamsters have provided evidence that both arsenite and arsenate can increase the incidence of lung adenomas and/or carcinomas (Ishinishi et al., 1983; Pershagen and Bjorklund, 1985; Pershagen et al., 1984; Yamamoto et al., 1987).

Most studies of animals exposed to arsenate or arsenite by the oral route have not detected any clear evidence for an increased incidence of skin cancer or other cancers (Byron et al., 1967; Kroes et al., 1974; Schroeder et al., 1968).

Application of arsenic acid to the skin of mice pretreated with dimethylbenzanthracene did not result in any skin tumours (Kurokawa et al., 1989), suggesting that arsenic does not act as a promoter in this test system.

Arsenic has sometimes been called a ‘paradoxical’ human carcinogen because of these negative findings (Jager and Ostrosky-Wegman,1997). The basis for the lack of tumorigenicity in animals is not known, but could be related to species-specific differences in arsenic distribution, and induction of cell

proliferation (Byrd et al.,1996). (This view has recently been contended by Tokar et al. (2010).[164])

Additional data

A study by the US National Toxicology Program provided clear evidence of the carcinogenicity for gallium arsenide after inhalation in rodents.[165] In female rats exposed via inhalation to several levels of gallium arsenide (GaAs) particulate (0, 0.01, 0.1,1.0 mg/m^3 for up to 2 years, dose related lung alveolar/bronchiolar tumors and adrenal medulla phaeochromocytomas occurred. In male rats, though, treatment-related tumours were not observed, a dose-related increase in the incidence of atypical hyperplasia of the lung alveolar epithelium occurred. In the female rats, increases also occurred in leukemia at the highest dose. In a separate component of this study, mice exposed via inhalation to several doses of gallium arsenide particulate for 2 years did not show treatment-related tumours, but both males and females showed exposure concentration-related increases in the incidence of lung epithelial alveolar hyperplasia.

Especially recent work with arsenical methylation metabolites MMA and DMA and early life exposures to inorganic arsenic has now become available.[166-168]

Arnold et al. (2003) evaluated the carcinogenicity of monomethylarsonic acid (MMA^V) in male and female Fisher F344 rats and B6C3F1 mice in 2-year feeding studies according to US EPA guidelines.[166] Rats were treated with 50, 100 or 1300 ppm MMA and mice were treated with 10, 50, 200 or 400 ppm MMA based on preliminary short studies. There was no treatment related mortality in the mice. The primary target organ for MMA-induced toxicity in rats and mice was the large intestine. Toxicity was more severe in rats compared to mice and in male rats compared to female rats. The maximum tolerated dose for chronic dietary administration of MMA in rats and mice was assessed as 400 ppm, and the no effect level with regard to intestinal toxicity was assessed as 50 ppm for rats and 200 ppm for male mice. There were no treatment-related neoplastic effects detected in either the rat or the mouse.

To evaluate the carcinogenic effects of DMA^V, a bioassay was conducted in rats given various doses of DMA (Yamamoto et al., 1995[167]). One-hundred twenty-four male F344/DuCrj rats were divided randomly into 7 groups (20 rats each for groups 1-5; 12 rats each for groups 6 and 7). Groups 2-5 were given various tumour initiators followed by 50, 100, 200, or 400 ppm DMA, respectively, in the drinking water. Groups 6 and 7, received 100 and 400 ppm DMA during weeks 6-30. All rats were killed at the end of week 30. In the

initiated groups (groups 1-5), DMA significantly enhanced the tumour induction in the urinary bladder, kidney, liver, and thyroid gland, with respective incidences in group 5 (400 ppm DMA) being 80, 65, 65, and 45%. Induction of preneoplastic lesions (glutathione S-transferase placenta! form-positive foci in the liver and atypical tubules in the kidney) was also significantly increased in DMA-treated groups. Ornithine decarboxylase activity in the kidneys of rats treated with 100 ppm DMA was significantly increased compared with control values ($p < 0.001$). The authors conclude that DMA is acting as a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats, but not of the lung.

To elucidate molecular mechanisms, an 18 month carcinogenicity study was conducted of DMA^V in p53 heterozygous (+/-) knockout mice, which are susceptible to early spontaneous development of various types of tumours, and wild-type (+/+) C57BL/6J mice (Salim et al., 2003[168]). Totals of 88-90 males, 7-8 weeks of age, were divided into three groups each administered 0, 50 or 200 ppm. DMA in their drinking water for 18 months. Mice that were found moribund or died before the end of the study were autopsied to evaluate the tumour induction levels, as well as those killed at the end. Both p53(+/-) knockout and wild-type mice demonstrated spontaneous tumor development, but lesions were more prevalent in the knockout case. Carcinogenic effect of DMA was evident by significant early induction of tumours in both treated p53(+/-) knockout and wild-type mice, significant increase of the tumor multiplicity in 200 ppm-treated p53(+/-) knockout mice, and by significant increase in the incidence and multiplicity of tumours (malignant lymphomas) in the treated wild-type mice. By the end of 80 weeks, tumor induction, particularly malignant lymphomas and sarcomas, were similar in treated and control p53(+/-) knockout mice. No evidence for organ-tumor specificity of DMA was obtained. Molecular analysis using PCR-SSCP techniques revealed no p53 mutations in lymphomas from either p53(+/-) knockout or wild-type mice. The authors conclude that DMA primarily exerted its carcinogenic effect on spontaneous development of tumours with both of the animal genotypes investigated here.

Several studies have investigated exposure during the perinatal period in rodents.[169,170] Waalkes et al. (2003)[169], exposed pregnant C3H mice to different levels of sodium arsenite (0, 42.5 and 85 ppm) in the drinking water from gestational days 8 to 18, allowed to give birth, and, at weaning, offspring groups of males (25, 25, 25) and females (25, 25, 25) were formed and observed for tumour formation. Over the next 90 weeks post partum, female offspring exposed to arsenic in utero developed dose-related increases in lung adenocarcinoma (0/25, 1/23, 5/24), benign ovarian tumors (2/25, 4/23, 8/24) and

combined benign or malignant ovarian tumours (2/25, 6/23, 9/24). Female offspring also developed arsenic dose-related uterine and oviduct preneoplasias after fetal arsenic exposure. After in utero arsenic exposure, male offspring showed dose-related increases in incidence of liver adenoma (9/24, 12/23, 19/21), hepatocellular carcinoma (3/24, 8/23, 10/21), liver adenoma or carcinoma (12/24, 14/23, 19/21), and adrenal cortical adenoma (9/24, 15/23, 15/21). Additionally, arsenic exposed male offspring showed arsenic-induced, dose-related increases in liver tumour multiplicity (tumors/mouse), which was maximally over 5.6-fold over control.

Reproduction toxicity

Effects on fertility-oral exposure

WHO/ATSDR/additional data

No studies on inhalation exposure were retrieved, only oral and parenteral studies were available (Wang et al. 2006)[171]). Arsenite exposure causes male reproductive toxicity when given through drinking water (Chinoy et al. 2004)[172]; Pant et al. 2001)[173], 2004[174]) or by ip injection (Sarkar et al. 2003[175]). As^{III} interferes with spermatogenesis (Pant et al. 2001[173], 2004[174], Sarkar et al. 2003[175]) and alters activities of spermatogenetic enzymes (Chinoy et al., 2004[172]; Pant et al. 2001[173], 2004[174]). Furthermore, As^{III} lowers levels of testosterone and gonadotrophin (Chinoy et al. 2004[172]; Sarkar et al. 2003[175]). In female mice and rats, inorganic arsenic suppresses ovarian steroidogenesis, prolongs diestrus, and degenerates ovarian follicular and uterine cells (Chattopadhyay et al. 2001[176]; Navarro et al. 2004[177]). It also increases meiotic aberrations in oocytes, and decreases cleavage and preimplantation development (Navarro et al., 2004[177]).

Pant et al. (2001)[173] administered arsenic to male mice via drinking water as sodium arsenite at doses of 53.39, 133.47, 266.95 and 533.90 µmol/L (4, 10, 20, 40 µg As/L respectively) for 35 days. There was no difference in the uptake of water in control and treated animals. Arsenic treated mice survived the treatment period without any signs of clinical toxicity and showed no significant change in the body weight and in the weight of testes, epididymis and accessory organs. A decrease in the activity of 17 ß-hydroxysteroid dehydrogenase (17 ß-HSD) along with increase in lactate dehydrogenase (LDH) and gamma-glutamyl transpeptidase (γ-GT) activity were observed at 533.90 µmol/L. The observed sperm count, motility and morphological abnormalities in sperm were similar to control at lower dose levels. However at 533.90 µmol/L (40 µg As/L) a

significant decrease in sperm count and motility along with increase in abnormal sperm were noticed. Significant accumulation of arsenic in testes and accessory sex organs may be attributed to the arsenic binding to the tissues or greater cellular uptake.

In a chronic study Pant et al. (2004)[174] administered sodium arsenite to male mice via drinking water at a dose of 53.39 µmol/L (4 µg As/L) for 365 days. The mice did not demonstrate any apparent symptoms of toxicity, or any change in food consumption or water intake. Arsenic caused a decrease in the absolute and relative testicular weight. However, epididymal and accessory sex organ weight was similar to control. The activities of marker testicular enzymes such as sorbitol dehydrogenase, acid phosphatase and 17ß-HSD were significantly decreased, but those of LDH and γ-GT were significantly increased. A decrease in sperm count and sperm motility, along with an increase in abnormal sperm, was observed in arsenite-exposed mice. A significant accumulation of arsenic in testes, epididymis, seminal vesicle and prostate gland was observed in treated animals.

In mice, in addition to spermatogenesis, cholesterol metabolism and testicular testosterone level were affected by As^{III} (Chinoy et al. 2004[172]). Male Swiss mice were given arsenic trioxide (As2O3) orally at 0.5 mg/kg for 30 days. Treated mice showed increased cholesterol levels and decreased protein levels in the testes. Testicular structural damage observed included degeneration of tubules and denudation of germinal epithelial cells. There was also a lack of sperm in the lumen of seminiferous tubules. In addition, testicular activities of 3β-HSD and 17*β*-HSD, and testosterone levels in the serum were decreased.

Chattopadhyay et al. (2001[176], 2003[178]) gave a subchronic treatment to mature female Wistar-strain albino rats in diestrous phase with sodium arsenite at a dose of 0.4 ppm/100 g body weight/rat/day via drinking water for period of 28 days (seven oestrous cycles). No differences in food consumption were seen in any of the groups of animals throughout the experimental schedule. The body weights of arsenic-treated rats did not differ significantly from the controls. Liver weights and activities of liver enzymes were increased. The treatment caused a significant reduction in the plasma levels of lutinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol along with a significant decrease in ovarian activities of 3ß-HSD and 17ß-HSD followed by a reduction in ovarian and uterine peroxidase activities. A significant weight loss of the ovary and uterus was also observed after this treatment, along with a prolonged diestrous phase and a high accumulation of arsenic in the plasma and these organs. Moreover, sodium arsenite was also responsible for ovarian follicular and uterine cell degeneration characterized by a high number of regressing follicles and a

reduction in the uterine luminal diameter, respectively, in comparison with the controls.

Effects on fertility – parenteral exposure

WHO/ATSDR/additional data

Sodium arsenite was given to Wistar rats via ip injections at 4, 5, or 6 mg/kg/day for 26 days (Sarkar et al. 2003[175]). At 5 and 6 mg/kg/day, relative testicular weight, accessory sex organ weights and epididymal sperm counts were decreased. The same was true for plasma concentrations of luteinizing hormone (LH), FSH, and testosterone. Massive degeneration of all the germ cells at stage VII was observed at 5 and 6 mg/kg/day.

Female CD-1 mice were ip injected with 0, 8, or 16 mg/kg sodium arsenite every 2 days for a total of 7 injections over 14 days (Navarro et al. 2004[177]). Superovulation was induced by injections of equine and human chorionic gonadotrophins overlapping the end of the arsenite treatment. Metaphase II oocytes from these arsenite-treated mice had increased meiotic aberrations, characterized by spindle disruption and chromosomal misalignment. Additionally, zygotes from arsenite-treated mice showed lower rates of cleavage, decreased morula formation, and decreased development to blastocysts. More apoptotic nuclei were seen in the blastocysts of arsenite-treated mice. Some of these effects of arsenic on oocytes were observed at 8 mg/kg, a previously established maternal NOAEL.[171]

Developmental effects-inhalation exposure

WHO/ATSDR/additional data

Studies in animals showed that arsenic caused reduced birth weight, a variety of foetal malformations (both skeletal and soft tissue), and increased foetal mortality. These effects have been noted following inhalation exposure of mice and rats, oral exposure of mice, rats, hamsters and rabbits, and intraperitoneal or intravenous exposure of mice, rats and hamsters.

Holson et al. (1999)[179] administered inorganic arsenic, as arsenic trioxide (As^{III}, As_2O_3), via whole-body inhalational exposure to groups of twenty-five Crl:CD(SD)BR female rats for six h per day every day, beginning fourteen days prior to mating and continuing throughout mating and gestation. Exposures were initiated prior to mating in order to achieve a biological steady state of As^{III} in

the dams prior to embryo-fetal development. In a preliminary exposure range-finding study, half of the females that had been exposed to arsenic trioxide at 25 mg/m^3 died or were euthanized in extremis. In the definitive study, intended exposure levels were 0.3, 3.0, and 10.0 mg/m^3. Maternal toxicity, which was determined by the occurrence of rales, a decrease in net body weight gain, and a decrease in food intake during pre-mating and gestational exposure, was observed only at the 10 mg/m^3 exposure level. Intrauterine parameters (mean numbers of corpora lutea, implantation sites, resorptions and viable fetuses, and mean fetal weights) were unaffected by treatment. No treatment-related malformations or developmental variations were noted at any exposure level. The NOAEL for maternal toxicity was 3.0 mg/m^3; the NOAEL for developmental toxicity was greater than or equal to 10 mg/m^3.

Mice were exposed by inhalation to 0.22, 2.2 and 22 mg As/m^3 (as As_2O_3) for 4 h on days 9-12 of gestation On day 18 the foetuses were removed and the number of dead fetuses, retardation in growth, osteogenesis and chromosomal aberrations were examined. The highest dose group (22 mg As/m^3) had significant increases in the percentage of dead foetuses, skeletal malformations, and the number of foetuses with retarded growth, while those exposed to 2.2 mg As/m^3 had only a 10% decrease in average fetal body weight, and those exposed to 0.20 mg As/m^3 had 3% decrease in fetal weight (Nagymajtenyi et al., 1985[180]).

Developmental effects-oral exposure

WHO/ATSDR/additional data

Schroeder and Mitchner (1971)[181] found a statistically significant increase in the incidence of small litters and a trend toward decreased number of pups per litter in all generations of a 3-generation drinking water study in mice. The average litter size in the exposed group amounted 8.2 in the F1 generation, 9.6 in the F2 generation and 8.1 in the F3 generation. In the control group the average litter size was 11 in the F1, 10.3 in the F2 and 10.5 in the F3 generation.

Hood et al. (1978)[182] administered sodium arsenate orally to mice at a dose of 120 mg/kg on one of gestation days 7-15. The litters were examined at gestation day 18 and compared with litters from dams which were treated ip with a reference dose of 40 mg/kg sodium arsenate known to result in reprotoxic effects, and with litters from negative controls (dams treated orally with the solvent H_2O). The two arsenate treatments (oral and ip) caused comparable maternal mortality. Foetuses from orally treated dams weighed statistically significantly less than those from negative controls when treatment was given on

gestation days 10, 11 or 15, and either less than (treatment days 11 and 15) or more than (day 9) those from positive controls. Oral arsenate treatment on gestation day 11 increased prenatal mortality over that in negative controls ($p < 0.01$). Oral treatment on days 8-15 suggested an increase in prenatal mortality but the difference with the negative controls was not statistically significant.

The intraperitoneal treatment with the reference dose of 40 mg/kg sodium arsenate resulted in a significantly higher prenatal mortality than the oral treatment when given on day 7, 11, 13 and 14.

Fetal malformations (oligodactyly, micromelia, kinked tail) were seen in litters from dams given oral arsenate on days 7, 8, 9, 10, or 11, but the percentages of affected foetuses (0.6-5.0 %) did not differ significantly from those from negative controls (0 %). Skeletal defects (fused ribs, fused vertebrae) were also seen in litters from orally arsenate treated dams, but only treatment on gestation day 9 was associated with an incidence of defects significantly different from negative controls. Litter from dams treated ip with the reference dose of 40 mg/kg sodium arsenate exhibited both gross and skeletal abnormalities. Histopathological evaluations of fetal tissues were negative.

Baxley et al. (1981)[183] treated CD-I mice with a single dose of 20, 40, or 45 mg/kg sodium arsenite by oral gavage on one of days 8-15 of pregnancy. Controls were given equivalent volumes of water or were untreated. On gestation day 18, the dams were weighed and sacrificed. Litters were evaluated for prenatal mortality, and all live fetuses were examined for gross malformations and weighed. The lowest dose of sodium arsenite (20 mg/kg) produced no discernible teratogenic or maternal toxic effects in 8 to 15 pregnant mice exposed per treatment day. With the 40 and 45 mg/kg arsenite doses, maternal mortalities were 19 and 36% respectively and prenatal effects were observed. At 40 mg/kg, a low incidence of gross malformations, consisting of exencephaly and open eyes, was noted in fetuses from dams treated on days 8 or 9. Similar malformations were observed in fetuses from the dams treated with 45 mg/kg, if exposure to arsenic was on the 8^{th}, 9^{th} or 10^{th} day of gestation.

Hood and Harrison (1982)[184] treated outbred golden hamsters of the Lak: LVG (SYR) strain with single gavage doses. Groups of at least 10 pregnant females were given a single dose of sodium arsenite. Treatment was given as gavage with a dose of 25 mg/kg (14 mg As/kg) on gestation days 8, 11 or 12, or a dose of 20 mg/kg (11 mg As/kg) on days 9 or 10. Controls received water. On day 15 mated females were sacrificed and their litters were examined for prenatal mortality, gross, visceral, and skeletal malformations and the foetal weight. Treatment of pregnant hamsters with a 20 mg/kg oral dose of sodium arsenite on one of gestation days 9 or 10, or with 25 mg/kg on day 11, had no significant

effect on prenatal growth or survival. When hamsters were treated similarly with the higher dose on days 8 or 12, however, prenatal deaths increased, and growth was inhibited in day 12 treated fetuses. No morphological defects were observed in any fetuses from orally treated mothers. Seven of 57 arsenite gavaged mothers died, compared with only 1 of 51 solvent controls.

Nemec et al. (1998)[185] evaluated potential effects of exposure to arsenic acid throughout major organogenesis in CD-1 mice and New Zealand White rabbits. The animals were gavaged with arsenic acid dosages of 0, 7.5, 24, or 48 mg/kg/d on gestation days (GD) 6-15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg/d on GD 6 through 18 (rabbits) and examined at sacrifice (GD 18, mice; GD 29, rabbits) for evidence of toxicity. In the high dose group (48 mg/kg/d) in mice two dams died. In the surviving dams a significant increase was detected in the number of resorptions per litter (42% vs. 4% in controls) and significant decreases in the number of live pups per litter (6.6 vs. 12.3 in controls) and mean fetal weight (1.0 g vs. 1.3 g in controls). No significant decreases in fetal weight or increases in prenatal mortality were seen at other dosages. Malformations occurred in all groups of mice, including controls with a similar incidence. In mice, 7.5 mg/kg/d was the maternal NOAEL; the developmental toxicity NOAEL was estimated to be 7.5 mg/kg/d.

At the high dose in rabbits (3.0 mg/kg/d), seven does died or became moribund, and prenatal mortality was increased; surviving does had signs of toxicity, including decreased body weight. Does given lower doses appeared unaffected. Fetal weights were unaffected by treatment, and there were no effects at other doses. These data revealed an absence of dose-related effects in both species at arsenic exposures that were not maternally toxic. In rabbits, 0.75 mg/kg/d was the NOAEL for both maternal and developmental toxicity.

Stump et al. (1999)[186] treated rats (25 per group) with a single gavage dose of 3.8, 7.6, 15.2 and 22,7 mg As/kg as arsenic trioxide on day 9 of gestation (GD 9). Seven of the animals in the 22.7 mg As/kg group died on GD 10-11; all other animals survived. Maternal food consumption (GD 9-10) was decreased in a dose-dependent manner across all treated groups. In the 22.7 mg As/kg group, body weight, body weight change, and net body weight change were significantly decreased In the 20 mg/kg (15.2 mg As/kg) group, only transient effects on body weight were seen. The dose of 22.7 mg/As/kg resulted in a significant increase in postimplantation loss and a decrease in viable fetuses per litter, while those treated with 15.2 mg As/kg showed no effects.

Holson et al. (2000)[187] administered arsenic trioxide orally beginning 14 days prior to mating and continuing through mating and gestation until gestational day 19. Groups of 25 Crl:CD1(SD)BR female rats received doses of 0, 1, 2.5, 5 or 10

mg/kg/day by gavage. The selection of these dose levels was based on a preliminary range-finding study, in which excessive post-implantation loss and markedly decreased foetal weight occurred at doses of 15 mg/kg/day and maternal deaths occurred at higher doses. Maternal toxicity in the 10 mg/kg/day group was evidenced by decreased food consumption and decreased net body weight gain during gestation, increased liver and kidney weights, and stomach abnormalities (adhesions and eroded areas). Transient decreases in food consumption in the 5 mg/kg/day group caused the maternal NOAEL to be determined as 2.5 mg/kg/day. Intrauterine parameters were unaffected by arsenic trioxide. No treatment-related foetal malformations were noted in any dose group. Increased skeletal variations at 10 mg/kg/day were observed (unossified sternebrae #5 or #6, slight or moderate sternebrae malalignment, 7th cervical ribs) that the researchers considered to be consequences of developmental growth retardation. The developmental NOAEL was thus 5 mg/kg/day.

Hill et al. (2008)[188] evaluated the developmental toxicity of oral exposure on embryonic day (E) 7.5 and E:8.5 to 4.8, 9.6, or 14.4 mg As /kg (given as sodium arsenate) in an inbred mouse strain, LM/Bc/Fnn. This strain does not exhibit an underlying rate of spontaneous neural malformations, but is highly sensitive to arsenic induced neural tube defects (NTD). Control and arsenic-treated dams (20 per treatment group) were weighed daily, and evaluated for signs of maternal toxicity. Foetuses were evaluated for soft tissue and skeletal malformations. There was no maternal toxicity as evidenced by changes in maternal body weight following As treatment. However, liver weights were slightly lower in all As-treated groups. The number of litters affected with a neural tube defect (NTD) (exencephaly) in each treatment group was: 0 in the control group and 1, 5, and 9 in the groups treated with 4.8, 9.6, or 14.4 mg/kg, respectively, which exhibited a dose-dependent positive linear trend. There was also evidence for trends between As dose and the number of litters displaying vertebral ($p < 0.001$) and calvarial ($P < 0.01$) abnormalities, components of the axial skeleton. Mean fetal weight of all As-treated groups was significantly less than in control. In this model, maternal oral treatment with As induced NTDs. It also significantly increased the frequency of axial skeletal anomalies in the offspring exposed in utero, and reduced mean fetal weight, without convincing evidence of maternal toxicity.

To characterize developmental and behavioral alterations induced by arsenic exposure, Rodriguez et al. (2002)[189] exposed Sprague-Dawley rats to arsenite (37 mg As/L) in drinking water from gestation day 15 (GD 15) or postnatal day 1 (PND 1), until approximately 4 months old. The pregnant or lactating dams received either the arsenic solution or regular drinking water and once pups were weaned, they continued receiving the same solution as drinking water. Animals

exposed from GD 15 showed increased spontaneous locomotor activity and both exposed groups showed increased number of errors in a delayed alternation task in comparison to the control group. The latter effects were also found in rats exposed from postnatal day one.

Xi et al. (2009)[190] exposed rats in utero and during early life to sodium arsenite in drinking water and evaluated developmental neurotoxicity. The pregnant rats or lactating dams, and weaned pups were given free access to drinking water, which contained arsenic at (elemental) concentrations of 0, 10, 50, 100 mg/L from GD 6 until PND 42. A battery of physical and behavioral tests was applied to evaluate the functional outcome of pups. Pups in arsenic exposed groups weighed less than controls throughout lactation and weaning. Body weight of 10, 50 and 100 mg/L arsenic exposed groups decreased signifcantly on PND 42, 16 and 12, respectively. Physical development (pinna unfolding, fur appearance, incisor eruption, or eye opening) in pups displayed no signifcant differences between control and arsenic treated groups. In the highest dose group (100 mg As/L), arsenic decreased the incidence of neuromotor reflexes (tail hung, auditory startle and visual placing) signifcantly compared to the control group ($p < 0.05$). In square water maze test, the trained numbers to finish the trials successfully in 50 and 100 mg/L arsenic exposed groups increased remarkably compared to control group, and there was a dose-related increase ($p < 0.01$) observed. Maternal effects were observed only in the group treated with 100 mg/L (annoyance, irritation, infuriated, dysporic, dystocia, labour elongation, bleeding, no lactation).

Developmental effects-parenteral exposure and in vitro experiments

WHO/ATSDR/additional data

The experimental data in mice, rats and hamsters (Ferm and Carpenter (1968)[191], Willhite et al. (1981)[192], Beaudoin et al. (1974)[193], Hood and Harrison (1982)[184], Carpenter et al. (1987)[194], Stump et al. 1999[186], DeSesso[195,196], WHO[128], ATSDR 2007[7]) revealed that inorganic arsenic caused malformations (including neural tube defects) in offspring when injected ip or iv into pregnant animals during early gestation at maternally toxic (and often nearly fatal) doses. The Committee evaluated the parenteral studies and considered them of no further relevance for the classification and human risk assessment process. In addition, the Committee evaluated a selected number of in vitro studies showing effect of inorganic arsenic on embryonic development (Hanna et al., 1997[197], Tabacova et

al.,1996[198], Wlodarczyk et al., 1996[199]) and considered them of no further relevance for the classification and human risk assessment process.

Lactation

WHO/ATSDR/additional data

No data on toxic effects on the pups via lactation could be retrieved from the literature.

Immunological effects

WHO/ATSDR data

Mice exposed to 250-1000 µg arsenic trioxide/m^3 aerosol for 3 hours had a concentration-related decrease in pulmonary bactericidal activity (presumably as a result of injury to alveolar macrophages) and a corresponding concentration-related increase in susceptibility to introduced respiratory bacterial pathogens (Aranyi et al., 1985). Mice exposed to arsenate in drinking water did not display any signs of immunotoxicity (Kerkvliet et al., 1980), and mice given intratracheal doses of sodium arsenite had decreased humoral responsiveness to antigens but no measurable decrease in resistance to bacterial or cellular pathogens (Sikorski et al., 1989). Reports that gallium arsenide suppresses immune function and increases the co-stimulatory activity of macrophages in rodents treated orally or by intraperitoneal injection (Caffrey-Nolan and McCoy, 1998; Flora et al., 1998; Lewis et al., 1998a, 1998b) are confounded by the use of gallium nitrate as an immuno-suppressing drug (Makkonen et al., 1995; Orosz et al., 1997).

Additional data

Patterson et al. (2004) hypothesized that arsenic may modulate hypersensitivity responses to cutaneous sensitizing agents by altering cytokine production, LC migration, and T-cell proliferation.[200] Therefore the induction and elicitation phases of dermal sensitization were examined. Mice exposed to 50 mg/L arsenic in the drinking water for 4 weeks demonstrated a reduction in lymph node cell proliferation and ear swelling following sensitization with 2,4-dinitrofluoro-benzene (DNFB), compared to control mice. LC and T-cell populations in the draining lymph nodes of DNFB-sensitized mice were evaluated by fluorescence-

activated cell sorting; activated LC were reduced in cervical lymph nodes, suggesting that LC migration may be altered following arsenic exposure. Lymphocytes from arsenic-treated animals sensitized with fluorescein isothiocyanate (FITC) exhibited reduced proliferative responses following T-cell mitogen stimulation in vitro; however, lymphocyte proliferation from nonsensitized, arsenic-treated mice was comparable to controls. Arsenic exposure also reduced the number of thioglycollate-induced peritoneal macrophages and circulating neutrophils. These studies demonstrate that repeated, prolonged exposure to nontoxic concentrations of sodium arsenite alters immune cell populations and results in functional changes in immune responses, in this case specifically leading to attenuation of contact hypersensitivity.

Neurological effects

WHO/ATSDR data

No studies were located regarding neurological effects in animals after inhalation and dermal exposure to inorganic arsenicals. With regard to oral exposure, Heywood and Sortwell (1979) noted salivation and uncontrolled head shaking in two monkeys given several doses of 6 mg As/kg bw/day as arsenate, while no such effects were noted in monkeys given 3 mg As/kg bw/day for 2 weeks. Nemec et al. (1998) observed ataxia and prostration in pregnant female rabbits treated with 1.5 mg As/kg bw/day repeatedly during gestation, but not in rabbits treated with 0.4 mg As/kg bw/day.

Additional data

No additional animal data on neurological effects of arsenic and arsenic compounds after inhalation were found.

A number of oral studies in rats and mice has reported no symptoms of overt systemic toxicity from inorganic arsenic, but observed more subtle - neurobehavioural effects (Rodriguez et al., 2003[201]). In rats the most consistent change in behaviour after high oral inorganic arsenic administration (10, 20 mg/kg bw per day by gavage for 2-4 weeks) was a decrease in locomotor activity. Additionally rats showed a delay in execution of various task tests reflecting learning and memory after oral exposure to arsenic (Rodriguez et al., 2001[202], 2002[189]). Effects on locomotor activity, grip strength and rota rod performance were also observed recently in rats exposed orally to 20 mg arsenite/kg bw/day

po for 28 days (Yadav et al., 2009)[203]. Mice were exposed to 1 and 4 mg/kg bw/day of As_2O_3 subchronically for 60 days in water and significant dose-dependent neurobehavioural changes associated with memory (Morris Water Maze test) were observed. Rats exposed to inorganic arsenic in drinking water at 68 mg/L for 3 months showed a significant decrease in their spatial memory, while neurons and endothelial cells presented pathological changes, and the gene expression of aspartate receptors in the hippocampus was downregulated. These effects were not seen at 2.72 and 13.6 mg/L (Luo et al., 2009[204]).

7.3 Summary and evaluation

Human data

Relatively little information is available on the local effects of arsenic and arsenic compounds, except for arsenic trioxide. This corrosive compound may cause local damage to the skin, eyes and respiratory tract.

No cases were located regarding death in humans from inhalation exposure to inorganic arsenicals following acute exposure, even at very high exposure levels (1-100 mg As/m^3) found previously in the workplace.

Acute ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.

In vitro studies with human lymphocytes and fibroblasts showed clastogenic effects of arsenic. Methylated trivalent arsenicals were more potent DNA damaging compounds than the other arsenicals.

In vivo, chromosomal aberrations were observed in peripheral lymphocytes after inhalation exposure. Furthermore, human studies involving people exposed to relatively high arsenic concentrations in drinking water showed chromosome aberrations and sister chromatid exchanges in different cell types.

Studies of populations occupationally exposed (primarily by inhalation) to arsenic, such as smelter workers, pesticide manufacturers and miners in many different countries, consistently demonstrated an excess lung cancer risk among the arsenic-exposed. Sufficient quantitative information from human studies on the levels of occupational arsenic exposure to ensure reliable assessment of the

exposure-response relationship was available for three copper smelter cohorts: Tacoma (USA), Anaconda (USA) and Rönnskär (Sweden).[4,1,2,3] Exposure-response relationships and high risks have been observed. Increased risks have been observed at relatively low cumulative exposure levels in the smelter cohort of Rönnskär (Sweden; arsenic exposure category of < 250 µg/m^3·year) and in the smelter cohort of Tacoma (USA; arsenic exposure category of < 750 µg/m^3·year). Furthermore, in the Tacoma smelter, daily exposure to 213 µg/m^3 arsenic for 30 years or more was associated with a statistically significant SMR of 238 for lung cancer [98]. Studies indicated that smoking had a synergistic effect on the lung cancer effects of arsenic exposure.

Long-term exposure to arsenic in drinking-water is causally related to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. The effects have been most thoroughly studied in Taiwan but there is considerable evidence from studies on populations in other countries as well. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been reported to be associated with arsenic exposure categories of ≤ 50 µg/L. Chronic arsenic exposure (via drinking water) in Taiwan has been shown to cause blackfoot disease, a severe form of peripheral vascular disease which leads to gangrenous changes. This disease has not been documented in other parts of the world, and the findings in Taiwan may depend upon other contributing factors. However, there is good evidence from studies in several countries that arsenic exposure causes other forms of peripheral vascular disease. Conclusions on the causality of the relationship between oral arsenic exposure and other health effects are less clear-cut. The evidence is strongest for hypertension and cardiovascular disease, suggestive for diabetes and weak for cerebrovascular disease, long-term neurological effects, and cancer at sites other than lung, bladder, kidney and skin.

Several studies have examined a number of reproductive end-points in humans. No effects of arsenic on fertility are observed upon inhalatory or oral exposure. In the older inhalatory and oral human studies (Nordström et al., 1978a[129], 1978b[130], 1979a[131], 1979b[132], Aschengrau et al., 1989[134]; Zierler et al., 1988[135]) the populations were exposed to a number of other chemicals beyond arsenic. These chemicals may have contributed to the observed effects (congenital malformations, spontaneous abortions, stillbirth) but a causal relationship is uncertain. However, the Committee observes that the recent human studies on arsenic exposure from drinking water in different parts of the world give strong indications that arsenic can not be excluded as a causal factor for spontaneous

abortion, stillbirth, preterm delivery and reduced birth weight and also neuro-psychological development.

Animal data

Relatively little information is available on the local effects of arsenic and arsenic compounds. Sodium arsenite and sodium arsenate were not allergenic in the guinea-pig maximisation test.

In a developmental study, 100% mortality in groups of 10 pregnant rats after 1 day of inhalation exposure to arsenic trioxide concentrations ≥100 mg/m^3 was observed (76 mg As/m^3). The acute dermal LD_{50} for the pentavalent arsenicals calcium arsenate and lead arsenate in the rat amounts to ≥ 2400 mg/kg bw (≥400 mg As/kg). LD50 values after oral and parenteral arsenic exposure range from 15 to 960 mg As/kg bw/day, depending on the compound and the animal species.

Arsenic may cause clastogenic effects in vitro. A significant increase in the number of micronuclei, chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells after exposure to dimethylarsinous acid and monomethylarsonous acid are observed. As^{III} and As^{V} induced chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells, but not micronuclei. Monomethylarsonous acid, dimethylarsinous acid and dimethylarsinic acid are capable of inducing DNA damage via formation of reactive oxygen species (ROS).

No point mutations were observed in bacteria or in mammalian cells after arsenic exposure.

In vivo, sodium arsenite (2.5 mg/kg bw) produced significantly high frequencies of chromosome aberrations in bone marrow cells in mice after 24 h exposure[161].

Several animal carcinogenicity studies on arsenic have been carried out, but limitations such as limited time of exposure and limited number of animals make these inconclusive. In a recent study, female C57B1/6J mice had an increased incidence in tumours involving mainly lung, liver, gastrointestinal tract and skin after exposure to 500 µg As^{V}/L drinking water for 2 years. One study has indicated that dimethylarsinic acid may cause cancer of the urinary bladder in male rats at high doses.

Perinatal exposure to inorganic arsenic between gestational day 8-18 in mice suggests that cancer may be induced in offspring.

In experimental animals effects on fertility of inorganic arsenic via the inhalatory route are not reported, but exposure to inorganic arsenic (As^{III} and ^{V}) via the oral and ip route has shown significant effects on fertility (a.o. interference with spermatogenesis, degeneration of follicular cells). Exposure of experimental animals to inorganic arsenic via inhalation, oral and parenteral routes caused, usually at relatively high maternally toxic doses, reduced birth weight, a variety of foetal malformations (both skeletal and soft tissue), and increased foetal mortality. Reproductive performance was not affected in female rats that received inhalation exposures to concentrations as high as 20 mg As/m^3 or gavage doses as high as 8 mg As/kg bw/day from 14 days prior to mating through gestation day 19. The Committee is aware that in none of the animal studies maternal toxicity can be unambiguously excluded. Only the study by Hill et al. (2008)[188] administering arsenate to an inbred mouse strain, supports the view that foetal malformations can develop in the absence of maternal toxicity.

Limited information is available with regard to immunological effects of arsenic and arsenic compounds in animals.

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

8.1.1 Air quality guidelines

Quantitative risk estimates for the occurrence of lung cancer after inhalation exposure of arsenic have been derived by the WHO[128] and the EPA[127].

The risk estimates of the WHO[128] for arsenic have been derived from studies describing the dose-response relationships between arsenic exposure and excess lung cancer mortality in workers at the Anaconda, Tacoma and Rönnskär smelter. Assuming a linear dose-response relationship, a safe level for inhalation exposure cannot be recommended. The unit risk for arsenic-induced lung cancer (excess risk estimate associated with lifetime exposure to a concentration of 1 µg/m^3) amounts to $1.5*10^{-3}$. This means that the excess lifetime risk level is 1:10,000, 1:100,000 or 1:1,000,000 at an air concentration of about 66 ng/m^3, 6.6 ng/m^3 or 0.66 ng/m^3, respectively.

The inhalation unit risk established by the EPA using the absolute-risk linear model amounts to $4.3*10^{-3}$ per 1 µg/m^3 based on the dose-response relationships between arsenic exposure and excess lung cancer mortality in workers at the

Anaconda and the Tacoma smelter. This means that the excess lifetime risk level is 1:10,000, 1:100,000 or 1:1,000,000 at an air concentration of about 20 ng/m^3, 2 ng/m^3 or 0.2 ng/m^3, respectively.[127]

8.2 Working population

Currently, the 8 hr time-weighted average (TWA) and short-term (15 min) occupational exposure limits for the combination of all inorganic arsenic compounds in the Netherlands are 0.05 and 0.1 mg As/m^3, respectively. The TWA (8 hr) and short-term (15 min) occupational exposure limits for the water-soluble inorganic arsenic compounds are 0.025 and 0.05 mg As/m^3, respectively. There is currently no limit value for exposure to arsenic and arsenic compounds at the European level. A number of EU member countries have set a limit for arsenic and arsenic compounds. Furthermore, the ACGIH, OSHA and NIOSH have set a limit/recommended standard for exposure to arsenic (see Table 7). None of the referred EU members or organisations have attached a skin notation to arsenic and arsenic compounds.

The Health and Safety Executive (HSE) has established an occupational exposure limit of 0.1 mg/m^3 for the United Kingdom.[206,207] This limit is based on cancer of the respiratory tract. Since it was not possible to identify a no-adverse-effect level (NOAEL), a maximum exposure limit was considered appropriate. According to the HSE, this maximum exposure limit was set at 0.1 mg/m^3 (8 hr TWA value), which was below the exposure level at which raised incidence of respiratory tract cancer had been observed, and below the no-effect level for respiratory tract irritation. For most industries there should be no difficulty in using engineering controls to maintain exposures below this level, but in a minority of cases respiratory protective equipment may be necessary. Lead arsenate is excluded from this limit.

According to the Deutsche Forschungsgemeinschaft (DFG), arsenic and inorganic arsenic compounds are carcinogenic in man.[209,210] After inhalation of the substance, carcinogenic effects are observed in the lungs and after ingestion, in the bladder, kidneys, skin and lungs. However, neither the studies with inhalation exposure to arsenic nor those with oral administration of the substance can be used to derive a no observed adverse effect level (NOAEL). As a NOAEL for carcinogenicity cannot be derived from the epidemiological studies, no occupational exposure limit value has been established for arsenic and inorganic arsenic compounds in Germany. Arsenic and arsenic compounds therefore

Table 7 Existing Occupational Exposure Limits (OELs) for arsenic and arsenic compounds.

Country / Organisation	Arsenic compound	Level (mg/m^3)	Time-relation	Remarks
The Netherlands[205]	Arsenic (combination of inorganic compounds, as As)	0.05 0.1	TWA - value (8 hr) Short term - value (15 min)	C[1]
	Arsenic (water-soluble inorganic compounds, as As)	0.025 0.05	TWA - value (8 hr) Short term - value (15 min)	
United Kingdom[206,207]	Arsenic and compounds (as As) (except lead arsenate)	0.1	TWA - value (8 hr)	C
Denmark[208]	Arsenic and inorganic compounds, as As	0.01	TWA - value	C
	Calcium arsenate	1	TWA - value	
Germany[209-211]	Arsenic and inorganic compounds	(no OEL established)	-	C
	Arsenic trioxide, arsenic pentoxide, arsenic acid	0.1 (inhalable fraction)	TRK (Technische Richtkonzentration) - value (8 hr)	
		0.4 (inhalable fraction)	TRK - Short term - value (15 min)	
Sweden[212]	Arsenic and inorganic compounds (as As)	0.03 (total dust)	TWA - value (8 hr)	C
Scientific Committee on Occupational Exposure Limits (SCOEL)[213]	Arsenic and inorganic compounds	(no OEL established)	-	-
ACGIH (TLV)[214]	Arsenic and inorganic compounds, as As	0.01	TWA - value (8 hr)	C
OSHA[215]	Arsenic, inorganic compounds	0.01	TWA - value (8 hr)	C
NIOSH[216,217]	Arsenic (inorganic compounds, as As)	0.002	Short term - value (15 min, ceiling)	C

[1] C: the substance is considered carcinogenic

remain in Carcinogenicity category 1. As a result of the mutagenic effects in somatic cells, the formation of genotoxic and systemically effective metabolites, the bioavailability of the substance in the gonads and the inadequate investigation in germ cells, arsenic and inorganic arsenic compounds are classified in Category 3A for germ cell mutagens.[209] Arsenic and arsenic

compounds are not designated as sensitisers.[209] Arsenic acid penetrates the skin in vivo and in vitro in amounts of less than 10 % and sodium arsenate from aqueous solution or as the solid *in vitro* in amounts of about 30% to 60 %. Dermal absorption under workplace conditions does not seem to be relevant, and arsenic and inorganic arsenic compounds are not designated with a skin notation.[209]

Based on the carcinogenic properties of arsenic trioxide, arsenic pentoxide and arsenic acid an 8 hr Technische Richtkonzentration (TRK) of 0.1 mg/m^3 (inhalable fraction) is established for these arsenic compounds in Germany. A TRK is the concentration of a chemical substance in air within a working area, which may be reached in accordance with the best available technology (state of art). Furthermore, a short-time TRK (15 min) is established at 0.4 mg/m^3.[211]

In Sweden, the 8 hr TWA limit value is 0.03 mg/m^3.[212] However, it is noted that in the planning of new facilities or the alteration of old ones, an effort shall be made to ensure that exposure to arsenic and inorganic compounds other than arsenic hydride in the course of a working day is acceptable with reference to a time-weighted average concentration of 0.01 mg/m^3 (as As).

The American Conference of Governmental Industrial Hygienists (ACGIH) has specified a threshold limit value (TLV) of 0.01 mg/m^3 (as As) (8 hr TWA value).[218] Furthermore, based on the weight of evidence from epidemiologic studies, arsenic and inorganic compounds are designated as an A1, Confirmed Human Carcinogen.

Numerous epidemiologic studies showed lung cancer excesses with occupational arsenic exposures of smelter workers and pesticide workers. The quantitative air monitoring data presented by Enterline et al., 1987[98] indicate a significant excess of lung cancer risk for workers exposed to a mean a level of 0.2 mg/m^3 of arsenic. This is based on an SMR of 213. According to the ACGIH this is the lowest level at which an excess risk of cancer in humans has been found. To allow some measure of safety, a TLV-TWA of 0.01 mg/m^3 (as As) is recommended.[214]

The permissible exposure limit (PEL) of the Occupational Safety and Health Administration (OSHA) for inorganic arsenic amounts to 0.01 mg/m^3 when averaged over any 8-hour work shift.[215]

The recommended standard of the National Institute for Occupational Safety and Health (NIOSH) was established in 1975[216](and reconfirmed in 2005[217]). In the criteria document for the recommendation, it was mentioned that studies suggested that exposure on an 8 to 24 hour TWA basis at concentrations of 2-3 µg/m^3 has resulted in increased cancer mortality. According to NIOSH, in the absence of information for a safe level of exposure to a carcinogen, protection of the worker should be effected by requiring that airborne concentrations not exceed minimally detectable levels. However, background atmospheric concentrations of arsenic up to 1.4 µg As/m^3 were reported. Therefore, to achieve the greatest practicable reduction in worker exposure while avoiding spurious sampling results produced by natural background concentrations of inorganic arsenic, NIOSH recommended that worker exposure be controlled to prevent exposure in excess of 2.0 µg/m^3 of air as determined by a 15 minute sampling period.

8.2.1 Biological monitoring

For the purpose of biological monitoring in the Netherlands, in 1984, a (tentative) concentration of arsenic in urine as an individual maximum of 40 µg arsenic/g creatinine was specified (provided that recent intake of sea food is excluded)[219]. A remark was made that this recommended value possibly should be corrected when new data would become available. This value was based on a reference of Lauwerys (1980) in which normal urine arsenic concentrations were specified as < 40 µg arsenic/g creatinine, mostly much lower.

In Germany, the Deutsche Forschungsgemeinschaft (DFG) investigates the relationships between the concentration of the carcinogen in the workplace air and that of the substance or its metabolites in biological material (EKA values, exposure equivalents for carcinogenic substances) and specifies the following EKA values for arsenic trioxide[210]:

Table 8 EKA values for arsenic trioxide.

Arsenic in air (mg/m^3)	Sampling time: end of exposure or end of shift – urine[a] Arsenic (µg/L)
0.01	50
0.05	90
0.10	130

[a] volatile arsenic compounds by hydrogenation

The DFG has also specified a 'Biologische Leitwerte' (BLW) value of 50 µg for inorganic arsenic and methylated metabolites in urine per L urine (sampling: for long term exposures: after several shifts, end of exposure or end of shift).[210]

According to the American Conference of Governmental Industrial Hygienists (ACGIH)[23], monitoring of the sum of inorganic arsenic plus its two major organic metabolites, monomethylarsonic acid and dimethylarsinic acid, collected at the end of the workweek in urine is the recommended method for biological monitoring of occupational exposure to elemental and soluble inorganic arsenic compounds.

Chapter 9

Hazard assessment

9.1 Assessment of the health hazard

Occupational exposure to arsenic may be significant in several industries. Absorption of arsenic from inhaled airborne particles is highly dependent on the solubility and the size of particles. Different types of occupational exposures may result in different uptakes of arsenic because of the bioavailability of the form of arsenic to which workers are exposed. Both pentavalent and trivalent soluble arsenic compounds are rapidly and extensively absorbed from the gastrointestinal tract ($\geq 70\%$). Dermal absorption appears to be much less than by the oral or inhalation routes. Arsenic and its metabolites distribute to all organs in the body. Arsenic readily crosses the placenta. Arsenic metabolism is characterised by alternation of two main types of reactions: (1) two-electron reduction reactions of pentavalent to trivalent arsenic, which may occur nonenzymatically via glutathione or enzymatically, and (2) oxidative methylation reactions in which trivalent forms of arsenic are converted to (mono-, di- or tri-) methylated pentavalent products, using S-adenosyl methionine (SAM) as the methyl donor and glutathione (GSH) as an essential co-factor. Arsenic and its metabolites are largely excreted via the renal route. Arsenic can be excreted in human milk.

9.1.1 *Acute and short term toxicity*

No deaths were reported in humans from inhalation exposure to inorganic arsenic-compounds following acute exposure, even at the very high exposure levels (1-100 mg As/m^3) found previously in the workplace. A few case reports of neurological effects after acute inhalation have been described (see Section 7.1.4.5). No more recent studies on neurological effects and other adverse health effects after acute inhalation exposure to arsenic were reported.

Acute ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.

With regard to animals, in a developmental study, 100% mortality in groups of 10 pregnant rats after 1 day of inhalation exposure to arsenic trioxide concentrations ≥100 mg/m^3 was observed (76 mg As/m^3) (Holson et al., 1998). Oral and parenteral lethal doses range from 15 to 960 mg As/kg bw/day, depending on the compound and the animal species.

9.1.2 *Long-term toxicity*

In epidemiological studies respiratory cancer is the only type of effect, convincingly associated with long term inhalation exposure to arsenic, whereas skin cancer and cancers of multiple internal organs (liver, kidney, lung and bladder) have been observed in populations with exposure to arsenic in drinking water. A difference between studies of occupationally and environmentally exposed cohorts is that for most of the environmentally exposed cohorts exposure starts at birth, whereas for the occupationally exposed cohorts exposure starts much later in life. Bates et al. (1992) (cited in Enterline et al.[4] and Lubin et al.[1]) suggested that differences in cancer risk after oral and occupational exposure were based on differences in cumulative exposure. From the study of Lubin et al.[1] it may be concluded that carcinogenic mechanisms associated with inhaled arsenic differ from those related to ingested arsenic.

Until recently there was a lack of clear evidence for carcinogenicity of any arsenic compound in animals. More recent work with arsenical methylation metabolites and early life exposures to inorganic arsenic has provided evidence of carcinogenicity in rodents after oral exposure. Only one long term inhalation study (2 yr) is available which indicates that gallium arsenide (NTP 2000)[165] is

carcinogenic in rats. No studies were located regarding cancer in animals after inhalation exposure to the other arsenicals.

9.2 Quantitative hazard assessment

9.2.1 Critical effect and approach to the quantitative hazard assessment

The Committee considers lung cancer as the critical effect in humans after inhalation exposure to arsenic and arsenic compounds. Studies of populations occupationally exposed (primarily by inhalation) to arsenic, such as smelter workers, pesticide manufacturers and miners in many different countries, consistently demonstrated an excess lung cancer risk among the arsenic-exposed. Sufficient quantitative information from human studies on the levels of occupational arsenic exposure to ensure reliable assessment of the exposure-response relationship was available for three copper smelter cohorts: Tacoma (USA), Anaconda (USA) and Rönnskär (Sweden).[4,1,3] Increased risks have been observed in relatively low cumulative exposure categories: exposure category of < 250 µg/m^3·year (Rönnskär, Sweden) and exposure category of < 750 µg/m^3·year (Tacoma, USA). Furthermore, in the Tacoma smelter, daily exposure to 213 µg/m^3 arsenic for 30 years or more was associated with a statistically significant SMR of 238.7 for lung cancer.[98] Studies indicated that smoking had a synergistic effect on the the development of lung cancer of arsenic exposure.

The Committee considers arsenic as a non-stochastic genotoxic compound (see Annex I and J). Clastogenic damage was observed in human and animal studies in vivo and in vitro. For point mutations, the results are largely negative. With regard to the mechanism which caused the genotoxic effects, there is evidence that arsenicals bind to thiol-groups in proteins which may lead to inhibition of e.g. DNA repair enzymes. There is also evidence that arsenic exposure can result in hypo- or hypermethylation of cellular DNA; these changes can be caused by e.g. an effect of arsenic on DNA methyltransferases. Furthermore, arsenic does not generate reactive oxygen by itself but inhibits the scavenging systems of reactive oxygen species, which indirectly leads to an increase of reactive oxygen species. Since all these processes support a non-stochastic mechanism of genotoxicity a NOAEL for arsenic and arsenic compounds should theoretically be derived using a threshold model. However, the available epidemiological studies do not allow derivation of such a threshold, i.e., a no-effect concentration.

Therefore the Committee decided not to pursue a threshold approach but to calculate excess lifetime cancer mortality risks (health-based calculated occupational cancer risk values (HBC-OCRV)), using mathematical modeling and extrapolation.

9.2.2 *Quality of the studies on occupational arsenic exposure*

The Committee selected four epidemiological studies on lung or respiratory cancer mortality among workers exposed to arsenic. The studies by Lubin et al. (2000)[1], Lubin et al. (2008)[2], Järup et al. (1989)[3] and Enterline et al. (1995)[4] were considered for quantitative hazard assessment. First, the Committee evaluated the quality of these studies using a number of characteristics including study design, execution, analyses etc. and their usefulness for derivation of HBC-OCRVs (see Annex L for detailed information).

The study of Jarüp et al. (1989)[3] had an exposure assessment component for which the description was limited and basic documentation was lacking. The way exposure has been calculated was not transparent, therefore making it difficult to analyse the risk per unit of increase by exposure. Information on exposure before 1945 was unclear, and again, it was not clear how exposure was assigned to certain job titles. Furthermore, no exposure-response relation was given. However, in this study loss-to-follow-up was low. An exposure-response relation could be calculated by the Committee. This study analysed lung cancer mortality. Calculations were based on a comparison with the general population (SMR study).

In the study by Enterline et al. (1995)[4] the description of the exposure assessment component was limited and basic descriptive information was lacking. Information about exposure before 1938 was lacking completely. It was not clear how exposure was assigned to certain job titles. Loss-to-follow-up was low, and an exposure-response relation was given. The study used lung cancer mortality. Calculations were based on a comparison with the general population (SMR study). The dose-response relationship was described using a power model.

In the studies by Lubin et al. (2000[1], 2008[2]) instead of lung cancer mortality, respiratory cancer mortality is used, which included larynx and trachea tumours. According to the information given in the Lubin et al. (2000) paper (Table 2), this may cause a deviation in mortality rate smaller than 4%. Therefore, the effect on association measures like risk ratio's is assumed to be negligible. Another potential limiting factor of this paper is the higher loss-to-follow-up, compared to the other studies under consideration. If it is assumed that this loss-

to-follow-up is non-differential across exposure, which seems a reasonable assumption, the Lubin et al. (2000) study is the strongest study with fewest limitations. To describe the dose-response relationships both a power model and a linear model were applied. The Lubin et al. (2008) study was a follow-up to the 2000 study, with a different modelling strategy. In the update, Lubin et al. used an exposure reduction factor in the higher exposure categories to account for the use of personal protection equipment. This is not common practice in risk calculations and this study was not further considered for risk assessment.

9.2.3 *Calculation of HBC-OCRVs*

Lubin et al. (2000)[1] used an internal exposure-response analysis (resulting in a relative risk, RR), whereas the studies by Jarüp et al. (1989) and Enterline et al. (1995) compared exposure-related mortality with mortality in the general population, which results in a standardized mortality ratio (SMR). The Committee reevaluated the exposure-response relationships in the three studies using both power and linear modelling (see Annex L).

The use of a power model to describe the data of Lubin et al. (2000)[1] did not significantly improve the fit compared to a linear model (see p. 557-558 of Lubin *et al.* 2000). Therefore the linear model was used (with risk function RR=1+0.19*cum.exposure) for further calculations. Life table calculations were performed. The Committee calculates (see Table 9) that the concentration of arsenic in the air, which corresponds to an excess cancer mortality of

- 4 per 1,000 ($4x10^{-3}$), for 40 years of occupational exposure, equals to 28 µg/m^3
- 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 0.28 µg/m^3.

The Committee also evaluated the studies of Jarüp et al. and Enterline et al. for quantitative hazard assessment.

An exposure-response relationship was not given in the original article by Jarüp et al. 1989.[3] Therefore the Committee calculated the relation based on the data given in the original article. This resulted in the following relations: RR = 1 + 0.1002(cum. Exp.) and RR = 1 + 1.69(cum. Exp.)$^{0.253}$ (RR = 1 means no difference in mortality when compared to reference group). The power-model calculated for the Jarüp et al. study has a better fit than the linear model. This also is said to be the case for the power model shown in Enterline et al.[4]

(SMR=100+10.5 (cum exp)^{0,279}, see p. 30 of the original article), although no model fit information is given.

For both these studies it cannot be excluded that the strong fit of the power models is artefactual and caused by the fact that there is a clear difference in risk between the exposed population and the comparison group, while there is a very weak association among the exposed only. These studies by Jarüp et al. and Enterline et al. are SMR studies and are thus the result of a comparison with the general population and potential systematic differences in mortality between the general population and the exposed workers in these cohort studies. When an attempt was undertaken to model the exposure response curve in the low exposure range (steep part of the curve) the fit of linear models was very poor for both studies indicating that there was no clear exposure response curve discernible in this range. This again suggests that the comparison with the general population may be problematic.

For comparison the risk estimates using the linear model are also given for the other two studies (see Table 9). In spite of these equations being derived from marginally fitting risk functions, the resulting risk estimates are in the same order of magnitude as those calculated from the Lubin et al. study.

Table 9 Health-based calculated occupational cancer risk values.

Study	Equation	ER*=4e-3	ER=4e-5
Lubin et al. 2000[1]	RR=1+0.19*cum.exposure	28 µg/m^3	0.28 µg/m^3
Jarup et al. 1989[3]	1+0.33*cum.exposure	16 µg/m^3	0.16 µg/m^3
Enterline et al.1995[4]	1+0.16*cum.exposure	33 µg/m^3	0.33 µg/m^3

Considering the quality of the papers and fit of the models, the Committee decides to use the outcomes of the Lubin et al. (2000) study and calculates that exposure to 28 µg As/m^3 for 40 years results in 4 additional death cases per per 1,000 (4x10^{-3}) deaths and exposure to 0.28 µg As/m^3 for 40 years result 4 additional death cases per per 100,000 (4x10^{-3}) deaths.

The Committee concludes that the concentration level of 28 µg/m^3 associated with a lifetime cancer risk level of 4 x10^{-3} is well below any health based occupational exposure limit derived from data other than carcinogenicity.

9.2.4 *Short Term Exposure Limit (STEL)*

Although arsenic, arsenic pentoxide and lead arsenate are labelled with Acute Tox. 3: H331 and H301 (‘toxic if inhaled’ and ‘toxic if swallowed’) and arsenic trioxide is labelled with Acute Tox. 2: H300 and Skin Corr. 1B (‘fatal if

swallowed' and 'causes severe skin burns and eye damage') according to EC Regulation 1272/2008[11], the available data do not warrant the setting of a Short Term Exposure Limit (STEL) or ceiling value according to the Committee.

With regard to local effects, relative little information is available; effects on the respiratory tract, skin and eyes have been observed in old reports, where exposure was to high levels of arsenic dust. No concentrations or exposure duration are given, but since no recent studies on these effects were reported, these results are probably of no relevance for current exposure levels.

Furthermore, no other countries/organisations have established a STEL for arsenic and arsenic compounds based on acute systemic or local toxicity (see chapter 8).

9.2.5 Skin notation

Absorption by the dermal route has not been well characterised, but is low compared to the other routes. The rate of absorption of arsenic and arsenic compounds through the skin does not warrant a skin notation.

9.3 Recommendation

The Committee calculates that the concentration of arsenic in the air, which corresponds to an excess cancer mortality of

- 4 per 1,000 ($4x10^{-3}$), for 40 years of occupational exposure, equals to 28 $\mu g/m^3$
- 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 0.28 $\mu g/m^3$.

The available data do not warrant the setting of a Short Term Exposure Limit (STEL) or ceiling value.

The rate of absorption of arsenic and arsenic compounds through the skin does not warrant a skin notation.

9.4 Groups at extra risk

WHO/ATSDR data

No studies were located regarding unusual susceptibility of any human subpopulation to arsenic. However, since the degree of arsenic toxicity may be

influenced by the rate and extent of its methylation in the liver (see Chapter 6), it seems likely that people may differ in susceptibility because of difference in methylating capacity and the existence of polymorphism has been hypothesised. While there is some evidence that methylation capacity does vary among individuals (e.g., Buchet et al., 1981; Foa et al., 1984; Tam et al., 1982), the basis of this variation and its impact on human susceptibility have not been established.

Additional data

Furthermore, smokers may be more susceptible as according to Hertz-Picciotto (1992)[101] arsenic and smoking act in a synergistic manner to produce lung cancer.

Chapter 10

Recommendation for research

No recommendations for research were made by DECOS.

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- G.J. Mulder, *chairman*
  Emeritus Professor of Toxicology, Leiden University, Leiden
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  Occupational Physician, Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam
- A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam

- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- G.M.H. Swaen
  Epidemiologist, Dow Benelux NV, Terneuzen
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R.A. Woutersen
  Toxicologic Pathologist, TNO Quality of Life, Zeist, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- G.B. van der Voet, *scientific secretary,*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter

Subject	: Submission of the advisory report *Arsenic and inorganic arsenic compounds*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7493/BvdV/fs/459-X67
Enclosed	: 1
Date	: December 11, 2012

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to *Arsenic and inorganic arsenic compounds*.

This advisory report is part of an extensive series in which health-based calculated occupational cancer risk values are derived for the concentrations of various substances in the workplace. The advisory report in question was prepared by the Health Council's Dutch Expert Committee on Occupational Safety (DECOS) and assessed by the Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,

(signed)
Prof. W.A. van Gool,
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in July 2012 for public review. The following organisations and persons have commented on the draft report:

- Mr. T.J. Lentz, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA
- Mr. H.W.C.M. Flipsen, Nederlandse Vereniging Diervoederindustrie (Nevedi), Rotterdam.

Annex **E**

Abbreviations

AAS	atomic absorption spectroscopy
AES	atomic emission spectroscopy
AFS	atomic fluorescence spectroscopy
AM	arithmetic mean
BEM	biological effect monitoring
BM	biological monitoring
bw	body weight
CI	confidence interval
G(L)C	gas liquid chromatography
GD	gestation day(s)
GM	geometric mean
GSD	geometric standard deviation
hr	hour
HBR-OEL	health based recommended occupational exposure limit
ICP	inductively coupled plasma
HPLC	high performance liquid chromatography
im	intramuscular
ip	intraperitoneal
IR	infrared
it	intratracheal

iv	intravenous
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAK	Maximale Arbeitsplatz Konzentration
MMAD	mean mass aerodynamic diameter
MS	mass spectrometry
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
OR	odds ratio
PEL	permissible exposure limit
ppb	parts per billion (v/v)10^{-9}
ppm	parts per million (v/v)10^{-6}
RR	relative risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio
STEL	short term exposure limit
tgg	tijdgewogen gemiddelde
TLV	threshold limit value
TWA	time-weighted average
UV	ultraviolet
XRF	X-ray fluorescence

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Safety
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OSHA	Occupational Safety and Health Administration (USA)
WHO	World Health Organisation

Annex **F**

WHO/ATSDR References

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Annex **G**

Human data

(additional data, if not further specified)

Table 10 Human in vitro studies with arsenic and arsenic compounds.

Human cell type	Procedure/Concentration tested	Effects	Reference
Human hepatocytes, human epidermal keratinocytes, human bronchial epithelial cells and human urinary bladder cells	MTT (thiazolyl blue) assay (cell viability) As^{III}, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid and dimethylarsinous acid at final concentrations of 0.05 to 20 µM for up to 24 hr. Capacities of cells to produce methylated metabolites Hepatocytes were exposed to 0.1-20 µM As^{III}, the other cell types to 0.05 µM As^{III}; incubation for 24 hr.	Monomethylarsonous acid species were the most cytotoxic in all cell types; dimethylarsinous acid were at least as cytotoxic as As^{III} for most cell types. Pentavalent arsenicals were significantly less cytotoxic than their trivalent analogs. Hepatocytes exhibited the greatest methylation capacity for As^{III} followed by epidermal keratinocytes, and bronchial epithelial cells. Cells derived from human bladder did not methylate As^{III}.	Styblo et al., 2000[56]

Table 11 Human case reports with regard to exposure to arsenic and arsenic compounds.

Humans involved/No. of humans	Procedure	Effects	References/ Remarks
Inhalation			
No data available			
Oral			
77-year old male	Suicide attempt by ingestion of 4 g arsenic trioxide (for dental devitalizations).	Between 5 h and 7 days he developed nausea, vomiting, gastritis and stomach ulcers, anaemia. He recovered after clinical intervention.	Yilmaz et al., 2009[77]
18-year old male	Suicide attempt by ingestion of arsenic trioxide (termiticide)	Multisystem organ failure, peripheral neuropathy. He recovered after clinical intervention.	Kim and Abel, 2009[78]
43-year old male	Suicide attempt by drinking 54 g arsenic trioxide	Vomiting, diarrhoea, thirst, pharyngeal constriction, paraesthesia of the legs. He recovered after timely clinical intervention but polyneuropathy remained.	Duenas-Laita et al., 2005[79]
25-year old woman	Case report: root canal treatment of the teeth by a private practitioner, an arsenical paste was applied to the teeth.	Arsenical necrosis of the jaws affecting the right and the left side of the maxilla. As a result of leakage into the tissues of an arsenical paste from the pulp chamber of endodontically treated teeth, bilateral oroantral fistula (OAF) occurred.	Yalcin et al., 2003[85]

Table 12 Human studies with regard to non-carcinogenic effects after long-term exposure to arsenic and arsenic compounds.

Study population	Exposure assessment	Effects (non-carcinogenic)	References/ Remarks
Inhalation			
86 exposed art glass workers and 54 controls (workers from tool makers). Individuals with liver or kidney diseases were excluded.	As_2O_3	A significant increase in the excretion of penta and uroporphyrins was demonstrated for workers exposed to As; As(III) was the species best correlated with urinary porphyrin excretion.	Apostoli et al., 2002[49] Major objective of this study was biomonitoring.
Oral			
Cases: subjects identified by dermatologists during 1994, with basal cell carcinoma (2 cases), Bowen's disease (squamous cell carcinoma of the skin, 19 cases) or hyperkeratosis/ hyperpigmentation (6 cases) from the blackfoot disease endemic area in southwestern Taiwan. Controls subjects matched by gender (12 female and 14 male pairs) and age within 3 years (average age: 63.4 years)..	Both cases and controls had been exposed to drinking water from contaminated well water for approximately 30 years but had changed to piped water for more than 10 years. Cases and controls had ingested similar concentrations of arsenic in drinking water (0.77 and 0.98 ppm, respectively (not statistically significant different (p = 0.117))) and excreted comparable urinary arsenic metabolite concentrations.	Results indicated that skin lesion cases had higher percents of inorganic arsenic (13.1 ± 3.7%), monomethylarsonic acid (16.4 ± 3.2%), lower percent of dimethylarsinic acid (70.5 ± 5.8%), and higher ratio of monomethylarsonic acid to dimethylarsinic acid (monomethylarsonic acid/dimethylarsinic acid, 0.24 ± 0.06) than matched controls (InAs: 11.43 ± 2.1%; monomethylarsonic acid: 14.6 ± 2.6%; dimethylarsinic acid: 73.9 ± 3.3%; monomethylarsonic acid/ dimethylarsinic acid: 0.20 ± 0.04). Individuals with a higher percentage of monomethylarsonic acid (>15.5%) had an odds ratio of developing skin disorder 5.5 times (95% confidence interval, 1.22-24.81) higher than those having a lower percentage of monomethylarsonic acid. This association was not confounded by hepatitis B surface antigen, cigarette smoking, or alcohol and tea consumption.	Yu et al., 2000[220] Cases and controls were very similar in terms of cigarette smoking, consumption of alcohol beverages for at least 1 year before the study, status of hepatitis B surface antigen and tea
Residents from Hetao plain of Inner Mongolia Autonomous Region, China; two areas: Wuyuan: 216 males (114 patients) and 217 females (80 patients) and Alashan: 610 males (222 patients) and 566 females (214 patients).	In the Wuyuan area, 96.2% of water samples from tubule-type wells contained arsenic above 50 µg/L and 69.3% in Alashan area; the highest value was 1354 µg/ L and 1088 µg/L, respectively.	The results showed the prevalence of arsenical dermatosis in the Wuyuan area was 44.8%, higher than 37.1% prevalence of arsenical dermatosis in the Alashan area. The prevalence of arsenical dermatosis was highest in the over 40-year-old age group. There was no sex difference in the prevalence.	Guo et al., 2001[221]

Cases: (persons with arsenic-induced skin lesions) and age- and sex-matched controls from participants in a 1995-1996 cross-sectional survey in West Bengal were selected. Participants were re-examined between 1998 and 2000. Consensus agreement by four physicians reviewing the skin lesion photographs confirmed the diagnosis in 87% of cases clinically diagnosed in the field.	The average peak arsenic concentration in drinking water was 325 µg/L for cases and 180 µg/L for controls. A detailed assessment of arsenic exposure that covered at least 20 years was used.	The average latency for skin lesions was 23 years from first exposure. A strong dose-response gradients with both peak and average arsenic water concentrations was found for arsenic-induced skin keratosis and hyperpigmentation.	Haque et al., 2003[222] -
Eighty-five seemingly normal subjects living in blackfoot disease (BFD)-hyperendemic villages in Taiwan and 75 external normal controls without exposure were recruited. All subjects were 30-75 years old, without possible causes of peripheral neuropathy and suffered from no symptoms of peripheral neuropathy.	Arsenic concentration in drinking water in the BFD-villages ranged from 0.70 to 0.93 mg/L; the shallow well water in other areas had an arsenic content between 0.00 and 0.30 mg/L with a median of 0.04 mg/L.	BFD residents had significantly 1.28-2.23-fold higher current perception threshold (CPT) than normal controls for all frequencies at the 3 nerves. If the mean values + 3 standard deviations (SD) derived from normal controls were used as cut-off points for defining abnormalities, 36 of the 85 (42.4%) residents in the BFD villages had at least one abnormal measurement. Stepwise regression analyses consistently showed that residency in BFD villages was significantly associated with higher CPT values after adjusting for age, sex, body height and body weight.	Tseng, 2003[223] Results showed that the two groups were comparable in age, sex, body height and body weight.
Inner Mongolia, China: 431 residents of an arsenic-affected village and 189 residents of an arsenic-free village in 1996.	An arsenic level of 50+ µg/L was found in 90.6% of wells in the arsenic-affected village.	Adjusted ORs of subjective symptoms, including coughs (odds ratio [OR] = 12.8, 95% confidence interval [CI]: 6.4-25.6), stomach-aches (OR = 5.8, 95% CI 3.6-9.4), palpitations (OR = 3.6, 95% CI 1.5-8.2), urination problems (OR = 14.7, 95% CI: 3.3-65.5) and spontaneous abortions (OR = 2.7, 95% CI 0.8-8.4), were markedly higher amongst residents of the arsenic-affected village, including those without arsenic dermatosis.	Guo et al., 2003[224] Information bias cannot be excluded.

199 male and 264 female adult residents who lived >6 months in the study area from the southwestern area of endemic arseniasis in Taiwan A total of 436 (94%) of the subjects completed the ultrasonographic assessment of extracranial carotid artery (ECCA).	Cumulative arsenic exposure (arsenic water well concentration * years living in a village) was grouped into three categories: 0, 0.1-19.9 and >/=20 mg/L-years.	Three indices of long-term exposure to ingested arsenic, including the duration of consuming artesian well water, the average arsenic concentration in consumed artesian well water, and cumulative arsenic exposure, were all significantly associated with prevalence of carotid atherosclerosis in a dose-response relationship. The biological gradient remained significant after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, alcohol consumption, waist-to-hip ratio, and serum levels of total cholesterol and LDL cholesterol. The multivariate-adjusted odds ratio was 3.1 (95% CI 1.3 to 7.4) for those who had a cumulative arsenic exposure of > or =20 mg/L-years compared with those without exposure to arsenic from drinking artesian well water.	Wang et al., 2002[225]
A total of 468 male and 613 female subjects living in the blackfoot disease-hyperendemic villages in Taiwan	History of arsenic exposure was estimated through a structured questionnaire and the arsenic content in artesian well water of the villages. Cumulative arsenic exposure (CAE) was calculated as the sum of the products multiplying the arsenic concentration in artesian well water (mg/L) by the duration of drinking the water (years) in consecutive periods of living in the different villages. Cumulative arsenic exposure (CAE) was grouped into three categories: 0, 0.1-14.9 and >/=15 mg/L-years.	Among the subjects, 78 cases (16.9%) were diagnosed as having IHD. The prevalence rates of IHD for the age groups of 30-39, 40-49, 50-59, and >/=60 years were 4.9, 7.5, 16.8, and 30.7%, respectively ($p < 0.001$). For those with CAE of 0, 0.1-14.9 and >/=15 mg/L-years, the prevalence rates of IHD were 5.2, 10.9 and 24.1%, respectively ($p < 0.001$). The odds ratios (95% confidence intervals) for IHD were 1.60 (0.48, 5.34), and 3.60 (1.11, 11.65), respectively, for those with CAE of 0.1-14.9 and >/=15.0 mg/L-years, when compared with those lacking drinking water exposure to arsenic after multivariate adjustment.	Tseng et al., 2003[226] Limitations: - no external control group; - diagnostic tool; (electrocardio-gram) was missing for about half of the cases.
Dermal No data available			

Table 13a Genotoxicity of arsenic compounds in humans (in vivo data).

Humans involved/ No. of humans	Study type	Exposure duration / Concentration tested	Effects	References/ Remarks
Exposure group: 422 inhabitants of North 24 Parganas and Nadia, West Bengal, India (244 skin-symptomatic and 178 non symptomatic) Control group 102 unexposed subjects from east and west Midnapore district, matched to the exposed group by age sex and socio-economic status	Chromosomal aberrations in lymphocytes, micronuclei formation in oral mucosa cells, urothelial cells and binucleated lymphocytes Identification of mutations in GSTT1, GSTM1, GSTP1	Exposure group (skin-symptomatic): mean level of arsenic in drinking water 242.06 µg/L Exposure group (asymptomatic): mean level of arsenic in drinking water 202.33 µg/L Control group: Mean level of arsenic in drinking water 7.16 µg/L	Symptomatic individuals had a higher level of cytogenetic damage compared to a symptomatic individuals. Asymptomatic individuals had significantly higher genotoxicity than unexposed individuals. GSTM1 was significantly higher in asymptomatic group than in symptomatic group.	Ghosh et al., 2006[89]
Exposure group: 45 residents of Baduria block in Atghara, North 24 Parganas Control group: 25 residents from Howrah, Kolkata, West Bengal	Micronuclei in oral mucosal cells, Chromosomal aberrations in lymphocytes	Exposure group: Mean (±SE) level of arsenic in drinking water 66.75±2.50 µg/L Control group: Mean (± SE) level of arsenic in drinking water 6.44 ± 0.21 µg/L	Chromosomal aberrations were 3.85 fold increased in the exposed population compared to those in the control group. Micronuclei in oral mucosa cells in the exposed group were significantly increased to 3.34 fold over levels in unexposed groep.	Chakraborty et al., 2006[90]
Exposure group: 105 individuals from the Antofagasta region (north Chile) Control group: 102 individuals from the area of Concepcion	Micronuclei in buccal cells	Exposure group: Mean level of arsenic in drinking water 0.75 mg/L Control group: Mean level of arsenic in drinking water 2 µg/L	Micronuclei in buccal cells were increased in the exposed group compared to the control group, although statistical significance was not reached.	Martinez et al., 2005[92] (same population as Martinez et al., 2004[91])
Exposure group: 111 individuals from the Antofagasta region (north Chile) Control group: 106 individuals from the area of Concepcion	Micronuclei in buccal cells	Exposure group: Mean level of arsenic in drinking water 0.75 mg/L Control group: Mean level of arsenic in drinking water 2 µg/L	Micronuclei in buccal cells were increased in the exposed group compared to the control group, although statistical significance was not reached.	Martinez et al., 2004[91]

Exposure group: 163 inhabitants of North 24 Parganas, West Bengal, India Control group 154 subjects residing in the East Midnapur district, India.	Cross-sectional biomarker study to evaluate and compare the frequencies of micronuclei in peripheral blood lymphocytes, oral mucosa cells, and urothelial cells.	Exposure group: in the district, North 24 Parganas, the mean level (± S.E.) of arsenic in drinking water (µg/L) was 214.72 ± 9.03 µg/L; Control group: in East Midnapur the mean arsenic content of water (µg/L) was 9.20 ± 0.32 µg/L.	Analysis revealed that micronuclei frequencies in the exposed group were significantly elevated to 5.33-fold over unexposed levels for lymphocytes, 4.63-fold for oral mucosa cells, and 4.71-fold for urothelial cells (increases in micronuclei frequencies significant at $p < 0.01$).	Basu et al., 2004[65]
Exposure group: 59 inhabitants of North 24 Parganas, West Bengal, India Control group: 36 healthy, asymptomatic individuals (age matched controls with similar socio-economic status) residing in districts--Midnapur and Howrah, India	Bio-monitoring study using chromosomal aberrations (CA) and sister chromatid exchanges (SCE) as end points to explore the cytogenetic effects of chronic arsenic toxicity. Exposure was assessed by standardised questionnaires and by detecting the levels of arsenic in drinking water, nails, hair and urine samples.	Exposure group: in the district, North 24 Parganas, the mean level (± S.E.) of arsenic in drinking water (µg/L) was 211.70 ±15.28; Control group: in Midnapur and Howrah (two unaffected districts) the mean arsenic content of water (µg/L) was 6.35 ± 0.45.	In the exposed group the mean arsenic concentrations in nails (µg/g), hair (µg/g) and urine (µg/L) samples were 9.04 ± 0.78, 5.63 ± 0.38 and 140.52 ± 8.82, respectively, which were significantly high ($p < 0.01$) compared to the corresponding control values of 0.44 ± 0.03, 0.30 ± 0.02 and 5.91 ± 0.49, respectively. Elevated mean values ($p<0.01$) of the percentage of aberrant cells (8.08%) and SCEs per cell (7.26) were also observed in the exposed individuals in comparison to controls (1.96% and 5.95, respectively).	Mahata et al., 2003[66]
Exposure group: Six symptomatic individuals with arsenic-related skin lesions and six age- and sex-matched As-exposed asymptomatic (no arsenic-related skin lesions) individuals from West Bengal, India Control group: Six control individuals with similar socio-economic status residing in non-affected districts of West Bengal	In vitro cytogenetic study was performed utilising chromosomal aberrations (CA) in lymphocytes treated with sodium arsenite (0-5 µM)	0-5 µM sodium arsenite The mean As content in nails and hair was 9.61 and 5.23 µg/g in symptomatic, 3.48 and 2.17 µg/g in asymptomatic and 0.42 and 0.33 µg/g in the control individuals, respectively.	Although both the exposed groups had chronic exposure to As through the drinking water, individuals with skin lesions accumulated more As in their nails and hair and excreted less in urine (127.80 versus 164.15 µg/L). The results showed that sodium arsenite induced a significantly higher percentage of aberrant cells in the lymphocytes of control individuals than in the lymphocytes of both the exposed groups. Within the two exposed groups As induced higher incidences of CA in the symptomatic than the asymptomatic individuals suggesting that asymptomatic individuals have relatively lower sensitivity and susceptibility to induction of genetic damage by As.	Mahata et al., 2004a[67]

Cases: 165 symptomatic (arsenic induced skin lesions) subjects from North 24 Parganas, West Bengal, India; Controls: 155 age-sex matched control subjects from Midnapur, West Bengal.	Case control study using chromosomal aberrations (CA) and sister chromatid exchanges (SCE) as end points to explore the cytogenetic effects of chronic arsenic toxicity.	Exposure group: in the district, North 24 Parganas, the mean level of arsenic in drinking water (µg/L) was 214.96 µg/L; Control group: subjects from Midnapur (unaffected district).	A significant difference ($p < 0.01$) in the frequencies of CA and SCE between the cases and control group was shown.	Mahata et al., 2004b[68]
Exposure group: 19 residents from Bayingnormen, located in Central West Inner Mongolia, China) Control group: 13 control residents from Bayingnormen, located in Central West Inner Mongolia, China)	A pilot study was undertaken to evaluate frequencies of micronuclei (MN), as measures of chromosomal alterations, in multiple exfoliated epithelial cell types	Exposure group: high levels of arsenic in drinking water (527.5 ± 24 µg/L); Control group: low levels of arsenic in drinking water (4.4 ± µg/L).	Analytical results from these individuals revealed that MN frequencies in the high-exposure group were significantly elevated to 3.4-fold over control levels for buccal and sputum cells, and to 2.7-fold over control for bladder cells (increases in MN frequency significant at $p < 0.001$ for buccal cells; $p < 0.01$ for sputum cells; $p < 0.05$ for bladder cells). When smokers were excluded from high-exposure and control groups the effects of arsenic were observed to be greater, although only in buccal and sputum cells; approximately 6-fold increases in MN frequency occurred in these tissues.	Tian et al., 2001[70]
Cases: 6 cases with bladder cancer (ages 25-74 years, from July 1, 1994 to June 30, 1998) from New Hampshire, United States. Controls: 10 controls (ages 25-74 years, from July 1, 1994 to June 30, 1998) from New Hampshire, United States.	Investigation to the association between nucleotide excision repair capacity expression and arsenic exposure.	Arsenic levels in toenails were grouped into two categories: ≤ 0.2 µg/g and ≥ 2 µg/g)	Toenail arsenic levels were inversely correlated with expression of critical members of the nucleotide excision repair complex, ERCC1 ($r(2) = 0.82$, $p < 0.0001$), XPF ($r(2) = 0.56$, $p < 0.002$), and XPB ($r(2) = 0.75$, $p < 0.0001$). The internal dose marker, toenail arsenic level, was more strongly associated with changes in expression of these genes than drinking water arsenic concentration.	Andrew et al., 2003[76]

Cases: 94 patients with transitional cell carcinoma of the bladder between 1996 and 2000 from Union Country, Cordoba, Argentina; 29 patients (between November 1994 and July 1996) with transitional cell carcinoma who were ascertained from a hospital-based case-control study previously conducted in Chile.	A case-case study was conducted in Argentina and Chile examining chromosomal alterations in bladder tumour DNA. - Smoking history and occupational history were included. - All statistical tests were two-sided.	Patients were placed into one of four arsenic exposure categories according to their average 5-year peak arsenic exposure. Category 1: between 0 and <10 µg/L per year (n=45); category 2, 10-99 µg/L per year (n=24); category 3, 100-299 µg/L per year (n=29); and category 4, ≥300 µg/L per year (n=25).	The total number of chromosomal alterations was higher in individuals exposed to higher arsenic levels (5.7 ± 5.1, 5.6 ± 5.1, 7.3 ± 7.4, and 9.1 ± 6.5 [mean ± standard deviation] chromosomal alterations per tumour with increasing arsenic exposure; p(trend) = 0.02, adjusted for stage and grade). The trend was stronger in high-grade (G2-G3) tumours (6.3 ± 5.5, 8.3 ± 4.7, 10.3 ± 7.8, and 10.5 ± 6.4 alterations per tumour; p(trend) = 0.01) than it was in low-grade (G1) tumours (3.5 ± 3.1, 1.1 ± 1.1, 2.5 ± 2.5, and 3.6 ± 3.2 alterations per tumour; p(trend) = 0.79). The mean number of chromosomal alterations also increased with tumour stage and grade (p(trend) < 0.001) independently of arsenic exposure but was not associated with smoking history. Deletion of part or all of chromosome 17p (p(trend)<0.001) showed the strongest association with arsenic exposure.	Moore et al., 2002[69]

Table 13b Genotoxicity of arsenic compounds in human cells (in vitro data).

Human cell type	Study type	Exposure duration/ Concentration tested	Effects	References /Remarks
Human peripheral lymphocytes	Challenge of lymphocytes with different arsenic compounds AsIII, AsV, MMAIII, MMAV, DMAV, TMAOV and testing the micronucleus formation (cytokinesis block micronucleus assay) followed by fluorescence in situ hybridization (MMAIII).	0.5-4 µM As^{III} 4-32 µm As^{V} 0.01-2 µM MMA^{III} 50-1000 µM MMA^{V} 50-250 µM DMA^{V} 100-1000 µM $TMAO^{V}$ Cytochalasin B blocked the cytokinesis process and cells were harvested after 72 hr	Trivalent compounds As^{III} and MMA^{III} enhanced micronuclei formation more than pentavalent compounds As^{V} and MMA^{V}. For DMA^{V} and $TMAO^{V}$ no genotoxicity was observed. MMA^{III} showed a aneuploidogenic property.	Colognato et al., 2007[227]
Human liver ferritin	Iron release from HLF by dimethylarsinic acid and dimethylarsinous acid with or without ascorbic acid.	The assay system (total volume 150 µl) contained HLF (2 µg), ferrozine (0.5 mM), dimethylarsinic acid/ dimethylarsinous acid (each 10 mM) with or without ascorbic acid (250 µM) in 10 mM phosphate buffered saline (pH 7.4).	Both dimethylarsinic acid and dimethylarsinous acid released iron from human liver ferritin (HLF) with or without the presence of ascorbic acid. With ascorbic acid the rate of iron release from HLF by dimethylarsinic acid was intermediate (3.37 nM/min, $p < 0.05$) and by dimethylarsinous acid was much higher (16.3 nM/min, $p < 0.001$). Relevance: Free iron causes redox cycling, production of ROS and oxidative stress.	Ahmad et al., 2002[158]
Human peripheral lymphocytes	A single-cell gel (SCG, "comet") (induction of DNA damage) (2 h incubation at 37 °C; 5% CO_2 in air)	As^{III}: 1 ?M - 1000 mM; As^{V}: 1 µM - 1000 µM; monomethylarsonic acid: 1 µM - 875 µM; dimethylarsinic acid: 1 µM - 1000 µM; monomethylarsonous acid: 1.25 µM - 80 µM; dimethylarsinous acid: 1.4 µM - 91 µM	Methylated trivalent arsenicals were much more potent DNA damaging compounds than any other arsenicals that were tested. On the basis of the slopes of the concentration-response curve for the tail moment in the SCG assay, monomethylarsonous acid and dimethylarsinous acid were 77 and 386 times more potent than As^{III}, respectively.	Mass et al., 2001[58]
Human lymphocytes	Supercoiled plasmid unwinding assay	synthetic monomethylarsonous acid and dimethylarsinous acid, as AsMeO and $AsMe_2I$	Nicking (unwinding) DNA, double-stranded breaks, and induction of alkaline labile sites (methylated arsenic species at concentrations much lower than inorganic As).	Mass et al., 2001[59] Remark: Evaluation of the results was limited since only an abstract is available.
Human lymphocytes	Single cell gel (comet) assay		As^{III} methylated species doubled the tail moment at concentrations 30 to 300-fold less than did inorganic As.	

HeLa S3 cells	Induction of oxidative DNA damage measuring frequencies of DNA strand breaks and lesions recognised by the bacterial formamidopyrimidine-DNA glycosylase (Fpg).	As^{III}, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, dimethylarsinous acid.	Incubations of 0.5-3 h with doses as low as 10 nM As^{III} induced high frequencies of Fpg-sensitive sites, the induction of oxidative DNA damage after 18 h incubation was rather low. Monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid and dimethylarsinous acid showed a pronounced induction of Fpg-sensitive sites in the nanomolar or micromolar concentration range, respectively, after both short-term and long-term incubations. Furthermore, monomethylarsonous acid and dimethylarsinic acid generated DNA strand breaks in a concentration-dependent manner.	Schwerdtle et al., 2003[94]
Human A549 cells	BPDE-induced DNA adduct formation and repair	As^{III}: 0 - 75 µM monomethylarsonic acid: 0 - 500 µM monomethylarsonous acid: 0 - 7.5 µM dimethylarsinic acid: 0 - 500 µM dimethylarsinous acid: 0 - 7.5 µM	Whereas only As^{III} and monomethylarsonous acid increased BPDE-DNA adduct formation, As^{III} (>/=5 µM), the trivalent (>/=2.5 µM) and the pentavalent (>/=250 microM) metabolites diminished their repair at non-cytotoxic concentrations.	Schwerdtle et al., 2003[93]
	Effects on the zinc finger domain of the human XPA protein (XPAzf)	1-1000 µM	All trivalent arsenicals were able to release zinc from XPAzf.	
	Effects on the *Escherichia coli* zinc finger protein Fpg	0-10 µM	Monomethylarsonous acid and dimethylarsinous acid inhibited the activity of isolated Fpg.	
Human fibroblast cells	SCE assay Comet assay Proteinase K assay	dimethylarsinic acid: 0, 125, 250 and 500 µM	SCE frequencies were significantly increased at all concentrations in treated cells in relation to controls ($p < 0.001$). In the standard alkaline comet assay, as well as in the control assay for proteinase K treatment, a significant dose-dependent reduction in tail moment was observed. Nevertheless, post-treatment with proteinase K induced the release of proteins joined to the DNA and consequently, a dose-dependent increment in DNA migration was observed ($p < 0.001$).	Mouron et al., 2005[95]
Human bladder cells (UROtsa)	DNA methylation	Monomethylarsonous acid (MMA^{III}) 50 nM	Malignant transformation after 12 weeks of exposure.	Wnek et al., 2010[96]

Table 14 Human carcinogenicity studies of arsenic and arsenic compounds (Inhalation).

Study population	Exposure assessment	Effects	References/Remarks
A cohort of 2802 Tacoma smelter workers who worked ≥ 1 year during 1940-1964. For 98.5%, it was possible to determine vital status at the end of 1986. Of 1583 known deaths, death certificates were obtained for 96.6%.	For departments for which no air data were available, exposure assessment based on urinary As measurements using the equation: air As (µg/m^3) = 0.0064 × (urinary As (µg/L))[1.942]. This equation was based on departments and years for which data from both air (1938 - 1984) and urinary arsenic (1948 - 1984) were available. Calculation of cumulative exposure: development of exposure matrix of arsenic in air by department and year from 1938 up to 1984 in combination with job histories for each worker. Cumulative exposure (µg/m^3·years) categories (mean exposure): 1) <750 (405) 2) 750-1,999 (1,305) 3) 2,000-3,999 (2,925) 4) 4,000-7,999 (5,708) 5) 8,000-19,999 (12,334) 6) 20,000-44,999 (28,356) 7) 45,000+ (58,957)	Respiratory cancer SMR[1] for respiratory cancer (total cohort, hired < 1940, hired ≥ 1940) for the exposure categories: 1) 154 - 65 - 178* 2) 176** - 68 - 256** 3) 210** - 246** - 170 4) 212** - 150 - 300** 5) 252** - 255** - 244* 6) 284** - 252** - 406** 7) 316** - 339* - not available *: $p < 0.05$; **: $p < 0.01$ [1] Calculation of expected deaths: only mortality of white men from Washington was used as all workers were men and nearly all were white. An earlier publication of the Tacoma copper smelter contained data on actual daily exposure concentrations, duration of exposure and the risk on lung cancer. In this study, an arsenic exposure category of < 400 µg/m^3 (mean 213 µg/m^3) was associated with a statistically significant SMR of 238.7 for copper smelter workers who were exposed to arsenic for 30 or more years.	Enterline et al., 1995[4] and Enterline et al., 1987[98] Smoking: According to the authors, smoking could be an important confounder in respiratory cancer and data on the histories of the study population for smoking were collected. The publication addressing this and other covariates is however not yet available.

A cohort of 8014 white males, who were employed in the Anaconda copper smelter for ≥ 12 months before 1957. Vital status was followed from 1 January 1938 to 31 December 1987; a total of 4930 (63%) were deceased, including 446 from respiratory cancer, and 1909 (24%) were known to be alive at the end of the follow-up. Lost to follow-up: 15% (assumed alive)	Work areas grouped as "light", "medium" or "heavy" exposure based on industrial hygiene data (702 measurements; 1943 - 1958). Based on estimates of workers' daily exposure time, time-weighted average exposures for each category were created. Estimated airborne exposures (time-weighted average) were 0.29, 0.58, 11.3 mg/m^3 in areas of light, medium and heavy exposure. Cumulative exposure: estimated from the time of working in different work areas and calculated as: $0.29 \times L + 0.58 \times M + \lambda (=0.1) \times 11.3 \times H$, where L, M, and H are years worked in areas where exposure was considered to be light (or unknown), medium, or heavy, respectively.	A significantly increased SMR (using the US population rate as the referent population) was found for respiratory cancer (SMR = 1.55 (1.41-1.70)). Internal analyses revealed a significant, linear increase in the excess relative risk of respiratory cancer with increasing exposure to inhaled airborne arsenic. The estimate of the excess relative risk per mg/m^3-year was $0.21/(mg/m^3$-year) (95% confidence interval: 0.10, 0.46).	Lubin et al., 2000[1] Smoking: Information on smoking was not available, according to the authors, however, it is noteworthy that mortality from smoking-related cancers, except for chronic obstructive pulmonary disease, was not excessive. In a sample of 1469 workers from the original cohort, there was a higher proportion of smokers compared with US white males. However, the authors stated that the proportion of cigarette smokers did not vary significantly by extent of exposure to airborne arsenic, indicating that it was unlikely that smoking confounded the assessment of lung cancer risk with arsenic exposure. Exposure assessment: Industrial hygiene measurements were available for less than half of the 29 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. The locations for sampling were not randomly selected.

A cohort of 3916 smelter workers who worked ≥ 3 months in the Rönnskär smelter between 1928 and 1967 and were followed for vital status 1947-1981. The vital status of all but 15 (0.4%) of them was verified.	Industrial hygiene measurements available from 1951; production figures used to extrapolate exposures before 1951. Cumulative exposure: each work site characterised by an exposure level during three consecutive time periods. Using this exposure matrix and detailed information of the work history, cumulative arsenic exposure could be computed for each worker. The cumulative exposure levels ($mg/m^3 \cdot year$) were categorised as follows: < 0.25 0.25-<1 1-<5 5-<15 15-<50 50-<100 100+	SMRs[1] (95% CI) for lung cancer for dose categories using age specific mortality rates from the country where the smelter was situated were as follows: < 0.25: 271 (148-454) 0.25-<1: 360 (192-615) 1-<5: 238 (139-382) 5-<15: 338 (189-558) 15-<50: 461 (309-662) 50-<100: 728 (267-1585) 100+: 1137 (588-1986) The slope rose steeply only in the two highest exposure categories. The overall trend was, however, highly significant ($p < 0.001$) with an overall SMR of 372 (304-450). [1] To enhance comparability the original SMRs were adjusted to the lowest intensity/shortest employment time group.	Järup et al., 1989[3] Smoking: See study of Järup et al, 1991 [100]
A cohort of 3916 smelter workers who worked ≥ 3 months in the Rönnskär smelter between 1928 and 1967 and were followed for vital status 1947-1981. The vital status of all but 15 (0.4%) of them was verified. - Cases: 103 subjects dying from lung cancer and an additional four cases during the observation period. - Controls: two deceased controls (deaths from all causes other than lung cancer) per case (214 controls in total) from the cohort; matched on year of birth.	- Estimation of arsenic exposure: see Järup et al, 1989 [3]. - Smoking histories: obtained by postal questionnaires sent to a next of kin and supplemented by telephone interviews. Individuals were assigned to a smoking category depending on the amount of tobacco smoked per day. Quantitative information on smoking habits was obtained for only 102 cases (95.3%) and for 190 controls (88.8%).	- Lung cancer risks were positively related to cumulative arsenic exposure ($mg/m^3 \cdot year$) with smoking standardised relative risks ranging from 0.7 to 8.7 in different exposure groups. A negative confounding by smoking was suggested in the highest exposure group.	Järup et al., 1991[100]

130 male lung cancer cases and 627 controls from a cohort of 7855 subjects employed at least 1 year between 1972 and 1974 in four tin mines in China. The cohort was followed up to the end of 1994. There were 91 miners (1.2% of whole cohort) considered lost to follow-up.	Cumulative total exposure to dust and cumulative exposure to arsenic calculated for each person based on industrial hygiene records. - Respirable concentration of arsenic in three tin mines in Dachang (μg/m^3): No: 1.1 (0.1-2.7) Low: 3.0 (1.0-4.9) Medium: 3.5 (2.4-4.7) High: 10.2 (1.9-38.3) - Respirable concentration of arsenic in the tin mines in Limu (μg/m^3): Medium: 0.5 (0.36-0.7) High: 1.2 (1.16) - Cumulative exposures to arsenic (μg/m^3·year) were derived: 0.1-99.9 100-499.9 500-999.9 ≥ 1000 Using questionnaires, information was obtained on tobacco smoking.	Increased risk of lung cancer was related to cumulative exposure to dust, duration of exposure, cumulative exposure to arsenic, and tobacco smoking. The ORs (adjusted for smoking) for different groups of cumulative arsenic exposure (μg/m^3·year) were: 0.1-99.9: 2.1 (95% CI 1.1 to 3.9) 100-499.9: 2.1 (95% CI 1.1 to 3.9) 500-999.9: 1.8 (95% CI 1.0 to 3.6) ≥ 1000: 3.6 (95% CI 1.8 to 7.3). It should be pointed that the percentages of smokers in both cases and controls were high (88.5% in lung cancer cases and 82.5% in controls). However, the adjusted ORs did not differ from non-adjusted ORs.	Chen and Chen, 2002 [228] A significant excess was found even in the lowest exposure category (mean arsenic concentration about 3.7 μg/m^3 and mean cumulative arsenic exposure about 46.6 μg/m^3·year). According to the authors, the carcinogenic effect of crystalline silica cannot be excluded in this study. High correlations between exposure to arsenic and exposure to dust or silica prevented from adjustment for any of these values. Furthermore, exposure assessment for arsenic began in the 1980s, while total dust concentration greatly decreased from the 1950s to the 1980s. Therefore, the cumulative exposure to arsenic may have been underestimated or overestimated in the earlier years. Because the aim of this analysis was to show an absence of an association between respirable silica and lung cancer and not to determine an exposure response association between arsenic exposure and lung cancer, the exposure assessment to arsenic should be interpreted more on a relative than an absolute scale.
A cohort of 12,011 males working for the Yunnan Tin Corporation, with complete exposure records, who were initially surveyed in 1976 and followed through 1988. There were 842 lung cancer deaths in this restricted group during the period of observation.	The two-stage clonal expansion model was used to analyse lung cancer mortality based on individual histories with multiple exposures to arsenic, radon, cigarette smoke, and pipe smoke.	Higher than expected lung cancer rates for Yunnan tin miners were found even in groups with no or low occupational arsenic exposure. Detailed analysis of exposure however, showed that for this population more factors may be associated with lung cancer. 15.8% of the lung cancer deaths was attributable to arsenic exposure. Arsenic was considered almost as potent as tobacco for the risk of lung cancer.	Hazelton et al., 2001[39] Confounding by: - environmental exposure to arsenic - arsenic in drinking water - poor nutritional intake (reduced intake of yellow and green vegetables and tomatoes) cannot be excluded.

Cases: 199 men with lung cancer (residents in the city of Buenos Aires; admitted for treatment in hospitals of Buenos Aires city during March 1994 to March 1996) Controls: 393 male subjects who had been hospitalised for conditions unrelated to tobacco use during the same period and were residents in the same area. Two male controls were matched with the exception of three cases that were matched with only one control subject.	A full occupational history was collected through interviewing. Exposure to arsenic, asbestos, chromium, dust, nickel, and polynuclear aromatic hydrocarbons was assessed by means of a job-exposure matrix.	A small, non-significant increased risk for lung cancer was observed after long-term exposure to arsenic.	Matos et al., 2000[229] The risk for occupational exposure was adjusted for hospital of admission, group of age, pack-years of cigarettes, and employment in other occupations / industries with increased risks. Selection bias and information bias cannot be excluded.
A cohort of 3979 smelter workers employed for at least 1 year between 1928 and 1979, and also exposed to lead and included in the Blood Lead Register that was started at the smelter in 1950. Two subcohorts were formed from the original cohort. One consists of 710 workers employed at the lead departments (Lead subcohort 1), and the other of 383 workers employed at the lead departments (Lead subcohort 2), but never at other works where an excess lung cancer risk was previously identified.	In the subcohorts, arsenic exposure in lung cancer cases was assessed in detail based on occupational hygiene information from the company. A detailed study of arsenic exposure in the 10 lung cancer cases in these two subcohorts revealed that all but one of these cases had a significant exposure also to arsenic.	Standardised Cancer Incidence Rates (SIR) 1958-1987 were calculated relative to county rates. Lung cancer incidence was raised in both subcohorts (Lead subcohort 1: SIR 2.4; 95% CI 1.2-4.5; Lead subcohort 2: SIR 3.6; 95% CI 1.2-8.3).	Englyst et al., 2001[230] It has not been possible to separate the carcinogenic effects of lead and arsenic, but a possible interaction between these metals may be involved in explaining the carcinogenic risks.
A cohort of 1462 males who had been employed in a UK tin smelter (Capper Pass, North Humberside) for at least 12 months between 1/11/1967 and 28/7/1995, followed up through 31/12/2001.	Exposure included lead, arsenic, cadmium.The mortality of the cohort was compared against that expected for both national and regional populations.	Lung cancer mortality was significantly elevated (SMR 161, 95% CI 124-206, $p < 0.001$, 62 deaths).	Binks et al., 2005[104] The risk of lung cancer has been enhanced by occupational exposure to one or more carcinogens, the effect of which diminishes with time since exposure

A cohort of 1462 males who had been employed in a UK tin smelter (Capper Pass, North Humberside) for at least 12 months between 1/11/1967 and 28/7/1995, followed up through 31/12/2001 (same cohort as in Binks *et al.* 2005)	Exposure matrices for arsenic, cadmium, lead were established. Lung cancer mortality was examined in relation to cumulative inhalation exposure.	No significant associations could be found between lung cancer mortality and simple cumulative exposure to any of the substances studied. When cumulative exposures were weighted according to time since exposure and attained age, significant associations were found between lung cancer mortality and exposure to arsenic, lead and antimony.	Jones et al., 2007[105] The excess relative risk diminishes with time since exposure and attained age.
A cohort of 625 male workers from a US cadmium recovery plant in Colorado; employees were employed for at least 6 month between 1 January 1940 and 31 December 1969 (same cohort as in Stayner *et al.* 1991)	Detailed work histories were available providing data on exposure to cadmium and arsenic.. Mortality (1940-2001) from lung cancer was compared with US national mortality rates.	There was a statistically significant ($p < 0.05$) negative trend in lung cancer standardized mortality ratios in relation to period from ceasing arseninc exposure.	Sorahan 2009[106] The findings are consistent with the hypothesis that arsenic is a late stage human carcinogen
A cohort of 625 male workers from a US cadmium recovery plant in Colorado; employees were employed for at least 6 month between 1 January 1940 and 31 December 1969	Air monitoring data on cadmium exposure were available. Detailed work histories were available. Cumulative exposure for each worker was estimated.	Excess in mortality from lung cancer was observed for the entire cohort (SMR 149, CI 95% 95-222	Stayner et al.,1992[107]
A cohort of 602 male workers from a US cadmium recovery plant in Colorado; employees were employed for at least 6 month between 1 January 1940 and 31 December 1969	Air monitoring data on cadmium exposure were available. Detailed work histories were available. Cumulative exposure for each worker was estimated.	Mortality from respiratory cancer and non-malignant gastrointestinal disease was significantly greater among the cadmium workers than would have been expected from US rates.	Thun et al., 1985[108]
A cohort of 8014 white males, who were employed in the Anaconda copper smelter for ≥ 12 months before 1957. Vital status was followed from 1 January 1938 to 31 December 1987; a total of 4930 (62%) were deceased (Same cohort as in Lubin *et al*. 2000).[69]	Reanalysis of the relationship between respiratory cancer mortality and cumulative inhaled arsenic exposure among copper smelter workers.	RR's for respiratory cancer increased linearly with cumulative arsenic exposure when analyzed for specific concentrations and concentration ranges of arsenic (0.29 mg/m^3; 0.30-0.39 mg/m^3; 0.40-0.49 mg/m^3; ≥ 50 mg/m^3). The slope of the linear exposure-response relationship increased with increasing arsenic concentration.	Lubin et al., 2008[2] This pattern implied that for equal cumulative arsenic exposure, the RR of respiratory cancer mortality was greater for cumulative arsenic exposure delivered at higher concentration for shorter duration compared with cumulative exposure delivered at lower concentration for longer duration.

Table 15 Human reproduction and developmental studies of arsenic and arsenic compounds.

Study population	Exposure assessment	Effects	References/Remarks
Inhalation			
No additional data available			
Oral			
424 infants from Antofagasta (Chili) and 420 from Valparaiso (Chili) (final study group). Pregnancy and birth information was obtained from medical records. The birth weight analysis was restricted to liveborn, singleton infants born between December 1998 and February 2000.	Drinking water arsenic levels: 40 µg/L (city: Antofagasta) and <1 µg/L (city: Valparaiso)	After controlling for confounders (gestational age, parity, infant sex, maternal age, maternal height, smoking, BMI, adequacy of prenatal health care and income), results of the multivariable analysis indicated that Antofagasta infants had lower mean birth weight (-57 g; 95% CI -123 to 9).	Hopenhayn et al., 2003[231]
533 women from areas in Bangladesh using tube wells with known arsenic concentrations	The range of measured arsenic concentration in tube well water ranged from non-detectable to 1710 µg/L.	Excess risks for spontaneous abortion and stillbirth were observed among the participants chronically exposed to higher concentrations of arsenic in drinking water after adjusting for participant's height, history of hypertension and diabetes, and (for neonatal death only) age at first pregnancy. Comparing exposure to arsenic concentration of greater than 50 µg/L with 50 µg/L or less, the ORs were 2.5 (95% CI =1.5-4.3) for spontaneous abortion, 2.5 (1.3-4.9) for stillbirth, and 1.8 (0.9-3.6) for neonatal death.	Milton et al., 2005[141] The study had some limitations: - recall bias with regard to documentation of pregnancy outcomes; - the available water samples reflected only a particular point in time and not the historical exposure (assumption: arsenic concentration from the tube wells had been relatively constant over time); - information for only 1 well for each woman was available, and so her cumulative duration of arsenic exposure could not include exposure from other wells, although 1 of the eligibility criteria for study participation was having lived in the study area since their marriage; - the amount of drinking water consumed was also not considered in this study.
Prospective cohort study, based on 1,578 mother-infant pairs, in Matlab, Bangladesh	Arsenic exposure was assessed by analysis of arsenic in urine collected at around gestational weeks 8 and 30.	Significant negative dose effects were found with birth weight and head and chest circumferences at a low level of arsenic exposure (<100 µg/L in urine).	Rahman et al., 2009[144]

Population-based cohort study with 4,436 pregnant women in Matlab, Bangladesh (an area with high-arsenic contaminated tube wells) and 1,799 infants born to these mothers.	Arsenic concentrations in spot urine specimens at 8 and 30 weeks of pregnancy were 81 µg/L (range 37-207) and 84 µg/L (range 42-230) respectively.	No significant effect of arsenic exposure during pregnancy on infant development (motor, PST score and behaviour rating) was detected.	Tofail et al., 2009[143] It is possible that other effects are as yet unmeasured or that effects will become apparent at a later age.

Annex **H**

Animal data

(additional data, if not further specified)

Table 16 Animal in vitro studies with arsenic and arsenic compounds.

Animal cell type	Procedure	Concentration tested	Effects	Reference
Rat hepatocytes (cultured)	Investigation to the effect of arsenicals on thioredoxin reductase (TR) (an NADPH-dependent flavoenzyme (plays an important role in the cellular response to oxidative stress)	As^{III}, monomethylarsonous acid: 1-50 µM; aurothioglucose (ATG, a competitive inhibitor of TR activity): 1-100 µM	The estimated IC(50) was >>100 µM for As^{III}, approx. 10 µM for ATG, and approx. 3 µM for monomethylarsonous acid. In hepatocytes exposed to 1 µM monomethylarsonous acid for up to 24 h, the inhibition of TR activity was maximal (approximately 40%) after exposure for 15 min. After exposure for 3 h [when most monomethylarsonous acid has been converted to dimethylarsinic acid], TR activity in these cells had returned to control levels. Notably, exposure of the cell to 50 µM dimethylarsinous acid did not affect TR activity. In hepatocytes exposed to 10 µM As^{III} for up to 24 h, the inhibition of TR activity was progressive; at 24 h, activity was reduced approximately 35%. Following exposure to As^{III} or monomethylarsonous acid, the extent of inhibition of TR activity correlated strongly with the intracellular concentration of monomethylarsonous acid.	Lin et al., 2001[57]

Table 17 Short-term animal toxicity studies of arsenic and arsenic compounds.

Species/Strain/ No. per Sex per Group	Exposure duration	Concentration tested	NOAEL[a]	LOAEL[b]	(Critical) effects	References/ Remarks
Inhalation						
New Zealand white rabbits/ n=6/sex/ concentration	8 hr/day, 7 days/ week for 8 weeks	Arsenic trioxide: 0.05, 0.1, 0.22, or 1.1 mg/m^3 (MMAD ranged from 3.2 to 4.1 µm)	1.1 mg/m^3	-	There were no significant clinical findings or consistent changes in body weight associated with any of the exposure group.	Beck et al. (2002)[38] Remark: Since the major objective of this study was toxicokinetics, only clinical findings and body weight were investigated.
Oral						
No data available						
Dermal						
No data available						

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table 18 Long-term animal toxicity studies of arsenic and arsenic compounds.

Species/Strain/ No. per Sex per Group	Exposure duration	Concentration tested	NOAEL[a]	LOAEL[b]	(Critical) effects	References/ Remarks
Inhalation						
No data available						
Oral						
Mice (C57Bl/6J)/ n=70 females/ dose	12 months	0, 100, 250 and 500 µg As^V/L (13, 32.5 and 65.0 µg/kg bw/ day) in drinking water as sodium arsenate ad libitum	65.0 µg/kg bw/day	-	No tumours were observed; no significant effect on the growth rate and on the water consumption of the treated animals compared to controls; no abnormal appearance or behaviour was observed.	Wu et al, 2004[50] Remark: Since the major objective of this study was to detect biomarker changes, no more toxicological investigations were performed.
Dermal						
No data available						

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table 19a Genotoxicity of arsenic and arsenic compounds in animals (in vitro data).

Test system	Procedure	Arsenic species/Dose/ Concentration	Result	References/ Remarks
ϕX174 RFI DNA (super-coiled)	A DNA nicking assay (2 h incubation at 37 °C) (pH 7.4);	As^{III}: 1 nM - 300 mM; As^{V}: 1 µM - 1 M; MAs^{v}: 1 µM - 3 M; dimethylarsinic acid: 0.1 mM - 300 mM; monomethylarsonous acid: 10 mM - 60 mM; dimethylarsinous acid: 40 µM - 10 mM	Both methylated trivalent arsenicals were able to nick and/or completely degrade ϕX174 DNA depending on concentration. monomethylarsonous acid was effective at nicking ϕX174 DNA at 30 mM; however, at 150 µM dimethylarsinous acid, nicking could be observed.	Mass et al., 2001[58]
Mice	Mouse lymphoma L5178Y TK+/- assay	Synthetic monomethylarsonous acid and dimethylarsinous acid, as AsMeO and AsMe2I	Mutation by AsMeO was seen down to 0.5 µM (30 to 100 fold more potent than arsenite)	Mass et al., 2001[59]
Strains TA98, TA100 and TA104	Ames assay	Synthetic monomethylarsonous acid and dimethylarsinous acid, as AsMeO and AsMe2I	-	Mass et al., 2001[59]
	Prophage-induction assay that detects SOS repair	Synthetic monomethylarsonous acid and dimethylarsinous acid, as AsMeO and AsMe2I	AsMe2I induced SOS repair at ~5 µM	Mass et al., 2001[59]
Isolated PM2 DNA	Induction of oxidative DNA damage measuring frequencies of DNA strand breaks and lesions recognised by the bacterial formamidopyrimidine-DNA glycosylase (Fpg).	As(III), monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, dimethylarsinous acid	Only dimethylarsinous acid (> or =10 µM) generated DNA strand breaks in the absence of Fpg-sensitive sites.	Schwerdtle et al., 2003[94]

Chinese hamster ovary cells	Cytotoxic effects by the trypan blue extrusion test;	As^{V}, As^{III}, monomethylarsonic acid, monomethylarsonous acid, dimethylarsinic acid, dimethylarsinous acid and $TMAO^{V}$: 0.1 µM to 10 mM for 30 min and 1 h	Viability was significantly decreased after incubation (1 h) of the cells with > or = 1 µM As^{III}, > or = 1 µM As^{V}, > or = 500 µM monomethylarsonous acid, > or = 100 µM monomethylarsonic acid, and 500 µM dimethylarsinic acid and > or = 0.1 µM dimethylarsinous acid. $TMAO^{V}$ was not cytotoxic at concentrations up to 10 mM.	Dopp et al., 2004[61]
	Genotoxic effects: micronucleus (MN) induction, chromosome aberrations (CA), and sister chromatid exchanges (SCE);		A significant increase of the number of MN, CA and SCE was found for dimethylarsinous acid and monomethylarsonous acid. As^{III} and ^{V} induced CA and SCE but no MN. $TMAO^{V}$, monomethylarsonic acid and dimethylarsinic acid were not genotoxic in the concentration range tested (up to 5 mM).	
	Intracellular arsenic concentrations were determined by ICP-MS techniques.		0.03% monomethylarsonic acid and dimethylarsinic acid, 2% monomethylarsonous acid, As^{III} and As^{V} and 10% dimethylarsinous acid were taken up by the cells. The total intracellular concentration of all arsenic compounds increased with increasing arsenic concentrations in the culture medium.	

Horse spleen ferritin	Iron mobilisation assays	The assay system (total volume 2.0 ml) contained horse spleen ferritin (100 mg), ferrozine (0.5 mM), and the test chemical (arsenate/arsenite/monomethylarsonic acid/ dimethylarsinic acid/ monomethylarsonous acid/ dimethylarsinous acid) (each 10 mM)) with or without of the ascorbic acid (1 mM) in 10 mM phosphate buffered saline (pH 7.4);	Dimethylarsinic acid and dimethylarsinous acid significantly released iron from horse spleen ferritin either with or without the presence of ascorbic acid, a strong synergistic agent. Ascorbic acid-mediated iron release was time-dependent as well as both dimethylarsinous acid and ferritin concentration-dependent. Iron release from ferritin by dimethylarsinous acid alone or with ascorbic acid was not significantly inhibited by superoxide dismutase (150 or 300 units/mL). However, the iron release was greater under anaerobic conditions (nitrogen gas), which indicates direct chemical reduction of iron from ferritin by dimethylarsinous acid, with or without ascorbic acid.	Ahmad et al., 2000[157]
	Bleomycin-dependent DNA damage	The assay system (total volume 1.0 ml) contained 50 mg ferritin and dimethylarsinous acid (0-5 mM) with or without ascorbic acid (50 mM) in 10 mM of phosphate-buffered saline (pH 7.4).	The release of ferritiniron by dimethylarsinous acid with ascorbic acid catalysed bleomycin-dependent degradation of calf thymus DNA	
Plasmid pBR322 DNA	Iron mobilisation assays	The assay system contained 500 ng pBR322 DNA, arsenic species (0-1.0 mM), HLF (10 µg), ascorbic acid (25 µM), DTPA (5 mM) or oxyradical scavengers (such as SOD (150 units), catalase (50 units), D-mannitol (5 mM), potassium iodide (5 mM), sodium azide (5 mM)) in 10 mM phosphate-buffered saline (pH 7.4).	No pBR322 plasmid DNA damage was observed from exposure As^{V}, As^{III}, monomethylarsonic acid, monomethylarsonous acid or dimethylarsinic acid alone. DNA damage was observed following dimethylarsinous acid exposure; coexposure to dimethylarsinous acid and HLF caused more DNA damage; considerably higher amounts of DNA damage was caused by coexposure of dimethylarsinous acid, HLF and ascorbic acid. Diethylenetriaminepentaacetic acid (an iron chelator), significantly inhibited DNA damage. Addition of catalase (which can increase Fe^{2+} concentrations) further increased the plasmid DNA damage.	Ahmad et al., 2002[158]

Table 19b Genotoxicity of arsenic and arsenic compounds in animals (in vivo data).

Test system	Dose / concentration	End point	Result	References
Mice (Swiss albino) / n=6 females/dose	0 and 2.5 mg/kg bw sodium arsenite (exposure for 24 h)	Total chromosome aberrations and number of chromosome breaks per cell (50 clear metaphase with normal chromosome number, 2n=40 were examined from each animal)	Sodium arsenite produced significantly high frequencies of chromosome aberrations as compared with negative control following exposure.	Poddar et al., 2000[161]
Mice (male ddY)/n= 4/dose	single gavage dose: 15.2 mg (11.5 mg As)/kg arsenite; 21.1 mg (11.5 mg As)/ kg dimethylarsinic acid	DNA oxidation	When the same dose of As as arsenite or dimethylarsinic acid was administered to ddY-strain mice, the amount of 8-oxodG (a biomarker of DNA oxidation) was significantly higher in the urine after 9 hour of mice exposed to dimethylarsinic acid.	Yamanaka et al., 2001[162]
Mice (male ddY)/n= 5/dose	400 ppm dimethylarsinic acid in drinking water (for 4 weeks)	DNA oxidation	The amount of 8-oxodG (a biomarker of DNA oxidation) was significantly increased not only in lung and liver, but also, though not significantly, in urinary bladder. No increase in 8-oxodG was observed in spleen or kidney.	
Mice (female HR-1 hairless)/n=5/dose	400 ppm dimethylarsinic acid in drinking water (for 2 weeks)	DNA oxidation	A significant increase in the amount of 8-oxodG in dorsal epidermis.	
Ovarian tissue in female rats	50, 100, 200 ppm sodium arsenite in drinking water for 28 days	DNA damage measure by comet assay	Decrease in mean comet length, height, comet head diameter and %DNA in comet head of high dose group. Dose-dependent increase in mean comet tail lengthe, %DNA in tail and tail moment in high dose groups.	Akram et al., 2009[160]
Mutamouse (a transgenic mouse model)	DMA 10.6 mg/day for 5 consecutive days	Mutation frequency in lacZ transgene and cII gene	Weak increase in mutation frequency in lung, but not in bladder and bone marrow	Noda et al., 2002[163]

Annex I

Evaluation of the Subcommittee on Classification of carcinogenic substances

Scope

On request of the Dutch Expert Committee on Occupational Safety of the Health Council, the Subcommittee on the Classification of carcinogenic substances evaluates the carcinogenic properties of arsenic and arsenic compounds.

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification with reference to the appropriate EU-directive (see Annex J). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substances in question.

The members of the Subcommittee on Classification of carcinogenic substances are listed at the end of this annex. The evaluation is based on the data summarized in the first part of the present report of DECOS.

Carcinogenicity of arsenic and arsenic compounds

Inorganic arsenic is the cause of human malignancies, and is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC).[1,2]

Inhalation is the primary route of occupational exposure to arsenic and occurs in industries such as mining, smelting, wood preservation, production and application of arsenic-based pesticides, and electronics. Non occupational exposure occurs mainly through food, but also in the drinking water in areas with high levels of arsenic (e.g. Taiwan, Bangladesh).[2]

Epidemiological studies of populations occupationally exposed to arsenic consistently demonstrate an excess lung cancer risk.[3,4] In addition, epidemiological studies consistently show that oral exposure to arsenic via drinking water increases the risk of skin and urinary bladder cancer.[2,3] Evidence also suggests a relationship between oral arsenic exposure via drinking water and cancer of kidney, liver and prostate, but these studies are not consistent.[2,3] Based on the compelling evidence from epidemiologic studies, the Subcommittee recommends classifying inorganic arsenic compounds as 'known to be carcinogenic to humans' (category 1A) according to the new classification system of the Health Council (comparable with EU category 1A according to the newly implemented Globally Harmonized System) (see Annex J).

Mechanism of genotoxicity

Several studies have been published on the genotoxic potential of inorganic arsenic and its methylated metabolites. Especially the trivalent metabolites monomethylarsonous acid and dimethylarsinous acid may play a role in the development of cancer.[2,8] From these studies various genotoxic processes could explain carcinogenic activity. For instance:

1 arsenicals can bind to thiol-groups in proteins which may lead to inhibition of e.g. DNA repair enzymes[5]
2 arsenic exposure can result in hypo- or hypermethylation of cellular DNA. These changes can be caused by e.g. an effect of arsenic on DNA methyltransferases[5]
3 arsenic does not generate reactive oxygen by itself but inhibits the scavenging systems of reactive oxygen species. This leads indirectly to the increase of reactive oxygen species.[6]

All three abovementioned processes support a non-stochastic genotoxic mechanism. Although inorganic arsenic is able to produce chromosomal effects (aberrations, sister chromatid exchange) in many in vitro and in vivo systems[3,7], no overt signs of stochastic genotoxic activity of inorganic arsenic have been found. Inorganic arsenic does not covalently bind to DNA[7] and does not induce point mutations in bacterial or mammalian test systems[3,7].

Therefore, the overall mechanistic evidence supports the view that genotoxicity is not caused by a direct effect of inorganic arsenic on the DNA, but via other processes which are triggered by arsenic. The Subcommittee concludes therefore that the genotoxic mechanism of inorganic arsenic should be considered as non-stochastic.

As arsenic and arsenic compounds have non-stochastic genotoxic mechanisms, an exposure limit should be derived using a threshold model. However, the epidemiological studies[3,4] on arsenic and cancer do not report exposure-effect relations that allow derivation of such a threshold. Therefore the Subcommittee advised to apply linear extrapolation to establish a health based reference value.

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Meeting date: 07 June 2010

Annex **J**

Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[232]

Annex **K**

Evaluation of the Subcommittee on the Classification of reprotoxic substances

As yet only one inorganic arsenic compound, lead arsenate, has been classified for reproduction toxicity according to EC regulations (Repr 1A; H360DF - may damage fertility or the unborn child).[1] However, for this compound, the reproduction toxicity is probably more related to lead than to arsenic. The ECHA website (European Chemicals Agency) recently reported that data on reproduction toxicity of diarsenic trioxide (As^{III}) and arsenic acid (As^{V}) are not sufficient for classification for reproduction toxicity (see website European Chemicals Agency, http://echa.europa.eu/). Nonetheless, the ECHA website reports for arsenic acid a 'self-classification' (suspected of damaging fertility or the unborn child).

Effects on fertility

In humans no effects of arsenic on fertility have been observed upon inhalatory or oral exposure. No fertility effects in experimental animals have been reported after inhalatory exposure. However, a number of oral and parenteral studies was available (Wang et al. 2006[2]) reporting effects on fertility in male and female animals.

Arsenic trioxide (As^{III}). In male mice, oral administration of arsenic trioxide (30 days) interferes with spermatogenesis (decreased sperm count, decrease of activity of 22 spermatogenetic enzymes 3ß- en 17ß-HSD, interference with cholesterol metabolism, 23 degeneration of tubules)(Chinoy et al., 2004[3]).

Sodium arsenite (As^V). Oral administration of sodium arsenite to male mice (35 days) resulted in a significant decrease in sperm count and motility, increase in abnormal sperm and interference with activity of spermatogenetic enzymes (17 ß-HSD)(Pant et al., 2001[4]). In a chronic study Pant et al. (2004)[5] administered sodium arsenite to male mice via drinking water for 365 days. This caused a decrease in the absolute and relative testicular weight, a decrease in activity of marker testicular enzymes (sorbitol dehydrogenase, phosphatase), and in increase of LDH and γ-GT activity. In addition, a decrease in sperm count and sperm motility, along with an increase in abnormal sperm, was observed. Intraperitoneal administration of sodium arsenite to male rats for 26 days (Sarkar et al. 2003[6]) resulted in a decrease in testicular weight, accessory sex organ weights and epididymal sperm counts, plasma luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone. In addition, massive degeneration of all the germ cells was observed.

Oral administration of sodium arsenite to female rats in diestrous phase for 28 days (seven oestrous cycles) (Chattopadhyay et al. 2001[7], 2003[8]) caused a significant reduction in the plasma levels of LH, FSH, and estradiol along with a significant decrease in ovarian activities of 3ß-HSD and 17ß-HSD followed by a reduction in ovarian and uterine peroxidase activities. A significant weight loss of the ovary and uterus was also observed.

The effects on fertility in experimental animals are observed in oral and parenteral studies at relatively high dose levels in the absence of general toxic effects. The Subcommittee concludes that the data support the view that inorganic arsenic should be classified ‘as presumed human reproductive toxicant’ (category 1B) for effects on fertility (H360F).

Effects on development

A huge number of human studies, both occupational and environmental, is reported addressing the effect of arsenic as a developmental toxicant. Several (older) human studies have reported an association between inhalation or oral exposure to inorganic arsenic and increased risk of adverse developmental effects (congenital malformations, low birth weight, spontaneous abortion)(WHO 2000[9], ATSDR 2007[10], Nordström et al. 1978a[11], 1978b[12], 1979a[13], 1979b[14], Aschengrau et al. 1989[15], Zierler et al. 1988[16]). However, the Subcommittee observed that these (older) populations were co-exposed to a number of other chemicals beyond arsenic and that these chemicals may have contributed to the observed effects an that not all studies were well analyzed and

designed (Aschengrau et al. 1989[15] and Zierler et al. 1988[16]). Therefore these studies did not unequivocally prove that arsenic is a developmental toxicant. More recent epidemiological studies however, especially in populations in areas of the world (Taiwan, Chile, Bangladesh/Bengal) with elevated levels of arsenic in drinking water, report associations between developmental effects and chronic exposure of women to arsenic in the drinking water.

*Taiwan***.** Yang et al. (2003)[17] suggested that arsenic exposure from drinking well water was associated, although not significantly, with the risk of preterm delivery and reduction in birth weight.

Chile. Associations have been found between late fetal mortality, neonatal mortality, and postneonatal mortality and exposure to high levels of arsenic in the drinking water (Hopenhayn-Rich et al. 2000[18]). Hopenhayn et al. (2003)[19] suggested that moderate arsenic exposures from drinking water (<50 μg/L) during pregnancy are associated with reduction in birth weight. Significantly increased SMRs were reported for lung cancer and bronchiectasis among subjects who had probable exposure *in utero* or during childhood to high levels of arsenic in the drinking water (Smith et al., 2006[20]).

Bangladesh/Bengal. Ahmad et al. (2001)[21] showed that adverse pregnancy outcomes in terms of spontaneous abortion, stillbirth, and preterm birth rates were significantly higher in an exposed group than those in the nonexposed group. Milton et al. (2005)[22] observed excess risks for spontaneous abortion and stillbirth among the chronically exposed study participants. Von Ehrenstein et al. 2006[23] observed that exposure to high concentrations of arsenic (≤ 200 μg/liter) during pregnancy was associated with an increased risk of stillbirth and neonatal death, while no association was found between arsenic exposure and spontaneous abortion. Rahman et al. (2009)[24] found negative dose effects with birth weight and head and chest circumferences at a low level of arsenic exposure (<100 μg/L in urine). Rahman et al. (2010)[25] reported that the odds ratio of spontaneous abortion was increased among women with elevated urine arsenic concentrations and that the rate of infant mortality increased with increasing arsenic exposure. Tofail et al. (2009)[26] assessed infants born to arsenic exposed mothers on two problem solving tests (PST) (the motor scale of the Baley Scales of Infant Development, and behaviour ratings). No significant effect of arsenic exposure during pregnancy on infant development (motor, PST score and behaviour rating) was detected.

The Subcommittee observes that these recent human studies have good quality when compared to the earlier ones, and give strong indications that exposure to arsenic may not be excluded as a causal factor for spontaneous abortion, stillbirth, preterm delivery and reduced birth weight and also

neuropsychological development. The actual chemical form of arsenic in these human studies, be it airborne or in solution (As^{III}, As^{V}), is never clearly reported.

Numerous studies in animals showed that arsenic caused reduced birth weight, a variety of foetal malformations (both skeletal and soft tissue), and increased foetal mortality following inhalation exposure of mice and rats, oral exposure of mice, rats, hamsters and rabbits, and intraperitoneal or intravenous exposure of mice, rats and hamsters. The Subcommittee focussed on the evaluation of inhalation and oral studies in animals.

Arsenic trioxide (As^{III}). Only two inhalation studies were reported, with controversial results. Holson et al. (1999)[27] administered as arsenic trioxide to rats. Maternal toxicity (rales, decrease in net body weight gain, a decrease in food intake during pre-mating and gestational exposure) was observed at the highest exposure level. No treatment-related malformations or developmental variations were noted at any exposure level. Nagymajtenyi et al. (1985)[28] exposed mice by inhalation to arsenic trioxide during gestation. The highest dose group had significant increases in the percentage of dead foetuses, skeletal malformations, and the number of foetuses with retarded growth. Stump et al. (1999)[29] treated rats with a single oral dose of arsenic trioxide during gestation. Maternal food consumption was decreased dose-dependently. In the highest dose group body weight, body weight change, and net body weight change were significantly decreased. The highest dose resulted in a significant increase in postimplantation loss and a decrease in viable fetuses per litter. Holson et al. (2000)[30] administered arsenic trioxide orally to rats beginning prior to mating and continuing through mating and gestation. Maternal toxicity in the highest dose group was evidenced by decreased food consumption and decreased net body weight gain during gestation, increased liver and kidney weights, and stomach abnormalities (adhesions and eroded areas). No treatment-related foetal malformations were noted in any dose group. Increased skeletal variations at the highest dose group were observed.

Sodium arsenite (As^{V}). Baxley et al. (1981)[31] treated pregnant mice by oral gavage and noted gross malformations (exencephaly, open eyes) in foetuses at the highest dose. Hood and Harrison (1982)[32] treated hamsters by oral gavage and observed no effect on prenatal growth or survival at the highest dose on gestation days 9-11. However, when treated with the highest dose on gestation day 12, prenatal deaths increased, and growth was inhibited in the foetuses. Rodriguez et al. (2002)[33] exposed rats to arsenite during gestation or postnatally and observed that animals showed increased spontaneous locomotor activity and increased number of errors in a delayed alternation task. Xi et al. (2009)[34]

evaluated the developmental neurotoxicity of arsenic in offspring rats by transplacental and early life exposure to sodium arsenite in drinking water and reported that tail hung reflex, auditory startle and visual placing showed signifcant decrease compared to the control group. In square water maze test spatial learning and memory were affected.

Arsenic acid /sodium arsenate (As^V). Nemec et al. (1998)[35] evaluated effects of oral exposure to arsenic acid in mice and rabbits. In the highest dose group in mice an increase was detected in the number of resorptions per litter and decreases in the number of live pups per litter, and mean fetal weight. At the highest dose in rabbits prenatal mortality was increased; surviving does had signs of toxicity, including decreased body weight. These data revealed an absence of dose-related effects in both species at arsenic exposures that were not maternally toxic. Hood et al. (1978)[36] administered sodium arsenate orally to mice on during gestation. Fetuses weighed significantly less than controls, prenatal mortality was increased and fetal malformations and skeletal defects were seen. Hill et al. (2008)[37] evaluated the developmental toxicity of oral exposure of arsenate during gestation in an inbred mouse strain, that does not exhibit spontaneous neural tube malformations. There was no maternal toxicity as evidenced by losses in maternal body weight following As treatment. However, liver weights were lower in all As-treated groups, suggesting hepatotoxicity due to As exposure. The number of litters affected with a neural tube defect (exencephaly) in each treatment group exhibited a positive linear trend (vertebral and calvarial abnormalities, components of the axial skeleton). Mean fetal weight of all As-treated groups was significantly less than in control. This is the only study proving that foetal malformations can develop in absence of maternal toxicity.

The Subcommittee concludes that the oral and inhalatory animal studies showed that arsenic, usually at maternally toxic doses, caused reduced birth weight, a variety of foetal malformations (both skeletal and soft tissue), and increased foetal mortality. In addition to the oral and inhalatory studies, the Subcommittee evaluated a number of parenteral animal studies and observed similar effects in offspring (Ferm and Carpenter, 1968[38]; Willhite et al., 1981[39]; Beaudoin et al., 1974[40]; Hood and Harrison, 1982[32]; Carpenter et al., 1987[41]; Stump et al., 1999[29]; DeSesso, 2001[42]; Desesso et al., 1998,[43]; WHO 2000[9]; ATSDR 2007[10]). The Subcommittee is aware that in none of the animal studies maternal toxicity can be unambiguously excluded. Only the study by Hill et al. (2008)[37], administering arsenate to an inbred mouse strain, supports the view that fetal malformations can develop in the absence of maternal toxicity.

The Subcommittee is of the opinion that, in view of the recent animal findings (Hill et al., 2008)[37], it can not be excluded that developmental effects can occur in the absence of maternal toxicity. Together with the recent epidemiological observations from different parts of the world on associations between developmental effects and chronic exposure, the Subcommittee recommends classification of inorganic arsenic (arsenate) as ' known human reproductive toxicant' (category 1A: H360D).

Effects on lactation

A small number of studies indicated that arsenic can be excreted in human milk. No data on the toxic effects from arsenic in human breast milk on the development of breastfed babies could be retrieved from the literature. No literature could be retrieved on toxic effects on pups via lactation.

Dang et al. (1983)[44] reported arsenic levels ranging from 0.2 to 1.1 ng/g in breast milk of nursing mothers 1-3 months postpartum (Bombay, India). Arsenic was detected in human breast milk at concentrations of 0.13-0.82 ng/g (Somogyi and Beck, 1993[45]). In human milk sampled from mothers on the Faroer Islands whose diets were predominantly seafood, arsenic concentrations were 0.1-4.4 ng/g (Grandjean et al., 1995[46]). Exposure to arsenic from the seafood diet in this population was most likely to organic arsenic. In a population of Andean women exposed to about 200 ng/g of inorganic arsenic in drinking water, concentrations of arsenic in breast milk ranged from about 0.8 to 8 ng/g (median 2.3 ng/g) (n=10)(Concha et al., 1998[47]). The arsenic concentration in the breast milk of 35 women in Izmir, Turkey, ranged from 3.24 to 5.41 ng/g, with a median of 4.22 ng/g (Ulman et al., 1998[48]). Samanta et al. (2009)[49] collected two hundred and twenty-six breast milk samples from lactating women in arsenic-affected districts of west Bengal. In only 39 (17%) samples arsenic was detected. The maximum arsenic concentration in breast milk was 48 µg/L. Women who had both high arsenic body burden and arsenical skin lesions also had elevated levels of arsenic in their breast milk.

Estimation of tolerable concentration for inorganic arsenic

The Subcommittee is of the opinion that the data on exposure via lactation are limited as yet. However, EFSA (2009)[50] calculates an average daily exposure to inorganic arsenic from breast milk for infants (up to 6 months) of 0.0275 µg/kg/day in the general European population (approx. 0.13 µg/L in breastmilk). The Subcommittee observes that this level is easily exceeded in specific area's in the world with elevated levels in drinking water. In order to protect up to 6-month-

old breastfed children from the effects of arsenic through intake of breast milk, the Subcommittee used the following default values:

- body weight infant: 4.5 kg
- intake human breast milk per infant per day: 900 mL
- an infant is as sensitive to the substance as an adult.

These assumptions are used for the calculation of a tolerable level of arsenic in human breast milk. The values are conservative figures estimated from the growth curves for the Netherlands (Fredriks et al. 2000[51]) and by the WHO (2006)[52], and breast milk intake (Butte et al. 2002[53]). Of note, however, are the indications that children might be more sensitive to the effects of arsenic than adults (ATSDR 2007[54]).

The following health-based values for inorganic arsenic have been recommended:

- ATSDR (2007)[10], Minimal Risk Level (MRL) for acute exposure (1-14 days): 5 µg/kg/day
- ATSDR (2007)[10], MRL for chronic exposure (1 year or longer): 0.3 µg/kg/day
- US-EPA (2007)[10], Reference Dose (RfD): 0.3 µg/kg/day.

(Unfortunately, the MRL for intermediate duration (15 days to 1 year) exposure is the most appropriate limit value for suckling infants but was unfortunately not derived by the ATSDR (2007)[54] due to lack of suitable data).

This corresponds to (MRL for acute exposure as limit):

- a tolerable intake of arsenic of 22.5 µg/infant/day
- a tolerable concentration of arsenic in breast milk of 25 µg/L.

(MRL for chronic exposure as limit):

- a tolerable intake of arsenic of 1.35 µg/infant/day
- a tolerable concentration of arsenic in breast milk of 1.5 µg/L.

The breast milk levels as reported by Samanta et al.[49] (48 µg/L) exceed the tolerable concentration of arsenic in both acute and chronic exposure situations. When the MRL for chronic exposure is chosen for the calculation, most of the breast milk levels reported by Grandjean et al.[46], Ulman et al.[48], Concha et al.[47], and Samanta et al.[49] will exceed the calculated tolerable concentration (of 1.5 µg/L). Supposing that the intermediate MRL is 2 or 3 µg/kg/day, this will lead to tolerable concentrations in breast milk of 10 and 15 µg/L. The Subcommittee estimates that 10 µg/L may be considered an upper tolerable concentration for

inorganic arsenic in breast milk and notes that this concentration is exceeded in specific area's in the world with elevated levels in drinking water (Samanta et al.[49]).

Therefore the Subcommittee recommends to label inorganic arsenic as a substance that 'may cause harm to breasfed babies'.

Proposed classification for effects on fertility

The Subcommittee is of the opinion that inorganic arsenic should be classified 'as presumed human reproductive toxicant' (category 1B: H360F) for effects on fertility.

Proposed classification for developmental toxicity

In view of the recent epidemiological and animal findings the Subcommittee is of the opinion that the developmental effects of inorganic arsenic allow classification as 'known human reproductive toxicant' (category 1A: H360D).

Proposed labeling for effects during lactation

The Subcommittee recommends classifiying inorganic arsenic as a substance that 'may cause harm to breastfed babies' (H362).

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Meeting date: July 6, 2012

Annex **L**

Derivation of health-based calculated occupational cancer risk values (HBC-OCRV)

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1 Mortality figures

Mortality has been calculated on the basis of national data on lung cancer mortality in five-year age bands obtained through Statistics Netherlands (Centraal Bureau voor de Statistiek, www.cbs.nl) and the Comprehensive Cancer Centres (Vereniging van Integrale Kankercentra, www.ikcnet.nl). Mortality data for the years 2000 to 2010 were used, separated by age and sex. Rates for women and men were averaged so that the calculations would describe the average risk for the population. To avoid an abrupt transition between age categories, the mortality data were 'smoothed'. These 'modelled' mortality data were employed in the Committee's analysis.

The mortality rates (deaths per 100,000 person-years) were used in a so-called survival analysis. Such an analysis may be thought of as involving two cohorts (in this case, of 100,000 people), that are followed from birth to death. For occupational arsenic exposure, it is assumed that exposure of the cohort starts at the age of twenty and lasts until the age of sixty. Every year the cohort reduces in size, through death as a result of the cause of death under study and other causes; the cohort is followed until it reaches the age of a hundred years.

The first cohort is not exposed; the second cohort is exposed to arsenic, and for this reason lung cancer mortality is higher in this cohort. Assuming a given average annual exposure to arsenic, every year that a person in the cohort is exposed to arsenic is another year contributing to their cumulative exposure. This approach employs cumulative exposure because studies of workers exposed to high levels of arsenic always work with cumulative exposure; the formulae employed are also based on cumulative exposure. Using this cumulative exposure, which is recalculated for each year, and the assumed exposure-response relationship between exposure to arsenic and death from lung cancer, the number of extra deaths is calculated for each year that the cohort ages. Using this information, first the additional risk of death per year associated with exposure to arsenic can be calculated and then the lifelong additional risk of death associated with exposure.

2 Quality of studies on occupational arsenic exposure

Four epidemiological studies on lung or respiratory cancer mortality among workers exposed to arsenic are of interest. These concern Lubin et al. (2000), Lubin et al. (2008), Jarüp et al. (1989), and Enterline et al. (1995). In Table A detailed information is given on study design, execution, analyses, and additional remarks made by the Committee. In short, all studies show some shortcomings, which differ between studies.

Lubin et al. (2000) and Lubin et al. (2008)

In these studies, instead of lung cancer mortality, respiratory cancer mortality is used. According to the information given in the 2000 paper (Table 2), this may cause a deviation in mortality rate smaller than 4%. Therefore, the effect on association measures like risk ratio's is assumed to be negligible. Another potential limiting factor of the 2000 paper is the higher loss-to-follow-up, compared to the other studies under consideration. If it is assumed that this loss-to-follow-up is non-differential across exposure, the Lubin et al. (2000) study is the strongest study with fewest limitations.

The Lubin et al. (2008) study was a follow-up to the 2000 study, with a different modelling strategy. In the update, Lubin et al. used an exposure reduction factor in the higher exposure categories to account for the use of personal protection equipment. This is not common practice in risk calculations and this study was not further considered for risk assessment.

Enterline et al. (1995)

In this study, description of the exposure assessment component was limited and basic descriptive information was lacking. Information about exposure before 1938 was lacking completely. It was not clear how exposure was assigned to certain job titles. Loss-to-follow-up was low, and an exposure-response relation was given. The study used long cancer mortality (including bronchus and trachea cancer). Calculations were based on a comparison with the general population (SMR study).

Järup et al. (1989)

This study had an exposure assessment component for which the description was limited and basic documentation was lacking. The way exposure has been calculated was not transparent, therefore making it difficult to analyse the risk per unit of increase by exposure. Information on exposure before 1945 was unclear, and again, it was not clear how exposure was assigned to certain job titles. Furthermore, no exposure-response relation was given. However, also in this study loss-to-follow-up was low and an exposure-response relation could be calculated by the Committee. This study analysed lung cancer mortality. Calculations were based on a comparison with the general population (SMR study).

3 Exposure response analysis

Lubin et al. (2000) used an internal exposure-response analysis (resulting in a relative risk, RR), whereas the other studies compared exposure related mortality with mortality in the general population, which results in a standardized mortality ratio (SMR).

As an exploratory analysis, exposure-response relations for the Järup et al. (1989) study were calculated based on the data given in the original article. Exposure-response relations based on joined data from the paper were calculated with PROC NLMIXED (SAS), resulting in the following relations (RR = 1 means no difference in mortality when compared to reference group):

- $RR = 1 + 0.1002(\text{cum exp})$
- $RR = 1 + 1.69(\text{cum exp})^{0.253}$.

The power-model calculated for the Järup et al. study has a better fit (AIC power model: 43.0; AIC additive model: 68.5). This also is said to be the case for the

power model shown in Enterline et al. (p. 30), although no model fit information is given. For both these studies it cannot be excluded that the strong fit of the power models is caused by the fact that there is a clear difference in risk between the exposed population and the comparison group while there is a very weak association among the exposed only. These studies are SMR studies and are thus the result of a comparison with the general population and potential systematic differences in mortality between the general population and the exposed workers in these cohort studies. When an attempt was undertaken to model the exposure response curve in the low exposure range (steep part of the curve) the fit of linear models was very poor for both studies indicating that there was no clear exposure response curve discernible in this range. This again suggests that the comparison with the general population may be problematic. Considering the quality of the papers and fit of the models, the Committee decides to use the study of Lubin et al. (2000).

In the study by Lubin et al. model fit did not improve significantly after fitting a power model (p. 557-558). Therefore the linear model was used , in line with previous reports, with an intercept RR=1.

It is not completely clear from the paper how the linear relation in the paper has been calculated. If the published function is used the following equation is obtained RR = 1 + 0.19 * cumulative.exposure. However, post hoc estimation on the basis of the categorical data leads to the equation RR=1+0.09*cumulative.exposure).

Risk calculations

Lifetable calculations were performed by means of the software package R (in Windows). For the derivation of the health-based calculated occupational cancer risk values (HBC-OCRV), an additional risk of one extra cancer death due to occupational exposure per 250 (4×10^{-3}) and 25,000 (4×10^{-5}) is used. The results are shown below.

Study	equation	ER*=40e-4	ER=40e-6
Lubin	1+0.19*cum.exposure	0.028	0.00028

For convenience risk estimates are also given for the other two studies. Note however that these equations were derived from marginally fitting risk functions:

Study	equation	ER*=40e-4	ER=40e-6
Järup	1+0.33*cum.exposure	0.016	0.00016
Enterline	1+0.16*cum.exposure	0.033	0.00033

Study characteristics

Reference	Enterline et al. (1995)	Lubin et al. (2000)	Lubin et al. (2008)	äarup et al. (1989)
Cohort name:	Tacoma	Lee-Fraumeni (Anaconda)	Lee-Fraumeni (Anaconda)	Rönnskär
Country:	US	US	US	Sweden
Cohort size:	2802	8014	8014	3916
Cohort definition/source:	Males working ≥ 1 year at the Tacoma copper smelter between 1940-1964	Workers employed for ≥ 12 months prior to 1957	Workers employed for ≥ 12 months prior to 1957	Male workers employed ≥ 3 months between 1928-1967
Number of cases:	182 (bronchus, trachea, lung cancer)	446 (respiratory cancers)	446 (respiratory cancers)	106 (lung)
Source of unexposed:	Mortality rates of white men in Washington State since 1941	US population rates	US mortality rates for resp. cancer in white males	Reference mortality rates from county, available from 1951
Exposure period:	1940-1964	1938-1957	1938-1957	1928-1967
Follow-up period:	-1986	One year after initial employment or 1938-1989	One year after initial employment or 1938-1989	- 1981
Loss to follow-up:	1.5%	15%	Not mentioned	15 lost
Exposure assessment:	- Before 1971: environment samples - After 1971 personal samples - Before 1938 no exposure data - Biomonitoring data for part of the employees - Strategy is not clearly described - Resulting in 7 exposure categories	- Employment records - 702 measurements of airborne arsenic between 1943 and 1958 - Resulting in low, medium, high categories with group-average estimation of exposure - Exposure estimation based on measurements and duration of exposure	- Employment records - 702 measurements of airborne arsenic between 1943 and 1958 - Resulting in low, medium, high categories with group-average estimation of exposure - Exposure estimation based on measurements and duration of exposure	- After 1945 measurement data + production numbers - Strategy unclear
Reported dose-response relationship *(give formula)*:	SMR = 100 + 10.5 (cum exp)$^{0.279}$	Power: RR = 1+1.00(cum exp)$^{0.43}$ Linear: RR = 1+0.19(cum exp)	RR = 1+0.115a	None given
Is the paper valid and usable for lifetable?	Yes	Yes, but relatively high loss to follow-up	Yes, but dose-response is adjusted for wearing air filtration masks	Yes, but exposure estimates before 1945 are problematic and no exposure model

Remarks:	- Weaker in exposure assessment - Exposure not clearly described - Data on before 1938 is missing	- Exposure most clear and transparent - Analysis based on limited data - No exposure measurements before 1943 - High loss to follow-up - After 1971 job history not complete, but only 10% was still working in 1977 - Health outcome unknown for 15% of the employees- could mean possible methodological limitations	- Exposure most clear and transparent - Results are adjusted for wearing air masks - No exposure measurements before 1943 - Calculated with duration and cumulative exposure- inconvenient for normal procedure - High loss to follow-up - After 1971 job history not complete, but only 10% was still working in 1977 - Health outcome unknown for 15% of the employees- could mean possible methodological limitations	- Too little information on exposure (esp. before 1945), hence difficult to judge risk per unit increase of exposure - Details are lacking - Not clear how sound analysis is - No general exposure-response relation given