

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): Trilead diarsenate

EC Number(s): 222-979-5

CAS Number(s): 3687-31-8

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

Substance Name(s): Trilead diarsenate

EC Number(s): 222-979-5

CAS number(s): 3687-31-8

- 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 1A¹ which corresponds to classification as carcinogen category 1².
- The substance is proposed to be identified as a substance meeting the criteria of Article 57 (c) of Regulation (EC) 1907/2006 (REACH) owing to its classification as reproductive toxicant 1A¹ which corresponds to classification as toxic to reproduction category 1².

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

Trilead diarsenate 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)³. 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 arsenic acid meets the criteria for classification as carcinogenic in accordance with Article 57(a) of REACH.

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 Regulation (EC) No 1272/2008, Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) and classified for reproductive toxicity in Repr. 1A (H360Df: May damage the unborn child. Suspected of damaging fertility.) The corresponding classification in part 3 of Annex VI, Table 3.2 (list of harmonized classification and labelling of hazardous substances

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).

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from Annex I of Council Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is toxic to reproduction, Repr. Cat. 1; (R61 May cause harm to the unborn child) and Repr. Cat. 3; (R62; Possible risk of impaired fertility).

This classification of the substance in Regulation (EC) No 1272/2008 shows that trilead diarsenate meets the criteria for classification and as reproductive toxicant in accordance with Article 57 (c) of REACH.

Registration dossiers submitted for the substance? YES

PART I

JUSTIFICATION

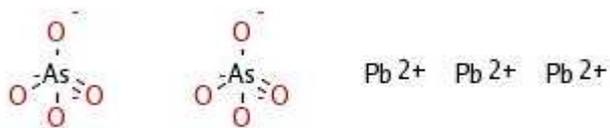
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	222-979-5
EC name:	Trilead diarsenate
CAS number (in the EC inventory):	3687-31-8
CAS number:	3687-31-8
CAS name:	Arsenic acid (H ₃ AsO ₄), lead salt (2:3)
IUPAC name:	Trilead (2+) diarsenate
Index number in Annex VI of the CLP Regulation	033-005-00-1 082-001-00-6
Molecular formula:	As ₂ O ₈ Pb ₃
Molecular weight range:	899.4
Synonyms:	Lead (II) arsenate, lead arsenate

Structural formula:



Source: ESIS

1.2 Composition of the substance**Name:** Trilead diarsenate**Description:****Degree of purity:** Information on purity is provided in the Confidential Annex.**Table 2: Constituents**

Constituents	Typical concentration	Concentration range	Remarks
Trilead diarsenate			

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	Solid, crystals or powder		ECHA website
Melting point	1042 °C		IARC 2006
Boiling point	Decomposes	No data for starting temperature for decomposition available	ECHA website
Vapour pressure			
Water solubility	Sparingly soluble	No specific data found	ECHA website
Partition coefficient n-octanol/water (log value)			
Dissociation constant			

2 HARMONISED CLASSIFICATION AND LABELLING

Trilead diarsenate is covered by Index number 033-005-00-1, “arsenic acid and its salts”, and by index number 082-001-00-6, “lead compounds with the exception of those specified elsewhere in this Annex”, 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	H350	GHS06	H350			A
				Acute Tox. 3 *	H331	GHS08	H331			
					H301	GHS09	H301			
				Acute Tox. 3 *	H400	Dgr	H410			
					H410					
082-001-00-6	lead compounds with the exception of those specified elsewhere in this Annex			Repr. 1A	H360Df	GHS08	H360Df		Repr. 2; H361f: C ≥ 2,5 % * STOT RE 2; H373: C ≥ 0,5 %	A 1
				Acute Tox. 4 *	H332	GHS07	H332			
					H302	GHS09	H302			
				Acute Tox. 4 *	H373	Dgr	H373			
					**		**			
				STOT RE 2 *	H400		H410			
	H410									
	Aquatic Acute 1									
	Aquatic Chronic 1									

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
082-001-00-6	lead compounds with the exception of those specified elsewhere in this Annex			Repr. Cat. 1; R61 Repr. Cat. 3; R62 Xn; R20/22 R33 N; R50-53	T; N R: 61-20/22-33-62-50/53 S: 53-45-60-61	Repr. Cat. 3; R62: C \geq 2,5 % Xn; R20/22: C \geq 1 % R33: C \geq 0,5 %	AE 1

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.

A summary of toxicity to reproduction of lead compounds is found in appendix 2.

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

Trilead diarsenate 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)⁴. 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 arsenic acid meets the criteria for classification as carcinogenic in accordance with Article 57(a) of REACH.

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 Regulation (EC) No 1272/2008, Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) and classified for reproductive toxicity in Repr. 1A (H360Df: May damage the unborn child. Suspected of damaging fertility.) The corresponding classification in 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 is toxic to reproduction, Repr. Cat. 1; (R61 May cause harm to the unborn child) and Repr. Cat. 3; (R62; Possible risk of impaired fertility).

This classification of the substance in Regulation (EC) No 1272/2008 shows that trilead diarsenate meets the criteria for classification and as reproductive toxicant in accordance with Article 57 (c) 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

Manufacture of trilead diarsenate is registered in the EU. Further information is provided in a Confidential Annex to this report.

1.2 Manufacturing process

Trilead diarsenate is present in complex raw materials from smelting and refining of nonferrous metals used for manufacture of copper, lead and a range of precious metals (Arsenic consortium, 2011, see furthermore Chapter 8). The raw materials are to some extent imported from countries outside the EU.

A flow sheet showing a typical metallurgical process involving raw materials containing trilead diarsenate is shown in the Confidential Annex.

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

The registered quantities of trilead diarsenate manufactured are in the range of 10-100 tons per year. More information on registered manufactured tonnages is provided in the Confidential Annex.

1.4 Trends

According to the Arsenic Consortium the decreasing markets and use 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). A decrease in the production of diarsenic trioxide will probably result in a larger part of the trilead diarsenate converted to calcium arsenate disposed of with waste, but would probably not influence the import of the trilead diarsenate with the complex raw materials.

Import of trilead diarsenate on its own has not been registered.

1.5 Import of the substance in mixtures

Trilead diarsenate is partly imported from countries outside Europe as part of complex materials i.e. byproducts from nonferrous metallurgical processes. The quantities of trilead diarsenate in the imported byproducts are described further in the Confidential Annex.

1.6 Releases from manufacture

No specific data on the releases of trilead diarsenate from the manufacturing process is available. The emission of arsenic from the process is monitored, however, the emission of arsenic from the process cannot be allocated to the different steps and/or different arsenic compounds in the raw materials. The total emission from manufacturing of the product, diarsenic trioxide is addressed in the next section.

2 USES OF THE SUBSTANCE

2.1 Use for the manufacturing of diarsenic trioxide

2.1.1 Function of the substance

Some of the arsenic from the trilead diarsenate ultimately ends up in diarsenic trioxide, and trilead diarsenate may according to the Arsenic Consortium (2011) be considered an intermediate in the production of diarsenic trioxide.

2.1.2 Quantities involved

The quantities involved are further described in the Confidential Annex.

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".

Table 8: Process descriptors and environmental releases categories for the use of trilead diarsenate

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, trilead diarsenate

2.1.3 Final use of the manufactured diarsenic trioxide

Some of the arsenic in the trilead diarsenate ultimately ends up in diarsenic trioxide.

The total estimated manufactured volume of diarsenic trioxide in the EU is about 1,800 t/y, but only a fraction of this is used in the EU. 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 tons while 1,200-1,300 is exported. About 200 tons was disposed of as waste.

3 RELEASES FROM THE USE OF TRILEAD DIARSENATE

3.1 Use for the manufacturing of diarsenic trioxide

3.1.1 Occupational exposure

No data on the occupational exposure to trilead diarsenate have been available. Arsenic is measured in different workplaces in the plant, but the exposure cannot be allocated to the specific manufacturing and use of trilead diarsenate.

4 RELEASES TO THE ENVIRONMENT

No data on the release of trilead diarsenate is available. The release of arsenic associated with the use of the trilead diarsenate is included in the total reported arsenic releases from the metallurgical processes. Information on the releases from the processes is reported in the Confidential Annex.

5 CONCLUSIONS ON MANUFACTURE, USES AND EXPOSURE

Based on the available information the following can be concluded:

- The trilead diarsenate is imported in complex byproducts from smelting and refining of nonferrous metals
- A part of the arsenic in the trilead diarsenate ultimately ends up in diarsenic trioxide (As_2O_3).
- Trilead diarsenate is not used by downstream users or present in any consumer products
- No data on the emission of trilead diarsenate or occupational exposure to trilead arsenate are available; emissions and workplace concentrations are measured as arsenic (As) and cannot be allocated to the individual arsenic compounds involved in the manufacturing of diarsenic trioxide and nonferrous metals

6 CURRENT KNOWLEDGE ON ALTERNATIVES

Trilead diarsenate is used as raw material in the manufacture of diarsenic trioxide from complex byproducts from metallurgical processes.

Trilead diarsenate constitutes a minor part of the arsenic in the raw materials and other arsenic compounds could be used for the manufacturing of the diarsenic trioxide as well. Large quantities of other arsenic compounds, generated as byproducts from metallurgical processes, are today disposed of as waste.

A substitution of diarsenic trioxide would result in a larger part of the arsenic in the trilead diarsenate ending up in waste from the metal recovery process.

6.1 Alternatives to diarsenic trioxide

The discussion on alternatives to trilead diarsenate 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 Sperimentale 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.

6.2 Conclusions on alternatives

Based on the available information the following can be concluded:

- Diarsenic trioxide may be manufactured without the use of trilead diarsenate as raw material
- A substitution of diarsenic trioxide would result in a larger part of the trilead diarsenate converted to calcium arsenate disposed of as waste.

7 REFERENCES

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.

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>. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, VOLUME 87
Inorganic and Organic Lead Compounds, Lyon, France 2004

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.

WMV (2010). Use of Diarsenic trioxide in the production of zinc. Comments from Wirtschaftsvereinigung Metalle (WMV) to ECHA background document on diarsenic trioxide.

8 ABOUT THE ARSENIC CONSORTIUM

As part of the stakeholder consultation for this Annex XV dossier 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 (Eurometex, 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.

9 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 $\text{As}_2\text{Ca}_3\text{O}_8$

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 AsH_3 .

Arsinic acid

An acid of general formula $\text{R}_2\text{AsO}_2\text{H}$, derived from trivalent arsenic; an example is dimethylarsinic acid, $(\text{CH}_3)_2\text{AsO}(\text{OH})$.

Arsonic acid

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

Monomethylarsonic acid (MMA^V)



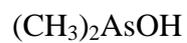
Monomethylarsonous acid (MMA^{III})



Dimethylarsinic acid (DMA^V):



Dimethylarsinous acid (DMA^{III}):



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 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	AsH ₃ O ₄	7778-39-4	302 g/L at 12.5 °C
Calcium arsenate	Ca ₃ (AsO ₄) ₂	7778-44-1	0.13 g/L at 25 °C
Trilead diarsenate	Pb ₃ (AsO ₄) ₂	3687-31-8	Sparingly soluble

¹data 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

	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 . A review of the documentation for the reproductive toxicity of lead compounds is presented in Appendix 2.

2 EXPOSURE

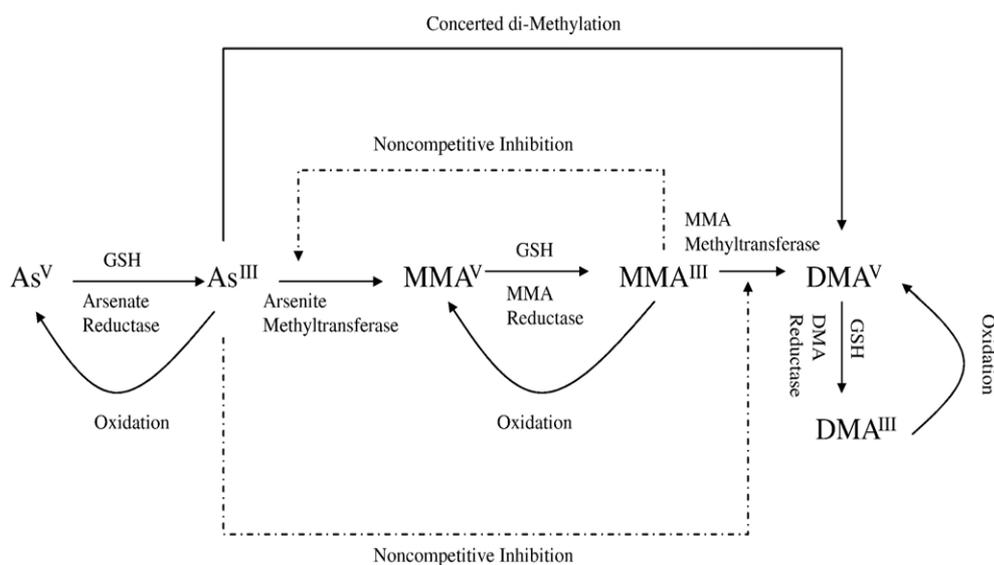
For the general population the main route of exposure is by the oral route, whereas occupational exposure is predominantly through inhalation and to a much lesser degree through dermal exposure.

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 arsenites (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 12.5°C; 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 water-solubility of calcium arsenate is 0.13 g/L at 25°C (IUCLID5) and it has been shown that after six days in 0.9% saline solution (50 mg As/L at 37°C) 23% of the added calcium arsenate was dissolved (Perschagen 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 toxicokinetics 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 administered 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 carcass. 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
$\text{Pb}_3(\text{AsO}_4)_2$	intratracheal	6.5±0.8	32.8±1.5	2.2±0.4	20.2±1.5	9.0±0.2
$\text{Pb}_3(\text{AsO}_4)_2$	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 readily 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 assays⁵ 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

⁵ 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 2010.

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 southwest 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

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.

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.

IUCLID 5: European Chemicals Agency. ECHA CHEM 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, Coglianò 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-Labeled 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

SUMMARY OF TOXICITY FOR REPRODUCTION OF LEAD COMPOUNDS

1 INTRODUCTION

A review of the documentation for the reproductive toxicity of lead compounds is presented below in order to support the proposal of trilead diarsenate as a Substance of Very High Concern since lead compounds are classified as toxic for reproduction, Repr.1A; H360Df, according to CLP. Lead compounds are easily distributed to blood, soft tissue and bone (IARC 2004). Information on toxicokinetics of trilead diarsenate is described in Appendix 1.

2 TRILEAD DIARSENATE

2.1 CLP classification

Trilead diarsenate is also covered by the entry: 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 in category 1A. The full classification is shown in Table 1.

Table 1. Classification of “Lead compounds with the exception of those specified elsewhere in this Annex”

Hazard Class and Category Code(s)	Hazard Statement Code(s)
Repr. 1A	H360Df
Acute Tox. 4	H332
Acute Tox. 4	H302
STOT RE 2	H373
Aquatic Acute 1	H400
Aquatic Chronic 1	H410

Repr. 1A, H360Df : May damage the unborn child. Suspected of damaging fertility

In addition to the group entry, several lead compounds are individually classified in the CLP Regulation, see Table 2.

Table 2. Specific lead compounds classified as Repr.1A (CLP)

International Chemical Identification	EC	CAS
lead hydrogen arsenate	232-064-2	7784-40-9
trilead bis(orthophosphate)	231-205-5	7446-27-7
lead hexafluorosilicate	247-278-1	25808-74-6
lead diazide, lead azide	236-542-1	13424-46-9
lead chromate	231-846-0	7758-97-6
lead di(acetate)	206-104-4	301-04-2
trilead bis(orthophosphate)	231-205-5	7446-27-7
trilead bis(orthophosphate)	231-205-5	7446-27-7
lead(II) methanesulphonate	401-750-5	17570-76-2
lead alkyls	-	-

http://echa.europa.eu/doc/restrictions/annex_xv_restriction_report_lead_en.pdf

2.2 Animal studies on reproductive and developmental toxicity of lead compounds

Fertility:

Several animal studies show fertility and developmental toxicity following lead exposure as reviewed in the Voluntary Risk Assessment Report On Lead and some Inorganic Lead Compounds (Lead Development Association International, LDAI, 2008a). For instance, Wadi and Ahmad (1999) investigated lead toxicity to the male reproductive system of sexually mature male CF-1 mice. Two concentrations of lead (0.25% and 0.5%) were administered to the mice via drinking water for six weeks. The low dose of lead significantly reduced the number of sperm within the epididymis, while the high dose reduced both the sperm count and percentage of motile sperm and increased the percentage of abnormal sperm within the epididymis. In another study, lead monoxide alloy powder used for storage battery electrodes was tested for effects on the reproduction of male BALBc mice. Two concentrations (25 and 50 mg/kg chow) were fed to males for 35 or 70 days starting at weaning. Some of the males were mated individually to two untreated females. The results showed a significant increase in post-implantation losses of embryos *in utero*, and a significant decrease in litter size of

surviving pregnancies. Light microscopic examination showed a general trend towards reduced spermatogenic activity (al-Hakkak ZS et al.1988).

Furthermore, experimental studies have demonstrated that lead can accumulate in different areas of the rat testes, including the seminiferous tubules, along the edge of the lumen, and in the tails of spermatozoa. Lead acetate was found to decrease motility of rat sperm and increase the number of abnormal sperm in male mice administered lead in the diet for 8 wk. Lead administered to mature female rats in drinking water in order to produce blood lead levels of 30 µg/dL caused irregular oestrous cycles. At higher exposure levels (blood levels of 53 µg/dL), animals developed follicular cysts and reduced numbers of corpora lutea (Goyer RA, 2001).

Development:

Low chronic exposure to lead compounds show abnormal neuronal development. Locomotor behavior in the absence and presence of amphetamine, running wheel activity, rotarod test, and dopamine utilization were examined in one year-old mice. Peak [BPb] were < 1 < or = 10, 24-27, and 33-42 microg/dL in control, low-, moderate- and high-dose gestational lead exposure (GLE) groups at postnatal day 0-10, respectively. One year-old male but not female GLE mice exhibited late-onset obesity. Similarly, male-specific decreased spontaneous motor activity, increased amphetamine-induced motor activity, and decreased rotarod performance were observed in one year-old GLE mice. GLE-induced alterations were consistently larger in low-dose GLE mice (Leasure JL et al., 2008).

2.3 Epidemiological studies showing fertility and developmental toxicity of lead compounds

Fertility:

As recently reviewed and reported in the background document to the RAC and SEAC opinions on the Annex XV dossier proposing restrictions on “Lead and its compounds in jewellery” (RAC/SEAC 2011), it is documented clear indications that high levels of lead in humans cause adverse effects on both male and female fertility. Less is known concerning effects on fertility following a chronic exposure to low levels of lead. However, if the PbB level is above 200 µg/L, an abortion or still-born baby risk exists and several studies reported that the length of gestation is affected at PbB level of 150 µg/L and above (ATSDR 2007). It was reported in 1999 that the risk of spontaneous abortion nearly doubles for every 5 µg/dL increase in blood lead levels (Borja-Aburto V. *et al.*, 1999). Effects on sperm may start to appear at blood lead levels of 400 µg/L. Moreover, a Finnish study has observed a significant increase of the risk of spontaneous abortion among the wives of men whose PbB level was 300 µg/L or higher during spermatogenesis (TNO, 2005; LDAI, 2008a).

Development:

Since lead can easily cross the placental barrier, the exposure of children starts *in utero* and lasts during the lactation period. PbB level is correlated to the serum calcium: the demineralization of the skeleton observed during pregnancy and lactation induces a migration of the lead accumulated in the mother's bone to the fetus and the infant. This transferred amount of lead is directly linked to lead accumulated by the mother (resulting from a cumulated exposure) rather than to the maternal exposure during pregnancy. The maternal and the fetal PbB levels are quite identical (LDAI, 2008a).

As a result, foetal exposure to lead can induce developmental neurotoxicity. It has been demonstrated that both maternal plasma and whole blood lead during the first trimester (but not in the second or third trimester) were significant predictors ($p < 0.05$) of poorer Mental Developmental Index (MDI) scores (ATSDR 2007). As a possible explanation, Hu H. *et al.* (2006) speculated that lead might be affecting the process of neuronal differentiation, which is primarily a first-trimester event. Another recent study of Schnaas L. *et al.* (2006) reported an association between prenatal lead exposure and intellectual function. According to the authors, IQ of 6 to 10-year-old children decreased significantly only with increasing natural-log third trimester PbB, but not with PbB at other times during pregnancy or postnatal PbB measurements. However, because their observations began after the 12th week of pregnancy, the effects of the first trimester PbB could not be examined. As with other studies, the dose-response PbB-IQ function was log-linear, with a steeper slope at PbB $< 100 \mu\text{g/L}$ (RIVM, 1995).

In a recent EFSA report (April 2010), environmental exposure of children to lead was evaluated. EFSA has calculated a BMDL01 (Benchmark Dose Limit) of $12 \mu\text{g/L}$, which corresponds to the PbB level at which a 1% change on human intellectual function (loss of one IQ point) will occur, due to an exposure to lead.

Table 3. Summary of the effects of an exposure to lead in children (RAC/SEAC. 2011).

	PbB ($\mu\text{g/L}$)					
	No threshold	56	100	400	700	800
Hematological effects			Inhibition of ALAD (i.e. haeme synthesis) : used as biomarker of lead exposure (LDAI 2008a)	↓ hemoglobin production in children (LDAI 2008a)	Anaemia (LDAI 2008a)	
Effects on kidney			Affection of the biological function Animals/humans : nephropathy (tubular atrophy) (LDAI 2008 a)			
Developmental neurotoxicity	Possible reduced IQ (WHO, 2003 ; JECFA, 2010 ; EFSA, 2010)					Encephalopathy Effect on the cognitive functions (development, maturation) (LDAI, 2008a)

Nervous system effects are documented in children at relatively low exposure levels and include hearing and IQ deficits (NRC,1993). According to all the effects observed in children (as shown in Table 3.) and particularly effects on the neurodevelopment which seem to occur with no threshold, it should be considered that a threshold for the effects of lead on children could not be identified.

3 CONCLUSION

Due to the classification of lead compounds, trilead diarsenate fulfils the criteria for identification of SVHC according to article 57 (c). Lead compounds are classified as Repr.1A H360Df based on the many animal studies and human epidemiological studies on reproductive and developmental toxicity. Lead compounds are easily distributed through blood and absorbed in soft tissue and bone. Accumulation of lead, especially in bone tissue, leads to low chronic exposure of lead *in utero* and may lead to neurodevelopment disorders. Information on toxicokinetics of trilead diarsenate is described in Appendix 1.

4 REFERENCES

- al-Hakkak ZS, Zahid ZR, Ibrahim DK, al-Jumaily IS, Bazzaz AA. (1988): Effects of ingestion of lead monoxide alloy on male mouse reproduction. *Arch Toxicol* 62 (1):97-100.
- ATSDR. (Agency for Toxic Substances and Disease Registry) (2007): Toxicological Profile for Lead. ATSDR. 582 p. <http://www.atsdr.cdc.gov/toxprofiles/tp13.pdf>
- Borja-Aburto VH, Hertz-Picciotto I, Rojas Lopez M, Farias P, Rios C, Blanco J (1999): Blood lead levels measured prospectively and risk of spontaneous abortion. *Am J Epidemiol* 150(6):590-7.
- EFSA. (European Food Safety Authority). (2010): Scientific Opinion on Lead in food on a request of the European Commission (Question N° EFSA-Q-2007-137). *EFSA Journal* 8(4):1570. <http://www.efsa.europa.eu/en/scdocs/doc/1570.pdf>
- Goyer RA (2005): *Patty's Toxicology CD-ROM*. NY, NY: John Wiley & Sons; Lead. Online Posting Date: April 16, 2001.
- Hu H, Tellez-Rojo MM, Bellinger D, *et al.* (2006): Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. *Environ Health Perspect*. 114(11):1730-5.
- IARC (2004): Inorganic and Organic Lead Compounds. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol. 87, Lyon, 10–17 February 2004.
- LDAI. (Lead Development Association International). (2008a): Voluntary Risk Assessment Report On Lead and some Inorganic Lead Compounds. Human health section. Final draft. http://echa.europa.eu/doc/trd_substances/VRAR/Lead/vrar_hh/vrar_annex8_hh.rtf#_Toc190630207
- Leasure JL, Giddabasappa A, Chaney S, Johnson JE Jr, Pothakos K, Lau YS, Fox DA (2008): Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice. *Environ Health Perspect* 116(3):355-61.
- NRC (1993): *Measuring Lead Exposure in Infants, Children and Other Sensitive Populations*. National Research Council, National Academy Press, Washington, DC.

RAC/SEAC (2011): Background document to the opinions on the Annex XV dossier proposing restrictions on Lead and its compounds in jewellery. ECHA/RAC/RES-O-0000001304-85-03/S1.

RIVM (National Institute for Public Health and the Environment) (1995): Human-Toxicological Criteria for Serious Soil Contamination: Compounds evaluated in 1993 & 1994. RIVM Report 715810009. 103 p. <http://www.rivm.nl/bibliotheek/rapporten/715810009.html>

Schnaas L, Rothenberg SJ, Flores MF, *et al.* (2006): Reduced intellectual development in children with prenatal lead exposure. *Environ Health Perspect* 114(5):791-7.

TNO (2005): Risks to Health and the Environment Related to the Use of Lead in Products. 102 p. http://ec.europa.eu/enterprise/sectors/chemicals/files/studies/tno-lead_en.pdf

Wadi, S.A. and Ahmad, G. (1999): Effects of Lead on the Male Reproductive System in Mice. *J Toxicol Environ Health Part A* 56:513-521.

APPENDIX 3: CONFIDENTIAL ANNEX