Annex XV dossier

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR 1A OR 1B, PBT, vPvB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN

Substance Name(s): Calcium arsenate

EC Number(s): 231-904-5

CAS Number(s): 7778-44-1

Submitted by: Climate and Pollution Agency, Norway

PUBLIC VERSION: This report does not include the confidential annexes referred to in the document.

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PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR 1A OR 1B, PBT, VPVB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN

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The substance is proposed to be identified as a substance meeting the criteria of Article 57 (a) of Regulation (EC) 1907/2006 (REACH) owing to its classification as carcinogen 1A[1] which corresponds to classification as carcinogen category 1[2].

Summary of how the substance meets the CMR (1A or 1B) criteria

Calcium arsenate is covered by index number 033-005-00-1, “arsenic acid and its salts”, in Regulation (EC) No 1272/2008, Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) and classified as carcinogen Carc. 1A (H350: “May cause cancer”). The corresponding classification in Annex VI, Part 3, Table 3.2 (the list of harmonised and classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is carcinogen, Carc. Cat. 1 (R45; ”May cause cancer”).

Consequently, this classification of the substance in Regulation (EC) No 1272/2008 shows that calcium arsenate meets the criteria for classification as carcinogenic in accordance with Article 57(a) of REACH.

Registration dossiers submitted for the substance? YES

1 Classification in accordance with Regulation (EC) No 1272/2008 Annex VI, part 3, Table 3.1 List of harmonised classification and labelling of hazardous substances.

2 Classification in accordance with Regulation (EC) No 1272/2008, Annex VI, part 3, Table 3.2 List of harmonised classification and labelling of hazardous substances (from Annex I to Council Directive 67/548/EEC).

PART I

JUSTIFI CATION

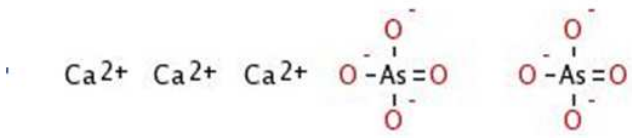
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	231-904-5
EC name:	Calcium arsenate
CAS number (in the EC inventory):	7778-44-1
CAS number:	7778-44-1
CAS name:	Arsenic acid (H3AsO4), calcium salt (2:3)
IUPAC name:	Tricalcium(2+) diarsenate
Index number in Annex VI of the CLP Regulation	033-005-00-1
Molecular formula:	$As_2Ca_3O_8$
Molecular weight range:	398,1
Synonyms:	Arsenic acid calcium salt; calcium orthoarsenate; tricalcium arsenate;

Structural formula:



Source: ESIS

1.2 Composition of the substance

Name: Calcium arsenate

Description:

Degree of purity: Information on purity is provided in the Confidential Annex.

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
Calcium arsenate			

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks

Table 4: Additives

Additives	Typical concentration	Concentration range	Remarks

1.3 Physico-chemical properties

Table 5: Overview of physicochemical properties

Property	Value	Remarks	Source
Physical state at 20°C and 101.3 kPa	Colourless amorph powder Calcium arsenate is a colourless to white, odourless, solid powder.		IPCS Health and Safety Guide No. 70 ECHA website
Melting point	1455°C		IPCS Health and Safety Guide No. 70
Boiling point	Decomposes	No data for starting temperature for decomposition available	IPCS Health and Safety Guide No. 70 ECHA website
Vapour pressure	0 mm Hg at 20 °C .		IPCS Health and Safety Guide No. 70 ECHA website
Water solubility	0.13 g/L in water at 25°C		HSDB ECHA website
Partition coefficient n-octanol/water (log value)			
Dissociation constant			

2 HARMONISED CLASSIFICATION AND LABELLING

Calcium arsenate is covered by Index number 033-005-00-1, “arsenic acid and its salts” in Part 3 of Annex VI, of Regulation (EC) No 1272/2008, as updated by Commission Regulation No 790/2009 (ATP01), as follows:

Table 6: Classification according to part 3 of Annex VI, Table 3.1 (list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
033-005-00-1	arsenic acid and its salts with the exception of those specified elsewhere in this Annex	-	-	Carc. 1A Acute Tox. 3 * Acute Tox. 3 * Aquatic Acute 1 Aquatic Chronic 1	H350 H331 H301 H400 H410	GHS06 GHS08 GHS09 Dgr	H350 H331 H301 H410			A

Table 7: Classification according to part 3 of Annex VI, Table 3.2 (list of harmonized classification and labelling of hazardous substances from Annex I of Council Directive 67/548/EEC) of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
033-005-00-1	arsenic acid and its salts with the exception of those specified elsewhere in this Annex	-	-	Carc. Cat. 1; R45 T; R23/25 N; R50-53	T; N R: 45-23/25-50/53 S: 53-45-60-61		AE

3 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

See section 2 on harmonised classification and labelling.

A summary of carcinogenic effects of arsenic acid and its salts is found in appendix 1.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier.

6 CONCLUSIONS ON THE SVHC PROPERTIES

6.1 PBT, vPvB assessment

Not relevant for this dossier.

6.2 CMR assessment

Calcium arsenate is covered by index number 033-005-00-1, “arsenic acid and its salts”, in Regulation (EC) No 1272/2008, Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) and classified as carcinogen Carc. 1A (H350: “May cause cancer”). The corresponding classification in Annex VI, Part 3, Table 3.2 (the list of harmonised and classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is carcinogen, Carc. Cat. 1 (R45; ”May cause cancer”).

Consequently, this classification of the substances in Regulation (EC) No 1272/2008 shows that calcium arsenate meets the criteria for classification as carcinogenic in accordance with Articles 57 (a) of REACH.

6.3 Substances of equivalent level of concern assessment

Not relevant for this dossier.

PART II

INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

1 MANUFACTURE, IMPORT AND EXPORT

1.1 Manufacturing sites

Calcium arsenate is manufactured in the EU. Names and locations of the companies are described in a Confidential Annex to this report.

1.2 Manufacturing process

A description of the manufacturing process is included in the Confidential Annex.

Generation of calcium arsenate waste in other processes
Calcium arsenate is generated in various metallurgical processes apart from those here described under manufacturing processes. According to information obtained from industry, the calcium arsenate all ends up in the waste stream and consequently is considered by the manufacturers to be waste as defined in Directive 2006/12/EC and beyond the scope of REACH. The quantities formed are further described in the Confidential Annex.

1.3 Manufacture, import and export of the substance on its own

The registered quantities of calcium arsenate manufactured are in the range of 100-1000 tonnes per year. More information on manufactured and imported tonnages is provided in the Confidential Annex.

Import or export of calcium arsenate on its own has not been registered.

1.4 Trends

According to information obtained from the Arsenic Consortium (see Chapter 7), the main objective of the metallurgical processes involving the manufacture and use of calcium arsenate is neither the manufacture of calcium arsenate nor manufacture of diarsenic trioxide, but the manufacture of a range of metals (Arsenic Consortium, 2011). The manufacturing of calcium arsenate and the use of the substance in the processes is governed by the arsenic content of the raw materials.

The trend in the use of diarsenic trioxide is decreasing. The decreasing market and uses of arsenic compounds over the last decades let most copper and lead producers decide to eliminate the arsenic impurity from the byproducts through the disposal of the waste (Arsenic

Consortium, 2010b). For the processes concerned, a decreasing market for diarsenic trioxide would probably result in larger amounts of calcium arsenate being disposed of as waste.

1.5 Import of the substance in mixtures and articles

Calcium arsenate is partly imported from countries outside Europe as part of complex byproducts from non-ferrous metallurgical processes. The exact quantities are described further in the Confidential Annex. Besides the registered quantities, calcium arsenate may be imported in complex byproducts where the calcium arsenate (or the arsenic content of the calcium arsenate) ultimately ends up in the waste stream and consequently has not been registered.

1.6 Releases from manufacture

No specific data on the releases of calcium arsenate from the manufacturing processes is available. Emission from the different process steps is monitored and reported as arsenic, and the arsenic emission cannot be allocated specifically to the manufacture or use of calcium arsenate (Arsenic Consortium, 2011). Total releases of arsenic from the processes involving calcium arsenate are reported in the Confidential Annex.

The majority of the generated calcium arsenate is disposed of as waste (Arsenic Consortium, 2011). The quantities are further described in the Confidential Annex.

1.6.1 Exposure by manufacture

No data on exposure by manufacture of the calcium arsenate have been available.

2 USES OF THE SUBSTANCE

Two uses of the substance have been identified on the basis of information obtained from industry

- Precipitating agent in copper smelting,
- Manufacturing of diarsenic trioxide (As_2O_3).

Both uses are part of the same integrated metallurgical processes and are described in the next section and the Confidential Annex.

2.1 Use in metallurgical processes

2.1.1 Use as precipitating agent

Function of the substance
The function of the substance as precipitating agent is further described in the Confidential Annex.

Formation of toxic arsine
It is reported that the use of diarsenic trioxide in the manufacturing of zinc may lead to the formation of toxic arsine (AsH_3) (ECHA, 2010a). According to information from a manufacturer and user of the calcium arsenate, the AsH_3 is not stable at the operating conditions used in the process (~1000 C°) and the formation of arsine is not an issue in this process. According to the manufacturer, AsH_3 can only be formed in aqueous medium under strong reducing conditions such as electro winning.

Quantities involved
The quantities of calcium arsenate used as precipitating agent are described in the Confidential Annex.

2.1.2 Use for manufacturing of diarsenic trioxide

Function of the substance
The function of the substance in the manufacturing of diarsenic trioxide is further described in the Confidential Annex.

Final use of the manufactured diarsenic trioxide
The total estimated manufacturing volume of diarsenic trioxide in the EU is about 1,800 tonnes, but only a fraction of this is used in the EU (Arsenic Consortium, 2010a). The volume used within the EU is in the range of 690-850 t/y (Arsenic Consortium, 2010a)

According to information from the Arsenic Consortium (2010b), the main ongoing uses of diarsenic trioxide include (with quantities used in the EU in brackets):

- Ultrapure arsenic metal for the (opto-)electronics industry (e.g. for gallium arsenide wafers) (30-40 t/y)
- Chemicals sector (60 t/y)
- Industrial application of special glass (100-150 t/y)
- Artisanal applications in hand made glass/crystal (8 t/y is consumed for the production of Murano glass in Italy)
- Zinc production sector (500-600 t/y)

The import of diarsenic trioxide is estimated at 500-600 tonnes while 1,200-1,300 is exported. About 200 tonnes was disposed of as waste.

2.1.3 Process descriptors and environmental release categories

Process descriptors and environmental release categories according to the public part of the registrations published on ECHA´s website are shown in Table 8 below**.** The sector of end-use is reported to be SU 14 "Manufacture of basic metals, including alloys". The registrations for the substance do not include chemical safety reports (CSRs).

Table 8: Process descriptors and environmental releases categories for the use of calcium arsenate

Process descriptor		**Environmental release category**		**Market sector by type of chemical product and sector of end use**	
PROC 3	Use in closed batch process (synthesis or formulation)	ERC 1	Manufacture of substances	PC 7	Base metals and alloys
PROC 4	Use in batch and other process (synthesis) where opportunity for exposure arises	ERC 6a	Industrial use resulting in manufacture of another substance (use of intermediates)	SU 14	Manufacture of basic metals, including alloys
PROC 22	Potentially closed processing operations with minerals/metals at elevated temperature. Industrial setting	ERC 5	Industrial use resulting in inclusion into or onto a matrix		

Source: ECHA website, information on Registered Substances, calcium arsenate

3 RELEASES FROM THE USE OF CALCIUM ARSENATE

3.1 Use in metallurgical processes

3.1.1 Occupational exposure

No data on the occupational exposure to calcium arsenate have been available. According to information from the Arsenic Consortium (2011), arsenic is measured in different workplaces, but the exposure cannot be allocated to the specific manufacturing and use of calcium arsenate. No data on exposure to the arsenic by manufacture of the calcium arsenate have been available.

3.2 Releases to the environment

No data on the release of calcium arsenate is available. The release of arsenic associated with the use of the calcium arsenate is included in the total reported arsenic releases from the

metallurgical processes involving the manufacture of calcium arsenate and diarsenic trioxide. Information on the releases from the processes is reported in the Confidential Annex.

4 CONCLUSIONS ON MANUFACTURE, USES AND EXPOSURE

Based on the available information, the following can be concluded:

- Calcium arsenate is manufactured, imported and used in the EU;
- Calcium arsenate is imported in complex byproducts from smelting and refining of nonferrous metals used as raw materials for the manufacture of non-ferrous metals;
- In addition to the registered manufacture and import of calcium arsenate, the substance is to some extent formed as a by-product in other metallurgical processes from which it is disposed of as waste;
- The majority of the manufactured calcium arsenate is disposed of as waste;
- The following uses has been identified: Precipitating agent in copper smelting and use in the manufacturing of diarsenic trioxide (As_2O_3).
- Calcium arsenate is not used in any consumer products;
- No data on the emission of calcium arsenate or occupational exposure to calcium arsenate are available. Releases and workplace concentrations are measured as arsenic (As) and cannot be allocated to the individual arsenic compounds involved in the entire process.

5 CURRENT KNOWLEDGE ON ALTERNATIVES

5.1 Alternatives to calcium arsenate

The calcium arsenate fed into the copper smelter is used as precipitation agent and more information can be found in the Confidential Annex. As an example, diarsenic trioxide is used as precipitating agent in the manufacture of zinc.

5.2 Alternatives to diarsenic trioxide

The discussion on alternatives to calcium arsenate may to some extent be linked to the discussion on alternatives to diarsenic trioxide. If diarsenic trioxide is substituted by non-arsenic alternatives, all arsenic in the flow sheet would ultimately be disposed of as waste.

According to the report by RPA (2009), alternatives exist to diarsenic trioxide used as fining agent and decolourising agent in glass production. The alternatives include sodium sulphate (used in lead crystal), antimony trioxide (used in lead crystal), sodium/potassium nitrates with antimony trioxides (used in special glasses) and cerium oxide. Furthermore, the report states that several major glass producers and computer companies are now promoting the use of arsenic-free glass in computer monitors (RPA, 2009).

Many of the alternatives to the use of arsenic in glass/enamel processing, e.g. antimony trioxide, may be considered potentially harmful to human health and the environment (ECHA, 2010a).

In their answer to the consultation on diarsenic trioxide, the Arsenic Consortium states that although in certain crystal applications diarsenic trioxide can be replaced by lead oxide (PbO), the use of diarsenic trioxide in special industrial glass remains so far essential. Alternatives to the application of diarsenic trioxide in artisanal glass/crystal are under investigation by a program of the Stazione Sperimetale del Vetro in Italy (Murano-Venezia) a government funded research institution. Several other salts (including phosphor and sulfur based) are under investigation (Arsenic Consortium, 2010a).

The background document for diarsenic trioxide (ECHA, 2010a) concludes that given the range and diversity of alternatives to the use of diarsenic trioxide, it might be expected that alternatives would be available with suitable technical and economic characteristics for most applications. However, although it is accepted that there are alternatives for most domestic (lead crystal) applications, the glass industry (CPIV, 2008 as cited by ECHA, 2010a) has highlighted a number of applications where there are technical difficulties in replacing arsenic in special glass (see ECHA, 2010a for more details).

No data on the availability of alternatives to diarsenic trioxide in zinc production have been reported.

5.3 Conclusions on alternatives

Based on the available information the following can be concluded:

- Arsenate may in principle be used as precipitating agent in the form of other arsenic compounds.
- If the use of diarsenic trioxide decreases, an increasing amount of calcium arsenate would end up in the waste stream.

6 REFERENCES

Some references to information from industry are indicated in the Confidential Annex.

Arsenic Consortium (2011): Personal communication with Sylvi Claußnitzer, Manager of the Arsenic REACH Consortium, March-May, 2011.

Arsenic Consortium (2010a): First commentary of the Arsenic Consortium for the Public Consultation on the Recommendation for Inclusion of Diarsenic trioxide and Diarsenic pentaoxide on the Annex XIV list under REACH.

Arsenic Consortium (2010b): Second commentary of the Arsenic Consortium for the Public Consultation on the recommendation for inclusion of Diarsenic trioxide and Diarsenic pentaoxide in the Annex XIV list under REACH.

BREF (2001): Reference Document on Best Available Techniques in the Non Ferrous Metals Industries. Integrated Pollution Prevention and Control (IPPC). European Commission, December 2001.

ECHA (2010a): Background document for diarsenic trioxide. Document developed in the context of ECHA's second Recommendation for the inclusion of substances in Annex XIV. 17 December 2010.

ECHA website, European Chemicals Agency database.
http://apps.echa.europa.eu/registered/registered-sub.aspx.

Eurometaux (2011): Arsenic. REACH Metals Gateway. Eurometaux, European Association of Metals, Brussels. Accessed August 2011 at: http://www.reach-metals.eu/index.php?option=com_substance&task=view&id=62&Itemid=68

HSDB (2011): Hazardous Substances Data Bank (HSDB®). National Library of Medicine's (NLM) Toxicology Data Network (TOXNET®).
http://www.nlm.nih.gov/pubs/factsheets/hsdbfs.html#

IPCS (1992): Inorganic arsenic compounds other than arsine. Health and Safety Guide 70.IPCS International Programme on Chemical Safety, Geneva.
http://www.inchem.org/documents/hsg/hsg/hsg070.htm

RPA (2009): Data on manufacture, import, export, uses and releases of: diarsenic trioxide (cas no: 1327-53-5); diarsenic pentaoxide (cas no: 1303-28-2); lead hydrogen arsenate (cas no: 7784-40-9); and triethyl arsenate (cas no: 15606-95-8), as well as information on potential alternatives to its use. ECHA/2008/02/SR3/ECA.220. RPA, DHI and Milieu for European Chemicals Agency.

7 ABOUT THE ARSENIC CONSORTIUM

As part of the stakeholder consultation for this Annex XV dossier concerning information on the use of the substance has been obtained from the Arsenic Consortium (2011). The Arsenic Consortium became effective in August 2009 with its members having a common interest in fulfilling the requirements laid down by the REACH Regulation. The Arsenic Consortium consists of a number of EU and international companies showing interest in joint activities regarding the registration of Arsenic and Arsenic compounds. Under the administrative lead of WirtschaftsVereinigung Metalle WVM, the German Non-ferrous Metals Association, the Consortium initially identified ten substances which are in common interest of the involved companies. According to the REACH Metals Gateway (Eurometeax, 2011) the Consortium coordinates joint activities regarding the following substances: Arsenic (metal), calcium arsenate, diarsenic trioxide, gallium arsenide, tricopper arsenide, and trilead diarsenate.

Arsenic acid is not included in the list and activities related to this substance are coordinated by another consortium, the Arsenic Acid Consortium managed by Patric Levy, Patrick Levy Consulting, France.

8 DEFINITION OF ARSENIC COMPOUNDS AND GLOSSARY

Arsenic and its compounds are ubiquitous in nature. They exhibit both metallic and nonmetallic properties. From both the biological and the toxicological points of view, arsenic compounds can be classified into three major groups: inorganic arsenic compounds; organic arsenic compounds; and arsine gas. Arsenic can exist in four valence states: –3, 0, +3 and +5. Under reducing conditions, the +3 valence state as arsenite (As^{III}) is the dominant form; the +5 valence state as arsenate (As^{V}) is generally the more stable form in oxygenated environments. Inorganic As^{III} and As^{V} are the major arsenic species identified in natural water, whereas minor amounts of monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) can also be present.

Glossary:

Arsenic acid

Formula H_3AsO_4 . Colourless crystals, soluble in water and alcohol.

Arsenate

Arsenate is a salt or ester of arsenic acid having a negative ion of AsO_4^{3-}, Example of an arsenate salt is calcium arsenate $As_2Ca_3O_8$

Arsenite

Arsenite is a salt or ester of arsenious acid having a negative ion of AsO_3^{3-} derived from aqueous solutions of As_4O_6. Example of an arsenite salt is sodium arsenite Na_3AsO_3.

Arsenide

Arsenide is a negative, trivalent binary arsenic compound having a negative ion of As^{3-}. Example of an arsenide is gallium arsenide (GaAs).

Arsine

A colorless, highly poisonous gas with an unpleasant odor with the formula $As H_3$.

Arsinic acid

An acid of general formula R_2AsO_2H, derived from trivalent arsenic; an example is dimethylarsinic acid, $(CH_3)_2AsO(OH)$.

Arsonic acid

An acid derived fromarsenic acid H_3AsO_4, the type formula is generally considered to be $RAsO(OH)_2$, an example is monomethylarsonic acid $CH_3AsO(OH)_2$

Monomethylarsonic acid (MMA ^{V})

$CH_3AsO(OH)_2$

Monomethylarsonous acid (MMA^{III})

$CH_3As(OH)_2$

Dimethylarsinic acid (DMA^{V}):

$(CH_3)_2AsO(OH)$

Dimethylarsinous acid (DMA^{III}):

$(CH_3)_2AsOH$

APPENDIX 1

SUMMARY OF CARCINOGENIC EFFECTS OF ARSENIC ACID AND ITS SALTS

1. INTRODUCTION

A review of the documentation for the carcinogenic effects of arsenic acid and selected salts is presented in this section to support the proposal of "Arsenic acid and its salts" as Substances of Very High Concern under REACH. The classification of arsenic acid and its salts are presented in table 6 according to criteria in the Regulation (EC) No 1272/2008 as amended (CPL regulation).

The substances listed in table 1 are the target for this review.

Table 1 Overview of arsenic compounds addressed by this review

Substance name	Molecular formula	CAS no.	Water solubility[1]
Arsenic acid	AsH3O4	7778-39-4	302 g/L at 12.5 °C
Calcium arsenate	Ca3(AsO4)2	7778-44-1	0.13 g/L at 25 °C
Trilead diarsenate	Pb3(AsO4)2	3687-31-8	Sparingly soluble

[1]data from IUCLID5

For all three substances arsenic is in the pentavalent (+5) state. The water solubility is presented in table 1.

Arsenic acid and the two mentioned arsenates are all covered by Index number 033-005-00-1, “arsenic acid and its salts” in Part 3 of Annex VI, of the CLP Regulation as amended with a harmonised classification as carcinogens in category 1A. The full classification is shown in table 2.

Table 2 CLP classification of arsenic acid and its salts

International Chemical Identification	Hazard Class and category code(s)	Hazard Statement Code(s)
arsenic acid and its salts	Carc. 1A	H350
	Acute Tox. 3 *	H331
	Acute Tox. 3 *	H301
	Aquatic Acute 1	H400
	Aquatic Chronic 1	H410

Trilead diarsenate is also covered by Index number 082-001-00-6 “Lead compounds with the exception of those specified elsewhere in this Annex” in Annex VI, of the CLP Regulation with harmonised classification as toxic for reproduction Repr 1A; H360Df .

2. EXPOSURE

For the general population the main route of exposure is by the oral route, whereas occupational exposure is predominantly through inhalation and to a much lesser degree through dermal exposure.

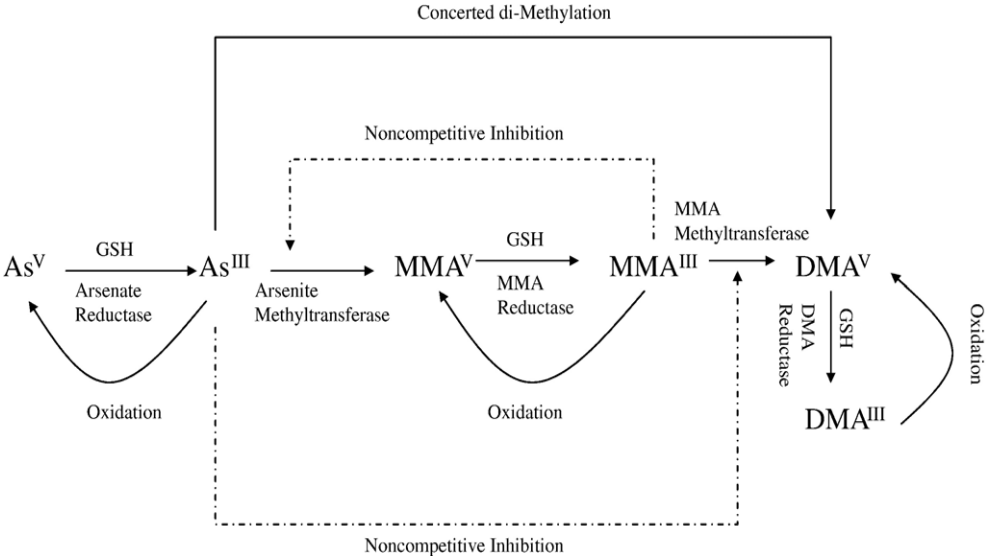
3. TOXICOKINETICS

3.1 Biotransformation of inorganic arsenic compounds

Soluble inorganic arsenic compounds are rapidly absorbed after oral exposure (about 70–90%) (Pomroy et al., 1980; Vahter and Norin, 1980; Freeman et al., 1995), but less well after inhalation (Beck et al., 2002), and dermal exposure (1 – 6%) (Wester et al., 1993). Absorbed arsenic is transported, mainly bound to SH groups in proteins and low-molecular-weight compounds such as glutathione (GSH) and cysteine, to different organs in the body (IARC, 2004).

Biotransformation of inorganic arsenic is characterized by two main types of reactions, i.e. reduction reactions where pentavalent arsenic is reduced to trivalent arsenic, and oxidative methylation where the trivalent arsenic forms are methylated to form mono- and dimethylated products. Once absorbed, arsenates in the pentavalent state (As^{V}) are rapidly reduced to arsenates (As^{III}) through a reaction requiring glutathione (GSH) and the distribution of As^{V} and As^{III} metabolites is therefore very similar as long as the methylation capacity is not exceeded (IARC, 2004). Inorganic arsenic is metabolized via methylation. The methylation occurs through alternating reductive and oxidative methylation reactions, that is, reduction of As^{V} to As^{III} followed by addition of one or two methyl groups. The methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) occurs mainly in the liver and S-adenosylmethionine is considered to be the main methyl donor (IARC, 2004). The glutathione (GSH) complexes formed in the reactions can decompose to yield GSH and MMA^{III} or DMA^{III}, which can then form MMA^{V} and DMA^{V} respectively. Limited short-term studies on humans indicate that the capacity to methylate inorganic arsenic is progressively, but not completely, saturated when daily intake exceeds 0.5 mg (WHO, 2003). An illustration of the biotransformation of inorganic arsenic is shown in figure 1.

Figure 1. Biotransformation of inorganic arsenic (from Clewell et al. 2007)



In humans most of the arsenic in blood is rapidly cleared, following a three-exponential clearance curve (Pomroy et al., 1980). The majority of arsenic in blood is cleared with a half-time of about 2 or 3 h. The half-times of the second and third phases are about 168 and 240 h, respectively (IARC 2004). In human subjects exposed chronically to arsenic, the hair and nails generally show the highest concentrations. Thus, arsenic appears to concentrate in tissues with a high content of cysteine-containing proteins like hair and nails, liver, kidney, blood, squamous epithelium of the upper gastrointestinal tract, epididymis, thyroid, skeleton and lens (IARC, 2004). Inorganic arsenic and methylated metabolites cross the placenta barrier, but do not readily cross the blood–brain barrier (IARC, 2004). In humans most inorganic arsenic is excreted in the urine as a mixture of As^{III}, As^{V}, MMA, and DMA and smaller amounts via faeces. The relative amounts of species in urine are generally 10–30% inorganic arsenic, 10–20% MMA_{total} and 60–80% DMA_{total} although there is a wide variation among individuals (Vahter and Concha, 2001). Hamsters are considered a suitable animal model for toxicokinetic studies since its urinary profile of arsenic species resembles that of humans following exposure to inorganic arsenic.

3.2 Toxicokinetics

Arsenic acid is readily soluble in water (302 g/L at 12.5ºC; IUCLID5). Very few studies on toxicokinetics of arsenic acid are available in the open literature. However, in one study by Odanaka and co-workers (1980) arsenic acid was administered orally to mice, hamsters and rats. In all species the absorption via GI was above 40% and DMA, MMA and inorganic-As compounds were measured in urine and faeces.

In the open literature there are few studies examining toxicokinetics of calcium arsenate. The watersolubility of calcium arsenate is 0.13 g/L at 25ºC (IUCLID5) and it has been shown that after six days in 0.9% saline solution (50 mg As/L at 37ºC) 23% of the added calcium arsenate was dissolved (Pershagen et al., 1982). In hamsters given weekly intratracheal instillations of calcium arsenate dust suspension (for 2 to 5 weeks), arsenic was measured in

both liver and hair. High concentration of calcium arsenate was retained in the lungs (Pershagen et al., 1982). Calcium arsenate has also been found in lungs after intratracheal instillations for a long period of time (50% retained after 7 days) in rats (Inamasu et al., 1982).

The available data for trilead diarsenate and toxicokinetic is sparse. Trilead diarsenate is sparingly soluble in water (IUCLID5). In a study by Marafante and Vahter (1987) the absorption and biotransformation following intratracheal and oral administration of radiolabel ^{74}As in trilead diarsenate (suspension) was examined. Groups of four hamsters were administrated 2 mg/kg bw intratracheally or orally. Three days after intratracheal administration, approximately 45% of the trilead diarsenate was retained in the lungs, while 1.2% was found in the carcase. The excretion via urine and faeces was 33% and 6%, respectively (Table 3). Hence, the absorption after intratracheal administration was approximately 40%. After oral administration, 70-80% of the dose was detected in the faeces. The faecal elimination following intratracheal administration was low (6%) and indicate that biliary and intestinal excretion most likely contribute little to the total elimination following oral administration. Twenty two percent of the dose was found in urine. The absorption of trilead diarsenate via the gastrointestinal tract is estimated to be between 20-30% (Table 3). After oral administration, most of the ^{74}As was measured as DMA metabolite in the urine (17%), while after intratracheal administration only 9% of the ^{74}As was found as DMA and 20% was detected as As^V.

Table 3 Faecal and urinary elimination of total ^{74}As and urinary excretion of ^{74}As-metabolites in hamsters during three days following intratracheal or oral administration of ^{74}As trilead diarsenate

				^{74}As-metabolites in urine		
Compound	**Administration route**	**Faeces**	**Urine**	**As^{III}**	**As^V**	**DMA**
$Pb_3(AsO_4)_2$	intratracheal	6.5±0.8	32.8±1.5	2.2±0.4	20.2±1.5	9.0±0.2
$Pb_3(AsO_4)_2$	oral	68.8±4.4	22.2±3.4	1.9±0.8	5.8±1.0	17.0±2.4

Figures represent percentage of the dose. Mean of four hamsters ± SE.

3.3 Summary of toxicokinetics

Arsenic acid is soluble in water and absorption following oral administration of experimental animals is high. Inorganic arsenic species and metabolites were measured in urine from arsenic acid exposed animals. Calcium arsenate is soluble in water and in saline solution *in vitro*. Its bioavailability is supported by the measurement of arsenic in hair and liver after intratracheal instillation in hamsters. Trilead diarsenate is sparingly soluble in water, but absorption via intratracheal or oral administration in hamsters was found to be 40% and 30%, respectively. The As-species As^{III}, As^V and DMA were measured in urine after trilead

diarsenate exposure in hamsters. These findings show that arsenic acid, calcium arsenate and trilead diarsenate are bioavailable and that exposure to these substances leads to systemic exposure to inorganic arsenic.

4. GENOTOXICITY

Arsenicals (inorganic and organic arsenic compounds) have not been shown to have mutagenic effects in Ames test (reviewed in IARC, 2004). The methylated forms of trivalent arsenic are the only arsenic species that cause DNA damage *in vitro.* Arsenicals do not react directly with DNA but oxidative damage is seen in cells treated with low concentrations of $As^{III.}$.

Kligerman *et al.* (2003) have evaluated the arsenicals As^{V}, As^{III} and their MMA and DMA counterparts, in different assays[3] and found that MMA^{III} and DMA^{III} were the most potent clastogens of the six arsenicals in human lymphocytes and the most potent mutagens at the Tk(+/-) locus in mouse lymphoma cells. The dimethylated arsenicals were also spindle poisons, suggesting that they may be ultimate forms of arsenic that induce aneuploidy. Although the arsenicals were potent clastogens, none were potent SCE inducers, similar to clastogens that act via reactive oxygen species. None of the six arsenicals (As^{V}, As^{III} and their MMA and DMA counterparts) were gene mutagens in Salmonella TA98, TA100, or TA104; and neither MMA^{III} nor DMA^{III} induced prophage. The results show that both methylated As^{V} compounds were less cytotoxic and genotoxic than As^{V}, whereas both methylated As^{III} compounds were more cytotoxic and genotoxic than As^{III}. The results support the view that MMA^{III} and DMA^{III} are candidate ultimate genotoxic forms of arsenic and that they are clastogens and not gene mutagens. The authors suggest that the clastogenicity of the other arsenicals is due to their metabolism by cells to MMA^{III} or DMA^{III}.

Other studies of micronuclei (MN) induced by As^{III} in human fibroblasts have shown that at lower (relatively non-toxic) doses, As^{III} acts as an aneugen, while at high (toxic) doses it acts as a clastogen and that aneuploidy is seen after treatment with As^{III} concentrations lower than those that cause chromosomal aberration (Sciandrello *et al.*, 2004). Studies of humans in West Bengal, India exposed to high concentrations of inorganic arsenic in drinking water also showed a significantly higher frequency of micronuclei in oral mucosal cells, bladder epithelial cells and peripheral lymphocytes (IARC, 2004).

Jensen *et al.* (2008) have demonstrated that malignant transformation of human urothelial cells by arsenicals is also associated with changes in histone acetylation and DNA methylation in gene promoter regions. Other studies have also shown altered DNA methylation in arsenic-exposed humans.

Fischer *et al.* (2005) have shown that As^{III} can act as a co-mutagen and enhance the mutagenicity of other agents like BaP. Other studies have shown that this may occur by interference with both nucleotide-excision repair and base-excision repair (BER). BER is crucial for development and for the repair of endogenous DNA damage. However, unlike nucleotide excision repair, the regulation of BER is not well understood. Arsenic is known to produce oxidative DNA damage, which is repaired primarily by BER, whilst high doses of

[3] Induction of chromosome aberrations, sister chromatid exchanges (SCE), toxicity in cultured human peripheral blood lymphocytes, mutagenicity in L5178Y/Tk(+/-) mouse lymphoma cells, the Salmonella reversion assay; and prophage-induction in Escherichia coli.

arsenic can also inhibit DNA repair. Studies by Sykora and Snow (2008) have shown that there is evidence that changes in BER due to low doses of arsenic could contribute to a non-linear, threshold dose response for arsenic carcinogenesis.

Arsenic induces cell transformation in Syrian hamster embryo cells (SHE), BALB/3T3 cells and in the rat liver cell line TRL1215. Inoculation of the latter cells into nude mice gave rise to malignant tumours (fibrosarcoma and metastases to the lung) (IARC, 2004). The SHE cell-transformation assay represents a short-term in vitro assay capable of predicting rodent carcinogenicity of chemicals with a high degree of concordance. Induction of malignant transformation in the normally non-tumorigenic rat liver epithelial cell line (TRL 1215), and the chronic arsenic-exposed cells produce invasive and metastatic tumours upon inoculation into nude mice (the immunodeficient nude mice do not reject tumour transplantations from other species).

5. CARCINOGENICITY

5.1 CLP classification

In Annex VI of the CLP regulation the arsenic compounds shown in table 4 are classified as carcinogenic in category 1A.

Table 4 Arsenic compounds classified as carcinogenic in category 1A

Index No.	CAS No.	Substance name
033-003-00-0	1327-53-3	diarsenic trioxide
033-004-00-6	1303-28-2	diarsenic pentaoxide
033-005-00-1	-	arsenic acid and its salts with the exception of those specified elsewhere in this Annex
601-067-00-4	15606-95-8	triethyl arsenate
028-038-00-3	13477-70-8	trinickel bis (arsenate)
082-011-00-0	7784-40-9	lead hydrogen arsenate
028-051-00-4	12068-61-0 [1] 27016-75-7 [2]	nickel diarsenide nickel arsenide

5.2 IARC Classification

Arsenic and arsenic compounds were evaluated previously as being carcinogenic to humans (Group 1) on the basis of sufficient evidence of an increased risk for skin cancer among patients exposed to inorganic arsenic through medical treatment, and an increased risk for lung cancer among workers involved in mining and smelting, who inhaled inorganic arsenic

(IARC, 1980, 1987). In a more recent report, IARC concluded that there is sufficient evidence in humans that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin (IARC, 2004).

In 2009 IARC reconfirmed the classification of arsenic and inorganic arsenic compounds as "carcinogenic to humans" (group 1) (Straif *et al.*, 2009; IARC monograph vol 100C, in press). The working group made the overall evaluation on a group "arsenic and inorganic arsenic compounds" rather than on some individual arsenic compounds, based on the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and data on the chemical characteristics, metabolism and modes of action of carcinogenicity. The common metabolic pathway of elemental and inorganic arsenic species was underlined.

5.3 Human information

There is evidence from a large number of epidemiological studies and case reports that exposure to inorganic arsenic increases the risk of developing cancer (reviewed in IARC, 2004; ATSDR, 2007). In humans exposed chronically to inorganic arsenic by the oral route, from food or drinking water, skin tumours are the most common type of cancer, but other internal tumours in bladder and lungs, and to a lesser extent, liver, kidney, and prostate are also reported from epidemiological studies and case reports.

In drinking water, arsenic in the form of arsenic acid (arsenate, As^{V}) and arsenous acid (arsenite, As^{III}) are considered the causative agents behind the carcinogenicity demonstrated in a broad range of epidemiological studies. Epidemiological studies form the basis for the classification of arsenic in drinking-water and they reveal a dose-response trend of ingested arsenic on skin and lung cancer risk. A few of the studies on exposure to arsenic in drinking-water and risk of skin or lung cancers are summarised in Table 5. Some central studies showing an association between exposure and cancer are shortly presented below.

Several studies conducted in arseniasis (i.e. chronic arsenic poisoning) endemic areas have found elevated risks for skin, lung and bladder cancer associated with levels of arsenic in drinking water. An ecological study from south-west Taiwan, Tseng et al. (1968) found an

eightfold difference in the prevalence of skin cancer lesions from the highest (> 600 µg/L) to

the lowest category (< 300 µg/L) of arsenic concentration in artesian wells, after an extensive

examination survey of 40 421 inhabitants in 37 villages. A more recent ecological study from

northern Chile showed that the relative risks for lung and bladder cancer peaked in the years 1980–2000 in a region that experienced strong increases in drinking-water arsenic contamination in the years between 1950-1970 (Marshall et al., 2007).

A case-control study from northern Chile revealed significantly increasing risks of lung cancer with increasing levels of arsenic in drinking-water (Ferreccio et al., 2000). Clear trends in dose-response were found when concentrations were averaged over the years 1930–1994 and also when the peak exposure period 1958–1970 was considered.

In a cohort from south-west Taiwan, Chen et al. (1986) observed a dose–response relationship between the duration of consumption of artesian well water containing high levels of arsenic and lung cancer mortality risk. A study of combined cohorts in south-west and north-east Taiwan found a synergistic interaction between arsenic in drinking water and cigarette smoking (Chen et al., 2004).

A summary of epidemiological studies of workers exposed to As_2O_3 in smelters is presented in the background document to the opinion proposing harmonised classification and labelling at community level for gallium arsenide (RAC, 2010).

Table 5 Summary of selected epidemiological studies of inorganic arsenic in drinking water and risk of skin or lung cancer. From RAC

Design	Country	Study size	Adjusted for confounders	Comment	Concentration µg/L water	*No. of observations,* Risk estimate#, (95% confidence interval)	Reference
Ecologic	Taiwan	40,421		Incidence of skin cancer was measured as a function of exposure level in over 40,000 people residing in 37 villages, and compared to a control group of 7,500 people with low arsenic exposure. No skin cancers were found in the control group.	>600	Skin cancer, *428* Prevalence rate (per 1000) Overall: 10.6, 21.4	Tseng et al. 1968
Cohort study	Taiwan	10,591	Adjusted for risk factors, including cigarette smoking	Relative risk of lung cancer was related to arsenic exposure level in 2503 residents in southwester and 8088 in northeastern arseniasis-endemic areas.	≥700 (village median)	Lung cancer, *139* Relative Risk Overall: 3.29 (1.60-6.78), Non-smokers: 2.21 (0.71-6.86)	Chen et al. 2004
Case-control study	Chile	570	Adjusted for risk factors, including cigarette smoking and working in copper smelting industry	Hospital based study using frequency-matched hospital controls. Relative risk of lung cancer was related to arsenic exposure level.	200-400 (average value 1930-94) ≥700 (average concentration 1958-1970; peak exposure period)	Lung cancer, *151* Odds Ratio 8.9 (4.0-19.6) 7.1 (3.4-14.8)	Ferreccio et al. 2000

2010.

5.4 Non-human information

In general, animal models seem to be less sensitive than humans to the carcinogenic effect of arsenic. However, mouse models with transplacental or whole life exposures induces tumours in multiple tissues including lung and liver that are known or suspected human target sites of inorganic arsenic compounds (reviewed in Tokar et al., 2010; Waalkes et al., 2003, 2004, 2006a, 2006b; Tokar et al., 2011). Furthermore, oral sodium arsenate in the drinking water enhanced lung tumour multiplicity and lung tumor size in male mice (Cui et al., 2006) and several animal studies on DMA, has demonstrated carcinogenicity (reviewed in Tokar et al., 2010). In two hamster studies, weekly intratracheal administration of calcium arsenate induced significant increase in lung tumours (adenomas and an adenocarcinoma) when the animals were observed over their life span (Pershagen and Björklund, 1985; Yamamoto et al., 1987).

5.5 Mechanism of carcinogenicity

The knowledge of arsenic biotransformation holds the trivalent methylated and non-methylated species accountable for most arsenic toxicity. Effects such as oxidative DNA damage, genomic instability, aneuploidy, gene amplification, epigenetic effects, DNA-repair inhibition leading to mutagenesis and cell proliferation, oxidative stress, co-carcinogenesis and tumour promotion have been suggested as mechanisms for the carcinogenic effects of arsenic (Straif *et al.*, 2009). Transgenic animal model deficient in methylation or in repair of oxidative DNA lesions have been used to study mechanisms of toxicity and carcinogenicity of arsenic compounds (Yokohira, 2011; Kinoshita, 2007). However, most of these mechanisms remain poorly understood with regard to the various organs affected by the inorganic arsenic compounds. A better understanding is also required with regard to the exact dose at which arsenic induces tumours *in vivo*.

Available data indicates a complex mode of action for the toxicity of inorganic arsenicals and no threshold has been established for the induction of cancer.

6. CONCLUSION

Several inorganic arsenicals, including arsenic acid and its salts are classified as carcinogenic to humans in category 1A (CLP regulation, Annex VI; IARC 1980, 1987, 2004) based on epidemiological studies of carcinogenicity from occupational inhalation exposure and exposure via drinking water. Although animal models seem to be less sensitive than humans to the carcinogenic effect of arsenic, recent rodent studies confirm the carcinogenicity of inorganic arsenicals.

There is no human data for the individual arsenates *per se*, but substantial documentation of carcinogenicity in humans of arsenic and arsenic compounds in the trivalent and pentavalent state is available. Results from animal cancerstudies are available for specific compounds including calcium arsenate. Furthermore, animal studies support that arsenic acid, calcium arsenate and trilead arsenate are bioavailable and lead to systemic release of arsenic species.

Due to the classification of, “Arsenic acid and its salts” as carcinogenic in category 1A it is recommended to identify arsenic acid, calcium arsenate and trilead diarsenate as SVHC's based on this classification .

7. REFERENCES

ATSDR (2007): Toxicological Profile for Arsenic, Public Health Statement. Available at http://www.atsdr.cdc.gov/ toxprofiles/phs2.html.

Beck BD, Slayton TM, Farr CH, Sved DW, Crecelius EA, Holson JF (2002): Systemic uptake of inhaled arsenic in rabbits. Hum Exp Toxicol 21(4):205-15.

Clewell HJ, Thomas RS, Gentry PR, Crump KS, Kenyon EM, El-Masri HA, Yager JW (2007): Research toward the development of a biologically based dose response assessment for inorganic arsenic carcinogenicity: A progress report. Toxicol Appl Pharmacol 222(3): 388-98.

Chen CJ, Chuang YC, You SL, Lin TM, Wu HY (1986): A retrospective study on malignant neoplasms of bladder, lung and liver in blackfoot disease endemic area in Taiwan. Br J Cancer 53:399–405.

Chen CL, Hsu LI, Chiou HY, Hsueh YM, Chen SY, Wu MM, Chen CJ (2004): Ingested arsenic, cigarette smoking, and lung cancer risk: a follow-up study in arseniasis-endemic areas in Taiwan. JAMA 292(24):2984-90.

Cui X, Wakai T, Shirai Y, Hatakeyama K, Hirano S (2006): Chronic oral exposure to inorganic arsenate interferes with methylation status of p16INK4a and RASSF1A and induces lung cancer in A/J mice. Toxicol Sci 91:372–81.

Ferreccio C, González C, Milosavjlevic V, Marshall G, Sancha AM, Smith AH (2000): Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology 11(6):673-9.

Fischer JM, Robbins SB, Al-Zoughool M, Kannamkumarath SS, Stringer SL, Larson JS, Caruso JA, Talaska G, Stambrook PJ, Stringer JR (2005): Co-mutagenic activity of arsenic and benzo[a]pyrene in mouse skin. Mutat Res 588:35–46.

Freeman GB, Schoof RA, Ruby MV, Davis AO, Dill JA, Liao SC, Lapin CA, Bergstrom PD (1995): Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam Appl Toxicol 28(2): 215-22.

HSDB (2011): Hazardous Substances Data Bank (HSDB®). National Library of Medicine's (NLM) Toxicology Data Network (TOXNET®). http://www.nlm.nih.gov/pubs/factsheets/hsdbfs.html#

IARC. (1980): IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 23, Some Metals and Metallic Compounds. Lyon: IARC Press, 39–143.

IARC. (1987): IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42, Lyon: IARC Press, 100–6.

IARC (2004): IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol. 84, Some Drinking-water Disinfectants and Contaminants, including Arsenic, Lyon, IARC. 41–267.

Inamasu T, Hisanaga A, Ishinishi N (1982): Comparison of arsenic trioxide and calcium arsenate retention in the rat lung after intratracheal instillation. Toxicol Lett 12(1):1-5.

IPCS (1992): Inorganic arsenic compounds other than arsine. Health and Safety Guide 70.IPCS International Programme on Chemical Safety, Geneva. http://www.inchem.org/documents/hsg/hsg/hsg070.htm

IUCLID 5: European Chemicals Agency. ECHA website database. (Internet). (Updated 31 Mar 2011; downloaded 1 Apr 2011). Available from: http://apps.echa.europa.eu/registered/registered-sub.aspx.

Jensen TJ, Novak P, Eblin KE, Gandolfi AJ, Futscher BW (2008): Epigenetic remodeling during arsenical-induced malignant transformation. Carcinogenesis 29: 1500-8.

Kinoshita A, Wanibuchi H, Morimura K, Wei M, Nakae D, Arai T, Minowa O, Noda T, Nishimura S, Fukushima S (2007): Carcinogenicity of dimethylarsinic acid in Ogg1-deficient mice. Cancer Sci 98:803–14.

Kligerman AD, Doerr CL, Tennant AH, Harrington-Brock K, Allen JW, Winkfield E, Poorman-Allen P, Kundu B, Funasaka K, Roop BC, Mass MJ, DeMarini DM (2003): Methylated trivalent arsenicals as candidate ultimate genotoxic forms of arsenic: induction of chromosomal mutations but not gene mutations (abstract). Environ Mol Mutagen 42(3): 192-205.

Marafante E, Vahter M (1987): Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. Environ Res 42(1):72-82**.**

Marshall G, Ferreccio C, Yuan Y, Bates MN, Steinmaus C, Selvin S, Liaw J, Smith AH (2007): Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. J Natl Cancer Inst 99(12):920-8.

Odanaka Y, Matano O, Goto S (1980): Biomethylation of inorganic arsenic by the rat and some laboratory animals. Bull.Environm.Contam. Toxicol 24:452-9.

Pershagen G, Lind B, Björklund NE (1982): Lung retention and toxicity of some inorganic arsenic compounds. Environ Res 29(2): 425-34.

Pershagen G, Björklund NE. (1985): On the pulmonary tumorigenicity of arsenic trisulfide and calcium arsenate in hamsters. Cancer Lett 27:99–104.

Pomroy C, Charbonneau SM, McCullough RS, Tam GK (1980): Human retention studies with 74As. Toxicol Appl Pharmacol 53(3):550-6.RAC (2010) (Committee for Risk Assessment): Annex 1. Background document to the Opinion proposing harmonised classification and labelling at Community level of gallium arsenide. ECHA/RAC/CLH-0000000792-73-03/A1.

Sciandrello G, Caradonna F, Mauro M, Barbata G (2004): Arsenic-induced hypomethylation affects chromosome instability in mammalian cells. Carcinogenesis 25:413-7.

Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Guha N, Freeman C, Galichet L, Cogliano V; WHO International Agency for Research on Cancer Monograph Working Group (2009): Special Report: Policy. A review of human carcinogens - Part C: metals, arsenic, dusts, and fibres. Lancet Oncology 10:453-4.

Sykora P, Snow ET (2008): Modulation of DNA polymerase beta-dependent base excision repair in cultured human cells after low dose exposure to As^{III}. Toxicol Appl Pharmacol 228:385-94.

Tokar EJ, Benbrahim-Tallaa L, Ward JM, Lunn R, Sams RL 2nd, Waalkes MP (2010): Cancer in experimental animals exposed to arsenic and arsenic compounds. Crit Rev Toxicol 40(10):912-27. Review.

Tokar EJ, Diwan BA, Ward JM, Delker DA, Waalkes MP (2011): Carcinogenic effects of "whole-life" exposure to inorganic arsenic in CD1 mice. Toxicol Sci 119(1):73-83.

Tseng WP, Chu HM, How SW, Fong JM, Lin CS, Yeh S (1968): Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J Natl Cancer Inst 40(3):453-63.

Vahter M, Concha G (2001): Role of metabolism in arsenic toxicity. Pharmacol Toxicol 89(1):1-5.

Vahter M, Norin H (1980): Metabolism of 74As-LabeIed Trivalent and Pentavalent Inorganic Arsenic in Mice. Environ Res 21:446-57.

Waalkes MP, Ward JM, Liu J, Diwan BA (2003): Transplacental carcinogenicity of inorganic arsenic in the drinking water: Induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. Toxicol Appl Pharmacol 186:7–17.

Waalkes MP, Ward JM, Diwan BA (2004): Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: Promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis 25:133–41.

Waalkes MP, Liu J, Ward JM, Powell DA, Diwan BA (2006a): Urogenital carcinogenesis in female CD1 mice induced by in utero arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. Cancer Res 66:1337–45.

Waalkes MP, Liu J, Ward JM, Diwan BA (2006b): Enhanced urinary bladder and liver carcinogenesis in male CD1 mice exposed to transplacental inorganic arsenic and postnatal diethylstilbestrol or tamoxifen. Toxicol Appl Pharmacol 215:295–305.

Wester RC, Maibach HI, Sedik L, Melendres J, Wade M (1993): In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol 20(3):336-40.

WHO (2003): Arsenic in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Geneva, World health Organisation (WHO/SDE/WSH/03.04/75).

Yamamoto A, Hisanaga A, Ishinishi LN (1987): Tumorigenicity of inorganic arsenic compounds following intratracheal instillations to the lungs of hamsters. Int J Cancer 40:220–3.

Yokohira M, Arnold LL, Pennington KL, Suzuki S, Kakiuchi-Kiyota S, Herbin-Davis K, Thomas DJ, Cohen SM (2011): Effect of sodium arsenite dose administered in the drinking water on the urinary bladder epithelium of female arsenic (+3 oxidation state) methyltransferase knockout mice. Toxicol Sci 121(2):257-66.

APPENDIX 2: CONFIDENTIAL ANNEX