# Risk Assessment Report
# Arsenic in foods
# (Chemicals and Contaminants)

Food Safety Commission Japan (FSCJ)

October 2013

# Contents

# Chronology of Discussions

Revision of Drinking-water Quality Standards

| | | | |
|---|---|---|---|
| 1 | July | 2003 | Risk assessment of the Revision of Drinking-water Quality Standards was requested from the Health Science Council, Ministry of Health, Labour and Welfare (Shokuan-No. 0701015 notified by the Ministry of Health, Labour and Welfare) |
| 3 | July | 2003 | Relevant documents were received. |
| 18 | July | 2003 | The 3rd Meeting of the Food Safety Commission (explanation of MHLW's request outline) |
| 8 | October | 2003 | Additional documents were accepted |

Self-tasking risk assessment

| | | | |
|---|---|---|---|
| 19 | March | 2009 | The 278th Meeting of the Food Safety Commission (decided to conduct as self-tasking risk assessments) |
| 11 | June | 2009 | The 5th Board Meeting of the Expert Panel on Chemicals and Contaminants (EPCC) |
| 20 | August | 2009 | The 3rd Meeting of the Division of Contaminants, EPCC |
| 27 | January | 2010 | The 4th Meeting of the Division of Contaminants, EPCC |
| 19 | January | 2011 | The 5th Meeting of the Division of Contaminants, EPCC |
| 10 | March | 2011 | The 6th Meeting of the Division of Contaminants, EPCC |
| 15 | February | 2012 | The 7th Meeting of the Division of Contaminants, EPCC |
| 4 | July | 2012 | The 8th Meeting of the Division of Contaminants, EPCC |
| 15 | March | 2013 | The 5th Meeting of the EPCC |
| 25 | June | 2013 | The 9th Meeting of the Division of Contaminants, EPCC |
| 30 | July | 2013 | The 10th Meeting of the Division of Contaminants, EPCC |
| 30 | August | 2013 | The 11th Meeting of the Division of Contaminants, EPCC |
| 30 | August | 2013 | The 11th Board Meeting of the EPCC |
| 28 | October | 2013 | The 492nd Meeting of the Food Safety Commission (Report) (Notice to the Minister of Health, Labour and Welfare dated the same date) |

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Taku Nagao
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Katsuyuki Murata
Akemi Yasui
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**Special Advisers, the EPCC**

The 7th Meeting of the EPCC
Takashi Kawamura
Ken'ichi Hanaoka
Hiroshi Yamauchi
Kenzo Yamanaka

The 8th Meeting of the EPCC
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The 9th Meeting of the EPCC
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Ken'ichi Hanaoka
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The 10th Meeting of EPCC
Takashi Kawamura
Kazuhiko Takahashi
Ken'ichi Hanaoka

The 11th Meeting of the EPCC
Takashi Kawamura
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# Executive Summary

FSCJ conducted risk assessment of arsenic in food, as a self-tasking assessment and as a risk assessment related to revision of the standards for beverages, based on data from various studies, epidemiological surveys and others.

The data used in the assessment were on toxicokinetics, acute toxicity, chronic toxicity, carcinogenicity reproductive and developmental toxicity, genotoxicity and others of inorganic arsenic compounds, organic arsenic compounds and synthesized organic arsenic compounds.

According to the epidemiologic surveys in the area with a long term intake of drinking water contaminated with arsenic, noncarcinogenic effects of inorganic arsenic exposure, such as skin lesion, neurodevelopmental, reproductive and developmental effects, were observed depending on the inorganic arsenic concentration in drinking-water. Among these, skin lesion was the adverse effect observed at the lowest concentration. The lowest observed adverse effect level (LOAEL) was 7.6-125 μg/L and the 95% confidence limit of benchmark concentration at which the incident rate increases 5%($BMCL_{05}$) was 19.5-54.1 μg/L.

Also, cancers such as lung tumor, urinary bladder tumor and others and genotoxicity such as chromosomal aberration due to inorganic arsenic exposure were observed in human. Although implication of genotoxicity in the carcinogenic effect of inorganic arsenic exposure is suggested, none of the currently available data is conclusive for the direct effect of arsenic on DNA. In addition, animal study in rodents suggested that the carcinogenesis by arsenic exposure is attributable to DNA aberration induced by indirect interaction of arsenic but not to the direct interaction of arsenic with DNA which produces an adduct such as point mutation. Although a threshold mechanism may be assumed on the basis of these points, the data is insufficient at this particular stage.

Hence, FSCJ concluded that threshold of carcinogenicity in arsenic exposure cannot be determined.

In this assessment, the no observed adverse effect level (NOAEL) (or LOAEL) and the 95% confidence limit of benchmark dose (BMDL) of inorganic arsenic were calculated by adding arsenic intake via diet to the intake by drinking-water based on the above mentioned epidemiologic surveys of long-term intake of arsenic contaminated water as follows. LOAEL of 4.3-5.2 μg/kg body weight/day and $BMDL_{05}$ of 4.0-4.2 μg/kg body weight/day for skin lesion; NOAEL of 3.0-4.1 μg/kg body weight/day for effect on nervous system (decreased IQ); NOAEL of 8.8-11.1 μg/kg body weight/day for effect on reproduction and development; NOAEL of 4.1-4.9 μg/kg body weight/day for lung tumor; NOAEL of 5.0-12.1 μg/kg body weight/day and $BMDL_{01}$ of 9.7-13.5 μg/kg body weight/day for tumor of urinary bladder .

However, in calculation of the NOAEL or BMDL based on the epidemiological surveys, daily intake of inorganic arsenic generally tends to be underestimated, because food items used to estimate arsenic intake were limited. Moreover, in some cases of high exposure through drinking water, the exposure through foods is even higher. Thus FSCJ considered the estimated values to be possibly with non-negligible variation.

Meanwhile, the estimated intake of inorganic arsenic in the cases of the people having normal life and not being exposed excessively by accident or contaminations was 0.130-0.674 μg/kg body weight/day as the average from several duplicated diet studies in Japan. The survey by FSCJ in 2013, which is one of these diet studies, showed the mean value of 0.315 μg/kg body weight/day and 95 percentile value of 0.754 μg /kg body weight/day.

Each value of NOAEL/LOAEL and the estimated intake of inorganic arsenic probably contain uncertainties. However, both values are close. Thus, some Japanese population with high exposure may have intake above the value of NOAEL or LOAEL that are estimated in this assessment.

It is necessary to examine whether it is appropriate to estimate the total inorganic arsenic intake from the arsenic concentration in drinking-water of the arsenic contaminated area. It is also necessary to examine whether it is appropriate to apply the result of dose-response assessment based on the surveys where exposure through drinking water is substantial to Japan where the people scarcely intake arsenic from drinking-water. Also, there are still many problems to be solved to conduct the detailed risk assessment in which tolerable daily intake (TDI) is evaluated by estimating NOAEL or BMDL and unit risk is calculated. For example, the effects from the several factors associated with the difference in dietary habit, environment, sanitation, medical care system, and lifestyles, may be related to the difference in health effects of inorganic arsenic exposure. The effects of smoking or other chemical compounds on the health effects which derived from inorganic arsenic exposure are mostly unknown.

If there is a lack of data on carcinogenic mechanism which is required for hazard assessment, and if the uncertainty in exposure assessment is high, unknown factors have been involved in the risk assessment even the latest scientific evidences are used. These situations are considered to be the cause of the gap between the estimation and the reality. If there is a discrepancy between the result of the assessment on a hazardous substance and the current situation in Japan, the real situation should be taken as a basis for the consideration.

Therefore, epidemiological studies on the population with arsenic exposure in an ordinary life and studies on mechanisms for the toxicity will be required to support the exposure assessment or the dose-response data, with evidences such as actual exposure situation in Japan

and the arsenic exposure through food intake. Also, further information on the toxicities of organic arsenic compounds are required because the data on toxicological effects of organic arsenic compounds for assessment of food safety risk are insufficient.

## I. Background

Food Safety Commission of Japan (FSCJ) conducts risk assessment at the requests from risk management organizations but occasionally it implements a risk assessment on its own decision, self-tasking risk assessments.

Candidate substances for FSCJ's self-tasking risk assessments are selected by the Planning Expert Panel based on priority in risk assessment on possible hazards in food, from among substances which are considered to have a major influence on public health, or which highly necessitate the identification of hazards, or whose assessment needs are particularly high. Substances for discretionary assessments are then determined by the FSCJ, after collecting opinions and information from the public and following other relevant procedures.

A risk assessment of arsenic in foods (organic arsenic and inorganic arsenic) as a chemical substance in the revision of the standards for soft drinks has been requested by the Minister of Health, Labour and Welfare in 2003. As arsenic is contained in various foods, it is required to target entire foods for assessment and to conduct risk assessment based on the forms of arsenic. Because of these reasons, FSCJ decided at the meeting of the 278th commission on March 19, 2009, to conduct self-tasking risk assessment of arsenic in foods and discussion and investigation were started.

## II. Outline of the Substances under Assessment

### 1. Physicochemical property

Arsenic is metalloid and generally exists as a simple substance (metallic arsenic) and as compounds binding with other elements in the environment (ATSDR 2007). Arsenic compounds are classified into organic arsenic compounds in which carbon directly binds to arsenic, and into inorganic arsenic compounds, the compounds other than organic arsenic compounds. In this report, arsenic as a simple substance is expressed as metallic arsenic. The names in Table 2 are used for inorganic arsenic compounds, and the abbreviations in Table 3 are used for organic arsenic compounds. Trivalent arsenic and pentavalent arsenic are expressed as As(III) and As(V), respectively.

In addition, the weight of an arsenic element calculated from the weight of arsenic compound is expressed as μg As.

#### (1) Metallic arsenic

Arsenic belongs to group 15 in the periodic table of elements and its chemical property is similar to that of phosphorus (EFSA 2009). Its property does not change in the air at room temperature. It has 3 allotropic forms of yellow, gray, and black (Iwanami rikagaku jiten 1998). Table 1 shows the physicochemical property of major metallic arsenic (NCBI 2004; NIH 1994; The Merck Index 2006; Iwanami rikagaku jiten 1998).

Table 1 Physicochemical property of metallic arsenic

| Substance name | Arsenic | | |
|---|---|---|---|
| | Yellow arsenic | Grey arsenic | Black arsenic |
| IUPAC name | Arsenic | | |
| CAS registered number | 7440-38-2 | | |
| Chemical formula | As | | |
| Structural formula | As | | |
| Molecular weight | 74.9216 | | |
| Form | Cubic crystal | Trigonal crystal | Amorphous |
| Color | Yellow | Gray with metallic lustre | Black |
| Odor | Garlic odor | - | - |
| Melting point (°C) | - | 818 (36 atm) | - |
| Boiling point (°C) | - | 615 (Sublimation point) | - |
| Density ($m^3$) | 1.97 | 5.778 (25°C /4°C) | 4.73 |
| Solubility | Carbon disulfide: Soluble 80 µg/g (20°C) | Water: Insoluble | - |
| Others | Metastable, produced by cryocondensation of arsenic steam, has a smell like garlic; is transparent, waxy and soft. It becomes gray arsenic by ultraviolet irradiation. | It is formed into diarsenic trioxide or arsenic acid by nitric acid or hot sulfuric acid. | |

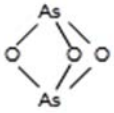
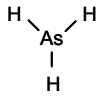
(Quoted from NCBI 2004; NIH 1994; The Merck Index 2006; Iwanami rikagaku jiten 1998)

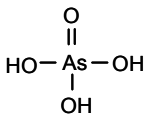
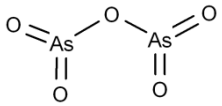
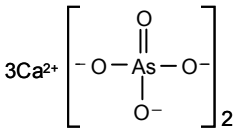
(2) Inorganic arsenic compounds

Among inorganic arsenic compounds, diarsenic trioxide is a major compound that is produced and used (Japan Oil, Gas and Metals National Corporation, 2011). Diarsenic trioxide turns into weak acid, arsenite ($H_3AsO_3$), when it is dissolved and exists as arsenite in foods and *in vivo*. Therefore, in this report, diarsenic trioxide is assessed as arsenite.

Table 2 shows physicochemical property of major inorganic arsenic compounds. (NCBI 2004; NIH 1994; The Merck Index 2006; Iwanami rikagaku jiten 1998).

Table 2 Physicochemical property of major inorganic arsenic compounds

| Valence | Trivalent | | | | |
|---|---|---|---|---|---|
| Substance name | Diarsenic trioxide (Arsenic trioxide) | | | Arsine (hydrogen arsenide) | Gallium Arsenide |
| | Arsenolight | Claudetite | Amorphous | | |
| IUPAC name | arsenic(3+); oxygen(2-) | | | arsane | gallanylidynearsane |
| CAS registered number | 1327-53-3 | | | 7784-42-1 | 1303-00-0 |
| Chemical formula | $As_2O_3$ | | | $AsH_3$ | GaAs |
| Structural formula |  | | |  |  |
| Molecular weight | 197.84 | | | 77.95 | 144.64 |
| Form | Cubic crystal | Monoclinic crystals | Amorphous with 8 rhombuses | Gas | Cubic crystal |
| Color | White | Colorless | Colorless | Colorless | Gray, Gray with metallic lustre |
| Odor | Odorless | - | - | Unpleasant garlic odor | Garlic odor when it is dampened |
| Melting point (°C°) | 275 | 313 | - | -117 | 1238 |
| Boiling point (°C) | 465 | - | - | -62.5 | - |
| Density ($g/cm^3$) | 3.86 | 3.74 | - | 3,186 μg/L (gas) | 5.3176 (25°C) |
| Solubility | Water: 20.5 g/L (25°C), 17 g/L (16°C)<br>Cold water: Dissolved slowly<br>15 parts boiling water, dilute hydrochloric, alkali hydroxide solution, carbonate aqueous solution: soluble<br>Alcohol, chloroform, ether : practically insoluble<br>Glycerin: soluble,<br>Claudetite, dilute acid,<br>alkali: soluble,<br>Ethanol: insoluble | | | Water: 0.28 g/L (20°C)<br>Absorbed in potassium permanganate solution or bromine water. | Water: < 1 g/L (20°C)<br>DMSO, 95% ethanol, methanol, acetone: < 1 g/g<br>hydrochloric acid: soluble |
| Others | - | | | Oxidized in the air and produces $As_2O_2$.<br>Decomposed to arsenic and hydrogen at 300°C.<br>Vapor density: 2.7 (air = 1) | - |

| Valence | Pentavalent | | |
|---|---|---|---|
| Substance name | Arsenic acid | Arsenic pentoxide | Calcium arsenate |
| IUPAC name | arsonic acid | - | - |
| CAS registered number | 7778-39-4 | 1303-28-2 | 7778-44-1 |
| Chemical formula | $AsH_3O_4$ | $As_2O_5$ | $As_2Ca_3O_8$ |
| Structural formula |  |  |  |
| Molecular weight | 141.94 | 229.84 | 398.07 |
| Form | Hygroscopic crystals (1/2 hydrate) | Amorphous mass or powder | Amorphous powder |
| Color | White translucent (1/2 hydrate) | White | White |
| Odor | - | - | Odorless |
| Melting point (°C) | 35 | - | (Dissolved) |
| Boiling point (°C) | 160 (1/2hydrate) | - | - |
| Density ($g/cm^3$) | 2.2 | 4.32 | 3.620 |
| Solubility | Water: 590 g/L, 3,020,000 μg/L (12.5 °C, 1/2 hydrate)<br>Water, alcohol, glycerin: easily soluble (1/2 hydrate) | Water: 658 g/L (20 °C), 2,300 g/L (20 °C)<br>Ethanol: soluble<br>Acid, alkali: soluble | Water: 0.13 g/L (25 °C)<br>Dilute acid: soluble<br>Organic solvent: insoluble |
| Others | Exists only as hydrate.<br>Exists only in solvent.<br>Hydrate loses water molecule at ≥ 160 °C. | Decomposed at 300 °C. | - |

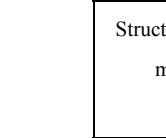
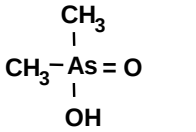
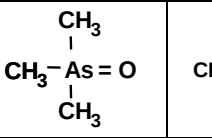
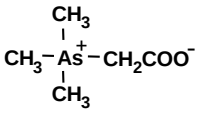
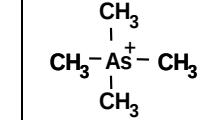
(Quoted from NCBI 2004; NIH 1994; The Merck Index 2006; Iwanami rikagaku jiten 1998)

(3) Organic arsenic compounds

(i) Organic arsenic compounds existing in animals and plants

In the world of nature, inorganic arsenic is methylated and monomethyl arsenic compound, dimethyl arsenic compound, trivalent methylated arsenic species, and tetramethyl arsenic compound exist in animals and plants. Table 3 shows physicochemical property of major organic arsenic compounds (NCBI 2004; NIH 1994; The Merck Index 2006; Kagakudaijiten 1963).

Table 3 Physicochemical property of major organic arsenic compounds

| | Monomethyl arsonic acid MMA(V) | Methyl arsonous acidMMA(III) | Dimethyl arsenic acid (Cacodylic acid) DMA(V) | Dimethyl arsinous acid DMA(III) | Trimethyl arsine oxide TMAO | Arsenobetaine AsBe | Arsenocholine AsC | Tetramethyl arsonium TeMA |
|---|---|---|---|---|---|---|---|---|
| IUPAC name | methylarsonic acid | methylarsonous acid | dimethylarsinic acid | dimethylarsinous acid | dimethylarsoryl methane | 2-trimethylarsoniumyl acetate | 2-hydroxyethyl (trimethyl) arsanium | Tetramethyl arsanium |
| CAS registered number | 124-58-3 | - | 75-60-5 | - | 4964-14-1 | 64436-13-1 | 39895-81-3 | - |
| Chemical formula | $CH_5AsO_3$ | $CH_5AsO_2$ | $C_2H_7AsO_2$ | $C_2H_7AsO$ | $C_3H_9AsO$ | $C_5H_{11}AsO_2$ | $C_5H_{14}AsO$ | $C_4H_{12}As$ |
| Structural formula |  | - |  | - |  |  |  |  |
| Molecular Wt. | 139.97 | 123.95 | 138.00 | 122.00 | 136.03 | 178.06 | 165.09 | 135.06 |
| Form | Monoclinic crystals, Leading frame shaped plate (from absolute alcohol) | - | Triclinic crystal | - | | - | - | - |
| Color | White | - | Colorless | - | | - | - | - |
| Odor | - | - | Odorless | - | | - | - | - |
| Melting point (°C) | 161 | - | 195-196 | - | | - | - | - |
| Boiling point (°C) | - | - | > 200 | - | | - | - | - |
| Density($g/cm^3$) | - | - | - | - | | - | - | - |
| Solubility | Water: 256 g/L (20°C) Ethanol: soluble | - | Water: 2,000 g/L (25°C) Acetic acid: soluble Ethanol: soluble diethyl ether: insoluble | - | | - | - | - |
| Others | Acidity sourness Strong diprotic acid | - | - | - | | Slightly soluble in acid | - | |

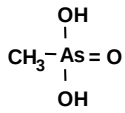
(Quoted from NCBI 2004; NIH 1994; The Merck Index 2006; Kagakudaijiten 1963)

(ii) Organic arsenic compounds in marine ecosystem

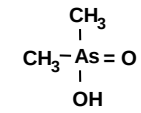
In a marine ecosystem, organic arsenic compounds exist in different chemical forms that are categorized into water-soluble and lipid-soluble organic arsenic compounds.

Liquid Chromatography Atomic Absorption Spectrometry (LC-AAS) and Liquid Chromatography-Inductively Coupled Plasma Atomic Emission Spectrometry (LC-ICP-AES) has been used for analyzing various complicated chemical forms of organic arsenic compounds contained in marine organisms. Recently, Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (LC-ICP-MS) with higher sensitivity has been developed and widely used (Inoue et al. 1996). Arsenic contained in marine organisms has been studied for many years as one of major sources for arsenic intake form food in Japan. Although these studies for years has accumulated information on arsenic in marine organisms, it is not sufficient yet. Figure 1 shows major organic arsenic compounds that exist in marine ecosystem (Hanaoka 2011). Among these, arsenosugar is known to exist as tertiary alkylarsine oxides and quaternary alkylarsoniums.

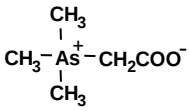
Water-soluble organic arsenic compounds mainly exist in marine organisms or in seawater



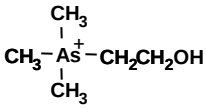
MMA(V)



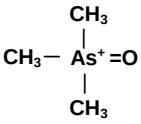
DMA(V)



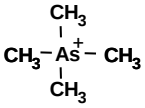
AsBe



AsC

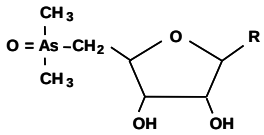


TMAO

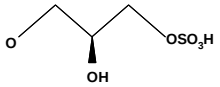


TeMA

Water-soluble organic arsenic compounds mainly exist in seaweed



Arsenosugar



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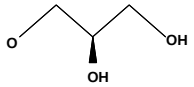

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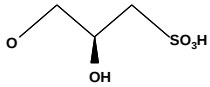

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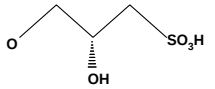

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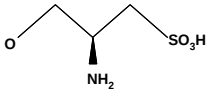

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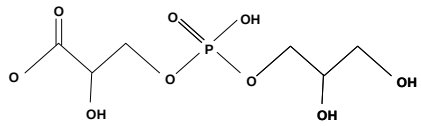
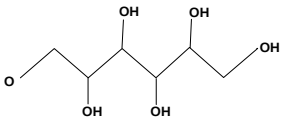
6 (R)



7 (R)



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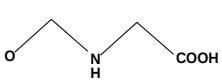

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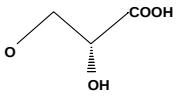

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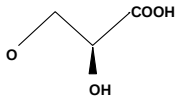

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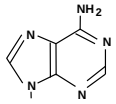

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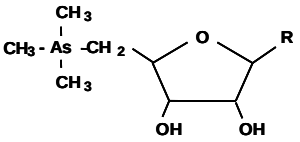
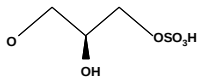


Arsenosugar



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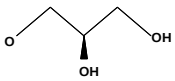

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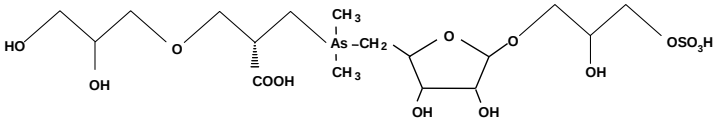

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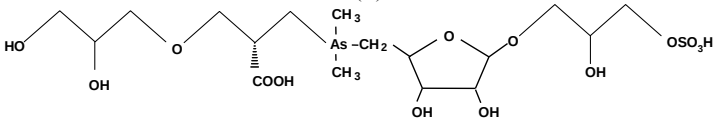


16 (R)

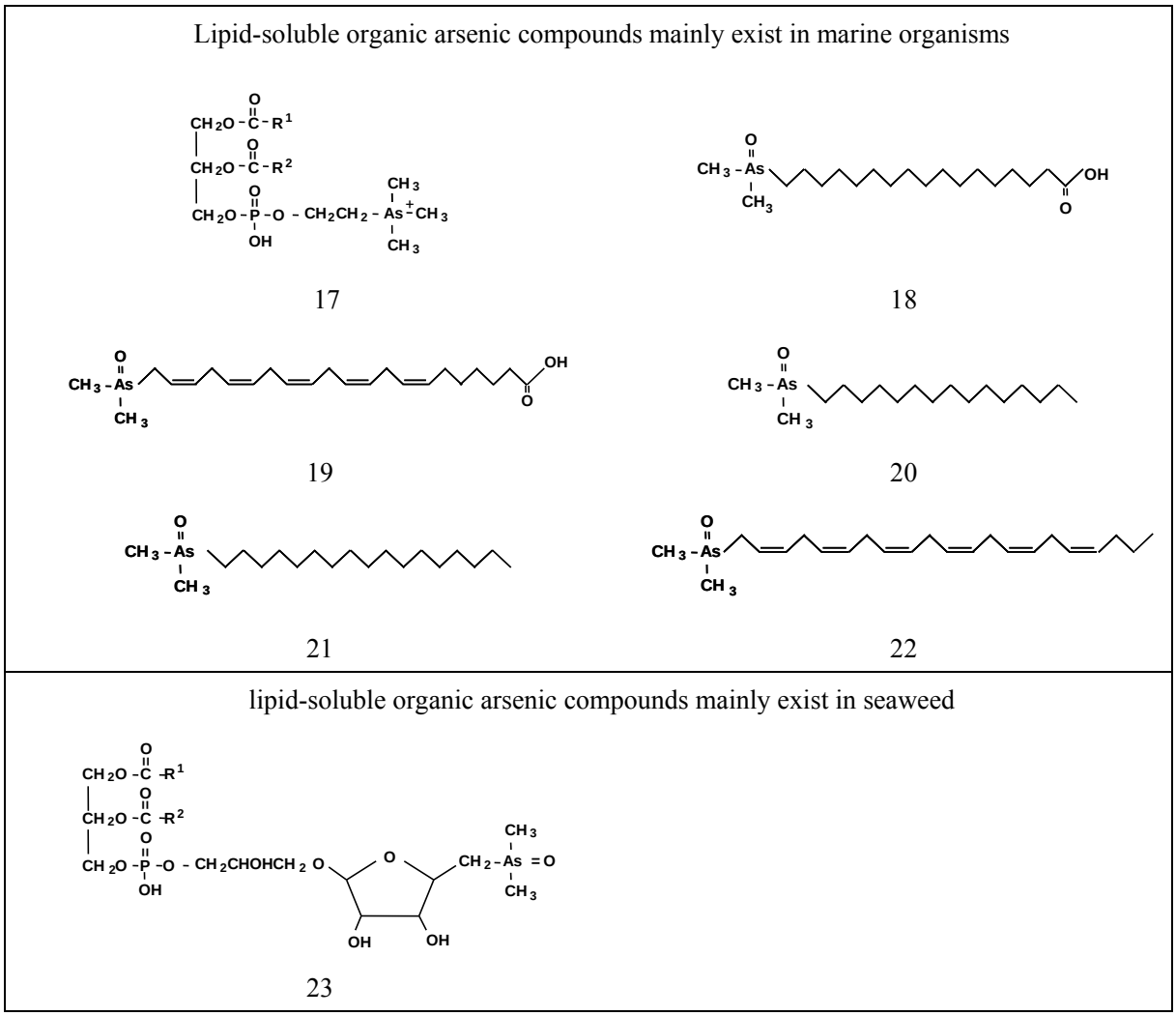



Figure 1 Major organic arsenic compounds in marine ecosystem

(Quoted from Hanaoka 2011)

(4) Analytical methods for arsenic

There are two main methods for analysis of arsenic: the method for total arsenic and the method for arsenic by different chemical forms. In both analysis, arsenic is detected mainly by atomic spectrometry such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), ICP-atomic emission spectrometry (ICP-AES), and ICP-MS

(i) Analytical methods for total arsenic

While chemical staining techniques such as the Gutzeit method or the silver diethyldithiocarbamate method have been used as the official analytical method for the total arsenic in beverages or crops, the Food Sanitation Act designated to enable the use of methods that are confirmed to have the same or higher efficiency. Thus, hydride generation AAS (HGAAS)

and hydride generation ICP (HGICP) -AES have been used for a long period as an analytical method for total arsenic in foods. Product standards for beverages designated HGAAS or flameless-AAS as test methods. In analysis of total arsenic in foods, generally the samples are decomposed by acidic cleavage to change all forms of arsenic into inorganic arsenic ion, and the arsenic ions are detected following hydride generation or without hydride generation. In addition, a direct analysis method by ICP-MS which is not coupled with hydride generation has been available more recently.

The selection of decomposition method for pretreatment of samples is very critical in the total arsenic analysis by HGAAS or HGICP. Some organic arsenic compounds are hardly decomposable to inorganic arsenic in ordinal temperature of acidic cleavage (up to 200°C). AsBe compounds cannot be decomposed by 100% unless the compounds are decomposed by heating with nitric acid, sulfuric acid, or perchloric acid at a temperature around 320°C (Narukawa et al. 2005). If acidic cleavage of a sample is insufficient, total arsenic analysis by HGAAS or HGICP will underestimate arsenic because undecomposed organic arsenic compounds do not form hydride. In contrast, the analysis method which does not depend on hydride generation does not require such complete decomposition.

a. Atomic absorption spectrometry (AAS)

In AAS, arsenic compounds in the samples are atomized in chemical flame or an electrothermal quartz tube or in a graphite furnace, and the absorption of light of a specific wave length which is absorbed by arsenic is measured. In HGAAS, inorganic arsenic in the sample is converted to gaseous arsine ($AsH_3$) by a strong reducing agent and is led into an electrothermal quartz tube where it is atomized and then atomic absorption by arsenic is measured. HGAAS is advantageous in terms of high efficiency of transferring gaseous substances into metal atomizer and of high sensitivity. Also it is advantageous to exhibit no spectral interference because it is separated from other sample matrix.

HGAAS has been the most common method for measurement of the total arsenic amount in foods since the 1970's to 1980's, and is still widely used. The lower detection limit for arsenic in food by HGAAS is approximately 0.02 μg As/g (dry weight).

Two types of methods for measuring arsenic in foods by HGAAS are used as the standard in Europe (CEN 2005; CEN 2006). These 2 types of methods are different only in their procedures for decomposition of samples.

Electrothermal atomic absorption spectrometry (ETAAS) is also called graphite furnace atomic absorption spectrometry (GFAAS). It is a method where arsenic in the small amount of sample solution (in general 10-20 μL) is atomized in an electrothermal graphite tube. Although ETAAS is generally regarded as a highly sensitive analysis method, a time-consuming

and careful pretreatment is required due to the effect of sample matrix. This method is recognized in Europe as a standard method of measuring total arsenic in marine products, and it has been used in a joint study where the arsenic in 8 types of marine product samples were detected within the range of 2.3-79 μg As/g (dry weight) (Julshamn et al. 2000). The lower detection limit of this analytical method is 0.1 μg As/g (dry weight) (Julshamn et al. 2000). In Japan, this method is used for analysis of tap water but it has not been applied to drainage with complicated sample composition, environment samples, or to foods.

b. Atomic fluorescence spectrometry (AFS)

In AFS, the fluorescence of a specific wavelength emitted from the atom in vapor, which is exited to a high energy state by the excitation wave of specific wavelength, is measured.

Combination of HGAFS with a hydride generation process provides highly sensitive quantitative measurement of arsenic at the concentration as low as 0.01 μg As/g or less (Vilano and Rubio 2001). However, this method is not popular in Japan and the use is limited.

c. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a method widely used for trace element analysis, although it is not particularly sensitive to arsenic, and is not commonly used for the measurement of arsenic in foods. Principle of atomic emission spectrometry is to detect light of the atom specific wave length which is emitted by the atom when the objective atom returns to a low energy state from a high energy state brought by thermal excitation in excitation source. Because of its high temperature, ICP is superior as an excitation source. It has been reported that ICP combined with hydride generation, HGICP, is highly sensitive and hardly affected by spectrum interference, and has a lower detection limit of approximately 0.015 μg As/g (dry weight) (Boutakhrit et al. 2005).

d. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) has been established as a principle method for trace element analysis in foods because of its high sensitivity and its wide and dynamic range. An element in samples is dissociated, atomized, and ionized by high energy argon plasma (8,000 K) and detected selectively by mass spectrometer. Arsenic is monoisotopic with a mass number of 75.

ICP-MS is widely used for the measurement of arsenic in foods. For example, from the result of statistical analysis in the recent study which analyzed arsenic in foods by ICP-MS, ICP-MS was considered to be relevant for analysis of elements (Julshamn et al. 2007). ICP-MS is a highly sensitive method for arsenic which can detect arsenic at the level of 0.01 μg

As/g (dry weight), and it is an easy and highly reliable method for analysis of arsenic in foods (EFSA 2009).

In general, the effect from matrix is not a big problem to the arsenic measurement by ICP-MS. However, the sample solution with total salt concentration of ≥ 0.1% (mass/volume) may have non-spectral interference and may cause spectral interference by coexisting chloride ion by forming $ArCl^+$ (*m/z* 75, same nominal mass of $As^+$) in argon plasma of ICP-MS. This interference can be eliminated by collision and reaction cell technologies and this technology was introduced to many ICP-MS in recent years. In order to eliminate the effect of chloride interference as much as possible, ICP-MS may be used with hydride formation. The lower quantitative limit can be obtained by HG-ICP-MS as compared to the conventional ICP-MS. However, not much improvement can be found as compared to the drastic improvement found in the comparison in sensitivity between HG-AAS and conventional AAS (EFSA 2009).

(ii) Chemical speciation

Since toxicity of arsenic largely differs by its chemical species, the determination of arsenic in foods by chemical species has been recognized to be necessary for years. Chemical speciation of arsenic has two steps: separation of arsenic compounds and detection of compounds which has been separated. There are 2 methods for separation, i.e. hydride generation and liquid chromatography. Generally, one of the atomic spectrometry mentioned in (i) is used for detection.

a. Chemical speciation by hydride generation method

A hydride generation method had been used for chemical speciation of arsenic in the 1970's and 1980's. Reduction of inorganic arsenic (arsenic acid, arsonous acid), MMA, and DMA with sodium tetraborate produces gaseous hydrides such as arsine ($AsH_3$), methylarsine ($CH_3AsH_2$), and dimethylarsine ($(CH_3)_2AsH$) respectively. Boiling points of these gaseous hydrides are -55°C, 2°C and 35.6°C respectively, and principle of HG method is based on this difference in the boiling point of each. In this chemical speciation method, hydride generated from sample solution is trapped at the liquid nitrogen temperature first, and then hydride of each form is led to the detector by gradual rising in temperature for quantification (Anawar 2012). TMAO also forms hydride. Instead of separation by boiling point, gas chromatograph may be used. Arsenic acid and arsenous acid can be separated by adjusting pH of the sample solution. However, it is disadvantage of this method that the optimal condition for hydridegeneration of each chemical species are different, and that actual biological and environmental samples have constituents which inhibit hydride generation. In addition, major arsenic compounds in foods such as AsBe or arsenosugar do not

produce hydride so that this method is not applicable to these arsenic compounds. Therefore, TMAO and DMA are only detectable indirectly as trimethylarsine and dimethylarsine respectively, after preprocessing such as alkalinolysis (Nakahara 1997). Because of these disadvantages, this method is hardly used except in the case where hydride generation method is used for the detection of arsenic after the separation by liquid chromatography as follows.

b. Chemical speciation by liquid chromatography

LC is adequate for the separation of water-soluble arsenic compounds. The flow rate of a mobile phase around 1mL/min employed commonly in LC matches well with flow rate for injection of sample solutions into the detector such as AAS, ICPAES, ICP-MS and others. Because of this advantage, the system can be easily set up by connecting outlet of LC column directly to a sample introducing system for AAS and other detector systems.

Currently, ICP-MS is the major detector because of its high sensitivity. However, since a mobile phase containing salt or organic solvent at a high concentration is not suitable for ICP-MS, this method has a certain limit for the selection of mobile phase and of separation mode. Thus, the method where arsenic compounds are converted to hydride after LC separation has been commonly used to detect arsenic with AAS or AFS of high sensitivity. However, as mentioned above, for the detection of major arsenic compounds in foods such as AsBe or arsenosugar, a system to mineralize AsBe and other compounds by microwave digestion or by photo-oxidation (PO) should be connected prior to the HG. Such an arrangement will make the system disadvantageously complicated like LC-PO-HG-AFS.

In order to analyze arsenic compounds in solid samples such as foods, arsenic compounds need to be extracted from samples without changing tis chemical form prior to the analysis. For example, arsenic compounds are extracted from marine animals and algae by supersonic wave in chloroform:methanol:water (Beauchemin et al. 1988), or in methanol:water (Milstein et al. 2003). Arsenic compounds are also extracted by decomposing the sample tissue by enzymes (trypsin, amylase and others) (e.g. Lamble & Hill 1996). Following methods have been also proposed. Such as, extraction of inorganic arsenic from seaweed by dilute hydrochloric acid (CEN, 2008; Nakamura et al. 2008), hot water extraction of arsenic compounds in rice (Narukawa et al. 2008); and by dilute nitric acid extraction (Hamano-Nagaoka et al. 2008).

The separation methods which are commonly used for the analysis of arsenic compounds are ion-pair reversed-phase chromatography and ion exchange chromatography. However, it is difficult to separate and quantify many species of arsenic compounds at one time under the same chromatographic condition since each arsenic compound exists in various forms such as cation, anion, or amphoteric ion in certain pH conditions. For separation of these

arsenic compounds, generally, anion exchange columnchromatography and cation exchange columnchromatography are employed depend on the chemical species. Arsenic acid, arsonous acid, MMA and DMA are separated by the former columnchromatography, and AsBe, AsC, and tetramethyl arsonium ion are separated by the latter columnchromatography (e.g. Larsen et al. 1993). Shibata & Morita (1989) separated and determined the total 15 species of arsenic compounds including inorganic arsenic, methylated arsenic and 6 types of arsenosugar, by using 3 different separation conditions such as cation-pair, reversed-phase chromatography, anion-pair reversed-phase chromatography, and gel filtration chromatography. The most popular condition which has been used for quantification of arsenic compounds contained in foods such as seaweed and rice in recent years in Japan is the condition using a C18 reversed-phase column with butanesulfonic acid as an ion pair reagent which is based on the condition used by Shibata and Morita (Narukawa et al., 2006; Hamano- Nagaoka et al., 2008).

Sensitivity of ICP-MS, though it is high in general, may not sufficient for the analysis of arsenic compounds of low concentration such as inorganic arsenic in a duplicate diet method. Although the system becomes complicated, a detection limit of less than 0.001 μg/g for inorganic arsenic in a duplicate diet study was achieved by LC-HEPO-HG-ICPMS system where a series of a high efficiency photo-oxidation (HEPO) system and a hydride generation method had been used (Oguri et al. 2011).

c. Analysis of lipid-soluble arsenic

Classically, the arsenic which was extracted from samples by solvents such as chloroform has been generically called lipid-soluble arsenic. For classifying their chemical species, chloroform-extracts used to be further partitioned into methanol/hexan soluble fractions which are designated as polar and nonpolarlipid soluble arsenic, respectively. Alternatively, their chemical species used to be assumed from relevant hydrolysate. Recently, by minimizing the flow rate of LC and by mixing oxygen into plasma, the introduction of solvent into ICP became possible. As the consequence, analysis of fish oil was achieved by LC-ICPMS (Schmeisser et al. 2005; Amayo et al. 2011) using normal-phase chromatography column or reverse-phase column. Moreover, structural analysis (Arroyo-Abad et al. 2010) of lipid-soluble arsenic compounds in the liver of cod based on the concomitant analysis with gas-chromatography (GC) -ICPMS or Helium Microwave-induced Plasma Atomic Emission Spectrometry, GC-MS, GC-TOFMS have been also conducted.

## 2. Major use and production

Metallic arsenic is mainly used for the synthesis of compound semiconductors such as gallium arsenide and others, and it is also used for synthesis of semiconducting glasses as well as additives to copper and lead. Arsenic compounds have been used for pesticide, rat poison and wood preservative. Arsine has been used as a material for epitaxial growth GaAs thin film on a GaAs substrate. Diarsenic trioxide has been used as a therapeutic agent for acute promyelocytic leukemia (APL). It is also used as a clarifier at the manufacturing of LC glass and lead glass.

The production of diarsenic trioxide in Japan is around 40 t/year (Japan Oil, Gas and Metals National Corporation 2012) (Table 4), but most of diarsenic trioxide used as materials for domestic demand were imported from China (Japan Oil, Gas and Metals National Corporation 2012). In addition, the disposition of high-purity metallic arsenic which has been used as material for semiconductor in 2010 was, 64.0 t for domestic production, 12.6 t for export, and 33.4 t was imported from Germany (Japan Oil, Gas and Metals National Corporation 2011).

Table 4 Major producing nation and production quantity of diarsenic trioxide (Unit: t)

| Country name | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|---|
| Japan | 40 | 40 | 40 | 40 | 40 | 40 |
| China | 30,000 | 30,000 | 25,000 | 25,000 | 25,000 | 25,000 |
| Chile | 11,500 | 11,800 | 11,400 | 10,000 | 11,000 | 11,500 |
| Morocco | 6,900 | 6,900 | 8,950 | 8,800 | 7,000 | 8,000 |
| Peru | 3,600 | 3,500 | 4,320 | 4,000 | 4,850 | 4,500 |
| Mexico | 1,650 | 1,750 | 1,600 | 513 | 500 | 1,000 |
| Kazakhstan | 1,500 | 1,500 | 1,500 | 1,500 | 1,500 | 1,500 |
| Russia | 1,500 | 1,500 | 1,500 | 1,500 | 1,500 | 1,500 |
| Belgium | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| Others | 1,960 | 1,760 | 590 | 360 | 465 | 460 |
| World total | 59,400 | 59,800 | 55,900 | 52,700 | 54,400 | 54,500 |

(Quoted from Japan Oil, Gas and Metals National Corporation 2012)

In the U.S., 4 aromatic arsenic compounds such as 4-aminophenylarsonic acid (*p*-Arsanilic acid, CAS registered number: 98-50-0), 4-Nitrophenylarsonic acid (nitarsone, CAS registered number: 98-72-6), *N*-Acetyl-4-aminophenylarsonic acid (arsacetin, CAS registered number: 618-22-4) and 4-Hydroxy-3-nitrophenylarsonic acid (roxarsone, CAS registered number: 121-19-7) have been used as feed additives for poultry or pigs. However, these compounds are not designated as feed additives in Japan.

In Japan, all of the organic arsenic compounds which had been registered as pesticides based on the Agricultural Chemicals Control Act were expired in 1998.

Diphenylarsinic acid (DPAA, CAS registered number 4656-80-8), an organic arsenic compound, had been used as the material for poison gas shells in former Japanese army. Generally, it does not exist in nature. However, the illegal dumping of waste containing diphenylarsinic acid in Kamisu, Ibaraki, contaminated the soil and well water, and caused a health hazard in the population (Nakashima et al. 2006; Ministry of the Environment 2008a).

## 3. Distribution and dynamics in the environment

(1) Air

Naturally occurring arsenic in the air is derived from weathering of mineral and from volcanic activity. Arsenic is also contained in seawater and in plants, so the blow-up of sea salt grain or a forest fire releases the arsenic into the air (ATSDR 2007). Meanwhile, anthropogenic arsenic is derived from industrial activities such as thermal power generation, metal refining, and incineration of waste containing antiseptic wood and others.

Both naturally occurring and anthropogenic arsenic compounds in the air are mainly inorganic arsenic and there are a few methylated compounds (Pacyna 1987; ATSDR 2000). Most of arsenic in the air is As(III) and mainly diarsenic trioxide (WHO 2001). A part of this As(III) becomes As(V) by oxidation and As(III) and As(V) are coexisting in the air (WHO 2001). The Pollutant Release and Transfer Register (PRTR) survey in Japan revealed that a large amount of fine particles were discharged in the refining process of non-ferrous metal. It is known that the arsenic in those fine articles is mainly diarsenic trioxide (Cheng and Focht 1979).

According to the review of Schroeder et al. (1987), concentration of arsenic in the air is reported to be 2-2,320 ng As/m³ in urban areas, 1.0-28 ng As/m³ in the other areas, and 0.007-1.9 ng As/m³ in the polar region, and the concentration is especially high in urban areas (Schroeder et al. 1987).

In Japan, the mean concentration of arsenic and its compound in the air were 1.6 ng/$m^3$ in fiscal year 2011 according to the “atmospheric pollution in fiscal year 2011 (data from monitoring survey on hazardous air contaminated substance)” published by the Ministry of the Environment (Ministry of the Environment 2013).

(2) Soil

Arsenic is known to present in soil of various earth crust widely at the concentration of approximately 3.4 μg/g (Wedepohl 1991). However, the arsenic concentration in the soil varies from several μg/g to ≥ 100 μg/g in the area where mineral deposit exist (ATSDR 2007). According to a survey in Japan by Oguri et al. (2013), the median value of total arsenic contained in soil which was collected from Japanese ordinary homes was 10 μg/g (4.6-36 μg/g, n = 38).

The same authors reported concentration of bioaccessible inorganic arsenic which is extractable with dilute hydrochloric acid (0.07mol/L), an imitation gastric juice, as follows. Inorganic arsenic (As(V)＋As(III)) was < 0.02 μg/g (< 0.02-0.33 μg/g, n = 20), As(III) was < 0.01 μg/g (< 0.01-0.02 μg/g, n = 20), As(V) was < 0.02 μg/g (< 0.02-0.32 μg/g, n = 20), methylarsonic acid was < 0.01 μg/g (n = 20), dimethyl arsenic acid was < 0.01 μg/g (< 0.01-0.12 μg/g, n = 20) (Oguri et al.2013).

As for chemical form in soils, arsenic exists mainly as diarsenic trioxide in anaerobic soil in general, butmetallic arsenic and arsine also exist (Bhumbla and Keefer 1994). These arsenic compounds become less soluble by binding to iron, aluminum, or manganese oxide, and remain on the surface of the earth. While in a reductive circumstance, it becomes water-soluble and infiltrates into ground water. In waters, inorganic arsenic mainly exists in oxidized form of As(V) and As(III). Moreover, arsenic concentration changes seasonally by the soil moisture (Bhumbla and Keefer 1994). Naturally occurring arsenic contamination in the soil is mainly due to weathering of minerals which contains arsenic, volcanic activity and biological activity, similarly to the situation in the air (WHO 2001).

In Soil Contamination Countermeasures Act (Act No. 53 of May 29, 2002), arsenic and its compounds are designated as a specific harmful substance and the soil elution standard of arsenic and the soil content standard for arsenic were ≤ 10 μg As/L and ≤ 150 μg As/g, respectively. In “enforcement situation of Soil Contamination Countermeasures Act and the result of the survey on soil contamination and countermeasurement cases in fiscal year 2011” (Ministry of the Environment 2013), it was reported that 281 cases did not meet the designated standard for arsenic and its compounds.

Also, the arsenic contamination of the water for agricultural use is an example of soil contamination by anthropogenic arsenic. Currently, although pesticides including arsenic are not used in Japan, arsenic herbicide (sodium methylarsonate (MSMA), Cacodylic acid, Sodium Cacodylate) or feed additives for poultry (roxarsone and others) are used in the U.S. The unchanged roxarsone contained in the waste from poultry such as feces or bedding straw, is converted to inorganic arsenic by microorganisms in soil (Stolz et al. 2007; Makris et al. 2008).

Also in Japan, there are 2 cases of contamination in soil. One is the case of the surrounding area of arsenous acid production in Toroku, Takachiho, Miyazaki, and the other is the case of a smelter in Sasagadani, Shimane (Meeting of specialized groups on Environmental geology, The Geological Society of Japan 1998).

(3)The waters, bottom sediment, ground water

In general, arsenic concentration in seawater is 2 μg/L and relatively stable (Andreae 1978). Naturally occurring arsenic in seawater is attributed to weathering of soil or rocks, and to flowing into the waters due to volcanic activity. Some arsenic is also attributed to naturalelution

from soil and following transit into ground water (Nriagu and Pacyna 1988). In addition, outflow of the dispersed pesticide from the soil into the water system (WHO 2001), and drainage of industrial effluent into river and sea waters are other potential sources of anthropogenic arsenic (Ministry of Economy, Trade and Industry and Ministry of the Environment 2005).

Regarding chemical form of arsenic in seawater, As(V) is the major form of arsenic in oxygen rich seawater and brackish water. While As(III) is considered hardly exceed 20% of the total arsenic amount. Marine sediments which are not contaminated with arsenic contain 5-40 μg/g dry weight. A bottom sediment that is under oxidative circumstance contains As(V) more than As(III), while a bottom mud under reductive environment contains As(III) more than As(V). Arsenic in a bottom sediment which contains a high amount of sulfur under a reductive circumstance form realgar. If the realgar contains sulfide of copper or zinc, the solubility and transitivity of the realgar to the seawater is decreased (Neff 1997).

The contamination of ground water with naturally occurring inorganic arsenic is reported all over the world, such as in India (West Bengal), Bangladesh, Taiwan, northern China, Hungary, Mexico, Chile, Argentina, U.S.A. (especially in the south west area), Thai, and in Ghana (Hagiwara et al. 2004).

Concentration of arsenic in river water has been reported to vary from 0.001 μg/g and less which is far below the values designated in the Guidelines for Drinking-water Quality by WHO (0.01 μg/g) to far above this level of the Guidelines (National Research Council 1985). When a high level of arsenic is detected in the river water, it is attributed to an unnatural pollution source such as drainage from mines or from hot springs besides natural sources (Tatsumi et al. 2002).

(4) House dust

Arsenic concentration in ordinary house dust has been reported by surveys conducted in different countries as follows: 2.1 μg/g (Seifert et al. 2000) in Germany, 7.3 μg/g (Butte and Heinzow 2002) in Canada, 12.6 μg/g (2.6-57 μg/g), 10.8 μg/g (1.0-49 μg/g) (Wolz et al. 2003), and 10.8 μg/g (1.0-172 μg/g) (Tsuji et al. 2005) in the U.S..

According to the survey of Oguri et al. (2013) which analyzed the arsenic compounds in house dust collected from ordinary homes in Japan, the median value of total arsenic was 2.5 μg/g (0.3-7.8 μg/g, n = 40). They reported also concentration of bioaccessible inorganic arsenic which is extractable with an imitation gastric juice, dilute hydrochloric acid, as follows. Inorganic arsenic (As(V)＋As(III)) was 0.24 μg/g ( 0.08-1.2 μg/g, n = 20), As(III) was 0.02 μg/g (< 0.01-0.25 μg/g, n = 20), As(V) was 0.21 μg/g ( 0.06-0.99 μg/g, n = 20), methylarsonic acid was < 0.01 μg/g (n = 20), dimethyl arsenic acid was 0.02 μg/g (< 0.01-0.05 μg/g, n = 20) (Oguri et al.2013).

(5) Arsenic circulation in ecosystem

Humans living on land consume organic arsenic compounds which are biosynthesized in a marine ecosystem, through consumption of marine animals and plants as foods or through land animals which had consumed organic arsenic compounds through their feed. Supposedly the human also intake arsenic compounds derived from sedimentary rock directly from the air or indirectly through consumption of plants where arsenic compounds transferred from soils formed by weathering of sedimentary rocks.

Arsenic content differs between marine organisms and terrestrial organisms. The arsenic concentration of marine organisms is considered to be several μg to 100 μg/g wet weight, while the concentration hardly exceeds 1 μg/g dry weight in terrestrial organisms. Also, there is a difference in chemical species of arsenic contained in marine organisms and in terrestrial organisms (Francesconi and Edmonds 1994). In this section, we describe the biological circulation of arsenic through marine and terrestrial ecosystem from the viewpoint of the difference in its chemical species and concentration between the two systems.

(i) Marine ecosystem

In the ocean, arsenic circulates through the complicated ecosystem of a food chain consisting of producer (phytoplankton or seaweed) and consumer which includes decomposer (microorganism such as bacteria) and abiotic environments as well. Figure 2 shows the hypothesis related to this biological circulation of arsenic (Hanaoka 2011).

As mentioned above, arsenic exists in seawater at the concentration of approximately 2 μg/L. The mean residence time which is (the total amount of the substance in the oceanic system / the rate of the substance input to the system) of arsenic is estimated to be several million years (Matsuo 1991; Nishimura 1998).

AsBe, an organic arsenic compound detected universally in marine animals, and AsC which is the precursor of AsBe in marine ecosystem are not detected directly in seawater. However, these organic arsenic compounds have been detected in minute suspended solid precipitated by concentrating seawater which passed through plankton net of 5 μm mesh (Hanaoka et al. 1997).

Most of arsenic exist in seawater is in the form of As(III) or As(V), and traces amount of MMA(V) and DMA(V) have been detected besides inorganic arsenic (Andreae 1983).

Arsenic acid in seawater is deoxidized to diarsenic trioxide by marine algae and then oxidized to organic compounds. Because of this biological activity, vertical distribution of arsenic acid shows a so-called nutrient type profile in which the distribution of arsenic acid is low in the marine surface layer and increasing in the middle and deep layer, while As(III), MMA (V) and DMA (V) are distributed in the surface to middle layer. Arsenic which has

been incorporated into marine phytoplankton and seaweed is accumulated and undergoes metabolic conversion through the food chain.

Inorganic arsenic in seawater which is mainly composed of As(V) is incorporated into marine phytoplankton or seaweed and is concentrated and undergoes organification. The resulted organic arsenic compounds undergo biochemical conversion sequentially through the food chain and accumulated in marine animals as AsBe. As a result of metabolic conversion through the food chain, various organic arsenic compounds exist in marine organisms and the amount of inorganic arsenic in marine organism tissue is low. As for another pathway, microorganisms synthesize *in vivo* AsBe from inorganic arsenic in seawater and accumulate it. Then the AsBe containing microorganisms are incorporated to marine animals through feed, thereby AsBe is accumulated in marine animals. AsBe returned to original inorganic arsenic by gradual decomposition by microorganisms following the death of the marine animals.

Organic arsenic compounds derived from algae contain MMA (V) in the most cases, and inorganic arsenic is a major component only in the tissue of some types of brown algae. Seasonal change of DMA (V) concentration in seawater is attributed to deoxidization of arsenic acid which has been accumulated by phytoplankton, bacteria, and yeast and to subsequent methylation (Neff 1997).

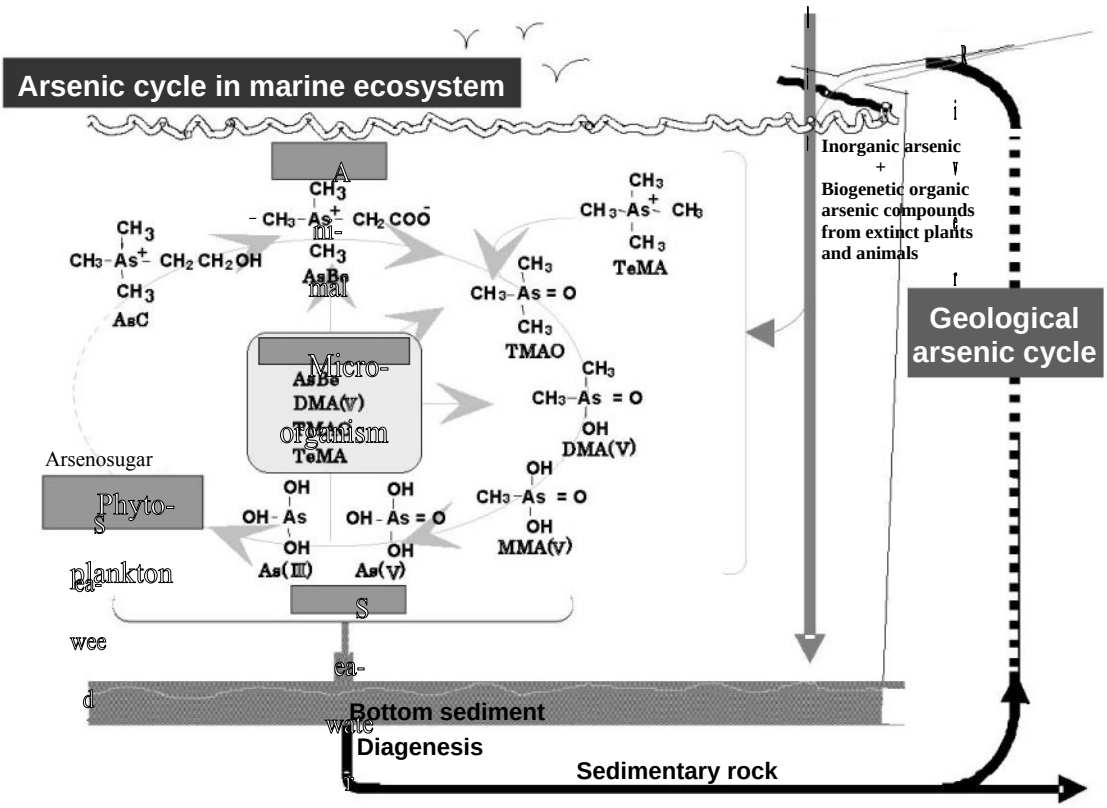



Figure 2 Hypothetical arsenic cycle in marine ecosystem
(Adapted from Hanaoka 2004; 2011)

(ii) Terrestrial ecosystems

Arsenic in terrestrial ecosystems is considered to be circulating along with changing chemical species mainly by deoxidization and methylation (Cullen and Reimer 1989; Ridley et al. 1977). However, some arsenic compounds in terrestrial ecosystems may be converted into the compounds with more complicated structure such as arsenosugar or AsBe like in marine organisms. In an analysis of mushrooms which contains arsenic at high concentration, it was reported that some mushrooms accumulate a high level of MMA (V) only, some accumulate As(III) and As(V) only, some mushrooms are mainly composed with DMA (V) and some contain AsBe (Byrne et al. 1995).

Arsenate-reducing bacteria and diarsenic trioxide-oxidizing bacteria have been suggested to exist in the environment with arsenic of high concentration (Oremland and Stolz 2003). Also, clostridium converts roxarsone into inorganic arsenic under anaerobic condition in the soil (Stolz et al. 2007). The role of the volatilization of arsenic through microorganisms is also considered to be important for arsenic in the air. A chain of such events has been visualized where dimethylarsinic acid scattered to soil was metabolized by microorganisms to

dimethylarsine which then volatilize and was further converted to DMA(V) by oxidation in the air, absorbed to dust, and floated in the air (Mukai et al. 1986).

## 4. Current regulations and others

As for regulation of arsenic in foods in Japan, Specifications and Standards for Food, Food Additives, Etc. (Ministry of Health and Welfare Notification No. 370, 1959) was established designating the requirement for good quality of food and food additives on December 28, 1959, following the Morinaga Arsenic Milk Poisoning Incident in 1955. Since then, arsenic various foods has been regulated by the Food Sanitation Act.

Table 5 shows the major regulations for arsenic contained in foods and non-food.

Table 5 Current regulations in Japan

| Name of law | Category of law | Relevant substances |
|---|---|---|
| Act on Confirmation, etc. of Release Amounts of Specific Chemical Substances in the Environment and Promotion of Improvements to the Management Thereof | Class I Designated Chemical Substances | Arsenic and its inorganic compounds |
| Poisonous and Deleterious Substances Control Act | Toxic substance | Arsenic, arsenic compounds |
| Food Sanitation Act | Standards for pesticide residues 1.0-3.5 μg As/g (Value may differ depends on the crop) | Arsenic and its compounds |
| | Standards and criteria for food: standards for ingredient in soft drinks should not be detected as As | Arsenic and its compounds |
| | Specifications and standards for apparatus, containers and packages: Leaching standards of metallic cans; 0.15 μg As/g | Arsenic and its compounds |
| | Material standards for polyethylene and polystyrene used for the area where milk and milk products directly touch the content : 1.51 μg As/g | Arsenic and its compounds |
| | Leaching standards of metallic used | Arsenic and its compounds |

| | | |
|---|---|---|
| | for the area where milk and milk products directly touch the content : 0.076 μg As/g | |
| | Material standards for synthetic resin contained in the processed aluminium foil which is used as a stopper where milk and milk products directly touch the content: 1.51 μg As/g | Arsenic and its compounds |
| | Standards criteria for toy: Leaching standards for the materials comprised mostly of shadowgraph, folding papers, vinyl chloride resin coating, or polyvinyl chloride; 0.076 μg As/g | Arsenic and its compounds |
| | Ingredient standards for washing agent: 0.038 μg As/g | Arsenic and its compounds |
| Water Supply Act | Water-quality standard: 10 μg As/L | Arsenic and its compounds |
| The Environment Basic Act | Environmental standard related to water pollution: 10 μg As/L | Arsenic |
| | Environmental standard related to water pollution of groundwater: 10 μg As/L | Arsenic |
| | Environmental standard related to soil contamination: 10 μg As/L (test liquid concentration for elution test: < 15 mg As/kg soil [1mol/L extracted in hydrochloric acid] only for agricultural land [limited to rice fields]) | Arsenic |
| Sewerage Act | Water-quality standard: 100 μg As/L | Arsenic and its compounds |
| The Water Pollution Prevention Act | Effluent standard: 100 μg As/L | Arsenic and its compounds |
| Agricultural Land Soil Pollution Prevention Law | Requirements for designation of the agricultural land with soil pollution control. : 15 mg As/kg (1mol/L extracted in hydrochloric acid) | Arsenic |
| | Soil Leachate Standard: 10 μg As/L | Arsenic and its compounds |

| | | |
|---|---|---|
| Soil Contamination Countermeasures Act | Standard of soil content: 150 μg As/g | Arsenic and its compounds |
| The Pharmaceutical Affairs Law | Poison | Arsenic, its compounds, and its products |
| | Prescription drug | Diarsenic trioxide |
| Labor Standards Act | Disease-related chemical substance | Arsenic and its compounds, arsine |
| | Carcinogenic chemical substance | Inorganic arsenic compounds |
| Industrial Safety and Health Act | Specified chemical substances (Group-2 substances, substance under special supervision) Dangerous and toxic substances subject to indicate their names and others. Standards for the harmful environment of business work which requires a health check. Administrative: 3 μg As/$m^3$ | Arsenic and its compounds (Except for Arsine and Gallium Arsenide) |
| | Hazardous and inflammable gas | Arsine |
| | Dangerous and Toxic Substances Subject to Notify Their Names | Arsenic and its compounds |

Ⅲ. Exposure in human

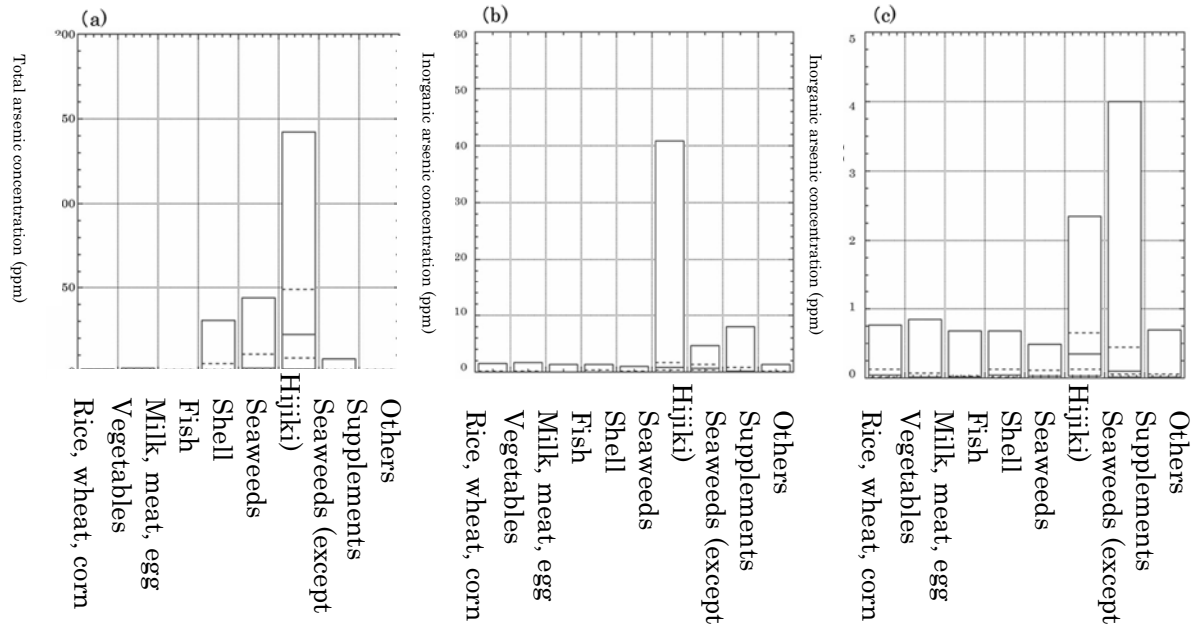
## 1. Oral exposure

Arsenic compounds are mainly taken in through the food and drinking water in general environment. Food contains inorganic and organic arsenic compounds and drinking water mainly contains inorganic arsenic. Intake of inorganic arsenic from the soil or house dust is so small and is negligible (Oguri et al. 2013).

(1) Exposure from food

A large amount of arsenic is contained in seaweed and seafood (Suzuki, 1993). Since Japanese have a traditional dietary habit of taking seaweed and seafood, people intake a large amount of arsenic through food in Japan compared to other countries. A large amount of organic arsenic compounds such as Arsenobetaine (AsBe) and arsenosugar are contained in marine animals and algae.

Regarding the amount of arsenic contained in foods, the total arsenic concentrations in rice, wheat, corn, and in vegetables were less than 1 μg/g at the 95 percentile, while the total concentration of arsenic in seaweed was approximately 20 μg/g at the 50 percentile and over 140 μg/g at the 95 percentile, which showed higher arsenic concentration. It is reported that the total concentration of arsenic was 30 μg/g and more in fish and 40 μg/g or more in shells at the 95 percentile, while the concentration of inorganic arsenic was approximately 0.1 μg/g at the 75 percentile (Figure 3) (Uneyama et al. 2007).



The foods with lower concentration of (a) total arsenic, (b) inorganic arsenic, (c) inorganic arsenic have been shown magnified

Graphs show 5-95 percentile. The lower broken line is 25, the center line is 50, and upper broken line is 75 percentile.

Figure 3 Arsenic contained in foods (percentile) (Revised from Uneyama et al. 2007)

### (I) **Marine Animals and Algae**

Concentration of arsenic contained in marine animals and algae is higher than that in terrestrial animals and plants, and its chemical forms are various (Figure 1).

The percentage of inorganic arsenic (As(III)＋As(V)) in the total arsenic of brown sole, yellowtails, mackerel, chub mackerel, pacific saury and Japanese sardine is 0-4%, and that for seaweed are approximately 60%, 3%, 7% in hijiki, laminaria japonica, and undaria, respectively (Table 6). The abundance ratio of fat-soluble arsenic to water-soluble arsenic in this table may be higher depending on the analytical method (Shiomi, 1992).

AsBe is a major organic arsenic compound existing commonly in seafood (Edmonds et al. 1977; Hanaoka et al. 1988; Francesconi and Edmonds 1994; Francesconi and Edmonds 1997; Shiomi 1994). AsC is contained in shrimp or trumpet shell and others. TMAO is contained in a kind of catfish. TeMA is contained in meretrix lusoria and others. These are the major arsenic compounds contained in these species. (Francesconi and Edmonds 1994; Francesconi and Edmonds 1997; Shiomi 1994).

Arsenosugar is a major arsenic compound in seaweed. However, arsenosugar is found not only in tridacninae, a symbiont of algae (Edmonds et al. 1982), but also in various bivalve such as mytilus edulis and scallop which eat phytoplankton or algae (Shibata and Morita 1992), in snails (Morita and Shibata 1987), and in zooplankton which eats phytoplankton (Shibata et al. 1996; Edmonds et al. 1997).

Various accumulation of arsenic depending on body part of fish has been reported. A large amount of inorganic arsenic were reported to be accumulated in the area around the fisheye and on the body surface (Lunde 1977), and arsenic accumulation was observed in optic nerve of skipjack (Kuroiwa et al. 1999). Relative content of AsBe in total arsenic in edible fish products is higher in the fish having higher freshness, and the relative content decreases in the order of frozen food and preserved food, suggesting that the accumulation of arsenic is affected not only by species or part of fish but also by processing and preservation method (Velez et al. 1995; 1996).

The amount of inorganic/organic arsenic compounds in marine animals and algae and water soluble/lipid soluble arsenic contained in marine animals and algae are shown in Table 6 (Shinagawa et al. 1983; Shiomi 1992).

Table 6 Inorganic /organic arsenic compounds and water soluble/lipid soluble arsenic contained in marine animals and algae

| Samples | | Analyzed part | Content of arsenic μg/g dry weight | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total arsenic | As(III) | As(V) | Organic | Water soluble | lipid soluble |
| Fishes | Brown sole | Muscle | 36.0 | 0.00 | 0.00 | 34.2 | 34.4 | 0.22 |
| | Yellowtails | Muscle | 5.0 | 0.05 | 0.12 | 4.2 | 4.2 | 0.24 |
| | Mackerel | Muscle | 25.6 | 0.00 | 0.06 | 24.0 | 24.3 | 0.18 |
| | Chub mackerel | Muscle | 5.4 | 0.00 | 0.00 | 5.1 | 4.6 | 0.54 |
| | Pacific saury | Muscle | 5.5 | 0.05 | 0.17 | 4.8 | 5.1 | 0.31 |
| | Japanese sardine | Muscle | 17.3 | 0.00 | 0.28 | 15.0 | 15.1 | 0.23 |
| Protochordata | Ascidian | Muscle | 25.0 | 0.00 | 0.05 | 24.3 | 17.3 | 7.6 |
| Echinodermata phylum | Japanese common seacucumber | Muscle | 12.4 | 0.00 | 0.10 | 11.3 | 7.2 | 1.0 |
| | Purple sea urchin | Gonad | 7.3*¹ | 0.16*¹ | 0.22*¹ | 7.0*¹ | 5.1*¹ | 1.8*¹ |
| Arthropods | Yellow sea prawn | Muscle | 41.3 | 0.00 | 0.00 | 39.2 | 39.8 | 1.0 |
| | spotted shrimp | Whole body | 7.6 | 0.07 | 0.00 | 7.2 | 6.0 | 1.0 |
| Mollusks | Turban shell | Muscle | 15.0 | 0.00 | 0.02 | 14.1 | 9.0 | 4.9 |
| | Japanese littleneck | Whole body | 17.5 | 0.04 | 0.01 | 15.9 | 11.7 | 5.0 |
| | North Pacific Giant Octopus | Muscle | 49.0 | 0.00 | 0.00 | 48.8 | 47.3 | 0.20 |
| | Japanese common squid | Muscle | 17.2 | 0.00 | 0.00 | 16.1 | 15.9 | 0.22 |
| | Argentine shortfin squid | Muscle | 9.5 | 0.00 | 0.00 | 9.0 | 9.0 | 0.26 |
| Annelida | Lugworm | Whole body | 5.1 | 0.00 | 0.00 | 5.1 | 3.3 | 1.5 |
| Phaeophyceae | Hijiki | Whole body | 61.3 | 36.7*² | | 15.2 | — | — |
| | Japanese sea-tangle | Whole body | 25.4 | 0.8*² | | 20.2 | — | — |
| | Wakame seaweed | Whole body | 8.3 | 0.6*² | | 6.5 | — | — |

*¹On a wet weight basis *²As(III) inorganic + As(V) inorganic

(Revised from Shiomi, 1992)

The total arsenic concentration in seaweed is generally the highest in brown algae, followed by algae and green algae. Also, in general, the major chemical species of arsenic in seaweed is arsenosugar. Arsenic compounds contained in seaweed are shown in Table 7 (Francesconi and Edmonds 1997).

Table 7 Arsenic compounds contained in seaweeds

| | Species | Arsenic concentration (μg/g) | | Water-soluble rate % | Arsenic compound[a] | | |
|---|---|---|---|---|---|---|---|
| | | Wet weight | Dry weight | | Significant | Minor | Trace |
| **Brown algae** | ***Ecklonia radiate* (Kajime)** | **10** | | **>80** | **3, 2, 7** | **-** | **-** |
| | *Sargassum fusiforme* (hijiki) | 10 | | >80 | Arsenic acid, 1 | 3 | 7, 5 |
| | *Laminaria japonica* (Makonbu) | 4 | | >80 | 3, 4 | 2, 7 | - |
| | *Sphaerotrichia divaricate* (Ishimozuku) | 2 | | 75 | 2 | 7, 3, 5 | - |
| | *Undaria pinnatifida* (Wakame) | 2.8 | | 71 | 23[b] | - | - |
| | *Sargassum thunbergii* (Umitoranoo) | 4 | | 51 | 1 | - | 13 |
| | *Sargassum lacerifolium* (No Japanese name) | 40 | | >80 | 1 | 7, 3, 4, 2 | 5, DMA(V), 6, 8, 15 |
| | *Spatoglossum pacificum* (Komongusa) | | 16.3 | 69 | 3 | 2, 7 | - |
| | *Pachydictyon coriaceum* (Sanadagusa) | | 16.7 | 72 | 3 | 2 7 | - |
| Green algae | *Codium fragile* (Miru) | 0.6 | | 67 | 2 | 7, DMA(V) | - |
| | *Ulva pertusa* (Anaaosa) | | 17.1 | 40 | 2 | 7 | UK |
| | *Bryopsis maxima* (Oohanema) | | 19.4 | 20 | 7 | 2 | UK |
| | *Caulerpa brachypus* (Heraiazuta) | | 11.6 | 32 | UK | - | - |
| Red algae | *Corallina pilulifera* (Pirihiba) | | 21.6 | 15 | 7 | 2, UK | - |
| | *Cyrtymenia sparsa* (Hijirimen) | | 44.8 | 69 | 7 | 2 | - |
| | *Ahnfeltia paradoxa* (Harigane) | | 11.7 | 58 | 7, UK | 2, 1 | - |
| | *Coeloseira pacifica* (Isomatsu) | | 23.1 | 35 | 7, UK | 2 | - |
| | *Laurencia okamurai* (Mitsudesozo) | | 19.2 | 47 | 2, 1 | 7, UK | - |

a Arsenic compounds are indicated by a number which corresponds to the number of structure in Figure 1.
Significant: 20% or more of the total water soluble arsenic; Minor: 1-19% of the total water soluble arsenic; Trace:1%or less of the total water soluble arsenic; UK: Unknown arsenic compound

b Lipid soluble arsenic

(Revised from Francesconi and Edmonds, 1997)

The proportion of inorganic arsenic such as arsenic acid is high in hijiki (*Sargassum fusiforme*), sargassum, and sargassum ringgoldianum which belong to the Class Phaeophyceae Order Fucales Family Sargassaceae (Francesconi and Edmonds 1997).

The mean total concentration of arsenic in marketed dried hijiki was estimated to be approximately 110 μg As/g and the maximum concentration was approximately 154 μg As/g (FSA

2004; Almela et al. 2006; Ogawa et al.2006). The proportion of organic arsenic such as arsenosugar is high in the seaweed which belongs to the group other than family Sargassaceae (Sakurai et al. 1997; Andrewes et al. 2004).

UK Food Standards Agency (FSA) measured the total arsenic and inorganic arsenic concentrations in 5 species (hijiki, sea trumpet, undaria, tangle weed and laver). The total arsenic was detected in all samples and especially hijiki contained high amount of arsenic. The mean concentration of inorganic arsenic in 9 samples of hijiki was 77 mg As/kg (range: 67-96 mg As/kg) and the content of inorganic arsenic in other 4 species of seaweeds were below the detectable limit of 0.3 mg As/kg. The authors reported that the total arsenic concentration and inorganic arsenic concentration in hijiki were higher than those in other seaweed foods (Rose et al. 2007). According to the survey by FSA in 2004, the maximum inorganic arsenic concentration in dried hijiki which has been soaked in water was 22.7 μg As/g (FSA 2004).

The arsenic concentration in hijiki which is consumed in Japan has been reported. Mohri et al. (1990) measured the arsenic concentration in marine animals and algae from the samples for duplicate diet study in 4 adults (2 males and 2 females), and reported that the total arsenic, inorganic arsenic, and DMA (V) in cooked hijiki were 1.204, 0.479, and 0.569 μg/g, respectively. Also, Nakamura et al. (2008) measured the content of inorganic arsenic, As(III), As(V), MMA(V), DMA(V), and arsenosugar in cooked hijiki (total 15 samples) collected from home or supermarket using LC-ICPMS, and reported that the arsenic concentration were 1.2, 0.031, 1.2, 0.010, 0.030, and 0.028 μg As/g (wet weight), respectively (Nakamura et al. 2008). According to the survey on hazardous chemical substance by Ministry of Agriculture, Forestry and Fisheries, the mean value of the total arsenic contained in marketed dried hijiki and water soaked hijiki in Japan measured by HGAAS were 93 μg/g (28-160 μg/g) and 6 μg/g (2.1-20 μg/g), respectively. Also, inorganic arsenic was measured by HPLC-ICPMS and the mean values were reported to be 67 μg/g (4.5-130 μg/g) and 3.6 μg/g (<0.5-17 μg/g), respectively (MAFF 2012).

Although several reports have been made on the arsenic concentration in hijiki in Japan, the results are rather inconsistent because of the different conditions of the samples.

(ii) Agricultural and animal products

The arsenic concentration in marine organisms are known to range from several to a hundred and some tens μg per gram, while the arsenic concentration in different terrestrial plants are similar. (Lunde 1973).

The Ministry of Agriculture, Forestry and Fisheries conducted survey of the total arsenic in domestic agricultural products, and the results have been published (Table 8). According to this survey, the mean total arsenic in brown rice was 0.17 μg/g (0.04-0.43 μg/g). Also, the mean inorganic arsenic concentration in rice was 0.15 μg/g (0.04-0.37 μg/g) (MAFF, 2012). Hamano-

Nagaoka et al. (2008) reported that the total arsenic and inorganic arsenic in brown rice were 0.12-0.26 μg/g dry weight and 0.11-0.23 μg/g dry weight respectively, indicating that inorganic arsenic was 62.2-96.3% of the total arsenic in brown rice.

Total arsenic concentration in vegetables and fruits has been reported approximately to be 0.01 μg/g (Yamauchi and Yamamura, 1980). Total arsenic (dry weight) and DMA (V) in mushrooms grown in the soil contaminated with arsenic were reported to be 1,420 μg As/g and 970 μg As/g, respectively (Larsen et al. 1998). Total arsenic concentration in meat of mammals was reported to be 0.024 μg/g in beef and 0.018 μg/g in pork (Yamauchi and Yamamura, 1980).

According to a report from FDA, apple juice and pear juice marketed in the United States from 2005 to 2011contained arsenic. The total arsenic, range from undetected level to 0.045 μg/g were contained in the 160 samples of apple juice and range from undetected level to 0.124 μg/g were contained in the 142 samples of pear juice analyzed. Inorganic arsenic, MMA, and DMA were detected in the pear juice which contained more than 23 ppb (0.023 μg/g) of the total arsenic, at the concentration of 0.005-0.06μg/g, undetected to 0.088μg/g, and undetected to 0.003 μg/g, respectively (FDA 2011; 2012).

Regarding animal products, a case of roxarsone, an organic arsenic compound used abroad as a growth promotant of poultry has been reported about its excretion where the most of the dose administered in chicken were excreted in the undegraded form (Morrison 1969). However, according to the survey conducted by the Institute for Agriculture and Trade Policy (IATP), arsenic was detected in approximately 55% of uncooked chicken purchased at the supermarket in the U.S., suggesting that chicken may be contaminated through arsenic containing feed additives such as roxarsone and arsanilic acid which have been approved in the U.S. (Wallinga 2006)

Table 8 Concentration of total arsenic in agricultural products in Japan (Products in FY 2003 to 2005)

| Crops | Number of analyzed samples | Detection limit mg/kg | Number of samples containing arsenic at below detection limit | | Number of samples containing arsenic at above detection limit | Minimum value mg/kg | Maximum value mg/kg | Mean value mg/kg |
|---|---|---|---|---|---|---|---|---|
| | | | | % of total samples | | | | |
| Rice (brown rice) | 600 | 0.01 | 0 | 0 | 600 | 0.04 | 0.43 | 0.17 |
| Wheat | 363 | 0.01 | 312 | 86 | 51 | <0.01 | 0.04 | 0.009 |
| Soybean | 300 | 0.01 | 278 | 93 | 22 | <0.01 | 0.04 | 0.008 |
| Sugar cane | 100 | 0.01 | 94 | 94 | 6 | <0.01 | 0.01 | 0.004 |
| Taro (With skin) | 98 | 0.01 | 59 | 60 | 39 | <0.01 | 0.04 | 0.01 |
| Taro (without skin) | 29 | 0.01 | 29 | 100 | 0 | - | - | 0.006 |
| Radish | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.004 |
| Carrot | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.004 |
| Solanum tuberosum | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.004 |
| Cabbage | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.003 |
| Broccoli | 100 | 0.01 | 99 | 99 | 1 | <0.01 | 0.01 | 0.003 |
| Chinese cabbage | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.003 |
| Lettuce | 99 | 0.01 | 99 | 100 | 0 | - | - | 0.003 |
| Spinach | 160 | 0.01 | 126 | 79 | 34 | <0.01 | 0.12 | 0.01 |
| Leek | 100 | 0.01 | 99 | 99 | 1 | <0.01 | 0.02 | 0.005 |
| Onion | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.005 |
| Cucumber | 101 | 0.01 | 98 | 98 | 2 | <0.01 | 0.02 | 0.006 |
| Eggplant | 100 | 0.01 | 97 | 97 | 3 | <0.01 | 0.01 | 0.006 |
| Tomato | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.004 |
| Bell pepper | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.004 |
| Pumpkin | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.003 |
| String beans | 100 | 0.01 | 100 | 100 | 0 | - | - | 0.003 |
| Shiitake mushroom | 100 | 0.01 | 45 | 45 | 55 | <0.01 | 0.14 | 0.02 |
| Strawberry | 100 | 0.01 | 99 | 99 | 1 | <0.01 | 0.01 | 0.005 |
| Apple | 99 | 0.01 | 96 | 97 | 3 | <0.01 | 0.03 | 0.004 |
| Tangerine (With skin) | 40 | 0.01 | 40 | 100 | 0 | - | - | 0.004 |
| Tangerine (Without epicarp) | 60 | 0.01 | 60 | 100 | 0 | - | - | 0.003 |
| Chinese citron (Without epicarp) | 30 | 0.01 | 30 | 100 | 0 | - | - | 0.003 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chinese citron (With epicarp) | 70 | 0.01 | 70 | 100 | 0 | - | - | 0.003 |
| Persimmon | 100 | 0.01 | 92 | 92 | 8 | <0.01 | 0.03 | 0.005 |
| Kiwi fruit (With skin) | 70 | 0.01 | 69 | 99 | 1 | <0.01 | 0.01 | 0.004 |
| Kiwi fruit (Without skin) | 30 | 0.01 | 30 | 100 | 0 | - | - | 0.003 |
| Pear | 100 | 0.01 | 99 | 99 | 1 | <0.01 | 0.02 | 0.004 |
| Peach | 100 | 0.01 | 98 | 98 | 2 | <0.01 | 0.01 | 0.004 |
| Grape | 100 | 0.01 | 99 | 99 | 1 | <0.01 | 0.01 | 0.004 |

Minimum value: The lowest value of analysis results in more than 1 sample. Value is not shown in case the all samples analyzed were below the detection limit.

Maximum value: The highest value of analysis results in more than 1 sample. Value is not shown in case the all samples analyzed were below the detection limit.

Mean value: Arithmetic mean of the analyzed results of more than 1 sample. The value was calculated according to the method suggested by GEMS/FOOD based on the analyzed value of the samples (WHO, 2003).

(Documented from MAFF 2012)

(iii) Drinking-water

Table 9 shows analytical data on the concentration of arsenic and its compounds in tap water detected in the samples from different sampling points in FY 2010. Although the maximum values in raw water from 28 sampling points were above the water quality standard for drinking-water as per the Waterworks Act (0.01 mg/L), most of the maximum values in raw water were 0.001 mg/L or less at 4,512 out of 5,319 sampling points. Also, most of the maximum values in clean water were 0.001 mg/L or less at 5,173 out of 5,679 sampling points.

People do not intake arsenic at the concentration beyond the water quality standard for drinking-water as per Waterworks Act (0.01 mg As/L) when the surface water is supplied as a source water to the tap water. However, when groundwater is used for drinking-water, all arsenic in groundwater is absorbed since people generally drink it directly. According to the analytical data on groundwater quality by Ministry of the Environment in FY 2011, approximately 2% of groundwater contains arsenic at the concentration above the water quality standard for drinking-water of Waterworks Act and the maximum value which has been detected from groundwater in Japan was 0.65 mg As/L (Ministry of the Environment 2012).

It has been reported that only As(III) and As(V), inorganic arsenics, were detected in 81 samples of hot spring water used for drinking in Japan. The mean concentration of total arsenic was reported to be 120.1 (0.116-1,024) μg As/L (Chiba et al. 2008).

Table 9 Concentration of arsenic and its compounds in tap water (Japan Water Works Association 2010)

| Clean water/ raw water | Water source | Number of sampling points | Frequency distribution in terms of target value | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ≤10% | >10% ≤20% | >20% ≤30% | >30% ≤40% | >40% ≤50% | >50% ≤60% | >60% ≤70% | >70% ≤80% | >80% ≤90% | >90% ≤100% | >100% |
| | | | up to 0.001 (mg/L) | up to 0.002 (mg/L) | up to 0.003 (mg/L) | up to 0.004 (mg/L) | up to 0.005 (mg/L) | up to 0.006 (mg/L) | up to 0.007 (mg/L) | up to 0.008 (mg/L) | up to 0.009 (mg/L) | up to 0.010 (mg/L) | from 0.011 (mg/L) |
| Raw water | Overall | 5,319[1)] | 4,512[1)] | 408[1)] | 158 | 65 | 64 | 30 | 19 | 16 | 11 | 8 | 28 |
| | Surface water | 1,077 | 878 | 124 | 35 | 15 | 8 | 3 | 2 | 2 | 0 | 2 | 8 |
| | Water from dam/ lake | 281 | 231 | 27 | 15 | 1 | 4 | 0 | 1 | 0 | 1 | 0 | 1 |
| | Groundwater | 3,100 | 2,674 | 187 | 84 | 40 | 42 | 22 | 13 | 11 | 8 | 5 | 14 |
| | Others | 839 | 709 | 68 | 24 | 9 | 10 | 5 | 3 | 3 | 2 | 1 | 5 |
| Clean water | Overall | 5,679[1)] | 5,173[1)] | 242 | 121 | 56 | 39 | 14 | 14 | 11 | 5 | 4 | 0 |
| | Surface water | 1,052 | 996 | 29 | 12 | 6 | 4 | 1 | 2 | 2 | 0 | 0 | 0 |
| | Water from dam/lake | 267 | 258 | 4 | 3 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | Groundwater | 3,005 | 2,650 | 170 | 81 | 44 | 30 | 11 | 6 | 7 | 4 | 2 | 0 |
| | Others | 1,340 | 1,254 | 39 | 25 | 5 | 5 | 2 | 5 | 2 | 1 | 2 | 0 |

(Data from survey in FY 2010)

(2) Estimation of oral exposure

(i) Total arsenic

A survey on the total arsenic intake from food and drinking-water in Japan was conducted by duplicate diet method or by market basket method.

Mohri et al. (1990) measured the mean total arsenic intake for 3 days in 12 adults (6 males and 6 females) by duplicate diet method and the mean daily intake of total arsenic was 201.6±142.9 μg (31.0-682.0 μg). Also, the mean daily intake of total arsenic in 4 adults (2 males and 2 females) from 7 days survey was reported to be 182.3±114.0 μg (27.0-376.0 μg).

Yamauchi et al. (1992) reported that the mean daily intake of total arsenic in 35 adults (12 males and 23 females) estimated by duplicate diet method was 195±235 μg (15.8-1039 μg).

In the Total Diet Survey (2009) by Ministry of Health, Labour and Welfare, all foods including drinking-water were divided into 14 groups and purchased from retail stores etc. based on

1) Documented as in the survey results of Japan Water Works Association 2010, although there are some discrepancy between total number of surface water, dam, lake water, groundwater, and others, and overall numbers.

the food intake estimated by National Health and Nutrition Survey. The food was cooked if necessary. Arsenic was measured by HGAAS for each food group, and a mean daily intake per person was estimated by market basket method. As a result, the total daily intake of arsenic in Japanese was estimated to be 177.8 μg (of that, drinking-water: 0.1 μg) for the period from 2002 to 2006.

FSCJ (2013) conducted the analytical survey of total arsenic using the food samples collected in duplicate diet study in the Survey of the Exposure to Dioxins and other chemical compounds in Humans (conducted between 2006 and 2010) by Ministry of the Environment. Among the diet samples from the subjects for 3 consecutive days, the total of 319 samples (94 from males and 225 from females) were collected individually on random days and thereafter, analyzed. As a result, the mean daily intake of total arsenic and 95 percentile value per body weight were reported to be 199±272 μg (3.14-2170 μg) and 8.88 (μg/kg bw/day), respectively.

(ii) Inorganic arsenic

Mohri et al. (1990) measured inorganic arsenic in 12 adults (6 males and 6 females) by atomic absorption spectrometry (AAS) using the samples for duplicate diet study for at least 3 days and reported that the inorganic arsenic intake per day was 13.7±7.8 μg (1.2-31.7 μg). In the same manner, the 7 days survey in 4 adults (2 males and 2 females) was conducted and the mean inorganic arsenic intake per day was 10.3±5.5 μg (1.8-22.6 μg). Yamauchi et al. (1992) measured inorganic arsenic in the samples for duplicate diet study from 35 adults (12 males and 23 females) by AAS and reported that the mean inorganic arsenic intake per day was 33.7±25.1 μg (8.34-101 μg).

Oguri et al. (2012) reported that the mean daily inorganic arsenic intake measured by duplicate diet method in 25 adult females living in metropolitan area was 6.52±11 μg (2.0-57 μg). The same authors estimated the daily intake from certified reference material (NIES CRM NO.27) which was prepared by mixing and uniforming the duplicate diet samples collected for 141 days from all over Japan. The estimated value was 27 μg according to the relevant report.

FSCJ (2013) analyzed inorganic arsenic in 319 samples (94 males and 225 females) that were collected in the duplicate diet study for the Survey of the Exposure to Dioxins and other chemical compounds in Humans (conducted between 2006 and 2010) by Ministry of the Environment. Consequently, FSCJ reported daily inorganic arsenic intake of 8.6±19.6 μg (2.18-161 μg) and 95 percentile value per body weight of 0.754 μg/kg bw/day.

Kawabe et al. (2003) estimated the exposure of Japanese to inorganic arsenic, by Monte Carlo method, based on the published representative value and distribution of total arsenic concentration in different medium. According to their estimation, the total exposure in a household where people drink tap water was 0.42 μg/kg bw/day, and of that, 94.3% are from food, 1.3% are from direct intake of soil, and 0.1% are from atmosphere inhalation.

(iii) Organic arsenic

Mohri et al. (1990) analyzed organic arsenic concentration in the samples for duplicate diet studies by the same method as was described above. The mean amount per day detected in the samples for 3 days and more from 12 adults (6 males and 6 females) were MMA 7.6±7.9 μg (0.6-36.0 μg), DMA 34.0±34.7 μg (0-110 μg), and trivalent methylated arsenic species 120.4±97.8 μg (0-425 μg). Methylarsonic acid 6.5±4.6 μg (0.6-19.0 μg), DMA 49.9±49.8 μg (2.8-183.6 μg), and trivalent methylated arsenic species 87.3±76.8 μg (10-271.4 μg) per day were detected in 7 days survey in 4 adults (2 males and 2 females).

According to the report by Yamauchi et al. (1992), in the measurement of samples for duplicate diet study in 35 adults (12 males and 23 females), the mean daily intake of organic arsenic, MMA, DMA, and trivalent methylated arsenic species were 2.25±2.5 μg (0.16-9.63 μg), 12.9±11.1 μg (0.36-38.0 μg), and 148±226 μg (1.95-946 μg), respectively.

Oguri et al. (2012) analyzed daily intake of MMA (V), DMA (V), and TMAO by duplicate diet method in 25 adult females living in metropolitan area. They reported that the mean of daily intake detected in the duplicate diet samples were <0.18 μg (<0.18-0.39 μg), 1.8±2.3 μg (0.35-12 μg), and <0.053 μg (<0.053-2.4 μg), respectively. In addition, they estimated daily intake from certified reference material to be 3.9 μg, 12 μg, and 5.9 μg, respectively. Also, the authors reported that daily intake of AsBe estimated from the certified reference material mentioned above was 140 μg.

FSCJ (2013) analyzed inorganic arsenic in 319 samples (94 males and 225 females) that were collected in the duplicate diet study for the Survey of the Exposure to Dioxins and other chemical compounds in Humans (conducted between 2006 and 2010) by Ministry of the Environment. Consequently FSCJ reported that daily intake per day of DMA and AsBe were 6.47±4.59 μg (1.97-39.7 μg) and 88.8±205.8 μg (2.05-1,680 μg), respectively.

## 2. Inhalation exposure

One of routes of exposure of human to arsenic is exposure through air inhalation. In Japan, NEDO (2008) estimated the daily intake of arsenic by inhalation exposure to be 0.22 μg As/person/day, based on atmospheric arsenic concentration of 0.011 μg As/$m^3$, and the daily intake per body weight in inhalation path to be 0.0044 μg As/kg bow/day (NEDO 2008).

Oguri et al. (2013) estimated the daily arsenic exposure through respiratory tract in Japanese adult to be 0.02 μg/day, from the national average (1.4 ng/$m^3$) of toxic air pollutant monitoring in FY 2010 by assuming 15 $m^3$ as amount of ventilation per day. Methylated arsenic is hardly detected in the air in general environment except in the area where the pesticide containing organic arsenic was scattered, and most of the detected arsenic has been estimated to be inorganic arsenic (especially As(V)) (Lewis et al. 2012). Therefore, the authors deemed the value

of 0.02 μg/day to be daily exposure of inorganic arsenic, thus exposure level through respiratory tract to be approximately 0.1％ of oral exposure.

When the pesticide containing organic arsenic was used, the maximum arsenic of 52 μg/g used to be contained in tobacco, but it decreased to 3 μg/g after the use was prohibited (Holland and Acevedo 1996; Becker and Wahrendorf 1993). The mean arsenic contained in 1 cigarette was reported to be 1.5 μg (Small HG Jr. and McCants CB 1962). It has been reported that the mainstream tobacco smoke contained 0-1.4 μg of arsenic per cigarette (Cogbill and Hobbs 1957) and sidestream smoke contained 0.015-0.023 μg (mean: 0.018 μg) of arsenic per cigarette (Landsberger and Wu 1995).

# IV. Summary of safety data

## 1. Pharmacokinetics

(1) Absorption

(i) Inorganic arsenic compounds

In most animal species, 80-90% of orally administered As(III) and As(V) are rapidly absorbed through the gastrointestinal tract (NEDO 2008). The absorption rate of 55-87% and of 40-50% have been reported in human (Buchet 1981; Crecelius 1977; Kumana 2002; Mappes 1977; Tam 1979; ATSDR 2007) and in hamsters (NEDO 2008) respectively. It is also reported that arsenite (As(III)) and arsenate (As(V)) in drinking-water is rapidly and almost completely, by approximately 95%, absorbed after intake (Zheng 2002). However, the absorption of inorganic arsenic in food depends upon the solubility of the arsenic compounds, the existence of other food components and nutrients in the gastrointestinal tract, and upon food matrix (EFSA 2009). Gastrointestinal absorption of low soluble arsenic trisulfide such as arsenic selenide and gallium arsenide is considered to be low (Mappes 1977; Vahter 2002; ATSDR 2007). According to JECFA (2011), gastrointestinal absorption of pentavalent arsenic compounds in mice after oral administration was examined by Odanaka et al. (1980) and Vahter and Norin (1980). Odanaka et al. reported a low rate of the urinary excretion, 48.5%, while Vahter and Norin (1980) reported a high urinary excretion rate of 89%. This discrepancy is attributed to the fact that the animals were fed freely in the former study while the animals were fasted for at least 2 hours prior to the administration to 48 hours after the administration of arsenic in the study of Vahter and Norin (1980) (JECFA 2011).

With *in vivo* model in pigs, Juhasz et al. (2006) examined bioavailability of arsenic. They compared the bioavailablity of arsenic contained in rice which was grown with water contaminated with disodium arsenate and was cooked with non-contaminated water to the bioavailability of arsenic contained in commercially available rice cooked with contaminated water. Bioavailability of arsenic contained in the former rice which contained mainly DMA (V) was low as 33.1±3.2%, while the latter which contained inorganic As(V) showed a high extent of bioavailability of 89.4±9.4%. The authors concluded that the extent of bioavailability of arsenic contained in rice provably depends on the chemical species of the arsenic and is also affected largely by the existence of arsenic in cooking water and its chemical species. Moreover, Juhasz et al. (2008) reported that while the bioavailability of inorganic arsenic contained in mung bean for pigs was almost 100%, the bioavailability of arsenic contained in lettuce and chard was 50%. Based on these data, they suggested effects of indigestible polysaccharide component of vegetables on gastrointestinal absorption of arsenic.

In addition, metabolism of arsenic in different strains of mouse (C57BL, C3H and B6C3F$_1$) was investigated and the difference in the gastrointestinal absorption rate of orally administered arsenic acid has been found (Hughes et al. 1999; Food Safety Commission of Japan (FSCJ) 2009).

(ii) Organic arsenic compounds

Little is known about gastrointestinal absorption of the orally administered organic arsenic in human (EFSA 2009). There is a study conducted by Buchet et al. (1981) where urinary excretion of either MMA (V) or DMA (V) which was administered to volunteers by a single oral dose (500 μg As) was measured. The study demonstrated that the urinary excretion of the arsenic for up to 4 days after the administration was 78% and 75% respectively, suggesting that gastrointestinal absorption of pentavalent organic arsenic compounds was higher than 75%.

Francesconi et al. (2002) reported that approximately 80% of arsenosugar was excreted in urine 4 days after the intake of the arsenosugar in a male volunteer. However, very large individual difference in absorption of arsenosugar has been suggested recently from data based on the urinary excretion (Raml et al. 2009).

As for AsBe, Brown et al. (1990) reported the absorption of $^{74}$As-AsBe administered into 6 volunteers, where the systemic content of $^{74}$As-AsBe 1 day after the administration was approximately 50% of the intake amount and this decreased gradually for a few days afterwards. However, Brown et al. (1990) suggested that most of the absorbed AsBe was excreted in urine on the following day of the administration (EFSA 2009). Similarly, Yamauchi and Yamamura (1984) reported that AsBe contained in fish and seafood was absorbed rapidly from the gastrointestinal tract in humans, and most of them were excreted in urine within 72 hours after the administration.

In rodents, more than 40% of the intake amount of compounds such as MMA(V) or DMA(V) in which arsenic exists as a form of pentavalent is absorbed from the gastrointestinal tract, while the absorption rate of trivalent organic arsenic compounds is considered to be low (Goodman and Gilman 1980; Vahter 1994; Hughes et al. 2005; EFSA 2009). Juhasz et al. (2006) reported that gastrointestinal absorptions of MMA (V) and DMA (V) in pigs are 17% and 33%, respectively, according to EFSA (2009).

(2) Distribution

(i) Inorganic arsenic compounds

It has been reported that As(III) is insoluble at physiological pH and incorporated into liver cells more rapidly than ionized As(V) (Lerman et al.1983). And affinity of As(III) to

thiol group is approximately 10 times high than that of As(V) (Jacobson-Kram and Montalbano 1985) (NEDO 2008).

The contents of arsenic in tissues of the victims (age, sex unknown) killed in the accident in Mumbai (old Bombay), India, has been reported, although there are big individual differences. The reported contents of arsenic were 3.9 ± 1.0 ng/g in the brain, 5.9 ± 3.9 ng/g in the blood, 12.4 ± 20.7 ng/g in the kidney, 14.5 ± 6.9 ng/g in the liver, 15.2 ± 16.6 ng/g in the spleen and 19.9 ± 22.7 ng/g in the lung (all expressed as per gram wet weight). Low level of arsenic in the brain was likely attributable to the effect of blood-brain barrier which might have prevented transition of arsenic to the brain (Dang et al. 1983). On the other hand, the study on the distribution of As and its metabolites in the tissues of Japanese adults (age: 36-79) who had died of cerebral hemorrhage, pneumonia, or of cancer reported that arsenic concentration in the brain was similar to that in the other tissues, and that big individual differences were observed in all tissues (Yamauchi and Yamamura 1983; NEDO 2008).

In addition, inorganic arsenic such as As(III), As(V)), MMA(III), MMA(V), DMA(III), DMA(V), AsBe and As has been detected in the plasma of the inhabitants of West Bengal which is the area contaminated with arsenic (Suzuki et al. 2002; NEDO 2008).

Rodriguez et al. (2005) studied arsenic concentration in the brain and liver in male CD1 mice after oral administration of disodium arsenate (As(V)) (0, 2.5, 5, 10 mg/kg bow/day) for 9 days. The data showed that the total arsenic concentration increased in the brain and liver in all dosed groups compared to the control group, and that it was approximately 2 and 3 times higher (in the brain and in the liver respectively) in the 10 mg/kg bw/day group, as compared to the controlled group. The arsenic which showed significant increase was DMA in the brain and inorganic arsenic in the liver, and the content of each in the 10 mg/kg bw/day group were approximately 9 times and 3 times higher than that in the controlled group, respectively (Rodriguez et al. 2005; ATSDR 2007). Kenyon et al. (2008) studied total accumulation of arsenic in tissues in female C57BL/6 mice after 12 weeks administration of disodium arsenate (As(V)) (0.5, 2, 10, 50 ppm as; 0.08, 0.35, 1.9, 7.0 mg as/kg bw/day) in drinking-water. Total accumulation of arsenic which was measured as a total of inorganic arsenic, MMA and DMA, was the highest in the kidneys, followed by the lungs, bladder, skin, blood, and then the liver, being the lowest. Among the detected metabolites of arsenic compounds, MMA was the highest in the kidney and DMA was the highest in the lung (Kenyon et al. 2008; EFSA 2009).

The excretion of arsenic in breast milk is known to be extremely low. According to the survey conducted in Bangladesh by Fangstrom et al. (2008), despite the high arsenic exposure through drinking-water where the median of urinary excretion amount was approximately 50 μg/L, the arsenic concentration in the breast milk sample was low showing median

value of 1 μg/kg, and the arsenic compound which transited to breast milk was mainly inorganic arsenic (EFSA 2009).

In mammals including humans, inorganic arsenic and its methylated metabolites, MMA and DMA, are known to pass through the placenta and to transfer to the fetus (Lindgren et al. 1984; Concha et al. 1998a; EFSA 2009).

(ii) Organic arsenic compounds

Vahter et al. (1984) studied the accumulation of organic arsenic, $^{74}$As-DMA, after oral administration in mice and rats. The detected accumulation within 6 hrs after administration was the highest in the kidney, followed by the lung, intestinal mucosa, stomach and the least in the testis. In many organs, DMA concentration decreased rapidly, but was retained for the longest time in the lung, thyroid gland and intestinal mucosa (EFSA 2009, Vahter et al. 1984). Accumulation of MMA (III) and MMA (V) each in female B6C3F1 mice after a single oral administration (0.4, 40 mg As/kg body weight) was examined by Hughes et al. (2005). As a result, MMA (V) was accumulated the most in the bladder and kidney and MMA (III) was accumulated the most in the lung as being measured as MMA and DMA. The proportion of DMA in tissues of the MMA (V) administered animals (0.4mg As/kg body weight) were 0% in blood and 19% in the lung, while 75% in blood and 100% in the bladder, kidney and lung in the MMA (III) administered animals.

(3) Metabolism

(i) Inorganic arsenic compounds

As(V) in drinking-water is detected as As(III) in blood in most animal species (NEDO 2008). Inorganic arsenic absorbed *in vivo* is metabolized to methylated compounds and mainly DMA (V), MMA (V), arsenic acid and arsonous acid are excreted in urine in humans (JECFA 2011). In this regard, it has been reported that arsenic detected in urine in the inhabitants showing symptoms of arsenic poisoning in the arsenic contaminated area in West Bengal, India, was mainly DMA (III) but not DMA (V) (Mandal et al. 2001). Metabolically methylated MMA (V) and DMA (V) have low acute toxicity, thus arsenic methylation has been considered to be mechanism for detoxication of arsenic *in vivo*. However, metabolic methylation and metabolism to trivalent methylated arsenic (MMA(III), DMA(III)) which are intermediate metabolites of As(V), is considered to be a process of metabolic activation rather than detoxication of inorganic arsenic in these years (EFSA 2009; Food Safety Commission of Japan (FSCJ) 2009).

Figure 4 shows the metabolic pathway for methylation of inorganic arsenic compounds (Aposhian et al. 2000). Generally, oxidative methylation where the methyl group is transferred along with oxidation of As(III) to As(V) has been suggested as a metabolic pathway

of arsenic (Challenger 1951; Aposhian et al. 2000). Also, a methylation mediated by formation of trivalent arsenic-glutathione conjugate shown in Figure 5has been also reported (Hayakawa et al. 2005; Thomas et al. 2007). Both methylations have been considered to be catalyzed by methyltransferase including trivalent arsenic methyltransferase (AS3MT) that requires *S*-adenosylmethionine (SAM) as the methyl donor in the redox cycle of arsenic (Thomas et al. 2007). It is also reported that in this process, reactive oxygen is produced and oxidative stress is induced as a result (Hu et al. 2002). On the other hand, the formation of free radicals such as the arsenic radical caused by the reaction of dimethylarsine which is generated through the process of reductive metabolism of DMA (III) with molecular oxygen has been reported (Yamanaka et al. 1990; Kitchin 2001). Moreover, sulfur-containing arsenic compounds such as dimethylarsine acid have been detected in urine and possible reactions of dimethylarsinic with endogenous sulfur containing compounds were suggested for the mechanism of the formation (Yoshida et al. 2003; Raml et al. 2007; Naranmandura et al. 2007).

(ii) Organic arsenic compounds

Concerning the metabolism of arsenic derived from marine products, only a few reports are available even from animal studies. A study on the chemical species of arsenic in urine and blood of sheep which lived on arsenosugar-rich seaweed revealed that the main metabolite in urine and blood was DMA (V) and the arsenic concentration in urine, blood, organs and in wool was higher than that of sheep which were not exposed with arsenic (Feldmann et al. 2000). Moreover, a study on biotransformation of arsenosugar using mice cecum flora and cecum tissue showed that 95% of arsenosugar were converted to thio arsenic in the reaction mixture for 1 hour at 37°C in which microbial flora was added, but the conversion rate in the reaction mixture composed of cecum tissue alone was low where arsenosugar converted for 48 hours at 37°C was as low as 77% (Conklin et al. 2006). Although thio-DMA(V), thio-dimethyl arseno ethanol (DMAE), and thio-arsenosugar besides DMA(V) were detected as metabolites in urine of humans who had taken in arsenosugar, these arsenic metabolites in urine except DMA(V) were found to be non-cytotoxic even with exposure to high concentration of 10 mM (Raml et al. 2005).

Arseno-lipid, which is a lipid-soluble organic arsenic compound, is metabolized to DMA (V) *in vivo* in humans (Raml et al. 2009; Schmeisser et al. 2006).

The organic arsenic compounds such as AsBe are hardly metabolized and are excreted more rapidly into the urine compared to inorganic arsenic (WHO 2001).

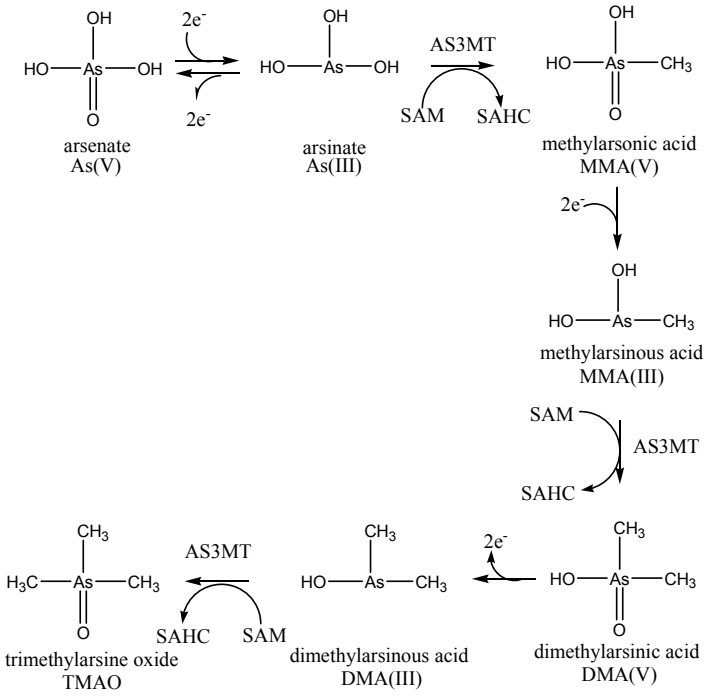
(iii) Species difference and individual difference in metabolism

Species difference is observed in metabolic methylation of inorganic arsenic. In marmosets, chimpanzees and guinea pigs, excretion of MMA (V) or DMA (V) into urine has not been observed since these species are deficient in arsenic methyltransferase in the liver (Food Safety Commission of Japan 2009). On the other hand, rhesus monkeys, rabbits, mice, rats and hamsters have arsenic methyltransferase in the liver therefore arsenic is metabolically methylated in these species (Goering et al. 1999). Moreover, the rate of urinary excretion of MMA (V) in these experimental animals has been reported to be extremely lower than that in humans, suggesting effective methylation from MMA (V) to DMA (V) occurred in these species (Vahter 2000).

The strain difference arsenic metabolism in mice has been investigated in C57BL, C3H and $B6C3F_1$. No strain difference in metabolic methylation was found, though there was a difference in gastrointestinal absorption of orally administered arsenic acid (Hughes et al. 1999).

Recently, effects of AS3MT on distribution and form of arsenic was suggested by a study where sodium arsenate (As(III)) was administered through drinking-water, at the dose of 1.73, 17.3, 43.3 ppm that were 1, 10, 25 ppm As, to 28 wild-type female mice (C57BL/6) (hereafter referred to as WT) and 28 AS3MT knock-out mice (hereafter referred to as KO) for 33 days and the total arsenic concentration in plasma was measured. The total arsenic concentration in plasma was higher in WT than in KO, but the total arsenic concentration in red blood cells was higher in KO than in WT. The total arsenic concentration in the liver, kidneys and lungs were higher in KO than in WT. In these tissues, the proportion of methylated arsenic (mono-, di-, tri-methylated arsenic compounds) in total arsenic was 80% in WT but lower in KO. Methyl arsenic was detected in the tissues of KO despite very low activity of the metabolic methylation. The authors attributed these methylated arsenic observed in the tissue of KO to the arsenic metabolism by intestinal flora (Chen et al. 2011).

Regarding the individual difference in humans, the relationship between genetic polymorphism of enzyme associated with arsenic metabolism such as AS3MT and urinary excretion of methylated arsenic has been investigated (Lindberg et al. 2007; Hernández et al. 2008a). It was reported that MMA (V) in urine increased by a single nucleotide polymorphism of Met287Thr in the AS3MT gene in Chilean (Hernández et al. 2008b).




SAM: *S*-adenosyl-L-methionine

SAHC: *S*-adenosyl-L-homocysteine

AS3MT: Trivalent arsenic methyltransferase

Figure 4 Metabolism of arsenic compounds (oxidative methylation) (Adapted from the Food Safety Commission of Japan (2009))

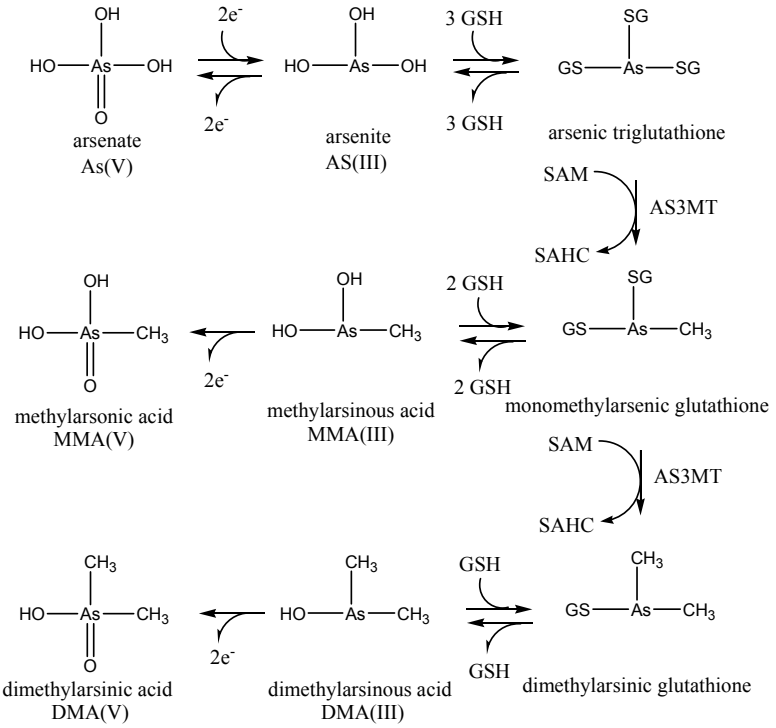



Figure 5 Metabolism of arsenic compounds (Methylation mediated by formation of trivalent arsenic-glutathione conjugate) (Adapted from the Food Safety Commission of Japan (2009))

(4) Excretion

(i) Inorganic arsenic compounds

Arsenic and its metabolites are mainly excreted in urine and bile. Arsenic and its metabolites tend to be excreted preferentially into bile in rats, but arsenic compounds are mainly excreted in urine in many mammals and humans (Schuhmacher-Wolz et al. 2009; EFSA 2009). Generally DMA (V) (40-75%), arsenic acid and diarsenic trioxide (20-25%) and MMA (V) (15-25%) are the arsenic compounds detected in human urine (ATSDR 2007). However, after eating algae and seafood which contain many organic arsenic compounds such as arsenosugar and AsBe, these organic arsenic compounds are excreted into urine.

Chemical speciation of urinary arsenic and sequential changes in its urinary concentration following intake of processed food of hijiki which contains a high amount of inorganic arsenic have been studied. The data showed that arsenic acid, diarsenic trioxide, MMA(V) and DMA(V) reached its maximum level 4, 6.5, 13 and 17.5 hours after intake of hijiki, respectively (Nakajima et al. 2006), and that 50-90% of arsenic was excreted 48-50 hours after intake of hijiki (Yamauchi and Yamamura 1979; Fukui et al. 1981).

Inorganic arsenic in human blood has been reported to disappear with the half-time consists of three phases, i.e. the first phase with a half-time of 1 hour, the second with a half-time of 3 hours and the third phase with a half-time of over 200 hours, and most of the inorganic arsenic disappeared from blood in the first phase (Mealey et al. 1959; Pomroy et al. 1980). A 2 phasic model has been considered appropriate for the excretion of arsenic absorbed in the lung, where 75% of the arsenic was excreted from the lungs with a half-life of 4 days and the other 25% was excreted from the lungs with a half-life of 10 days (Thorne et al. 1986). In addition, the half-life of insoluble arsenic compounds has been reported to be extended considerably (Brune et al. 1980).

(ii) Organic arsenic compounds

In human, MMA and DMA are excreted mainly into urine by 75-85% of the intake within a day. This is also the case in mice, rats and hamsters, except that in animals MMA and DMA are excreted into feces as well (ATSDR 2007). It has been reported that MMA and DMA rapidly disappeared from the body in mice and hamsters. For example, 85% of the administered DMA were excreted with a half-life of 2.5 hours. In contrast, 45% of administered DMA is excreted with a half-life of 13 hours and 55% is excreted with a half-life of 50 days in rats (Quoted from Vahter et al. 1984; ATSDR 2007 for a review).

A survey of 210 Japanese volunteers has revealed that the median value of AsBe in urine and that of DMA (V) were as high as 61.3 μg As/L and 42.6 μg As/L respectively (Hata et al. 2007). Generally, most of AsBe is rapidly excreted into urine right after intake without being metabolized. However, it has been reported that arsenosugar is metabolized to DMA (V) and DMAE which are carcinogenic in some animals (Ma and Le 1998; Francesconi et al. 2002; Heinrich-Ramm et al. 2002).

(iii) Species difference in excretion

Ninety percent of inorganic arsenic administered intravenously in mice is excreted in 2 days (Vahter and Marafante 1983), while the biological half-life in humans is 4 days (Buchet et al. 1981). In rats, the affinity of hemoglobin to As(III) is higher than that of human hemoglobin (IARC 2012), thus a metabolite, DMA (V), is retained in red blood cells. Therefore, urinary excretion of arsenic in rats is slower and arsenic is retained in the body for longer compared to other animals such as humans, mice or hamsters (Vahter 1981; Marafante et al. 1982; Lerman and Clarkson 1983).

## 2. Effect of inorganic arsenic compounds

(1) Effect in humans

The demand of arsenic compounds has been consistently high since 20th century to date. Health impairment due to the occupational exposure in copper smelters, nonferrous metal smelters, the glass industry, and in the semiconductor industry has been a concern.

Meanwhile, contamination of arsenic compounds in foods has been reported inside and outside the country. Approximately 12,000 infants have developed subacute poisoning in Japan. Overseas, the effect of inorganic arsenic exposure through drinking-water has been reported.

(i) Acute and subacute effects

Intake of inorganic arsenic compounds can be fatal in human beings. Toxicity of arsenic differs by its solubility and chemical species. In general, As(III) is considered to have higher toxicity than As(V) (JECFA 2011). The lethal dose of diarsenic trioxide in a human adult has been estimated to be 100-300 mg/person as internal absorption based on past cases of accidents, and human adults are considered more sensitive than animals (Food Safety Commission of Japan, Cabinet Office, Government of Japan. 2009). Also, it was reported that the minimum lethal dose of orally administered diarsenic trioxide and sodium arsenite was 2 mg/kg body weight and the minimum oral toxic dose in children was 1 mg/kg body weight (RTECS 1998).

The symptom of acute arsenic poisoning includes fever, diarrhea, asthenia, loss of appetite, vomiting, excitation, rash, alopecia and other various symptoms. First, oral and/or esophagus membrane irritation develop and secondly, burning pain in esophagus and dysphagia develop, then abdominal symptoms such as nausea, vomiting, abdominal pain and diarrhea develop a few minutes to a few hours later. If the poisoning is severe, strong abdominal pain, severe vomiting, serous diarrhea, shock led by dehydration, muscle spasms, myocardial and renal disorder occur and he/she dies within 24 hours in the earliest case. Also, polyneuritis with cardinal symptom of abnormal sensation develops as peripheral nerve disorder 2 to 3 weeks after intake (Inoue et al. 1987). In infants, the signs appear within a few weeks after taking powder milk (equivalent to the dose, 1.3-3.6 mg/day) which has been contaminated with inorganic arsenic compounds. Even in adults, similar signs appear within 2 to 3 weeks after the intake of arsenic compounds of 3 mg/day (Food Safety Commission of Japan, Cabinet Office, Government of Japan. 2009).

The Wakayama curry-poisoning case of acute toxicity and Morinaga arsenic milk poisoning case of subacute toxicity are the examples of poisoning in Japan.

In acute poisoning by inhalation (respiratory) exposure where the dust with a large amount of arsenic compounds is inhaled, oral contamination occurs and arsenic is absorbed through

the gastrointestinal tract by swallowing. Hence, similar to oral intake, digestive symptoms such as nausea, diarrhea, abdominal pain, and central and peripheral nervous system disorders may be observed (U.S. DHHS 1998). When high concentrated diarsenic trioxide is inhaled, irritation to nasal mucosa, cough, and dyspnoea are developed due to irritation to the respiratory system and corrosivity, and the patient may die because of pulmonary edema (Inoue et al. 1987).

a. The Wakayama curry-poisoning case

On July 25, 1998, 67 people developed the symptoms of acute arsenic poisoning and 4 people died after approximately 12 hours in Sonobe, Wakayama. Sixty-three people, 29 males and 34 females survived, where 20 people were in the range from 1 to 12 years old and 43 people were in the range from 13 to 67 years old. The cause of the poisoning was diarsenic trioxide, and the mean estimated intake (absorbed amount) in the 63 survivors was 53 mg, and 4 people had taken 100 mg or more and 25 people had taken 50-99 mg. Among them, the maximum intake was 141 mg, and the minimum intake was 18 mg. Arsenic intake of the 20 people aged 12 or younger was 48.5 ± 23 mg, and that of 43 people aged 13 or older was 55.5 ± 26.3 mg.

Diarsenic trioxide added in curry was mostly dissolved by remaining heat, and ionized. Some were taken as crystals. Abdominal symptoms were observed approximately 5 to 10 minutes after intake of the curry. Patients showed common symptoms such as feeling queasy and vomiting, and diarrhea or abdominal pain developed subsequently. However, diarrhea developed only in approximately half of the patients indicating that diarrhea was not a common symptom of acute arsenic poisoning. Hypotension persisted for a few days and tachycardia, and shock were also observed in the moderate and severe patients. Circulatory disturbance was the major cause for death. A disorder of the central nervous system such as headache, feelings of weakness, convulsion, and mental disorder were observed in severe patients. Symmetrical bilateral peripheral neuropathy developed in the distal portion of the extremities in moderate to severe patients approximately 2 weeks later, and sensory aberrations and pain were observed. In severe patients, painless erythematous rash appeared in the abdominal region, axilla, and on the back of the neck as a skin disorder about the same time. Moreover, Mees line (white line) gradually developed on the nails. Besides this, conjunctivitis, face edema, stomatitis, desquamation and loss of hair appeared in a few patients. In the patients who had taken crystalline diarsenic trioxide, punctate shadow of arsenic was observed in the gastrointestinal tract as a radio-opaque material by plain abdominal radiography.

Sixty-three patients were misdiagnosed with cyanide poisoning, so were not treated with BAL (British Anti Lewisite), a chelating agent for the basic treatment for acute arsenic poisoning.

Metabolic methylation activity of children is higher than that of adults, and arsenic was effectively excreted in urine in the early stage of arsenic intake. This strongly affected the outcome of poisoning. The majority of the children were on the process of recovery approximately 1 week to 10 days after the intake, while the poisoning symptoms in adults exacerbated. Some patients still did not show recovery from peripheral nervous disorder, even after approximately 10 years (Yamauchi et al. 2002).

b. Morinaga arsenic milk poisoning case

Morinaga arsenic milk poisoning case was the most serious case of food contamination with inorganic arsenic inside and outside the country. In 1955, dibasic sodium phosphate for industrial use which was to be added to milk powder was contaminated with inorganic As(V) by about 10% in the Tokushima factory of Morinaga Milk Industry Co., Ltd. The Okayama prefecture Inst. of Public Health measured arsenic concentration in the milk powder by the Gutzeit method and reported the concentration of diarsenic trioxide to be 20-60 μg/g though the values depend upon the manufacturing batch (Kitamura and Kasuyama 1955).

Powder milk for infants manufactured by Morinaga Milk Industry Co., Ltd. was widely distributed around Western Japan and arsenic contaminated powder milk had been taken for approximately 3 months. About 12,000 infants suffered subacute arsenic poisoning and 133 infants died. Arsenic intake from powder milk was estimated to be 1.3-3.6 mg/infant per day and the total intake was estimated to be 90-140 mg (Hamamoto 1955).

Comprehensive summary of reported clinical findings indicated that the symptoms of subacute poisoning observed in the patients included fever, cough, rhinorrhea, conjunctivitis, vomiting, diarrhea, melanoderma, hepatomegaly, and abdominal distension. In addition, the laboratory test abnormalities such as anemia, granulocytopenia, abnormal electrocardiogram, and a band-like shadow in the X-ray image of epiphyseal area of long bone were indicated (NAS 1977).

The follow-up study which was conducted from 15th year on after the case by Faculty of Medicine, Osaka University observed growth retardation, leukomelanodermia, keratosis, hypacusis, mental retardation, and brain disorder such as epilepsy (NAS 1977).

The Osaka Medical Center for Cancer and Cardiovascular Diseases conducted a prospective cohort study (1982-2006) on the current condition, more than 50 years after the case, of 6,104 victims (3,738 males and 2,366 females). The mean starting age of observation was 27.4 years old and the mean observation period was 24.3 years. A total of 258 victims died and the standardized mortality ratio (SMR) through the observation period by all causes of death were 1.1 in total (95% Confidence Interval (95%CI): 1.0-1.2), 1.0 for males (95%CI: 0.9-1.2), and 1.2 for females (95%CI: 1.0-1.6). No significant excess of the total mortality

was observed. However, significant excess deaths due to nervous system disease was observed (SMR: 3.7, 95%CI: 1.9-6.2). The risk of death of 408 male victims who had been unemployed at the initiation of observation was significantly higher in all causes of death (SMR: 2.8, 95%CI: 2.1-3.6, $p < 0.01$) and a significant increase was also observed in nervous system disease (SMR: 25.3, 95%CI: 10.8-58.8, $p < 0.01$), respiratory system disease (SMR: 8.6, 95%CI: 3.1-16.8, $p < 0.01$), circulatory system disease (SMR: 3.2, 95%CI: 1.6-5.2, $p < 0.01$) and in external factors (car accident, suicide etc.) (SMR: 2.6, 95%CI: 1.4-4.1, $p < 0.01$). The authors reported that the death risk due to nervous system disease in the victims of this case was significantly higher compared to the general population (Tanaka et al. 2010).

(ii) Chronic effect

Chronic arsenic poisoning is caused mainly by oral intake of arsenic and is observed in the cases of long-term oral intake of drinking-water contaminated with inorganic arsenic in Asia, Central and South America, and in North America and others.

The latent time to onset of chronic arsenic poisoning depends on exposure. The sign of toxicity potentially increases when the arsenic concentration in drinking-water exceeds 100 μg As/L in the group where the people are chronically exposed to arsenic by using well water in daily life (Grantham and Jones 1977) (JECFA 1989).

Since the majority of the data of the arsenic effect on humans by oral intake concern the exposure through drinking-water containing arsenic, we focused on these data in this document.

a. Carcinogenicity

In 2012, IARC summarized the evaluation of carcinogenicity of arsenic exposure. According to it, sufficient evidences are available which indicate that arsenic in drinking-water cause bladder cancer, lung cancer and skin cancer, all in a dose-dependent manner (IARC 2012). The summary was mainly based on the results from the investigation on the effect of well water which was contaminated with inorganic materials and its compounds. Although carcinogenicity of arsenic exposure at high concentration has been considered consistently in many studies, a concentration of arsenic to exert carcinogenicity at low level has not been specified (NEDO 2008). Recent study by Baastrup and his colleagues also did not find relation between exposure to low-concentrated arsenic in drinking-water and risk of carcinogenicity (Baastrup et al. 2008). Concerning carcinogenicity of arsenic in foods, a risk of cancer was estimated on the basis of arsenic intake from hijiki cooked in Japan and found to exceed the permissible level (Nakamura et al. 2008). Moreover, a recent study suggested that arsenic intake in Japanese tends to have a dose-dependent relationship with the risk of lung cancer in current male smokers (Sawada et al. 2013).

On the other hand, cases of inhaled (respiratory) exposure such as the cases of occupational exposure in copper smelters, nonferrous metal smelters, or in pesticide factories have been known. The excess death due to lung cancer, liver cancer and colon cancer was confirmed in the cohort study conducted from 1949 to 1971 on 839 workers in a copper smelter in Sagano-seki, Oita in Japan (Tokudome and Kuratsune 1976).

(a) Skin cancer

IARC summarized the reevaluation of inorganic arsenic in drinking-water and confirmed the causal relationship between arsenic intake and skin cancer (particularly squamous cell carcinoma) in 2012. The reevaluation was based on surveys including an ecological survey conducted in Taiwan, mainly in south west area where many arsenic was observed, using incidence rate and mortality of skin cancer as index (Tseng et al.1968; Chen et al. 1985, 1988a; Wu et al. 1989; Chen and Wang, 1990; Tsai et al. 1999), and an ecological survey of mortality of skin cancer in Chile (Rivara et al. 1997; Smith et al. 1998). A cohort study in Taiwan (Chen et al. 1988b; Hsueh et al. 1995, 1997) was also investigated. IARC reported that consistent and significant increase in risk of skin cancer caused by inorganic arsenic exposure was suggested in all the above mentioned surveys (IARC 2012).

A geographic information system (GIS) analysis in the European Prospective Investigation into Cancer and Nutrition (EPIC) conducted in Denmark by Baastrup (2008) did not find relation between arsenic in drinking-water, non-melanoma skin cancer, and melanoma after adjustment of data with the physiographic factor. However, subjects who had arsenic over 2 μg/L were only a few.

(b) Bladder cancer

In the assessment of carcinogenicity of arsenic exposure conducted in 2012, IARC reported that the relationship between inorganic arsenic in drinking-water and bladder cancer was not due to coincidence or biased data while the dose-dependent relationship was found. Therefore, IARC concluded that arsenic was carcinogenic in bladder. The assessment included the ecological study in Taiwan (Chen et al. 1985,1988a; Wu et al. 1989; Chen and Wang 1990; Chiang et al. 1993; Tsai et al. 1999), Chile (Rivara et al. 1997; Smith et al. 1998; Marshall et al. 2007), and in Argentina (Hopenhayn-Rich et al. 1996, 1998), and a case-control study in Taiwan (Chen et al. 1986), cohort studies in Taiwan (Chen et al. 1988b; Chiou et al. 1995, 2001; Chen and Chiou 2001), Japan (Tsuda et al. 1995), and in the U.K. (Cuzick et al. 1992). Consequently, IARC confirmed dose-dependent effect, the effects of arsenic of high concentration and of long term exposure on bladder cancer risk from arsenic. (IARC 2012).

Kurttio et al. (1999) investigated the relationship between bladder cancer or kidney cancer and arsenic exposure through well water in 144,627 inhabitants living in the area with no

waterworks in Finland between 1967 and 1980. The final assessment was conducted on the targeted population which included; 61 patients who had been diagnosed with bladder cancer (50 males and 11 females), 49 patients (24 males and 25 females) who had been diagnosed with kidney cancer both from 1981 to 1995, and 275 subjects (163 males and 112 females) in the control group who had been matched on age and sex. The sample water was collected from the well which had been used between 1967 and 1980. Arsenic concentration in well water in the control group was low where the median value was 0.1 μg/L.

Regarding kidney cancer risk, neither arsenic concentration in well water, daily arsenic exposure, nor cumulative arsenic exposure was found to correlate with the risk. Regarding bladder cancer, the risk was found to correlate with arsenic concentration in well water 3 to 9 years before the diagnosis. The relative risk (RR) which had been adjusted with age, sex, and smoking status were 1.53 (95%CI: 0.75-3.09) for 0.1-0.5 μg/L exposure group and 2.44 (95%CI: 1.11-5.37) for ≥ 0.5 μg/L exposure group, compared to that for < 0.1 μg/L exposure group. However, when the cumulative arsenic exposure was used as an index, the relative risk (RR) between bladder cancer risk and arsenic exposure in ≥ 2,000 μg exposure group (RR = 1.50 (95%CI: 0.71-3.15)) was not different compare to <500 μg/L exposure group.

Bates et al. (1995) investigated the relationship between exposure to low-concentrated arsenic in drinking-water and bladder cancer using the data of National Bladder Cancer Study which was conducted on case group of 117 subjects consisted of 97 males and 20 females with mean age of 64.2, and on control group of 266 subjects consisted of 194 males and 72 females with mean age of 61.1, in Utah, U.S.A. in 1978. In the investigation, they used two indices for cumulative arsenic exposure, i.g. total arsenic cumulative exposure (index 1) and total arsenic intake concentration that reflected arsenic in urine to which bladder wall was exposed (index 2). The arsenic concentration in drinking-water was 0.5-160 μg/L with mean of 5.0 μg/L. Based on cancer risk adjusted with age, sex, smoking status, the duration of drinking surface water with chlorine disinfection, history of bladder infection, education, urbanization in the longest settled area, and engagement in high risk occupations, odds ratio (OR) of bladder cancer for the 19,000-33,000 μg (equivalent to 50-90 μg/day) exposed smokers group tended to increase, when index 1 was used as an index (OR: 3.33 (90%CI:1.0-10.8). When index 2 was used as an index after the same adjustment, odds ratio (OR) of bladder cancer for ≥ 13,000 (μg/L・years) exposure group who had been exposed 30 to 39 years before diagnosis (OR: 3.07 (90%CI: 1.1-8.4)) showed a tendency to increase.

Steinmauset al. (2003) conducted a case-control study in the population in 6 counties in Western Nevada and Kings County in California in the U.S., who had been exposed to nearly 100 μg/L of arsenic in drinking-water in the past, and investigated the relationship between bladder cancer and arsenic intake (2003). The case group was of 181 patients consisted of 34 males and 147 females, with mean age of 69.8 (20-85), who had first been diagnosed with

primary bladder cancer from 1994 to 2000. The control group was of 328 subjects consisted of 76 males and 252 females, mean age of 70.3 years, who had been matched on age and sex. Arsenic exposure was estimated by phone questionnaire survey on the source of drinking-water, the amount of drinking, occupation, and on smoking etc. The increase in bladder cancer risk was not observed even in the group exposed to cumulative arsenic of ≥ 82,800μg after adjusting for age, sex, occupation, history of smoking, income, education and race (OR: 0.73, 95%CI: 0.45-1.17). In smokers, who were exposed to arsenic of high concentration (≥ 80 μg/day, median value: 177 μg/day) more than 40 years ago, a significant increase of risk was observed after adjusting the values according to age, sex, occupation, income, education, and race (OR: 3.67, 95%CI: 1.43-9.42, $p < 0.01$).

From 1996 to 2000 in Cordoba, Argentina, Bates et al. (2004) conducted a case-control study of bladder cancer in 114 case-control pairs based on age, sex, and states. The case group was consisted of 94 male pairs and 20 female pairs with mean age of 68.9. The control group was consisted of 94 male pairs and 20 female pairs with mean age of 68.3. Water samples were collected from subjects' residences which have been in use for the last 40 years. Statistical analyses showed no correlation between exposures which was estimated based on arsenic concentrations in drinking-water and bladder cancer. However, when well-water consumption itself was used as an index, the use of well water more than 50 years ago (51-70 years ago) adjusting for sex, years of birth, the use of mate tea with bombilla (a metal straw for mate), education, and the maximum number of cigarettes smoked per day, was associated with an increase of bladder cancer risk in smokers (OR: 2.5, 95%CI: 1.1-5.5) but not in other groups.

Chen et al. (2010a) followed up with a total of 8,086 inhabitants of northeast Taiwan for 12 years and assessed bladder cancer risk, effect of arsenic concentration of arsenic contaminated well water and of the duration of exposure through drinking. Incidence of bladder cancer was ascertained through linkage with the national cancer registry. The data were analyzed using Cox proportional hazards regression models. The analysis revealed that there were 450 incidences of bladder cancer and that a rate of the incidence monotonically increased in proportion to the increase in arsenic concentration ($p < 0.001$). The age- and sex-adjusted RR, when compared with the < 10 μg/L group, were 4.18 (95%CI: 1.37-12.8) for the 50-99.9 μg/L exposure group, and 7.73 (95%CI: 2.69-22.3) for the group of exposure to arsenic as high as >100 μg/L. The RR for the high exposure group was more than 5 times higher compared with the < 10 μg/L group. The age- and sex-adjusted RR of 5,000-10,000 (μg/L・years) cumulative arsenic exposure group compared to 400μg/L・years cumulative arsenic exposure group was 3.88 (95%CI: 1.18-12.7).

However, excessive risk of bladder cancer was not confirmed in some studies. For example, Baastrup et al. (2008) estimated individual arsenic exposure for the period from 1970 to 2003

in 56,378 inhabitants, consisted of 26,876 males and 29,502 females with the median age of 56 at the time of enrollment, of Copenhagen and Aarhus who had been registered to prospective cohort study in Denmark between 1993 and 1997, and investigated the relationship between exposure to low concentration arsenic in drinking-water and cancer risk using Cox's proportional hazards regression models. The mean arsenic exposure concentration was 1.2 μg/L (0.05-25.3 μg/L) in the cohort. No significant association was found in the time-weighted average arsenic exposure and the risk of bladder cancer ($p = 0.75$) after the adjustment for smoking, education, body mass index (BMI), drinking alcohol, occupation and others.

Meliker et al. (2010) conducted a case-control study in 411 patients consisted of 315 males and 96 females who had been diagnosed with bladder cancer during the period from 2000 to 2004, and 566 controls consisted of 418 males and 148 females in Michigan, U.S.A., and restructured the individual life-time exposure profiles. As a result, 90% of bladder cancer patients and controls were exposed to arsenic within the range of 0.02-25 μg/L. After adjusted the risk of bladder cancer for history of smoking, education, history of high risk occupation, family history of bladder cancer, age, race and sex, the risk was not increased in the group with> 10 μg/L of the time-weighted average life-time exposure compared to the risk in the < 1 μg/L group (OR:1.10 (95%CI: 0.65-1.86)). Similarly, in smokers, an increase in risk of bladder cancer was not observed in the > 10 μg/L group after the adjustment for education, history of high risk occupation, family history of bladder cancer, age, race, and sex (OR: 0.94 (95%CI: 0.50-1.78)).

Mink et al. reviewed on arsenic and bladder cancer (Mink et al. 2008). Some discrepancies are attributed to weakness of the statistical power for detecting moderate effect from low level exposures.

(c) Lung cancer

In 2012, IARC judged inorganic arsenic exposure through drinking-water to be carcinogenic in the lungs in the carcinogenicity assessment of arsenic exposure. IARC concluded that there were enough evidences of carcinogenicity of inorganic arsenic in lung (IARC 2012), based on the result from ecological studies in Taiwan (Chen et al. 1985, 1988a; Wu et al. 1989; Chen and Wang 1990; Tsai et al. 1999), Chile and Argentina (Rivara et al. 1997; Smith et al. 1998, 2006; Hopenhayn-Rich et al. 1998), the result from case-control studies in Chile (Ferreccio et al. 2000) and Bangladesh (Mostafa et al. 2008), and from a cohort study in Taiwan (Chen et al. 1986),.

Ferreccio et al. (2000) conducted a case-control study in 151 patients (72% were males, mean age: 61) who had been diagnosed with lung cancer in 1994 to 1996 at northern Chile where arsenic concentration in drinking-water during the period from 1958 to 1970 had been

860 μg/L, and in 419 inpatients (61% were males, mean age: 61) with disease unrelated to arsenic. The subjects were interviewed on the source of drinking-water, smoking status and others. The logistic regression analysis showed that increase in OR of lung cancer associated with the increase of arsenic concentration in drinking-water. After adjustment for sex, age, cumulative life-time smoking, occupational exposure to copper, and socio-economic status, OR for each exposure group compared to the < 10 μg/L exposure group were 1.6 (95%CI: 0.5-5.3), 3.9 (95%CI: 1.2-12.3), 5.2 (95%CI: 2.3-11.7), and 8.9 (95%CI: 4.0-19.6) for 10-29 μg/L, 30-49 μg/L, 50-199 μg/L and 200-400 μg/L exposure group respectively. For the interaction of arsenic exposure in drinking-water and smoking, the OR for the ≥ 200 μg/L exposure group in smokers was 32.0 (95%CI: 7.22-198.0) compared to the ≤ 49 μg/L arsenic exposure group in non-smokers indicating a synergistic effect.

Arsenic exposure in utero and in early childhood and the mortality of respiratory disease were investigated in a cohort study (Smith et al. 2006). For the period from 1958 to 1970, arsenic concentration in waterworks in Antofagasta and its environs was high as approximately 1,000 μg/L. Mortality in the period from 1989 to 2000 of the population who were born from 1958 to 1970 therefore exposed in utero and in early childhood to high concentration of arsenic through waterworks and mortality in the same period of the population who were born just before the peak exposure period (1950-1957) therefore not exposed in utero, were compared to the mortality of the control subjects who were born in other areas in Chile. The SMR in early childhood exposure group was 7.0 ((95%CI: 5.4-8.9), $p < 0.001$) for lung cancer and 12.4 ((95%CI: 3.3-31.7), $p < 0.001$) for bronchiectasis. The SMR for the subjects who exposed in utero and in early childhood was 6.1 (95% CI, 3.5–9.9; $p < 0.001$) for lung cancer and 46.2 (95% CI, 21.1–87.7; $p < 0.001$) for bronchiectasis.

Chen et al. (2004) conducted a follow-up study in 2,503 subjects (1,154 males and 1,349 females) in southwest Taiwan and 8,088 subjects (4,053 males and 4,035 females) in northeast Taiwan for approximately 8 years. Information on arsenic exposure, smoking, and other risk factors were collected at the enrollment from a standardized questionnaire interview. The incidence of lung cancer was ascertained through linkage with national cancer registry profiles in Taiwan (1985- 2000). There were 139 newly diagnosed lung cancer cases during a follow-up period of 83,783 person-years. After adjustment for smoking, age, sex and other risk factors, lung cancer risk was associated with arsenic exposure concentration in drinking-water ($p < 0.001$) and RR were 2.28 (95%CI: 1.22-4.27) and 3.29 (95%CI: 1.60-6.78) for the 100-299 μg/L exposure group and ≥ 700 μg/L exposure group, respectively, compared to the < 10 μg/L exposure group. A synergistic effect of arsenic exposure and smoking on lung cancer risk was observed.

A total of 8,086 residents in northeast Taiwan were followed for 11.5 years by Chen et al (2010b) and 6,888 residents (3,481 males and 3,407 females, mean age:59.1) remained in the

final analysis after the excluding of 1,198 residents with an unknown arsenic concentration. The 178 incidents of lung cancer were ascertained through linkage with the national cancer registry profiles in Taiwan. Mean arsenic concentration in well water was 117.2 μg/L, the mean duration of well water use was 42.0 years, and the mean cumulative arsenic exposure was 3,523.5 μg/L‧years. The analyses were conducted by Cox's proportional hazards regression models. A significant dose-response relationship between lung cancer risk and arsenic concentration was found (p = 0.001). The relative risk was 2.25 (95%CI: 1.43-3.55) for ≥ 300 μg/L exposure group compared to < 10 μg/L exposure group after adjustment for age, sex, education, smoking, and drinking. The synergistic effect of arsenic exposure and smoking was found in squamous and small cell carcinomas, but not in pulmonary adenocarcinoma.

On the other hand, in the prospective cohort study by Baastrup et al. (2008) in Denmark as was mentioned in the previous section (b)　bladder cancer, the evidence for the relation between the time-weighted mean arsenic exposure and increase in lung cancer incidence was not observed (p = 0.78).

Sawada et al. (2013) conducted a follow-up study in 90,378 Japanese (42,029 males and 48,349 females, age: 45-74 years) for approximately 11 years in 10 areas in Japan. Individual arsenic intake was estimated based on the Food Frequency Questionnaire (FFQ). Arsenic intake from foods and drinking-water was estimated for 12 groups (75 items) of arsenic-containing foods which were categorized from the examined 138 items. The estimation gave the mean total arsenic intake of 170.0 μg/day for this cohort. Since the arsenic concentration in tap-water was limited to ≤ 0.01 mg/L, intake from drinking-water was not considered. Though the validity of FFQ was evaluated in the comparison to a diet record, the biomarker was not measured in this study. Age, residential area, history of smoking, drinking alcohol, BMI, and exercise situation were used for adjustment as confounding factors. During the follow-up period, 7,002 subjects (4,323 males and 2,679 females) were diagnosed with cancer. They were analyzed by quartile arsenic intake. In the analysis by body part in male subjects, a dose-response relationship was found between total arsenic intake and incidence of lung cancer　(trend p = 0.07), between inorganic arsenic intake and incidence of lung cancer (trend p = 0.05) and incidence of kidney cancer (trend p = 0.06)、though statistically insignificant. Hazard ratio of cancer risk comparing risk in group quartile 4 (total arsenic intake247.5 μg/day, inorganic arsenic 99.1 μg/day) to that in group quartile 1 (total arsenic intake: 88.3 μg/day, inorganic arsenic: 40.5 μg/day) were 1.23 (95%CI = 0.96-1.57) for risk of lung cancer from total arsenic, 1.28 (95%CI: 1.00-1.62) for lung cancer from inorganic arsenic and 2.05 (95%CI: 1.05-4.03) for kidney cancer from inorganic arsenic. In addition, the investigation of synergistic effect of arsenic exposure and smoking on lung cancer revealed a negative relationship between total arsenic

intake and incidence of lung cancer in males with no smoking history ($p = 0.01$). The HR of the risk in quartile 4 to that in quartile 1 was 0.49 (95%CI: 0.27-0.86).

However, in male group of current smokers, a proportional increase in HR of incidence of lung cancer to the increase in total arsenic intake was observed ($p = 0.03$) where HR of quartile 4 compared to quartile 1 was 1.37 (95%CI: 1.06-1.77). Similarly, association between inorganic arsenic intake and smoking was observed (HR: 1.38 (95%CI: 1.07-1.77, $p = 0.01$)). In females, a correlation between inorganic arsenic intake and incidence of lung cancer in the group with no smoking history was observed ($p < 0.01$), and HR of the risk in quartile 4 to that in quartile 1 was 1.57 (95%CI: 1.12-2.20). Consequently, the authors considered that a positive relationship between arsenic exposure observed in the current male smokers and the risk of lung cancer incidence suggests a causal relation between arsenic exposure and lung cancer risk, based on the fact that the results are consistent with other studies and that mechanisms to explain it are available, although the positive relationship might be a result from multihypothesis test method. Also, the authors suggested that smoking was a modification factor (Sawada et al. 2013).

Risk ratio observed in this report was relatively small being around 1.3, and it may not have been adjusted by the effect of smoking which was a strong risk factor. Also, the negative correlation between total arsenic intake and incidence of lung cancer in group of male with no smoking history was not sufficiently explained. This is attributable to the difficulty of estimating individual intake from FFQ.

(d) Other cancer

In the evaluation of carcinogenic risk to humans in 2012, IARC reported that although a causal relationship between inorganic arsenic and liver cancer, kidney cancer, prostate cancer and other cancers was suggested in some studies, the relationship was also attributable to coincident and bias. Despite some ecological surveys which have suggested the relationship between inorganic arsenic in well drinking-water and kidney cancer (Chen et al. 1985, 1988a; Wu et al. 1989; Chen and Wang 1990; Tsai et al. 1999; Rivara et al. 1997; Smith et al. 1998; Hopenhayn-Rich et al. 1996, 1998), data from cohort study were limited (Chiou et al. 2001). Regarding prostate cancer and liver cancer, the investigation of causal relationship between inorganic arsenic in drinking-water and the cancer is restrained by the fact that the outcome data of the cancers depend on the data of death but not on the data of onset. A significant dose-response relationship between exposure to inorganic arsenic in drinking-water and mortality of prostate cancer was observed in the study conducted in Taiwan (Chen et al. 1985, 1988a; Wu et al. 1989; Chen and Wang, 1990; Tsai et al. 1999). However, the validity of the

data was limited due to very high incidence of hepatitis B in the relevant cohort. No relationship between inorganic arsenic exposure and the mortality of prostate cancer was found in the study conducted in Chile by Rivara et al. (1997) (RR = 0.9: 95%CI: 0.54-1.53). Data from the studies on liver cancer were not consistent. Although the relationship was confirmed in a study conducted in Taiwan (Chen et al. 1985, 1988a; Wu et al. 1989; Chen and Wang, 1990; Chiang et al. 1993; Tsai et al. 1999), no significant relationship was found in a study conducted in Chile (Rivara et al. 1997). Regarding this discrepancy, a working group in IARC (2012) suggested that the sensitivity to arsenic was possibly higher in the cohort of Taiwan where hepatitis B was frequently observed than in the cohort of South America. Therefore, IARC considers the strong causal relationship between arsenic exposure and liver cancer which was suggested by these studies is still ascribable to a coincidence and a bias.

b. Effects on skin

The Environmental Protection Agency (EPA) (US EPA 2005a) suggested that the Lowest Observed Effect Level of chronic arsenic poisoning due to oral exposure or inorganic arsenic contamination in drinking-water to be 700-1,400 μg/day. If the exposure to this level of arsenic lasts for several years, raindrop-like pigmentation and depigmentation will be observed on the abdomen and trunk as the first symptom, and then keratosis (5-6 years) will develop on the palms or soles of the feet. If the daily exposure is as high as 3-5 mg/day, the symptoms don't develop in stages and keratosis develops at the same time along with the development of pigmentation and depigmentation. Bowen disease and skin cancer develop in some patients.

Skin lesion such as skin hyperpigmentation and palmoplantar hyperkeratosis is a sensitive marker of the effects of chronic inorganic arsenic oral intake. These effects on skin are pointed out in most surveys in humans including repeated oral exposure through arsenic contaminated drinking-water. In many of the surveys conducted in Bangladesh (Ahsan et al. 2006; Rahman et al. 2006; Chen et al. 2006), India (Haque et al. 2003), and in the Inner Mongolia Autonomous Region of China (Guo et al. 2006; Xia et al. 2009), an increased incidence rate of skin lesions has been reported to be developed in areas where the arsenic concentration in drinking-water is below 100 μg/L.

Ahsan et al. (2006) investigated the dose-response relationship between arsenic exposure through drinking-water and premalignant skin lesions, using baseline data of 11,746 participants (714 males and 10,724 females) who were recruited for the Health Effects of Arsenic Longitudinal Study (HEALS) conducted in Araihazar, Bangladesh from 2000 to 2002. A dose-response relationship was observed consistently in 3 different regression models, such as logistic regression analysis, logarithmic binomial logistic regression analysis, and Poisson regression analysis. When time-weighted arsenic concentration in well water was used as an

index, the odds ratio (OR) of adjusted prevalence of skin lesions by age, sex, BMI, education, cigarette smoking, hookah smoking, sun exposure (male), and landownership (socio-economic index) were 1.91 (95%CI: 1.26-2.89), 3.03 (95% CI: 2.05-4.50), 3.71 (95% CI: 2.53-5.44), and 5.39 (95% CI: 3.69- 7.86) for the 8.1-40.0 μg/L group, 40.1-91.0 μg/L group, 91.1-175.0 μg/L group, and for the 175.1-864μg/L group, respectively, compared to the 0.1-8.0 μg/L group. When the cumulative As index was used as an index, OR was 1.83 (95%CI: 1.25-2.69) for the 48,200-226,400 μg group compared to the 100-48,100 μg group.

Rahman (2006) assessed the prevalence of arsenic-induced skin lesions by age and sex in the population who were exposed to arsenic through well water in Matlab, Bangladesh. A total of 504 cases of arsenic-induced skin lesions were identified through a procedure with 3 steps: Step 1 - screening of the entire population of 166,934 above 4 years of age (74,408 males and 92,526 females); Step 2 - diagnosis by physicians; Step 3 - diagnosis by physicians and confirmation by experts based on the photograph. As for controls, 2,201 subjects were randomly selected from the population in Matlab, and 1,830 subjects (833 males and 997 females) who had matched on water drinking condition were selected. Individual history of arsenic exposure was estimated based on the interview on their water sources after 1970 and arsenic concentration in all artesian well water by AAS. As a result, arsenic concentration in well water after 1970 was higher for the skin lesion patients (200 μg/L for males and 211 μg/L for females) than for the controls (143 μg/L for males and 155 μg/L for females). When cumulative arsenic exposure was used as index, the OR adjusted by age and economic index was 1.94 (95%CI: 1.10-3.42) for the female group with the exposure of 1,000-4,999 μg/L· years. When the mean arsenic exposure was used as an index, the OR adjusted by age and economic index was 3.25 (95%CI: 1.43-7.38) for the male group exposed to 10-49 μg/L of arsenic. In quintile of mean arsenic exposure, OR of skin lesions in the maximum exposure group compared to the control group was significantly higher in males, whose OR was 10.9 (95%CI: 5.80-20.4), compared to OR of 5.78 (95%CI: 3.10-10.8) in females ($p = 0.005$).

Using the baseline data from 11,062 subjects (4,721 males and 6,314 females) in the HEALS in Araihazar, Bangladesh, Chen et al. (2006) conducted a cross-sectional analysis to examine whether the association between arsenic exposure through drinking-water and the risk of skin lesions is modified by smoking, excessive sunlight, the use of fertilizer or pesticides. A time-weighted well arsenic concentration was estimated from the individual history of well use. The adjusted OR by age, BMI, education, use of drinking-water, use of Areca catechu, the use of pesticides, the use of fertilizer, sun exposure (male) compared to the ≤ 28.1μg/L exposure group was 2.3 (95%CI: 1.1-4.5) for female non-smokers group with exposure of 28.1-113.0 μg/L, and was 2.6 (95%CI: 1.5-4.5) for male smokers with exposure of 28.1-113.0 μg/L. A synergistic effect of smoking and the exposure more than 113.0 μg/L was

observed in the male group. Excessive sunlight exposure increased the risk of skin lesions in all arsenic exposure groups.

Haque et al. (2003) selected a group of the patients with arsenic-induced skin lesions and a group of age- and sex-matched controls from a 1995-1996 cross-sectional survey in West Bengal, India. Haque et al. conducted a detailed assessment of arsenic exposure that covered at least 20 years. The selected subjects were reexamined in 1998 and 2000. The OR adjusted for age, sex, smoking, BMI, sociodemographic factors (subject's education, education of household head, subject's occupation), and type of resident was 3.3 (95%CI: 1.7-6.4) in the 50-99 μg/L exposure group when the mean life-time exposure was used as an index. The average latency for skin lesions from first exposure was 23 years. The strong dose-response relationship was found between arsenic exposure and skin lesions.

Guo et al. (2006) investigated the association between keratosis or pigmentation and arsenic exposure concentration in 227 patients who were diagnosed with skin lesions between 1996 and 1998, and in 221 non-patients in the village of Hetao Plain in Inner Mongolia, China. The targeted patients included 162 patients of keratosis (69 males, 93 females, mean age: 42.5 years, mean use of well water: 15.6 years), 65 patients of pigmentation (47 males, 18 females, mean age: 52.4 years, mean use of well water: 15.2 years). The targeted non-patients consisted of 93 males and 128 females of mean age: 37.6 years, and mean use of well water was 15.2 years. Well-water which the subjects had been drinking was collected to analyze the arsenic concentration. A logistic regression analysis conducted after the adjustment for age, sex, and smoking, showed OR=5.25, 95%CI=1.32-83.24 for the 50-199 μg/L group; OR=10.97, 95%CI=1.50-79.95 for the 200-499μg/L group; and OR=10.00, 95%CI=1.39-71.77 for the ≥ 500μg/L group ($p = 0.000$), indicating that along with the increase of arsenic concentration in drinking-water, the risk of pigmentation also increased. However, the correlation between risk of keratosis and arsenic concentration was not significant ($p = 0.346$).

Xia et al. (2009) studied on arsenic exposure in the residents of the Bayingnormen region of Inner Mongolia, China, who have been exposed to arsenic-contaminated well water for over 20 years, where they evaluated the incidence of arsenic-associated skin lesions diagnosed by physicians and by self-assessment. Skin lesions such as keratoses, pigmentation, and depigmentation were identified in 632 residents which were more than 5% of the 12,334 targeted residents. In 11,416 subjects with completed data, OR for skin lesions adjusted for alcohol consumption, smoking, education, sex, farm work, income, water source, and age was 2.52 (95%CI: 1.47-4.30) in the 5.1-10 μg/L low exposure group compared to the 0-5 μg/L group , indicating a strong correlation between skin lesions and arsenic contained in well water ($p < 0.01$). There was also an association between prevalence rate of skin lesions and self-assessed cardiovascular disease.

c. Effects on reproduction and development

Studies on poisoning from drinking-water contaminated with inorganic arsenic have reported risks of spontaneous abortion, still-birth and preterm birth (Ahmad et al. 2001; Hopenhayn-Rich et al. 2003; Milton et al. 2005; von Ehrenstein et al. 2006; Kwok et al. 2006; Rahman et al. 2007, 2009; Cherry et al. 2008), and a decrease in body weight at birth (Hopenhayn-Rich et al. 2003; Rahman et al. 2009). However, in many cases, the information on the health effects was obtained by interviews conducted certain years after pregnancy. In this case, the mother who was aware of own status of the exposure may report harmful effects more than the mother who does not know her exposure status.

Ahmadet al. (2001) studied the pregnancy outcomes in terms of live birth, stillbirth, spontaneous abortion, and preterm birth in a group of 96 females of reproductive age (15–49 years) who were chronically exposed to arsenic through drinking-water in Bangladesh. They compared the pregnancy outcomes of the exposure group with that of a non-exposure group composed of 96 females of reproductive age (15–49 years) matched for age, socio-economic status, education, and age at marriage. In the exposure group, 98% of the group had taken drinking-water with arsenic concentration of ≥ 100 μg/L. Skin symptoms due to chronic arsenic exposure were detected in 22.9% of the females in the exposure group. Adverse pregnancy outcomes in terms of spontaneous abortion, stillbirth, and preterm birth rates were significantly higher in the exposure group than those in the non-exposure group ($p = 0.008$, $p = 0.046$, and $p = 0.018$, respectively).

Milton et al. (2005) conducted a cross-sectional study to investigate association between arsenic in drinking water and adverse pregnancy outcomes (spontaneous abortion, stillbirth, and neonatal death). They measured the arsenic concentration in 223 wells in the arsenic contaminated area in Bangladesh. A total of 533 female non-smokers who used these wells and had history of pregnancy with the age of 15 to 49 were interviewed. Information was obtained through a structured pretested interview using a questionnaire. After adjusting for the participant's height, history of hypertension and diabetes, and for age at first pregnancy in the case of neonatal death only (within 28 days after birth), logistic regression analysis was performed.

As a result, the odds ratio of the group with exposure to arsenic of concentration higher than 50 g/L (51-100 μg/L: 10 females, 101-500 μg/L: 37 females, ≥ 500 μg/L: 20 females) comparing to the low exposure group which was exposed to arsenic lower than 50 g/L was 2.5 (95% CI:1.5–4.3) for spontaneous abortion, 2.5 (95% CI:1.3–4.9) for stillbirth, and 1.8 (95% CI:0.9–3.5) for neonatal death.

In 2001 to 2003, von Ehrenstein et al. (2006) investigated the reproductive histories among 202 married females aged 20-40 years old (median: 31) in West Bengal, India using structured interviews. And, arsenic concentration in the water from 409 wells which had been used during the pregnancy was measured. Logistic regression analysis was performed for pregnancy outcomes in 644 cases where arsenic concentration in the well water they used had been obtained. As a result, the odds ratio for the risk of still birth in the group with highly-concentrated arsenic exposure (≥ 200 μg/liter) was 6.07 (95% CI: 1.54-24.0; p = 0.01) after adjusted for potential confounding factors. Twelve females who were diagnosed with skin lesions caused by arsenic toxicity had a substantially increased risk of stillbirth and the OR was 13.1 (95% CI: 3.17-54.0; p = 0.002).

Cherry et al. (2008) investigated the epidemiological pattern of stillbirth and the effect of arsenic contamination in well water in approximately 600 villages in Bangladesh, using the data collected by Gonoshasthaya Kendra, a large non-governmental organization providing health care to these areas. The arsenic concentration in drinking-water in each area was obtained from National Hydrochemical Survey using the data of 30,984 pregnancies and pregnancy outcomes (live birth and still birth) between 2001 and 2003, and socio-economic data, data of smoking and pregnancy history. The overall stillbirth rate was 3.4% (1,056 subjects). The stillbirth rate in the group with exposure to arsenic of 10-49.9 μg/L was 3.79% which was higher than 2.96% in the group with exposure to arsenic concentrations of less than 10 μg/L, but was insignificant. The stillbirth rate was significantly increased to 4.43% in the group with exposure to arsenic ≥ 50 μg/L ($p < 0.005$). In the logistic regression analysis after adjustment for confounding of socio-economic and health factors, OR of still birth to arsenic concentration of < 10 μg/L was 1.23 (95%CI: 0.87-1.74) for 10-50 μg/L and 1.80 (95%CI: 1.14-2.86) for the ≥ 50 μg/L group.

Hopenhayn-Rich et al. (2003) conducted a prospective cohort study in 2 cities in Chile to investigate the effect of arsenic in drinking-water on fetus development. Arsenic concentration in drinking-water in each city was 40 μg/L in Antofagasta and 1 μg/L in Valparaiso. Target subjects had answered in detail at an interview and submitted urine samples. The information of pregnancy and birth was obtained from medical records. In the analysis of body weight at birth, among infants who were born between December 1998 and February 2000, still births and multiple births were excluded. As a result, 424 infants from Antofagasta and 420 infants from Valparaiso were selected for the final analysis. After adjustment for each confounding factors, multivariate statistics were performed. The mean body weight at birth of infants in Valparaiso was 57 g less than that of Antofagasta but the difference was insignificant (95%CI=− 123 to 9g).

Although the number of cohort studies which have reported the individual exposure data was limited, large scale studies conducted in Bangladesh have been reported. Kwok et al.

(2006) investigated the relationships between arsenic exposure level and pregnancy outcomes (live birth, still birth, spontaneous abortion) in 2,006 pregnant women (mean age:26.4) in 2002 chronically exposed to naturally-occurring concentration of arsenic in drinking-water in three upazilas in Bangladesh. To obtain the information about pregnancy, participants had an interview at home based on the record of the Community Nutrition Center, and the arsenic concentration of the major source of drinking-water was analyzed. The median arsenic concentration in 3 upazilas were 0.073 μg/g (detected limit to 0.528 μg/g), 0.139 μg/g (detected limit to 0.635 μg/g), and 0.024 μg/g (detected limit to 0.668 μg/g). Logistic regression analysis indicated a statistically significant association between arsenic exposure and birth-defects (neural tube defect) only (OR=1.005 (95%CI: 1.001-1.010)). However, other outcomes, such as still birth (OR = 0.999 (95%CI: 0.996-1.002)), low birth weight (OR = 0.999 (95%CI: 0.997-1.000)), childhood stunting (OR = 1.000 (95%CI: 1.000-1.001)), and childhood underweight (OR = 1.000 (95%CI: 0.999-1.001)) were not associated with arsenic exposure. However, OR was low for all confounding factors, indicating that the effect of many confounding factors such as a genetic effect and environmental factors were unlikely to be excluded sufficiently.

Rahman et al. (2007) conducted a prospective study on the effect of arsenic exposure during pregnancy on fetal and infant mortality in a cohort of 29,134 pregnancies identified by the health and demographic surveillance system in Matlab, Bangladesh, in between 1991 and 2000. Arsenic exposure, reflected by drinking-water history and by arsenic concentrations in well water used by women during pregnancy, was assessed in a separate survey conducted between 2002 and 2003. Pregnancy outcomes and infant death (within 12 months after birth) were investigated by home visits every month. The risk of fetal loss and infant death in relation to arsenic exposure was analyzed by a Cox proportional hazards model. Drinking the well water with arsenic concentration of 164-275 μg/L (median: 224), 276-408 μg/L (median: 339) and ≥ 409 μg/L (median: 515) significantly increased the RR to 1.19 (95%CI: 1.00-1.42), 1.29 (95%CI: 1.08-1.53) and 1.19 (95%CI: 1.00-1.41), respectively after the adjustment for a calendar year. Significant dose-response relations of arsenic exposure and risk of infant death were observed ($p = 0.02$).

Moreover, Rahman et al. (2009) conducted a prospective cohort study in 1,578 mother-infant pairs, in Matlab, Bangladesh, between 2002 and 2003 to investigate the association between prenatal arsenic exposure and size at birth (birth weight, birth length, head and chest circumferences). Arsenic exposure was estimated by the urinary concentration of inorganic arsenic and the methylated metabolites at around gestational weeks of 8 weeks and 30 weeks. The association between arsenic exposure and size at birth was evaluated by linear regression analyses. As a result, no dose-response relationship was found between arsenic exposure and birth size in the full range of exposure (6-978 μg/L). Meanwhile, birth

weight and head and chest circumferences at birth showed a negative dose-response relationship to arsenic exposure in a group with exposure to arsenic of low concentration such as < 100 μg/L in urine (51% of mothers). Birth weight, head, and chest circumferences decreased by 1.68 g, 0.05 mm, and 0.14 mm, respectively for each 1 μg/L increase of arsenic in urine. No association between birth size and exposure was observed in the exposure to highly-concentrated arsenic of ≥ 100 μg/L.

d. Effect on neural development

Considering huge species difference in arsenic metabolism in animals and the period of the brain development which is longer in humans than in experimental animals, the conclusive neurotoxicity dose may be lower in humans than in experimental animals. Effects of inorganic arsenic exposure on intellectual functions of infants and children have been reported in Bangladesh, Shanxi Province, China, and in West Bengal, India.

Wasserman et al. (2004) conducted a cross-sectional study to investigate the association between arsenic exposure and intellectual function in 201 children of 10 years of age randomly selected from 11,749 children (98 males, 103 females, mean age: 10.0 ± 0.4) who had participated in the ongoing prospective cohort study examining health effects of arsenic exposure in Araihazar, Bangladesh.. The concentration of arsenic and manganese in well water at each child's home was obtained from the survey for all wells in the study areas. A medical check was performed and Children's intellectual function on tests drawn from the Wechsler Intelligence Scale for Children was assessed. Arsenic concentration in drinking-water was 0.094-790 μg/L (mean: 117.8 μg/L) and the mean manganese concentration was 1,386 μg/L. The arsenic concentration in drinking-water was associated with reduced intellectual function dose-dependently after adjustment for sociodemographic covariates and water manganese concentrations. Full scale score and performance scale score in groups of arsenic concentration of 50.1-176 μg/L or 177-790 μg/L significantly decreased compared to the group of arsenic 0.1-5.5 μg/L.

Wasserman et al. (2007) investigated association between arsenic exposure and intellectual function in 301 children of 6 years of age (150 males and 151 females, mean age: 6.1 ± 0.18) randomly selected from 11,749 parents who participated in the prospective cohort study examining health effects of arsenic exposure in Araihazar, Bangladesh. . They measured the arsenic and manganese concentration in the sample water from the well being used in each family, and performed a survey of the family environment and physical examination of targeted children between 2004 and 2005. Intellectual function was assessed by the subscale of the Wechsler Intelligence Scale for Children. Arsenic concentration in drinking-water ranged from 0.10 to 864 μg/L with mean of 120.1 μg/L, and the mean manganese concentration was

1,302 μg/L. Arsenic exposure in drinking-water was associated with the decrease in intellectual function before and after the adjustment for manganese in drinking-water, lead level in blood, and for sociodemographic characteristics.

In a study by Wang et al. (2007) conducted in 720 children between 8 and 12 years of age (376 males, 344 females, mean age:10) in Shanyin county, Shanxi province, China, the effects of arsenic and fluoride in drinking-water on children's intelligence and growth were investigated.. IQ scores by "Combined Raves Test" and somatometry (height, body weight, chest measurement, lung capacity) data of the intermediately-concentrated arsenic group (91 children, 142 ± 106 μg/L), the highly -concentrated arsenic group (180 children, 190 ± 183 μg/L), and highly-concentrated fluoride group (253 children, fluorine 8,300 ± 1,900 μg/L and arsenic 3 ± 3 μg/L) were compared to that of the control group (196 children, arsenic 2 ± 3 μg/L and fluorine 500 ± 200 μg/L). IQ scores of 100.6 ± 15.6 ($p < 0.05$) in the moderately-concentrated arsenic group, 95.1 ± 16.6 ($p < 0.01$) in the highly-concentrated arsenic group, and 100.5 ± 15.8 ($p < 0.05$) in the highly-concentrated fluoride group were significantly low compared to 104.8±14.7 in the control group. In the control group, height is significantly higher than that in the highly-concentrated fluoride group ($p < 0.05$), body weight is significantly heavier than that in the highly-concentrated arsenic group, ($p < 0.05$), and lung capacity is significantly larger than that in the intermediately-concentrated arsenic group ($p < 0.05$).

Von-Ehrenstein investigated the possible impairment of children's intellectual function in relation to arsenic exposure in a cross-sectional study conducted in 351 children (male: 54%, female: 46%, median age: 9) aged 5 to 15 years who were selected from a source population of 7,683 people in West Bengal, India, in 2001–2003.. Intellectual function was assessed with 6 subscales from the Wechsler Intelligence Scale for Children. Arsenic concentration was measured in urine and in sample water from 409 wells. By stratifying urinary arsenic concentrations into tertiles, arsenic concentration was associated with declines in the adjusted scores of the vocabulary test, the object assembly test, and the picture completion test. Relative decline of these scores in the upper tertile of urinary arsenic were 12% in the vocabulary test, 21% in the object assembly test, and of 13% in the picture completion test. However, an association between test results and arsenic concentrations in domestic water which was considered to show the long-term exposure amount was not found.

e. Effect on cardiovascular system

Tseng et al. (1996) investigated the correlation between cumulative arsenic exposure and peripheral arterial disease (PAD) in a population of 582 inhabitants (263 men and 319 women, mean age: 52.6 ± 10.6 years) who discontinued the use of well water after being exposed to the well water contaminated with highly-concentrated arsenic for more than 2 decades in black foot disease endemic area in Taiwan. Sixty-nine subjects were diagnosed with PAD

based on the clinical standard of ankle-brachial index of < 0.90 on either side. Three indices were used for the estimation of cumulative arsenic exposure: (1) duration of residence in a black foot disease endemic area, (2) duration of drinking well water, (3) history of residential addresses, and duration of drinking well water and the arsenic concentration in well water. The association between PAD and arsenic exposure was assessed by multiple logistic regression analysis. After the adjustment for age, sex, BMI, smoking, serum cholesterol and neutral fat as confounding factors, dose-response relationship was observed between long-term arsenic exposure and the prevalence rate of PAD, and the odds ratio was 4.28 (95%CI: 1.26-14.5) in the group with exposure to arsenic ≥ 20,000 μg/L・years.

The association between cardiovascular disease and arsenic exposure through contaminated drinking-water has been investigated in many studies. Navas-Acien et al. (2005) conducted a systemic review of the epidemiologic evidences, from thirteen epidemiologic studies in which drinking-water was an exposure source (of them, 8 references were from Taiwan). They selected the outcome of cardiovascular system such as black foot disease, morbidity of peripheral arterial diseases, mortality and morbidity of coronary heart disease, morbidity of myocardial infarction, mortality and morbidity of stroke as endpoints. In one case-controlled study among 3 studies in Taiwan where the morbidity of black foot disease was used as an endpoint, OR of the group which used well water for or more than 30 years was 3.47 (95%CI: 2.20-5.48) compared to the group which did not use well water (Chen et al. 1988b). In another small scale case-control study (number of cases: 20, versus controls: 20) where urinary arsenic was measured, OR of 1.66 was obtained though it was not statistically significant (Lin and Yang 1988). In another small scale study in the accident victims (number of cases: 31, number of non-cases: 30), it was found that arsenic exposure measured in arterial tissue was statistically higher in the patients with black foot disease (Wang and Chang 2001). This review did not include the ecological survey (Tseng 2008) in the southwest part of Taiwan which showed an increase of black foot disease associated with arsenic concentration in drinking-water in the village.

f. Other effects

Association of oral exposure to inorganic arsenic with abnormal glucose metabolism and diabetes has been reviewed by EFSA (2009). The risk of diabetes from exposure to arsenic of high concentration was shown in a study in Bangladesh, Taiwan, and in China. However, the data were not adjusted for BMI in many of these studies. On the other hand, study of general population with low to moderate exposure found no association between arsenic exposure and diabetes. Navas-Acien et al. (2009) have reported that the prevalence rate of type II diabetes was increased in the high exposure group in 80$^{th}$ or above percentile to of urinary arsenic concentration compared to low-exposure group in 20$^{th}$ percentile or below of urinary

arsenic concentration, using the data of the National Health and Nutrition Examination Survey (NHANES) which were adjusted for AsBe. However, Steinmaus et al. (2009) reported that association between arsenic exposure and diabetes was not shown when total arsenic excepting AsBe was considered to be inorganic arsenic exposure, using the same data. EFSA concluded that although excess risk of diabetes from arsenic was suggested from the studies on the population with highly-concentrated arsenic exposure, association between arsenic exposure and diabetes remained uncertain and data are insufficient to suggest the dose-response relationship with low level exposure (EFSA 2009; JECFA 2011).

(2) Effect on experimental animals etc.

(i) Acute Toxicity

The median lethal dose ($LD_{50}$) of diarsenic trioxide (As(III)) in single oral administration was 26-39 mg As/kg body weight for mice (C3H, C57H46, Dba2, Swiss-Webster) and 15-145 mg As/kg body weight for rats (Sprague-Dawley, Sherman, wild Norway) (Dieke and Richer 1946; Gaines 1960; Harrison et al. 1958). When calcium arsenate (As(V)) was orally administered to Sherman rats, $LD_{50}$ was 112 mg As/kg body weight and $LD_{50}$ of lead arsenate (As(V)) was 175 mg As/kg body weight (Gaines 1960).

The dispersion in $LD_{50}$ of inorganic arsenic is attributable to the difference in animal species, strain, administered compounds, and laboratories, then many experimental animals have been said to die 1 day after the administration in acute toxicity studies (EFSA 2009).

(ii) Repeated-dose toxicity

In general, As(III) was considered to have stronger toxicity than As(V) and repeated oral administration of inorganic arsenic compounds is considered to largely affect circulation, respiratory system, digestive organ, myelopoietic organ, immune organ, genital organ, and on nerves (WHO 2001; ATSDR 2007).

**a.** Subacute toxicity study

(a) Four-week subacute toxicity study in mice

Subacute toxicity of arsenic was studied in mice by Yokohira et al. (2011), where sodium arsenite (As(III)) (0, 1.73, 17.3, 43.3, 86.5 ppm: 0, 1, 10, 25, 50 ppm As) in drinking water was administered to female AS3MT defect mice (hereafter referred to as KO) and wild-type (C57BL/6) mice (hereafter referred to as WT) (female, 7 mice in each group).

As one KO mouse administered with 50 ppm arsenic died 5 days after the initiation of the study, all mice of the 50 ppm administered group were dissected for examination the day after. All of 6 KO mice showed moderate simple hyperplasia in the bladder epithelium while

5 out of 7 WT mice showed mild simple hyperplasia. Moderate simple hyperplasia was observed in 1 WT mouse.

Four weeks after the start of study, all the survived mice were dissected for examination. Hyperplastic changes in the bladder were observed in KO and WT mice that were administered with arsenic of 10 ppm and more. However the hyperplasia in KO was more severe than in WT mice. Among 25 ppm arsenic administered KO mice, mild simple hyperplasia in the bladder epithelium was observed in 5 out of 7 mice and moderate simple hyperplasia in the other 2 mice. Similarly, mild simple hyperplasia was observed in the WT group but moderate or severe hyperplasia was not observed. Hydronephrosis was observed in 1 out of 7 KO mice administered with 10 ppm arsenic , in 3 out of 7 KO administered with 25 ppm arsenic. The KO administered with 25 ppm arsenic showed mild acute inflammation in the liver. However, neither hydronephrosis nor mild acute inflammation was observed in WT mice.

The authors concluded that the effect of arsenic oral administration on bladder epithelium was observed in both KO and WT mice in a dose-response dependent manner, and that KO mice were more sensitive to it than WT. In addition, they suggested that no observable effect level (NOEL) of sodium arsenite to be 1 ppm As both for KO and WT mice (Yokohira et al. 2011).

(b) Four -week subacute toxicity study in rats

A 4-week study with administration of sodium arsenite (As(III)) at the dose of 0, 2, 5, 10, and 25 ppm(0, 0.12, 0.3, 0.6, and 1.5 mg As/kg bw/day as converted by ATSDR) in drinking-water in Sprague-Dawley (SD) rats (sex unknown, 3-5 rats for each dose) was conducted by Lee et al. (2002).

Increase in platelet aggregation was observed in the animals administered with sodium arsenite of 5 ppm and more. Increase in P-selectin positive cells in platelet and shortening the time for ligation of the pulmonary vessels by iron chloride in angioplasty were observed in the 10 ppm or 25 ppm administered group.

Based on these results, the authors suggested that the no observed adverse effect level (NOAEL) to be of sodium arsenite 0.38 mg/kg bw/day (5 mg/L × 0.024 L (daily water intake) /0.31 kg (body weight)). ATSDR (2007) suggested that the NOAEL of this study to be 0.12 mg As/kg bw/day.

(c) Two- and four-week subacute toxicity study in rats

According to ATSDR (2007), Bekemeier and Hirschelmann (1989) reported a study with 2- and 4-week (5 days per week) oral administration of diarsenic trioxide (As(III)) (15 mg/kg bw/day: 11 mg As/kg bw/day; converted by ATSDR) in Wistar-Barby rats (female, number of rats in the group is unknown).

A decrease of vascular responsiveness to norepinephrine was observed in rats received 2- and 4-week administration, and gastrointestinal tract stimulation as a clinical symptom was observed in the 2-week administration group.

(d) Twenty-eight-day subacute toxicity study in rats.

Subacute toxicity of sodium arsenite was studied with 28-day administration of sodium arsenite (As(III)) at dose of 0and 0.4 ppm: (0 and 0.14 mg As/kg bw/day; as was converted by ATSDR) in drinking-water into Wistar rats (female, 18 rats in each dose group) (Chattopadhyay et al. 2003).

The rats administered sodium arsenite showed significant decrease in absolute weight of the ovaries, uterus and liver, significant decrease in activities of $\Delta^5$, 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD in the ovaries, decrease in follicle stimulating hormone, progesterone and estradiol in plasma, also decrease in peroxidase activity in the ovaries and uterus. Significant increase in the activities of alkaline phosphatase, acid phosphatase, glutamic oxaloacetic transaminase and glutamic-pyruvic transaminase in the liver and kidneys were also observed (ATSDR 2007).

(e) Six-week subacute toxicity study in rats

Effects of 6-week administration of disodium arsenate (As(V)) were studied in CD rats (male, 18 rats in each dose group) administrating As(V) at the dose of 0, 20, 40, 85 ppm (0, 3, 6, 12 mg As/kg bw/day; converted by ATSDR) in drinking-water (Fowler et al. 1977).

In the 85 ppm dose group, growth rate was significantly decreased due to a delayed increase in body weight. Mitochondrial swelling and lowering of the density in liver cells were observed in the 40 ppm and 85 ppm arsenic administration groups. In the 85 ppm dose group, huge fat droplets in liver cells, and fascicular connective tissue between liver cells were observed.

ATSDR (2007) determined the NOAEL of this study in terms of effect on the body weight gain as 6 mg As/kg bw/day, and as 3 mg As/kg bw/day in terms of histologic change in liver.

(f) Sixteen-week subacute toxicity study in rats and guinea pigs.

According to ATSDR (2007), Kannan et al. (2001) reported about a 16-week administration study of As(III) of 0, 10, 25 ppm in rats and in guinea pig, which correspond to 0, 0.92, 2.3 mg As/kg bw/day in rats and 0, 0.69, 1.7 mg As/kg bw/day in guinea pig, converted by ATSDR. Arsenic was administrated in drinking-water in male rats and in male guinea pigs, although strain of both animals and number in each dose group is unknown.

In rats, number of red blood cell, number of leukocyte and the mean red blood cell hemoglobin concentration were decreased in the 10 ppm dose group. In the 25 ppm dose group, changes in neurotransmitter levels (dopamine, norepinephrine, and serotonin) were observed.

In guinea pigs, decrease in the number of red blood cell, number of leukocyte, δ-aminolevulinic acid dehydratase activity in blood, and increase in the mean corpuscular volume, mean red blood cell hemoglobin level, and δ-aminolevulinic acid synthase activity in liver were observed in the 10 ppm dose group. In the 25 ppm group, changes in neurotransmitter (dopamine, norepinephrine, and serotonin) levels were observed.

ATSDR (2007) determined the lowest observed adverse effect level (LOAEL) of this study for the changes in number of red blood cell and leukocyte in rats as 0.92 mg As/kg bw/day, and 0.69 mg As/kg bw/day for changes in number of red blood cells and leukocyte and changes in liver in guinea pigs. Also ATSDR determined the NOAEL of 0.92 mg As/kg bw/day and of 0.69 mg As/kg bw/day for the changes of neurotransmitter in rats and in guinea pigs, respectively.

(g) Two-hundred-day subacute toxicity study in rats.

A 200-day subacute toxicity study was conducted in Wistar rats administrating sodium arsenite (As(III)) or disodium arsenate (As(V)) at dose of 0 and 50 mg/L in drinking-water, using 8 males in each dose group.(Yang et al. 2007).

In all sodium arsenite and disodium arsenate administered rats, systolic blood pressure was significantly increased from 80 days after the administration onwards. This change was more prominent in the sodium arsenite group than in the disodium arsenate group. In the sodium arsenite group, significant increase of superoxide dismutase activity and significant decrease of catalase activity in plasma were observed throughout the administration period, while significant decreases of glutathione peroxidase activity and of catalase activity in plasma were observed in disodium arsenate group. No significant change in angiotensin converting enzyme (ACE) which is the most common index for hypertension was observed in both administration groups. However, expression of CYP4A protein in the liver and kidney was significantly increased in both groups. From these results, the authors suggested a more important role for CYP4A than for ACE in arsenic induced hypertension (EFSA 2009).

**b.** Chronic toxicity study and carcinogenicity study

(a) Forty-eight-week chronic toxicity study in mice.

Chronic toxicity of arsenic was studied in metallothionein (MT) gene deficit MT-I/II knock-out mice (MT-null) and in wild type mice, where sodium arsenite (As(III)) at dose of 0, 7.5, 22.5, 45 ppm: (for 22.5, 45 ppm, 5.6, 11.1 mg As/kg bw/day; converted by ATSDR) or disodium arsenate (As(V)) at dose of 0, 37.5, 75 ppm: (for 75 ppm, 18.5 mg As/kg bw/day;

converted by ATSDR) in drinking-water were administered into 4 to 6 male and female mice in each dose group, for 48 weeks (Liu et al. 2000).

There was no effect of sodium arsenite or disodium arsenate administration on body weight. In the 75 ppm disodium arsenate dose group, relative organ weights in the kidneys were significantly increased both in MT-null mice and in wild type mice.

In the disodium arsenate 75 ppm dose group, significant increase of blood urea nitrogen (BUN) which is an index of nephropathy was observed both in MT-null and in wild type mice. The increase of BUN was more significant in MT-null mice than in wild type mice. However, in the sodium arsenite 45 ppm dose group, significant increase of BUN was observed only in the MT-null mice group. Serum cytokine was not changed by the administration of sodium arsenite 45 ppm. In the sodium arsenite 22.5 ppm dose group, histopathological test revealed vacuolar degeneration of tubular epithelial cell in the kidneys, glomerulus swelling, atrophy of interstitial nephritis and tubular epithelial cells, and fibrosis of stroma in both the MT-null mice group and the wild type mice group. Although severe hepatic necrosis was not observed in the liver, fatty degeneration in liver cells and inflammatory cellular infiltration associated with focal hepatic necrosis were observed in the administered animals. MT-null mice showed histopathological changes in the kidneys and in the liver which were observed more frequently and were more severe compared to that in the wild type mice.

From these results, the authors inferred that chronic exposure of inorganic arsenic induced multiorgan failure, and that generally MT-null mice had higher sensitivity to arsenic induced toxicity than wild type mice regardless of administration method, and that metallothionein was a cell factor which had protective effects on chronic arsenic toxicity.

ATSDR (2007) determined the NOAEL of sodium arsenite and disodium arsenate administered in drinking-water in this study as 11.1 mg As/kg bw/day for change in body weight, and 18.5 mg As/kg bw/day for histological change in liver of mice. LOAEL of sodium arsenite and disodium arsenate in effect on the kidneys were determined as 5.6 and 18.5 mg As/kg bw/day, respectively.

(b) Two-year chronic toxicity study in rats and dogs.

Chronic toxicity was studied in rats and in dogs with 2-year feed studies of sodium arsenite (As(III)) and disodium arsenate in drinking water (Fowler et al. 1977). For the study in rats, sodium arsenite (As(III)) at the dose of 31.25, 62.5, 125, 250 ppm, which correspond to 2, 4, 9, 20 mg As/kg bw/day as was converted by ATSDR or disodium arsenate (As(V)) at dose of 31.25, 125, 250, 400 ppm which correspond to 2, 9, 20, 30 mg As/kg bw/day as was converted by ATSDR was administered into Osborne-Mendel rats (male and female, 25 rats in each dose group). Also, a 2-year feed study of sodium arsenite (As(III)) or disodium arsenate (As(V)) was conducted in beagles (male and female, 3 beagles for each dose group) with

the dose of 50 and 125 ppm which correspond to 1 and 2.4 mg As/kg bw/day as was converted by ATSDR.

In rats administered sodium arsenite, increases in body weight in both male and female were inhibited at the dose of 62.5 ppm. Swelling and proliferation of the bile duct were observed in the 125 ppm administered group, transient mild decrease in hemoglobin and hematocrit value, and pigmentation in tubular epithelial cell in the kidneys were observed in rats administered 250 ppm. In the rats administered arsenate, increase in body weight in male rats was inhibited in the 31.25 ppm group. Swelling of the bile duct, pigmentation in tubular epithelial cells and small sacculation in the kidneys were observed in the 250 ppm group.

In the beagles administered sodium arsenite, all male and female beagles died by 19 months after the administration of 125 ppm. Decrease in body weight by 44% to 61%, very mild to moderate anemia, hemorrhage in the gastrointestinal tract, and hemosiderosis in hepatic macrophage were observed. In the beagles administered 125 ppm disodium arsenate, one female died 13.5 months after the administration showing severe reduction of body weight gain, mild anemia, and hemosiderosis in hepatic macrophage.

ATSDR (2007) determined the NOAEL of sodium arsenite in this study to be 2 mg As/kg bw/day for reduction of body weight gain of rats, 4 mg As/kg bw/day for pathological changes in the liver, 9 mg As/kg bw/day for hematological and histological changes in the kidneys, 20 mg As/kg bw/day for changes in respiratory system, circulation, and digestive organ. ATSDR (2007) also determined the NOAEL of sodium arsenite in this study to be 1 mg As/kg bw/day for reduction of body weight gain, hematological changes, gross changes of the gastrointestinal tract, and histological changes in the liver, and 2.4 mg As/kg bw/day for changes in the kidneys and respiratory system, and circulation in dogs.

The NOAEL of disodium arsenate in this study was determined to be 9 mg As/kg bw/day for histological changes in the liver and kidneys in rats, 30 mg As/kg bw/day for blood, respiratory system, circulation, and digestive organ in rats, 1 mg As/kg bw/day for reduction of body weight gain, hematological changes, histological changes in the liver in dogs, and 2.4 mg As/kg bw/day for reduction of body weight gain, respiratory system, circulation and digestive organ in dogs for reduction of body weight gain,

(c) Twenty-seven-month chronic toxicity study in rats.

According to ATSDR (2007), Kroes et al. (1974) reported about a 27-month feed study on chronic toxicity of disodium arsenate (As(V)) or lead arsenate (As(V)) in Wistar rats. Although sex of the used animals and number of animals in each dose group was not reported, reduction of body weight gain was observed in rats administered disodium arsenate of 7 mg

As/kg bw/day. Meanwhile, in rats administered lead arsenate of 30 mg As/kg bw/day , increase of mortality, reduction of body weight gain, very mild anemia, swelling of the bile duct associated with severe expansion and inflammation were observed.

ATSDR (2007) determined the NOAEL of disodium arsenate in this study to be 7 mg As/kg bw/day for changes in blood, liver, kidneys, respiratory system, circulation, digestive organ, endocrine organs, and muscle/bone, LOAEL to be 7 mg As/kg bw/day for reduction of body weight, and NOAEL of lead arsenate as 7 mg As/kg bw/day for changes in body weight, blood, and the liver, and as 30 mg As/kg bw/day for changes in the kidneys, respiratory system, circulation, digestive organ, endocrine organs, and muscle/bone.

(d) Eighteen- month chronic toxicity study in rats, and 10-month subacute toxicity study in rabbits.

Chronic toxicity of sodium arsenite (As(III)) or disodium arsenate (As(V)) at the concentration of 50 mg As/L in drinking water had been studied with an 18-month administration in Wistar rats. (Male, number of rats in each dose group is unknown) and with 10-month administration of sodium arsenite (As(III)) (0, 50 mg As/L) in drinking-water in New Zealand rabbits (female, number of rats in each dose group is unknown) (Carmignani et al. 1985).

In rats and rabbits both administered with 50 mg As/L sodium arsenite, decrease in cardiac stroke volume and cardiac output per minute and increase in vascular resistance were observed. Decrease in coronary flow per minute was also observed in rats. Elevation of blood pressure by a vasoconstrictor tyramine (250 μg/kg body weight) in rats, and by an adrenergic drug phenylephrine (20 μg/kg body weight) in rabbits were significantly inhibited.

Administration of 50 mg As/L disodium arsenate was found to significantly inhibit an elevation of blood pressure by vagotomy, a decrease in blood pressure by a ganglion blocker hexamethonium (2.5 μg/kg body weight), and an elevation of blood pressure by tyramine (250 μg/kg body weight) (WHO 2001).

(e) Eighteen-month carcinogenicity study in mice.

Carcinogenicity of disodium arsenate (As(V)) was studied in an 18-month administration study in A/J male mice, a strain that exhibits development of spontaneous lung adenomas, where disodium arsenate at the dose of 0, 1, 10 and 100 ppm in drinking water were administered using30 mice of 5-weeks old at the initiation in each dose group (Cui et al., 2006).

After 18 months, the lungs from all animals were examined histopathologically and RNA and DNA were collected. Epigenetic effects of disodium arsenate (As(V)) on $p16^{INK4a}$ and on DNA methylation pattern of RASSF1A gene were investigated by methylated specific PCR. The mRNA and change in protein level of $p16^{INK4a}$ and RASSF1A gene were investigated by RT-PCR and by immunohistochemistry.

A dose-dependent accumulation of total arsenic in lung tissue was observed after the administration. In the administrated mice, increase in the number and size of lung tumors, and increase in the incidence rate of anaplastic pulmonary adenocarcinoma were observed compared to the non-administrated mice. Moreover, a dose-dependent increase in frequency of DNA-methylation in lung tumors was observed in the administrated group. Expression of $p16^{INK4a}$ and RASSF1A gene were decreased or abolished in lung tumor tissue of mice in the administrated group. This decrease in gene expression and gene hypermethylation were consistent.

The incidence number of lung adenoma in 0, 1, 10 ppm dose groups were 0. However, 4 mice in the 100 ppm group developed lung adenoma and the incidence rate of pulmonary adenocarcinoma increased by 9, 10, 11, 19 in the 0, 1, 10, 100 ppm dose groups, respectively.

The authors inferred that epigenetic changes in $p16^{INK4a}$ and RASSF1A gene, tumor suppressor genes, were associated with the development of lung tumors induced by As(V) (IARC 2012).

(f) A hundred-four-week carcinogenicity study in rats.

A 104-week administration study of sodium arsenite As(III) (0, 50, 100, 200 mg/L) in drinking-water was conducted in SD rats (male and female, 50 rats in each dose group, 8 weeks-old at the initiation). The observation was continued until the last animal died a natural death at 167 weeks-old. All lesioned parts, organs, and tissues were collected after the autopsy and histopathologic examination was performed (Soffritti et al. 2006).

Body weight, water and food intake decreased dose-dependently in both male and female groups. Benign and malignant renal tumors were observed in 5 out of 50 male rats (10.0%) to which 100 mg/L and 200 mg/L of As(III) had been administered. This incidence rate was not statistically significant compared to that of 1 out of 50 rats in the control group. The tumor observed in the 200 mg/L group included renal adenoma in 2 rats (4.3%), renal adenocarcinoma in 2 rats (4.3%), and renal pelvis carcinoma in 1 rat (2.2%). The tumors observed in the 100 mg/L group included renal adenoma in 3 rats (6.0%), renal adenocarcinoma in 1 rat (2.0%), and transitional cell papilloma of renal pelvis in 1 rat (2.0%). The increase in the tumor incidence in males was not significant as much as in females (IARC 2012).

(g) Studies on transplacental carcinogenicity and on carcinogenicity of life-time exposure in mice.

Waalkes and his colleagues have reported results from a series of studies on carcinogenicity of transplacental and life-time exposure to sodium arsenite in mice.

In one study, Waalkes et al. (2003) administered sodium arsenite (As(III)) into C3H mice (10 females in each dose group) on 8 to 18 days of the gestation period. The doses of As(III)

administered were 0, 42.5 and 85 ppm: (42.5 ppm for male offspring and 85 ppm for female offspring) that correspond to 9.55, 19.13 μg As/kg bw/day as was converted by ATSDR, in drinking-water. Their offspring were divided into each study group (male and female, 25 mice in each dose group) at 4 weeks-old and then, male offspring were raised for 74 weeks and female offspring were raised for 90 weeks till examinations

The change in body weight and water intake of dam animals was not observed during the administration period of sodium arsenite, and change in body weight was not observed also in offspring.

In male offspring, a decrease in the number of survivors due mainly to malignant liver tumors was observed after 52 weeks. Incidence rate of hepatocellular carcinoma and adrenocortical adenoma significantly increased in the 42.5 ppm and 85 ppm administered groups, and the number of developments of hepatocellular carcinoma and adrenocortical adenoma significantly increased in the 85 ppm dose group. Also, frequency of development of all neoplastic lesions and malignant tumors significantly increased in the 42.5 ppm and 85 ppm group.

Number of survivors in male offspring did not change during the study period. Incidence rate of ovarian tumors (benign and all neoplastic lesions), proliferative lesions in lung cancer and the oviducts (hyperplasia, all proliferative lesions) significantly increased in the 85 ppm dose group. Incidence rate of proliferative lesions in the uterus (hyperplasia, all proliferative lesions) significantly increased in the 42.5 ppm and 85 ppm dose group. Also, Incidence of malignant tumors significantly increased in the 42.5 ppm and 85 ppm groups (IARC 2004; EFSA 2009; ATSDR 2007).

In another report (Waalkes et al., 2004a) they studied transplacental carcinogenicity of As(III), examining the effects of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) which is a tumor promotor, in C3H mice. They administered As(III) with the dose of 0, 42.5 and 85 ppm into C3H mice (10 females in each dose group) on 8 to 18 days of the gestation period in drinking-water. Their offspring were divided into each study group which composed of male and female, 25 mice for each dose, at 4 weeks-old. To these offspring, TPA at the concentration of 2 μg/0.1 mL acetone,(control group: acetone only) was applied twice a week on the animal's shaved back skin for 21 weeks, and then the offspring were raised for 104 weeks.

In the animals received a single dose with sodium arsenite, the incidence rate of ovarian tumors (ovarian adenoma, all ovarian tumor) in female offspring (42.5 ppm, 85 ppm), and the incidence rate of hepatocellular adenoma and/or cancer, neoplastic lesions of hepatic cells (85 ppm) and adrenocortical adenoma (42.5 ppm, 85 ppm) in male offspring were significantly increased. Multiple doses of sodium arsenite and TPA resulted significant increases in the incidence rate of several tumors which had not been observed with the single doses.

Those were the incidence rate of neoplastic lesions of hepatic cells and number of tumors, lung adenoma in female offspring (85 ppm); significant increase in incidence rate of lung adenoma and neoplastic lesions of the lungs in male offspring (85 ppm). Either the single dose or the multiple dose of sodium arsenite and TPA in female offspring significantly increased hyperplasia lesions of the uterus (42.5 ppm, 85 ppm) and oviduct (85 ppm) which were considered as pre-neoplastic lesions. Although TPA did not affect the development of skin tumors, it promoted the development of liver tumors which was induced by arsenic in female offspring and lung tumors in male and female offspring (EFSA 2009; ATSDR 2007; IARC 2012).

In another series of study, Waalkes et al. (2004b) examined transplacental effects of As(III) administered with the dose of 0 ppm and 85 ppm into female C3H mice, 10 mice in each dose group, on 8 to 18 days of the gestation period in drinking-water. After raised their male offspring, for 74 weeks, the livers from the 0 ppm group (5 mice), 85 ppm group (8 mice), and from the control group which is untreated C3H mice ( 10 male and female each) were removed , and cryopreserved or formalin fixed for the following tests. In addition, in this study, human liver was obtained from 3 males who had skin lesions (keratosis, pigmentation)evoked by exposure to highly-concentrated arsenic in China, and from 5 males s who had surgery in the U.S. as controls. These human tissues were cryopreserved for the same tests. The test results of these samples were reported as follows.

Quantitative analysis of gene expression was performed by the real-time RT-PCR method on Estrogen Receptor -α (ER-α) and cyclin D1 gene in the liver of mouse and humans. Expression of CYP2A4, CYP2B9 and CYP7B1 gene in the liver of mice was also analysed, using β-actin gene as a control gene in all anlyses. Frequency of methylation of cytosine phosphate guanine (CpG) island in the ER-α promoter domain of genome DNA was studied in the cryopreserved liver of mice. Immunohistochemical examination using anti-ER-α and anti-cyclin D1 antibodies was performed in the histopathological specimen prepared from formalin fixed tissues.

In the liver of mice exposed to arsenic in-utero, expression of ER-α and cyclin D1 mRNA was significantly increased. Positive reaction to anti-ER-α and anti-cyclin D1 antibodies in immunohistochemical detection was also increased by the in-utero exposure compared to the control mice. In addition, significant increase in the expression of CYP2A4 and CYP2B9 mRNA, significant decrease in the expression of CYP7B1 mRNA, and significant decrease of methylation of CpG in the ER-α promoter domain were observed in the liver of the mice exposed to arsenic in-utero. Similarly, in the liver of humans who had exposure to highly-concentrated arsenic, expression of ER-α and cyclin D1 mRNA was significantly increased compared to that in the non-exposed subjects. From these results, the authors concluded that the abnormality of estrogen signaling pathway may be a factor which induced hepatocellular

carcinoma by in-utero exposure to arsenic, and that especially over expression of ER-α in the liver may be caused through decreased methylation in the promoter domain and relating to the carcinogenicity of arsenic.

Moreover, Waalkes et al. (2006a, b) studied transplacental carcinogenicity of As(III) in CD1 mice, where 85 ppm of As(III) was administered in female mice in drinking-water on 8 to 18 days of the gestation period. The offspring were divided into each study group (which consists of 35 male for each dose group after weaning, and received diethylstilbestrol (DES) (2 μg/mouse/day) which induced urogenital neoplasm or tamoxifen (TAM) (10 μg/mouse/day) by subcutaneous administration 1 to 5 days after the birth and were raised for 90 weeks. As a control of DES or TAM, corn oil solvent were administered. A single administration of As(III) resulted increase in the incidence of hepatocellular carcinoma, hepatocellular adenoma, and all neoplastic lesions in the liver compared to the control group. Also increases of pulmonary adenocarcinoma, adrenocortical adenoma and renal cyctichyperplasia of tubular epithelium were observed with the single administration compared to that in control animals. Multiple doses of arsenic and DES resulted an increase in the incidence rate of neoplastic lesions in the liver, and the number of neoplastic lesions was significantly increased compared to the single arsenic administration group. Incidence of urinary bladder transitional epithelium cell tumors **(**papilloma and cancer) which had not been observed in the animals administered single dose of arsenic was also increased. Increase of proliferative bladder lesions (tumor and hyperplasia) was observed in the arsenic and DES administered animals or the animals additionally administered with TAM compared to the control animals or the animals received a single dose of arsenic. Lesions in the bladder and hepatocellular carcinoma observed with the multiple doses of arsenic and DES or with the additional dose of TAM were associated with the overexpression of ER-α which indicates abnormality in an estrogen signaling pathway, suggesting that the overexpression of ER-α may be one of factors which potentiate carcinogenic response (EFSA 2009; ATSDR 2007; IARC 2012).

A study on the lifelong administration of As(III) was performed in CD1 mice by the same group based on the result of the above mentioned transplacental carcinogenicity study. The administration of As(III) of 0,6, 12 and 24 ppm in drinking-water was started 2 weeks before mating and continued for 104 weeks through the gestation period, lactation period, and after weaning, using 30 male and female mice for each dose.

Lifelong arsenic exposure increased incidence of pulmonary adenocarcinoma in male and female, hepatocellular carcinoma in male and female, gallbladder neoplasm in male and uterus cancer significantly and dose-dependently. Dose-dependent increase in the incidence of ovarian tumors including ovarian cancer and adrenal tumors in male and female by arsenic exposure were observed with the lowest dose onward. Lifelong exposure to arsenic elicited

carcinogenic activity in mice at target tissues which were almost same to that in mice exposed to arsenic through the placenta in dam animals in the series of transplacental carcinogenicity studies. However, oncogenesis in the mice with lifelong exposure to arsenic was observed at the dose lower than that in mice with exposure in-utero. In addition, the tumor was more malignant and its incidence rate was higher than in the mice exposed to arsenic in-utero in dam animals in the series of transplacental carcinogenicity studies.

In the uterine adenocarcinoma of lifelong exposed female mice, expression of ER-α was remarkably higher than that in the spontaneously developed tumors in the control group. Moreover, expression of gene which were controlled by estrogen α such as cyclin D1, NF-κB and Cox-2, was also increased. The authors considered the contribution of the pathway mediated by ER as one of the carcinogenic mechanisms of lifelong arsenic exposure (Tokar et al. 2011).

[References]

(h) Combined carcinogenic test in mice.

Combined carcinogenicity of arsenic and ultraviolet irradiation was studied in SK-1-*hr*BR hairless mice. Female SK-1-*hr*BR mice were divided into the control (5 mice), a group of 5 mice dosed with a single sodium arsenite (10 mg/L) administration in drinking-water, a group of 15 mice for a single dose of ultraviolet rays (UVR) (1.7 KJ/$m^2$: UVB85%, UVC < 1%, UVA4%, 3 times/week), and a group of 15 mice for the combined dose of sodium arsenite in drinking-water and UVR irradiation, and were observed for 26 weeks (Rossman et al. 2001).

No effect on body weight gain was observed in the mice with a single sodium arsenite in drinking-water compared to the control mice. Development of skin tumors was not observed in the control mice and in the mice dosed single sodium arsenite in drinking-water. The first oncogenesis was observed 8 weeks after UVR irradiation in the animals exposed to sodium arsenite administration in drinking-water combined with UVR irradiation and tumor development was not observed up to 12 weeks after UVR radiation in the single UVR irradiation group. The combined exposure to sodium arsenite and UVR irradiation caused oncogenesis statistically and significantly earlier than the single UVR irradiation. At least 1 tumor was developed in all animals by the single UVR irradiation within 26 weeks. However, the rate of tumor development by the single UVR irradiation was 33% compared to 100% by the sodium arsenite combined with UVR irradiation 19 weeks after UVR irradiation. The total number of tumors observed in the animals with the combined exposure to sodium arsenite and UVR irradiation was 127, and 53 in the single UVR irradiation group. A very strong invasive skin squamous cell carcinoma was observed in 64 out of 127 (50.4%) cases in the animals with the combined exposure, while 14 out of 53 (26.4%) tumors in the UVR single

irradiation group. Difference in the number of development of squamous cell carcinoma between these two groups was statistically significant (IARC 2004; EFSA 2009; IARC 2012).

(i) Combined carcinogenic test in mice.

Combined carcinogenicity of arsenic and ultraviolet irradiation was also studied in Skh1 hairless mice (sex and number of mice unknown). Sodium arsenite at the concentration of 0.0, 1.25, 2.5, 5.0 and 10 mg/L in drinking-water was administered in the Skh1 hairless mice from 21-days-old for 29 weeks and ultraviolet ray (UVR) (1.0, 1.7 kJ/$m^2$) was irradiated 3 times a week from 42-days-old for 182 days (Burns et al. 2004).

Number of the development of skin squamous cell carcinoma was increased in the group exposed to the combined sodium arsenite and UVR irradiation compared to the single UVR irradiation group (EFSA 2009; IARC 2012).

(j) Combined carcinogenic test in mice.

Combined carcinogenicity of arsenic in drinking-water with 9,10-Dimethyl-1,2-benzanthracene (DMBA) was also studied in Swiss-bald hairless mice. The study was conducted in Swiss-bald hairless mice divided into 4 groups of 10 males for each. One was an untreated group, the second was a single DMBA administration group in which the mice were painted with 9,10-Dimethyl-1,2-benzanthracene (DMBA) (25 μL/mouse) twice a week for 2 weeks, the third was a group of animals exposed to disodium arsenate of 25 mg/Lin drinking-water for 25 weeks, and the fourth was the animals for the combined exposure to disodium arsenate and DMBA (Motiwale et al. 2005).

No development of skin tumors was observed in the untreated mice and in mice with the disodium arsenate administration in drinking-water. However, development rate of tumors and the number of tumor developments per mouse increased and the development of large-sized skin papilloma with a diameter of above 3 mm significantly increased in the animals with the combined exposure compared to that in the animals with the single DMBA administration (EFSA 2009; IARC 2012).

(iii) Neurotoxicity

According to EFSA, lack of apparent systemic toxicity of inorganic arsenic has been confirmed by many studies in rats and mice. However, mild neurobehavioral effects have been observed (Rodriguez et al. 2003; EFSA 2009).

a. A study with sixty-day administration in drinking-water in mice.

Neurotoxicity of inorganic arsenic was studied in mice (sex unknown), where diarsenic trioxide (As(III)) of 0, 1and 4 ppm in drinking-water was administrated for 60 days, using 6 or 10 mice for each dose (Wang et al. 2009a).

Dose-dependent prolonged latency was observed in a Morris water maze test. Expression of crucial genes related to the Creb dependent long-term depression (LTD) in cerebellum was analyzed, and significant decrease in expression of $Ca^{2+}$/calmodulin-dependent protein kinase IV (Camk4), Fos and Jun among 25 genes was detected in the animals dosed with 4 ppm of As(III).

Since administration of antioxidant such as taurine or vitamin C with As(III) could not inhibit decrease in Camk4 expression, the authors attributed the decrease in Camk4 expression by arsenic to a pathway which was independent of oxidation.

b. A study with administration in drinking-water for 4 months in mice.

A study on neurotoxicity of sodium arsenite with 4-month administration of As(III) was conducted in mice, where As(III) at the doses of 0, 0.05, 0.5, 5.0 and 50 mg/L which correspond to 0.034-0.044, 0.043-0.057, 0.122-0.156, 1.56-1.62 and 6.58-7.34 mg As/kg bw/day in drinking-water were given to C57BL/6J mice of male and female(number of mice in each dose group is unknown) (Bardullas et al. 2009).

Spontaneous behavior was measured every month. In male mice, locomotor activity (horizontal movement, stereotyped behavior: repetition of movement at the same place) increased significantly after 4 months of administration at the dose of 0.05 mg/L and 0.5 mg/L , but significant increase and significant decrease were both observed in horizontal movement only in the 50 mg/L dosed group. In female mice, significant increase in horizontal movement was observed 2 months after the administration in the animals dosed with As(III) above 0.5 mg/L, and 3 and 4 months after the administration in all treatment groups. Stereotyped behavior was significantly increased in the animals administered with 0.5 mg/L and 5.0 mg/L dose 1 and 2 months after the administration, and in the animals with all doses3 and 4 months after the administration. No effect on dopamine in the striatum and in the hypothalamus was observed in male mice. Administration of As(III) at 0.5 mg/L and above into the female mice decreased dopamine in the striatum, and administration of As(III) with all doses significantly decreased dopamine in the hypothalamus in female mice. Administration of As(III) with all doses decreased expression of mRNA of tyrosine hydroxylase and of thioredoxin-1 in striatum of males and in nucleus accumbens of females.

From these data, the authors considered that chronic exposure to arsenic caused gender-dependent changes of locomotor activity, dopaminergic nervous system, and the thioredoxin system which had an antioxidative effect on the central nervous system in mice. The authors also suggested that female is more sensitive to arsenic than male is.

c. Fifteen-day and four-week study with oral gavage in rats.

Neuroroxicity of inorganic arsenate was studied with 15-day (study 1) and 4-week (study 2) oral gavage of As(III) in SD rats, where 8 male rats were used for each dose of 0, 5, 10 and 20 mg/kg bw/day for study1, and of 10 mg/kg bw/day for study 2 (Rodriguez et al. 2001).

A significant decrease in locomotor activity was observed in Week 1 and Week 2 in the 20 mg/kg bw/day dosed animals in Study 1. A significant decrease was observed only in duration time of vertical activity in Week 1 among other index of locomotor activities in the 10 mg/kg bw/day dosed animals. In study 2, decrease in locomotor activity in Week 2 and Week 3 was observed in the administration group. Among 3 tasks in study 1 and study 2 (T-maze task, redial-arm maze task, spatial learning task), decrease in the number of errors in the T-maze task was observed in the administration group in Study 2.

d. Four to twelve-week oral gavage study in rats.

Toxic effects of inorganic arsenate on the nervous system was studied with oral gavage of As(III) in Wistar rats, where dose of 0, 3 and 10 mg/kg body weight were administered into 3 male each for four to twelve-week. In ischiadic nerves, no change was observed in the expression of neurofilament subunit proteins medium chain (NF-M) or of neurofilament subunit proteins heavy chain (NF-H). However, the expression of neurofilament subunit proteins light chain (NF-L) significantly decreased by the gavage of all doses for 4, 8 and 12 weeks depending on the dose and duration of administration. No significant change in the expression of microtubule-associated protein (MAP) -tau was observed (Vahidnia et al. 2008a).

Since oral gavage of the all doses caused significant increases in the expression of μ-calpain and m-calpain proteins, the authors suggested that the disintegration of NF-L was probably progressed. The authors also considered that hyperphosphorylation of NF-L and MAP-tau likely occurred which consequently resulted unstableness and destruction of cytoskeletal framework, and eventually induced degeneration of axons. The authors concluded that this study importantly demonstrated the pathological changes induced by arsenic in the peripheral nervous system, showing the decrease in the expression of NF-L which is the only NF protein that can form and coassemble itself *in vivo*.

e. Four to twelve-week oral gavage study in rats.

Neurotoxicity of As(III) was also studied with oral gavage in Wister rats where 10 male rats were exposed to each dose of 0, 3.33, 6.66, 13.3 and 26.6 mg/kg bw/day of As(III) for 4- to 12-week (Schulz et al. 2002).A significant decrease in walking and grooming was observed in Week 4 in an open field study in the 26.6 mg/kg bw/day dose group, but no significant decrease was observed in Week 8 or Week 12.

f. Twenty-eight-day oral administration study in rats.

A twenty-eight-day oral administration study of sodium arsenite (As(III)) (0, 20 mg As/kg bw/day) in Wistar rats (female, 5 rats in each administration group) was conducted (Yadav et al. 2009).

Oral administration of As(III) caused significant decrease in the locomotor activity (moving distance, moving time, rest period, stand-up motion), and in a result of a gripping force and rotor rod test **(**coordination of motor function).

g. Three-month study with administration in drinking-water in rats.

Neurotoxic effects of As(III) administered for 3 months in drinking-water at the dose of 0, 2.72, 13.6, and 68 mg/L were studied in SD rats using 20 male rats for each dose (Luo 2009).

Spatial perception in the Morris water maze test was significantly decreased (disability in obtaining platform) in the animals administered with As(III) of 68 mg/L. Pathological changes of neuron and endothial cells, and dose-dependent decrease in gene expression of NR2A mRNA of the aspartate receptor in hippocampus were also observed after the administration of As(III).

h. Four-month study with administration in drinking-water in rats.

Neurotoxicity of As(III), that was administered in drinking-water at the dose of 0 and 36.7 mg As/L from 15 days of gestation or from 1 day after the birth till approximately 4 months, was studied in SD rats using 4 females for each dose (Rodriguez et al. 2002).

Locomotor activity was increased in the animals in which arsenic was administered from 15 days of gestation, and test scores on the delayed alternation task in T-maze was reduced (error increased) in the animals administered both doses compared to the control animals.

[References] *in vitro*

EFSA (2009) referred on the effect of As(III) and As(V) *in vitro* as follows:

The expression of NF-L gene was not affected by treatment *in vitro* with sodium arsenite (As(III)) or with disodium arsenate (As(V)) at the dose of 0, 0.3, 1 and 3 μM for 24 or 48 hours in culture (Vahidnia et al. 2007b). However, cellular calcium was increased by As(III) *in vitro* (Florea et al. 2007). Consequently calcium induced Calpine activity may decomposep35 protein to p25 protein. Hyperphosphorylation of cytoskeletal protein including MAP-tau caused by the decomposition was considered to be highly likely (Vahidnia et al. 2008b).

Moreover, the loss of normal cytoskeleton of axon by arsenic has been attributed to the hyperphosphorylation and deregulation of MAP-tau as a consequence of hyperphosphorylation. (Vahidnia et al. 2007a).

[References] Mechanism of neurotoxicity

According to EFSA (2009), arsenic is considered to enter into the brain through a yet unknown pathway and accumulate in the choroid plexus more than in other parts of the brain. Arsenic affects cholinergic, glutamic, and monoaminergic neurotransmitter in adult rodents and it affects the dopaminergic system the most. Since the structure of arsenate is similar to that of inorganic phosphate arsenite, it causes substrate competition which inhibits conversion of 3,4 dihydroxyphenylalanine (l-DOPA) to 2- (3,4-dihydroxyphenyl) ethylamine (dopamine). Since arsenite interacts with the thiol group, it may disturb the enzyme function related to carbohydrate metabolism such as succinic acid or pyruvate dehydrogenase (Rodriguez et al. 2003; Vahidnia et al. 2007a).

Moreover, it is known that inorganic arsenic induces oxidative stress to which the brain cells are particularly sensitive. Hence, oxidative stress induced by arsenic is considered to be a molecular mechanism of inorganic arsenic-induced neurotoxicity *in vivo* (Mishra and Flora 2008; Hong et al. 2009).

(iv) Immunotoxicity

a. Three-week subacute toxicity study in mice.

Subacute toxicity of arsenic was studied in White Swiss cross mice with a 3-week administration of As(III) at the dose of 0, 0.5, 2.0 and 10.0 ppm in drinking-water using 8 to 10 male mice for each dose (Blakley et al. 1980).

A decrease in plaque forming cell response in spleen cells to sheep erythrocyte was observed in 0.5 ppm, 2.0 ppm, 10.0 ppm dose groups, and inhibition of humoral immunity which inhibits both primary and secondary immune responses was observed (EFSA 2009).

b. Five-week subacute toxicity study in mice.

A 5-week study with administration of As(III) at the dose of 0.1, 1, and 50 μg/L in drinking-water was conducted in C57/BL6 mice using 3-4 male mice for each dose (Andrew et al. 2007).

At the end of the administration in drinking-water for 5 weeks, a comprehensive gene analysis using mouse lung was performed. Significant changes in gene expression associated with angiogenesis, lipid metabolism, oxygen transfer, apoptosis, cell cycle, and immune response were detected. Similar changes in some part of these responses were confirmed by semi-quantitative RT-PCR or immunoblotting techniques.

From this result, the authors suggested that the factors which were affected in this study may be a useful biological marker on searching the disease associated with arsenic and are useful for the assessment of disease risk (EFSA 2009).

c. Five- to six-week subacute toxicity study in mice.

Subacute toxicity of arsenic was studied in C57BL/6J mice with administration of As(III) in drinking-water or in feed at the dose of 10 and 100 ppb using 4 to 6 male mice for each dose (Kozul et al. 2009).

A comprehensive gene analysis using mice lung was performed at the end of the study. Significant changes in many genes relating to cell adhesion, cell division, channel, receptor, differentiation, proliferation, and innate immune response were observed in the animals of administration group. Some parts of these changes were similarly observed by semi-quantitative RT-PCR or by immunoblotting techniques.

Since the effects observed in the results were that on the factors related to natural immunity, the authors suggested that arsenic may especially associate with the increase of the disease risk of the lung (EFSA 2009).

d. Ten to twelve-week subacute toxicity study in mice.

Subacute toxicity study of disodium arsenate (As(V)) was conducted in C57BL6/6 B6 mice with administration of As(V) in drinking-water at the dose of 2.5, 25 and 100 ppm (20 mg As/kg bw/day for 100 ppm as converted by ATSDR using male mice (the number in each dose group is unknown) (Kerkvliet et al. 1980).

Abnormality in immune function, liver, and in the kidneys was not observed in disodium arsenate administered mice.

ATSDR (2007) determined the NOAEL in terms of immune function, liver, and the kidneys as 20 mg/kg bw/day.

e. Twelve-week subacute toxicity study in mice.

Subacute toxicity of disodium arsenate (As(V)) was studied with 12-week administration of As(V) at the dose of 0.5, 5, and 50 mg As/L in drinking-water in C57BL/6J/Han mice (female, 10 mice in each dose group) (Arkusz et al. 2005).

In examination of the peritoneal macrophage which was isolated from mice at the end of the administration for 12 weeks, significant decrease was observed in the production of reactive oxygen species by phorbol myristate stimulation and the production of nitric oxide by lipopolysaccharide (LPS) stimulation. No change was observed in the production of LPS stimulation-induced nitric oxide synthase, TNF-α production and mRNA expression.

The authors suggested that exposure to As(V) may affect the progress of infection and tumor cells based on the finding that the production of reactive oxygen species or of nitric oxide in peritoneal macrophage was decreased by the exposure (EFSA 2009).

(v) Toxicity on reproduction and development

Although it is known that inorganic arsenic poses fetal toxicity or teratogenicity in experimental animals, many studies have been conducted with doses as high as that with maternal toxicity (Golub et al. 1998; Wang et al. 2006).

In recent studies on the toxicity of arsenic, mostly on As(V), with a dose which did not have maternal toxicity, growth delay in fetus, neurotoxicity, and changes in lung structure were observed depending on the oral dose (Wang et al. 2006; Hill et al. 2008). Exposure in-utero or exposure at the postnatal early stage of mice to arsenic in drinking-water, which contains arsenite at the concentration of 100 μg/L or less, was found to cause a change in airway response to methacholine stimulation in 28-day-old mice. This change was accompanied with morphological structural change around the respiratory tract which was related to protein and gene expression (Lantz et al. 2009).

(vi) Developmental neurotoxicity

The brain is fragile especially at the development and arsenic exposure of fetus and arsenic exposure right after the birth are known to induce neurotoxicity which leads to behavioral changes as a result (Rodriguez et al. 2003; Wang et al. 2006). According to the report from EFSA (2009), in-utero exposure of fetuses to inorganic arsenic through oral administration into the mother caused neurotoxicity including neural tube aplasia, fetal growth retardation, changes in motor activity and of spatial learning in offspring. Arsenic exposure in-utero also caused changes in neuroendocrine markers associated with depression-like behaviors in offspring. In addition, inhibition of methylation by arsenic was reported to enhance the developmental neurotoxicity. However, EFSA also suggested that it was difficult to extrapolate these findings to humans since the specie difference was large and information was insufficient.

a. Study with perinatal administration in drinking-water in mice.

Developmental neurotoxicity of As(V) was studied with the administration in drinking-water with the dose of 0 and 50 μg/L in C57BL/6J mice. Administration of As(V) of each dose into 5-12 female mice was initiated before the gestation and continued until the birth of offspring. (Martinez et al. 2008).

An unenthusiastic behavior in learning, immobility in a forced swimming task and change in neuroendocrine markers related to depressive and depressive-like behaviors

were observed in offspring in the As(V) administered animals. Significant increase in serum corticosterone, significant decrease of CRFR1 protein in hippocampus, and significant increase in binding affinity of serotonin 5HT1A receptors and the receptor-effector coupling in the dorsal hippocampus were observed. But no such a change was observed in the ventral hippocampus, entorhinal cortex, cortex of frontal lobe, and in colliculus inferior.

The authors reported that perinatal exposure to arsenic inhibited regulatory interaction between the HPA axis and serotoninergic system in the dorsal hippocampus which related to depression-like behavior in children. It was also suggested that exposure to arsenic of low concentration in the developmental stage had adverse effects for long periods on the neurobiological markers which associated with the changes in neuronal system and behavior.

b. Study with perinatal administration in drinking-water in rats.

Administration with sodium arsenite 100 mg/L in drinking-water into pregnant rats from 6th day in gestation to 42nd post-parturition day was reported to cause changes in learning and memory behavior and in several reflex reactions (Xia et al. 2009).

3. Effect of organic arsenic compounds

The toxicity of methylated organic arsenic has been considered to be lower than that of inorganic arsenic, and the methylation *in vivo* has been considered to be a mechanism for detoxication of inorganic arsenic. However, trivalent methylated arsenic such as MMA (III) or DMA (III) which are highly reactive and strongly carcinogenic are known to be formed through the methylation process. Based on these findings, it has been suggested that MMA (III) or DMA (III) likely has adverse effects on organisms (Jomova et al. 2011).

(1) Effect on humans

Toxicity of organic arsenic compound is compound specific among several types of it, thus it is difficult to compare toxicity of each compound equally. Toxic risk of each organic arsenic compound should be evaluated separately (ATSDR 2007; EFSA 2009).

AsBe is not metabolized *in vivo* in humans and excreted as an unaltered substance. Since little toxicity of it has been reported in humans and experimental animals, AsBe has been considered not toxic (EFSA 2009). Although arsenosugar and arsenolipid which is a lipid-soluble organic arsenic compound are metabolized to DMA (V) *in vivo* in humans (Raml et al. 2009; Schmeisser et al. 2006), no information about toxicity of arsenosugar or arsenolipid has been available.

(i) Acute effect

According to ATSDR (2007) and EFSA (2009), any investigation of acute poisoning and mortality caused by oral intake of organic arsenic compounds in humans has not been reported.

(ii) Chronic effect

a. Cutaneous effects

No epidemiologic study about cutaneous effect caused by oral administration of organic arsenic compound alone is available. However, it has been reported that the risk of arsenic-induced skin lesions is high in the people who had inorganic arsenic exposure and excretes MMA (V) with a high proportion, since whose OR of skin lesions was 1.5 to 2.8 times higher than that in the people who excrete inorganic arsenic with a lower proportion (Ahsan et al. 2007; McCarty et al. 2007; Lindberg et al. 2008). However, it was pointed out that MMA (III) and MMA (V) were not distinguished at the measurement in these reports (EFSA 2009). Also, Valenzuela (2005) et al. reported that, among the people who had inorganic arsenic exposure through drinking-water, the people with skin lesions had mean urinary concentration of MMA (III) significantly higher than that of the people without skin lesions.

b. Carcinogenicity

According to IARC, although organic arsenic such as AsBe and AsC are considered to be contained in seafood, no epidemiologic data which is applicable to the assessment of the cancer risk of organic arsenic derived from seafood is available for the moment (IARC 2012).

c. Effects on nervous system

EFSA (2009) reported that there are only few reports about neurotoxicity of chronic exposure to organic arsenic compounds in human peripheral nervous system, and it is still difficult to distinguish neurotoxicity in peripheral nervous system caused by inorganic arsenic from that by organic arsenic in human pathology. Also, neurotoxicity in peripheral nervous system caused by organic arsenic compounds in foods such as AsBe and AsC has not been observed in humans. Similarly, no neurotoxicity of various arsenic metabolites such as MMA (methylarsonate) and DMA (III)) has been clarified in the clinical area (EFSA 2009).

(2) Effects on experimental animals.

(i) Acute Toxicity

According to ATSDR (2007), the oral $LD_{50}$ for MMA(V) was reported to be 1,800 mg MMA(V)/kg body weight in mice and the oral $LD_{50}$ for DMA(V) was reported as 1,200 mg DMA(V)/kg body weight in mice (Kaise et al. 1989).

Arsenosugar has been considered not to have acute toxicity like that organic arsenic hAs (Sakurai et al. 1997; Andrewes et al. 2004).

(ii) Repeated-dose toxicity

a. Subacute toxicity

When DMA (V) was orally administered to Fischer344 rats 5 times a week for 4 weeks, 50% of the males and 20% of the females died with the dose of 57 mg/kg bw/day (Murai et al. 1993). With the administration in drinking-water for 8 weeks, 100% of F344 male rats died with the dose of 17 mg/kg bw/day (Wanibuchi et al. 1996). With a 13-week feeding administration of DMA (V), 100% of F344 male rats died with the dose of 190 mg/kg bw/day (Crown et al. 1987).

b. Chronic toxicity

MMA (V) is known to have effects on the gastrointestinal tract, kidneys, thyroid gland and genital organ system (ATSDR 2007). The effect which is most sensitive to MMA (V) is diarrhea and it is reported that diarrhea occurs associated with the extension of the treatment period even in the lower dose in rats, mice, rabbits and dogs. Pathological change in the gastrointestinal tract was observed more frequently with higher doses than with lower doses which normally cause diarrhea. According to Arnold et al. (2003), the lowest NOAEL in the feeding study was 3 mg/kg bw/day in a 2-year feeding study in rats and LOAEL in terms of diarrhea was 25.7 mg/kg bw/day (Arnold et al. 2003).

DMA (V) has effects on the bladder, kidneys, thyroid gland, and fetus development, and the effect with the highest sensitivity to DMA (V) has been considered to be carcinogenicity in the bladder (ATSDR 2007).

(iii) Carcinogenicity

a. DMA (V)

While carcinogenicity of DMA (V) in the bladder of rats has been confirmed, carcinogenicity of DMA (V) in the bladder of mice has not been found.

(a) Mice

Carcinogenicity of DMA (V) was examined in OGG1 KO and wild type mice, where DMA (V) in drinking-water was administered with the dose of 0 and 200 ppm for 72 weeks. The animals were 14 weeks-old male and female at the initiation of the study, and 10 OGG1 KO mice and 12 wild type mice were used for each dose. As a result, incidence rate and number of lung tumors in mice deficit in OGG1, an enzyme that repairs oxidative damage of DNA, were significantly increased by DMA (V) when compared to the control animals. In contrast, the development of lung tumors was not observed in wild type mice. From these results, the authors suggested that DMA (V) is carcinogenic to the lungs in OGG1 deficit mice (Kinoshita et al. 2007a).

Effects of DMA (V) administered in AJ mice in drinking-water was reported as follows. DMA (V) of 0, 50, 200 and 400 ppm in drinking-water was administered in AJ male mice of 5 weeks old at the initiation for 50 weeks using 24 mice for each dose. Although administration of the maximum dose of 400 ppm significantly increased the number of tumors per mouse, no clear dose-response relationship was observed between the administered concentration of DMA(V) and indexes such as number and size of lung tumors, number of mice in which oncogenesis was observed (Hayashi et al.1998). The authors suggested that it is difficult to judge the presence or absence of carcinogenicity of DMA (V) in mice only from this study since AJ mice are the strain in which lung tumor is very easily developed, and that studies in other strains of mouse using more number of animals are required to judge it.

There is another report of a feeding study of DMA(V) in B6C3F1 mice, where 0, 2, 10, 40 and 100 ppm of DMA(V) in feed was administered to 56 male and female mice of 5 weeks-old at the initiation for 104 weeks. Neither preneoplasia in the bladder which is considered to be associated with DMA (V) administration nor oncogenesis was observed in both males and females in this study (Arnold et al. 2006).

(b) Rats

Carcinogenicity of DMA (V) in rats was studied with administration of 0, 12.5, 50 and 200 ppm of DMA (V) in drinking-water for 104 weeks, using 36 male F344 rats of 10 weeks old for each dose. In the 50 ppm administered group, bladder cancer was developed in 19% of the animals and tumors including papilloma were developed in 26% of the group. In the 200 ppm administered group, bladder cancer was developed in 39% of the animals (among them, 2 cases were accompanied with papilloma), and no development of tumors was observed in the 12.5 ppm administered group and in the control group. Carcinogenicity was observed only in bladder in this study (Wei et al. 1999; Wei et al. 2002).

A feeding study of DMA (V) with the dose of 0, 2, 10, 40 and 100 ppm for 104 weeks was conducted in male F344 rats of 5 weeks old at the initiation, using 60 in each dose group. In

the bladder of male rats, papilloma was observed in 1 case each in the 10 ppm and in the 40 ppm dose groups, and bladder cancer was observed in 1 case in the 2 ppm dose group and 2 cases in the 10 ppm dose group. In female rats, 4 cases of papilloma and 6 cases of bladder cancer were observed in the 100 ppm dose group. No tumor development was observed both in the males and females of the control groups. Carcinogenicity was observed only in the bladder (Arnold et al. 2006).

b. MMA (V)

No carcinogenicity was observed in the administration study of MMA (V) in experimental animals.

(a) Mice

A feeding study of MMA (V) with the dose of 0, 10, 50, 200 and 400 ppm for 104 weeks in B6C3F1 mice of 6 week-old at the initiation was conducted using 52 male and female mice in each dose group. Significant oncogenesis associated with the MMA (V) administration was not observed, and it was reported that although a decrease in body weight was observed in the 400 pm group, the survival rate was not changed significantly (Arnold et al. 2003).

(b) Rat

Effects of MMA (V) administered in drinking-water at the dose of 0, 50 and 200 ppm for 2 years were examined in F344 rats of 10 weeks old at initiation, using 42-45 males for each dose. No significant increase associated with MMA (V) administration was observed in any index of body weight, food intake, water intake, and survival rate. Although autopsy revealed oncogenesis in the liver or in the bladder in all dose groups including the control group, these were histologically similar to the tumors which spontaneously formed in F344 rats. These results indicated that significant oncogenesis was not caused by 2-year administration of MMA (V) in the liver or in other organs (Shen et al. 2003a).

In another feeding study conducted in F344 rats of 6 weeks-old at the initiation, MMA (V) was administered with the dose of 0, 50, 400 and 1,300 ppm in male rats for 104 weeks, using 60 rats in each dose group. No significant oncogenesis associated with administration was observed from this study. On the other hand, the maximum dose of 1,300 ppm was changed to 1,000 ppm in week 53 and further to 800 ppm in week 60, because of the rapid increase in the mortality (Arnold et al. 2003).

c. TMAO

In a study conducted in F344 rats of 10 weeks-old at the initiation, TMAO was administered in drinking-water with the dose of 0, 50 and 200 ppm in male rats for 2 years, using 42-45 rats in each dose group. The incidence rate and the number of incidence of hepatic adenoma increased significantly in the 200 ppm dose group compared to the control group. Although oncogenesis was also observed in other organs, these tumors were observed even in the control group and were histologically similar to tumors spontaneously developed in F344 rats (Shen et al. 2003b).

(iv) Carcinogenicity of simultaneous exposure

a. DMA(V)

(a) Mice

Tumor promoting activity of DMA (V) was examined in mice that were administered with 4-nitroquinoline 1-oxide, a tumor initiator, then administered with DMA (V) in drinking-water at the dose of 0, 200 and 400 ppm for 25 weeks in ddY mice of 6 weeks-old at the initiation using 9-13 male mice in each dose group. Prior to the DMA (V) administration, animals were administered with 4-nitroquinoline 1-oxide of 10mg/kg body weight subcutaneously. The incidence rate of lung tumors in the DMA (V) dose group tended to increase compared to the control group, and the incidence number of lung tumors significantly increased in the 400 ppm dose group (Yamanaka et al. 1996).

In another study, 200 ppm of DMA (V) mixed in cream was applied to K6/ODC transgenic mice of 10-14 weeks-old twice a week for 18 weeks prior to which the mice were administered with a tumor initiator DMBA, using 7-8 male mice in each administration group. The number of skin tumors in the mice which had DMA(V) application after DMBA was two times higher than that in the mice which had only DMBA pretreatment, indicating that DMA(V) had skin tumors promoting activity similar to that of TPA. Although skin tumors were not developed in the animals that were not treated with the initiator DMBA regardless of the application of DMA(V) (Morikawa et al. 2000), it was considered to be difficult to interpret appropriately since the study was conducted in only 2 DMBA non-treated animals (IARC 2012).

(b) Rats

A medium-term multi-organ carcinogenicity of DMA (V) was studied in F344 rats. Prior to the administration of DMA(V), F344male rats of 7 weeks-old at the initiation were administered with 5 different carcinogens such as diethylnitrosamin (DEN), methylnitrosourea (MNU), N-Butyl-N-butan-4-ol-nitrosamine (BBN), 1,2-dimethylhydrazine(DMH), N-bis (2-hyroxypropyl)nitrosamine (DHPN) (DMBDD for short) for 4 weeks followed by 1 week of

washout period. After this washout period, DMA (V) was administered in drinking-water with the dose of 0, 50, 100, 200 and 400 ppm for 25 weeks using 20 rats in each dose group. The carcinogenesis in the bladder was enhanced by DMA (V) at 50 ppm and above that in the liver and the kidneys was enhanced by DMA (V) of 200 ppm and above. Furthermore, tumor promoting activity of DMA (V) in the thyroid gland was observed from 400 pm. In contrast, carcinogenesis was not observed even after 25 weeks of DMA (V) administration in rats which were not pretreated with DMBDD (Yamamoto et al. 1995).

Tumor promoting activity of DMA (V) to carcinogenesis by BBN, a carcinogen of bladder cancer, was examined in F344 rats (Wanibuchi et al.1996). After 4 weeks of treatment with BBN, DMA(V) in drinking-water was administered with the dose of 0, 2, 10, 20, 50 and 100 ppm for 32 weeks in F344 male rats of 6 weeks-old at the initiation using 20 rats for each dose.,. As a result, incidence of bladder tumors was significantly increased already with 10 ppm DMA(V),and tumor promoting activity of DMA(V) in the bladder at the dose of and above 25 ppm was significantly different from that in control animals.

As a comparative study, the effects of an administration of 0, 10, 25 and 100 ppm of DMA (V) in drinking-water for 8 weeks were studied without the initiation with BBN in rats of 6 weeks-old at the initiation. The cell proliferation in bladder epithelium increased dose-dependently by DMA (V). The authors suggested that DMA (V) had tumor promoting activity in the bladder of rats, and that the stimulation of cell proliferation in bladder epithelium was one of the mechanisms.

b. DMA(V), MMA(V), TMAO

Effects of DMA(V), MMA(V) and TMAO on the initiation activity of DEN was studied in male F344 rats of 6 weeks old at the initiation of the study. The animals were administered with 0 and 100 ppm of DMA (V), MMA (V) and TMAO in drinking-water after 2 weeks of washout following the initiation treatment with DEN. The number and area of GST-P positive foci in the liver increased significantly in MMA and TMAO administered animals compared to the control animals, and it was revealed that that MMA(V) and TMAO accelerated the development of precancerous lesions in the liver of rats (Nishikawa et al. 2002).

(v) Neurotoxicity

Organic arsenic compounds in foods including AsBe and AsC have been considered not to relate to peripheral or central neurotoxicity (EFSA 2009). The administration of MMA(V) with the dose of 72.4 mg/kg bw/day to rats or MMA(V) with the dose of 67.1 mg/kg bw/day to mice have been reported not to cause any clinical symptoms or brain lesions (Arnold et al. 2003). Similarly, the chronic administration of DMA (V) with the dose of 7.8 or 94 mg/kg

bw/day has been reported not to cause any clinical symptoms or histologic changes (Arnold et al. 2006; ATSDR 2007; EFSA 2006).

Kuger et al. (2009) investigated the effects of MMA (V) and MMA (III) on the synaptic function in hippocampus, where the field excitatory postsynaptic potential (fEPSPs) in Schaffer collateral in the slice preparation of hippocampus from juvenile (14-21 days-old) and adult (2-4 months-old) rats were recorded. The slice preparations were treated with MMA (V) or MMA (III) prior to the recording of fEPSPs. The synaptic function in the slice preparation from neither adult nor juvenile rats was affected by MMA (V). However, MMA (III) at the dose of 50 and 25 μmol/L strongly suppressed the synaptic potential in the slice preparation from both adult and juvenile rats. MMA (III) also strongly suppressed amplitude of long-term potentiation (LTP) at the dose of 25 μmol/L and 10 μmol/L, both in the slice preparation from adult and juvenile rats. On the contrary, treatment with 1 μmol/L MMA (III) enhanced the LTP amplitude in the slice from juvenile rats. This effect was attributed to the enhancement of *N*-Methyl-D-Aspartic Acid (NMDA) receptor and lack of an antagonistic effect of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid**.** The authors suggested that these impairments of excitatory synapse in CA1 region of hippocampus was caused by the activity of MMA(III) to postsynaptic glutamate receptor and may be a cause of cognitive impairment by arsenic (Kruger et al. 2009; EFSA 2009).

(vi) Immunotoxicity

Effects of oral administration of organic arsenic compounds on the immune system or lymphoid organs associated with immune function have not been observed.

Exposure of rats and mice to MMA(V) of 72.4, 67.1 mg/kg bw/day (Arnold et al. 2003) and to DMA(V) of 7.8, 94 mg/kg bw/day or (Arnold et al. 2006) were found to cause no histologic changes in the immune system. Oral administration of MMA (V) in Finch chicks with the dose of 4-72 mg/kg bw/day for 20 days has been reported to have no effect on immune function (Albert et al. 2008).

(vii) Reproductive and developmental toxicity

According to EFSA (2009), reproductive toxicity of AsBe has not been observed, and there is little information on early toxicity of MMA (V) and DMA (V) (EFSA 2009).

A study on reproductive and developmental toxicity of MMA(V), DMA(V) that were orally administered in Sprague-Dawley rats and New Zealand White Rabbits reported that developmental toxicity associated with the administration was not observed with the exposure dose which did not cause maternal toxicity. With the repeated oral administration of MMA(V) 0, 10, 100 and 500 mg/kg bw/day in rats, and 0, 1, 3, 7 and 12 mg/kg bw/day in rabbits, and the repeated oral administration of DMA(V) 0, 4, 12 and 36 mg/kg bw/day in

rats and, 0, 3, 12 and 48 mg/kg bw/day in rabbits, during the organogenesis period (gestation day (GD) 6-15 for rats, GD7-19 days for rabbits), maternal toxicity and fetal toxicity were observed with the highest dose which was 500 mg/kg bw/day for rats and 12 mg/kg bw/day for rabbits, but developmental toxicity associated with the administration was not observed in lower doses. Teratogenicity was not observed with MMA (V) administration. With DMA (V) administration, maternal toxicity and developmental toxicity were observed with the dose of 36 mg/kg bw/day in rats. In rabbits, the DMA (V) administration of 48 mg/kg bw/day resulted spontaneous abortion in most mother rabbits and evident maternal toxicity where no live fetus for assessment existed. The dose which did not cause maternal toxicity or developmental toxicity associated with the administration was 12 mg/kg bw/day and less in rats and rabbits (Irvine et al. 2006; EFSA 2009).

(viii) Developmental neurotoxicity

In mice, similar to inorganic arsenic, DMA (V) is transferred from mother's body to fetus through the placenta, easily passes through the immature blood-brain barrier and is distributed widely in the fetus brain (Jin et al. 2006).

**4. Effect of synthetic organic arsenic compounds**

Based on ATSDR (2007) and EFSA (2009), we have summarized the toxicological data of man-made organic arsenic compounds, but it was very limited.

(1) Effect on humans

(i) Acute effect

According to ATSDR (2007), several cases of acute poisoning from organic arsenic compounds caused by the administration of pesticide have been reported. Vomiting, shock disease, liver and kidney failure were observed in a male who received 1,714 mg MSMA/kg body weight of organic arsenic compounds (Shum et al. 1995), and vomiting, abdominal pain, and diarrhea were observed in the patients who received organic arsenic compounds 78 mg DMA/kg body weight (Lee et al. 1995).

(ii) Chronic effect

Organic arsenic compounds have been used as therapeutic agents. Arsphenamine (3,3'-diamino-4,4'-dihydroxy-arsenobenzene) used to be the therapeutic drug for syphilis, and currently melarsoprol ((2-(4-(4,6-diamino-1,3,5-triazin-2-ylamino)phenyl)-1,3,2-dithiarsolan-4-yl) methanol) has been used for the treatment of human African trypanosomiasis (sleeping sickness) in the overseas countries of disease outbreak. Although it has been

known that acute central neurotoxicity were caused by these compounds, a few cases of peripheral neuropathy in the patients with syphilis or with trypanosomiasis were reported (Gherardi et al. 1990). For example, in a few syphilis patients who were treated with arsphenamine, the acute symptom (hemorrhagic encephalitis, brain purpura) called arsphenamine encephalitis which was characterized by brain hemorrhage is known to occur with the common symptoms such as paralysis, attack, vomiting, headache, fever and delirium. It has been reported that attack was observed in an examination of brain, and that hypostasis of blood vessels, necrosis, demyelinating in meninges, moderate to severe tigrolysis of neurons were observed in some patients (Roseman and Aring 1941; Globus and Ginsburg 1933). It has been reported that treatment with melarsoprol developed severe reactive arsenic encephalopathy, and may develop acute nonfatal mental disorder without evident neurological signs, rapidly progressing coma without seizure, or duplicate attack of seizure associated with acute brain swelling (Haller et al. 1986; Adams et al. 1986; EFSA 2009).

Contamination of well water with DPAA and DPAA poisoning with major symptoms of the cerebellum due to the drinking of the contaminated well water occurred in Kamisu town (currently Kamisu city), Ibaraki, in 2003 (Ishii et al. 2004). Highly-concentrated arsenic of 4,500 μg As/L was detected in the well water for drinking and DPAA was detected in urine by HPLC-ICP-MS analysis. Although the early symptoms such as staggering was observed and was considered to be the health effect of DPAA, no relationship between symptom development and exposure was found. However, the toxicity was estimated to be observed at the concentration of 1,100 μg As/L (140-2,400 μg As/L) from time series analysis of the data. In addition, it is reported that no DPAA was detected in urine of the non-exposed subjects (Nakajima et al 2006).

(2) Effect on experimental animals

(i) Acute Toxicity

ATSDR (2007) reported that oral $LD_{50}$ of roxarsone as 244 mg/kg body weight in mice (NTP 1989), 81 mg /kg body weight (NTP 1989) and 155 mg /kg body weight (Kerr et al. 1963) in rats. Also, oral $LD_{50}$ of MSMA which is an organic arsenic pesticide to be 2,449-3,184 mg MSMA/kg body weight in rats (Gur and Nyska 1990) and 102 mg MSMA/kg body weight in rabbits (Jaghabir et al. 1988).

(ii) Repeated-dose toxicity

a. Subacute toxicity

(a) Fourteen-day subacute toxicity study in mice.

A 14-day feeding study of roxarsone, with the dose of 0, 60, 120, 250, 500 and 1,000 ppm, was conducted in B6C3F1 mice using 5 male and female for each dose.

Two out of 5 male mice and 5 out of 5 female mice in 1,000 ppm dose group died. The final body weight decreased 34% in males of the 1,000 ppm group and 10% in females of the 500 ppm group. Also, significant decrease in activity was observed in the mice dosed with 250 ppm and over (NTP 1989).

ATSDR (2007) determined the NOAEL for body weight decrease and NOAEL for decrease in activity in this study as 84 mg/kg bw/day (500 ppm) and 20 mg/kg bw/day (120 ppm), respectively.

(b) Thirteen-week subacute toxicity study in mice

A 13-week feeding study of roxarsone with the dose of 0, 50, 100, 200, 400 and 800 ppm was conducted in B6C3F1 mice using 10 male and female mice each for each dose.

Six out of 10 male mice and 8 out of 10 female mice in the 800 ppm dose group, and 1 male and 1 female mouse out of 10 mice each in the 400 ppm group died. In the 800 ppm dose group, the final body weight decreased 18% in male mice and 11% in female mice compared to the control group. Decrease in absolute liver weight was observed in male mice with the dose of 800 ppm (NTP 1989).

Subsequently, the authors conducted another 13-week feeding study of roxarsone in B6C3F1 mice where 30 male and 30 female mice were fed with each of 0, 100 and 400 ppm of roxarsone. However, no effect of the roxarsone administration was observed (NTP 1989).

(c) Fourteen-days subacute toxicity study in rats

Subacute toxicity of roxarsone at the dose of 0, 100, 200, 400, 800 and 1600 ppm was examined with a 14-day feeding study in F344 rats where 5 males and 5 females were fed with each dose of roxarsone.

Three out of 5 male mice and 5 out of 5 female mice died in the 1,600 ppm dose group. The final body weight decreased 22% in males and 5% in females in the 400 ppm dose group compared to the control group. The decrease of food intake was observed in male mice with the dose of 1,600 ppm and in female mice with the dose of 800 ppm. Also, mild hypoactivity was observed with the dose of 400 ppm or above (NTP 1989).

(e) Thirteen-week subacute toxicity study in rats

Subacute toxicity of roxarsone was examined in a 13-week feeding study in Holtzman rats where 6 male and female mice each were fed with each of 0, 25, 50, 100, 200 and 400 ppm of roxarsone.

Ten out of 12 male rats and 10 out of 10 female rats in the 400 ppm dose group died (Kerr et al. 1963; ATSDR 2007).

(e) Thirteen-week subacute toxicity study in rats

A 13-week subacute toxicity study of roxarsone was also conducted in F344 rats administrating each of 0, 50, 100, 200, 400 and 800 ppm of roxarsone in feeds in 10 males and females each.

Three out of 10 male rats and 2 out of 10 female rats in the 800 ppm dose group died. The final body weight decreased in the 200 ppm, 400 ppm, and 800 ppm dose group by 14%, 26%, and 50% in male rats and by 8%, 11%, and 33% in female rats, respectively. Relative weight of the liver was increased with the dose of 50, 100, 200 and 400 ppm in males and with the dose of 800 ppm and over in females (NTP 1989).

Subsequently, the authors conducted another 13-week feeding study of roxarsone with the dose of 0, 100 and 400 ppm in F344 rats where 30 males and females each were fed with each dose of roxarsone.

The decrease in an absolute weight of the liver and increase in a relative weight of the liver were observed in female rats of the 400 ppm dose group. The decrease in an absolute weight of the liver was observed in the males with the dose of 100 ppm and over. An increase in a relative weight of the kidney, mild renal toxicity such as mild degeneration of tubular epithelial cell, tubular cast and focal calcification was observed in males in the 400 ppm dose group. No effect associated with the administration was observed in female rats (NTP 1989).

ATSDR determined the NOAEL for the decrease in body weight in this study as 8 mg/kg bw/day (100 ppm), NOAEL for the effect on the liver as 4 mg/kg bw/day (50 ppm), and NOAEL for the effect on the kidneys as 16 mg/kg bw/day (200 ppm) (ATSDR 2007).

b. Chronic toxicity

(a) Two-year chronic toxicity study in mice

A 2-year feeding study of roxarsone was conducted in B6C3F1 mice where 50 male and female mice each were fed with the dose of 0, 100, 200 ppm; that is 0, 21, 43 mg/kg bw/day for males, and 0, 27, 54 mg/kg bw/day for females. No effect of the administration was observed on the respiratory system, cardiovascular system, digestive system, blood, musculoskeletal, liver, kidneys, skin, eyes, body weight, immune system, reproduction and development, and carcinogenicity (NTP 1989; ATSDR 2007).

ATSDR determined the NOAEL as 43 mg/kg bw/day in this study (ATSDR 2007).

(b) Two-year chronic toxicity study in rats

A 2-year feeding study of roxarsone was conducted in F344 rats, where 50 males and 50 females were fed with each dose of 0, 50 and 100 ppm; that is 0, 2.1 and 4 mg/kg bw/day.

The frequency of development of pancreas adenoma tended to increase in male rats in the 100 ppm dose group.

No effect of the administration on respiratory system, cardiovascular system, digestive system, blood, musculoskeletal, liver, kidneys, skin, eyes, body weight, immune system, reproduction and development, and carcinogenicity was observed (NTP 1989; ATSDR 2007).

ATSDR determined the NOAEL as 4 mg/kg bw/day in this study (ATSDR 2007).

(iii) Carcinogenicity

It was reported that no clear evidence for carcinogenicity of roxarsone in mice or rats () was observed in a 2-year feeding study conducted by NTP (1989) where the dose of 100 and 200 ppm that is 21 and 43 mg/kg bw/day for male mice, 27 and 54 mg/kg bw/day for female mice, and the dose of 50 and 100 ppm; that is 2.1 and 4 mg/kg bw/day were administered (NTP 1989; ATSDR 2007; EFSA 2009).

(iv) Neurotoxicity

ATSDR (2007) reported that pigs were the most sensitive to neurotoxicity of roxarsone in the animal studies as the effect on nervous system was observed with the oral administration of 6.3 mg/kg bw/day for 30 days. (Rice et al. 1985; Edmonds and Baker 1986; Kennedy et al. 1986; ATSDR 2007; EFSA 2009).

(v) Immunotoxicity

According to ATSDR (2007), no effect of oral administration of organic arsenic compounds on the immune system and on the lymphoid organ associated with immune function has been observed. In a 2-year chronic toxicity study in mice and rats conducted by NTP (1989), exposure of mice to roxarsone 200 ppm that is 43 mg/kg bw/day and of rats to 100 ppm that is 4 mg/kg bw/day caused no histological changes in the immune organ (NTP 1989; ATSDR 2007).

(vi) Reproductive and developmental toxicity

There was no report about an animal study regarding reproductive and developmental toxicity associated with oral administration of synthetized organic arsenic compounds.

## 5. Genotoxicity

(1) Effect on humans

(i) Gene mutation

Ostrosky-Wegman et al. (1991) investigated gene damage caused by arsenic exposure in a pilot survey in Mexico, collecting the blood and urine samples from 11 subjects (2 males and 9 females) of high exposure group who had been chronically exposed to arsenic in the well water with arsenic concentration of 390 μg/L (As(V) 98%, As(III) 2%) and from 13 subjects (2 males and 11 females) of low exposure group who were exposed to arsenic the well water with arsenic concentration of 19-60 μg/L. () The mean age of the subjects was 38 years old (range: 21-62 years old) in the high exposure group, and 38 years old (range: 21-58 years old) in the low exposure group, and the mean duration of residence was 37 years (range: 21-55) in the high exposure group and 34 years (range: 5-56 years) in the low exposure group. The frequency of mutation of *HPRT* locus in peripheral blood lymphocyte was higher in the high exposure group than the low exposure group, but with no significant difference (IARC 2004).

As per the data of occupation exposure, Harrington-Brock et al. (1999) investigated the relationship between arsenic exposure and mutagenesis of *HPRT* locus of 15 male workers in a copper refining factory in Chile. The 15 subjects were 24 to 66 years old with the mean working period of 43 months in the relevant factory. Exposure conditions were confirmed by analyzing arsenic level in urine and were classified into 3 groups, i.e., low (mean: 120μg/L), middle (mean: 190 μg/L), and high (mean: 260 μg/L). The *HPRT* mutant frequencies for the three exposure groups were $9 \times 10^{-6}$ in the low exposure group, $11 \times 10^{-6}$ in the moderate exposure group, and $24 \times 10^{-6}$ in the high exposure group. No significant difference was observed in the mutation of *HPRT* locus in peripheral blood lymphocyte by arsenic exposure (IARC 2004).

(ii) Chromosome aberration

a. Chromosome aberration test

Gonsebatt et al. (1997) investigated cytogenetic effects of arsenic exposure in the population of the similar socio-economic status in the same area but with different inorganic arsenic exposure in drinking-water in Mexico. The exposure group (35 subjects) who have been drinking the well water of the mean arsenic concentration of 408 μg/L (range: 396-435 μg/L) was compared to the control group (34 subjects) who have been drinking the well water of the mean arsenic concentration of 29.9 μg/L (range: 7.4-62 μg/L). The mean age was 41 years old (range: 24-68) in the exposure group and 39 years old (range: 22-66) in the control group, and duration of residence was 3-65 in the exposure group and 10-64 years

in the control group. The sex ratio of the exposure group and the control group was equivalent. The subject who was exposed to suspected genotoxicity substance for a professional reason or the subjects who had some kind of treatment were excluded from the study. The chromosome aberration frequency in human peripheral blood lymphocyte in the exposure group was 7.1% (male: 8.9%, female: 5.7%) and was 3.0% (male: 2.8%, female: 3.1%) in the control group, indicating that the frequency was significantly increased by arsenic exposure Since the smoking rate was almost equivalent in exposure group (29%) and in the control group (33%), IARC considered that the smoking effect on the analysis result of the frequency of chromosome aberration was unlikely. In addition, IARC attributed the difference between the effect observed in males and in females of exposure group to the fact that water intake of men was higher than that of women because men in the targeted area worked in the field under the dry weather (IARC 2004).

Mäki-Paakkanen et al. (1998) investigated the relationship between chromosome aberration and arsenic exposure in 42 subjects who were exposed to arsenic contaminated well water in Finland. 32 out of 42 subjects, whose mean age was 52 ranging 15 to 83, had been using the well water contaminated with arsenic for the mean duration of 8 years ranging 1-29 yeas. The median value of arsenic concentration in drinking-water for these 32 subjects (exposure group) was 410 μg/L (range: 17-510 μg/L), and the median value of cumulative arsenic exposure for life-time was calculated to be 455 mg (range: 48-6,869 mg). Meanwhile, the other 10 out of 32 subjects (past exposure group), whose mean age was 46 ranging 17-68, and whose mean duration of exposure was 10 years ranging 5-30 years, had discontinued the use of contaminated well water 2 to 4 months before sampling. The median value of arsenic concentration in drinking-water for these 10 subjects of the past exposure group was 296 μg/L (range: 20-980 μg/L) and the median value of cumulative arsenic exposure concentration for life-time was calculated to be 828 mg (117-5,902 mg). The arsenic concentration in drinking-water of 8 subjects with mean age of 50, ranging 37-76, in the control group who lived in the same area, was less than 1 μg/L. Smoking habit, sex, intake of algae and seafood, and history of residence were used as confounding factors. The mean chromosome aberration frequency in peripheral blood lymphocyte was 6.9 ($p = 0.02$) for the exposure group, 4.2 for the past-exposure group, 8.6 for the control group without the adjustment for confounding factors, and 3.5 ($p = 0.1$) for the exposure group, 1.9 for the past-exposure group, 3.6 for the control group after the adjustment. No effect of smoking on chromosome aberration was observed. However, a significant relationship was observed between arsenic concentration in urine and chromosome aberration by generalized linear model (GLM) regardless of the adjustment for confounding factors (before adjustment: $r^2 = 0.25$, $p = 0.008$, after adjustment: $r^2 = 0.27$, $p = 0.04$). Also, significant relationship was

observed between cumulative arsenic exposure and chromosome aberration (before adjustment: r2 = 0.21, p = 0.008, after adjustment: r2 = 0.24, p = 0.25) (IARC 2004).

Mahata et al. (2003) examined cytogenetic effect by chronic arsenic exposure using chromosome aberration as the endpoint. The study was conducted in 59 subjects (male 37, female 22, mean age: 37 (range: 15-71)) of the exposure group who had skin symptoms from arsenic and 36 subjects of the control group (male 27, female: 9, mean age: 33 (range: 18-60)) who had matched on age and socio-economic status, of in West Bengal, India. The mean arsenic concentration in drinking-water was 212 μg/L (range: 60-580 μg/L) and 6.4 μg/L (3.0-12.6 μg/L) in the exposure group and in the control group, respectively. Also, cigarette smokers were included in the exposure group. The mean duration of arsenic exposure through drinking-water in the exposure group was 15.1 years (range: 3-35). The arsenic concentrations in nails, hairs, and in urine in the exposure group were significantly higher than those in the control group. The chromosome aberration frequency in peripheral blood lymphocyte was significantly higher in the exposure group (8.1%) than in the control group (2.0%). No effect of smoking was observed.

Ghosh et al. (2006) investigated the cytogenetic damage in three groups of subjects, in West Bengal in India, that were a group of 244 subjects with skin symptoms (male: 141, female: 103, smoking rate: 38.9%) who were taking drinking-water containing arsenic with the mean concentration of 242 μg/L, 178 subjects without skin symptoms (male: 77, female: 101, smoking rate: 27.53%) taking drinking-water with mean arsenic concentration of 202 μg/L, and 102 control subjects (male: 51, female: 51, smoking rate: 33.3%) taking drinking-water with mean arsenic concentration of 7.2 μg/L. The mean number of chromosome aberration per cell in peripheral blood lymphocyte for the group with the skin symptoms, for the group without the skin symptoms and for the control group were 0.094, 0.07 and 0.024, respectively. The number of chromosome aberration for the group with skin symptoms and the group without skin symptoms were significantly higher than that for the control group. Ninety-five percent confidence interval (CI) of each difference to the control group were 0.063-0.076 (p = 0.001) and 0.041-0.050 (p = 0.001), respectively.

Chakraborty et al. (2006) conducted a cytogenetic survey in 45 subjects (exposure group) consisted of 12 males and 33 females with mean age of 34 who had been taking drinking-water of mean arsenic concentration of 66.8 μg/L for at least 10 years in the past, and 25 subjects (control group) consisted of 6 males and 19 females with mean age of 34 who were matched on sex and age and who had been taking drinking-water of the mean arsenic concentration of 6.4 μg/L. The socio-economic status in both groups was almost equivalent. The chromosome aberration in oral mucosal epithelial cells was observed in the exposure group with significantly higher rate (mean 4.9%) compared to the rate in the control group (mean 1.3%). The authors concluded that the significant increase of cytogenetic damage

was observed in oral mucosal epithelial cells as well as in peripheral blood lymphocyte by arsenic exposure through drinking-water.

b. Micronucleus test

Warner et al. (1994) investigated the micronucleus frequency in exfoliated bladder cells and in oral mucosal epithelial cells in the population who had chronic arsenic exposure through well water in Nevada, U.S.A. Subjects of the exposure group were 8 males and 10 females, whose mean age was 38 ranging 14-74 years old. The mean arsenic concentration in the drinking-water for the exposure group was 1,310 μg/L (2,260 μg As/day), and the mean duration of residence was 4 years ranging from 1 to 13 years. The control group consisted of 8 males and 10 females with the mean age of 37 years old ranging from 16 to 70 years, who were matched on age, sex, and history of smoking. The mean arsenic concentration in the drinking-water for the control group was 16 μg/L (36 μg As/day), and the mean duration of residence was 5years ranging from 1 to 13 years. The micronucleus frequency per 1,000 bladder cells were in the exposure group (2.79) was 1.8 times higher than that in the control group (1.57) (90%CI: 1.06-2.99, p = 0.09). There was a positive relationship between micronucleus frequency in bladder cells and arsenic concentration in urine. Meanwhile, the micronucleus frequencies per 1,000 oral mucosal epithelial cells were 2.49 in the exposure group and 2.50 in the control group. The ratio of the frequency in the exposure group to that in the control was 1.0 (90%CI: 0.65-1.53, p = 0.5), indicating no increase in the frequency associated with arsenic exposure (IARC 2004, 2012).

Biggs et al. (1997) examined an association of the micronucleus frequency of bladder cells by arsenic exposure with the urinary concentration of arsenic comparing the subjects from the high exposure group and the low exposure group in Chile. The high exposure group consisted of124 subjects that are 70 males and 54 females, with a mean age of 41.1 ranging from 18 to 81 and a smoking rate of 29%. The low exposure group consisted of 108 subjects that are 55 males and 53 females, with a mean age of 41 ranging from 19 to 75 and a smoking rate of 31%. The subjects in the low exposure group were matched on sex, age composition, and smoking habit. Subjects in the high exposure group had been taking drinking-water of the maximum arsenic concentration of 670 μg/L (range: 560-670 μg/L) for the mean duration of residence of 20.0 years, while subjects in the low exposure group had been taking drinking-water of the mean arsenic concentration of 15 μg/L (range: 12-17 μg/L) for 27.7 years (range: 0.2-73). When urinary concentration of arsenic was classified into 5 classes from lower to higher concentration, micronucleus frequency of bladder cells was the lowest in the group of the lowest urinary concentration (Group 1) and increased proportionally to the increase in the urinary concentration up to Group 4 of which urinary concentration of arsenic was 53.9-729 μg/L. However, micronucleus frequency of bladder cells in group 5, where

arsenic concentration in urine was the highest as 729 μg/L and over, was even lower than that in group 1. Although the authors attributed the decrease in micronucleus frequency in the highest exposure group (group 5) to the cytotoxicity or cytostasis, they also suggested that further investigation of its mechanism was desirable.

Gonsebatt et al. (1997) investigated micronucleus in oral mucosal epithelial cells and in urothelial cells in addition to chromosome aberration in peripheral blood lymphocyte in the above mentioned study. The mean micronucleus frequency per 1,000 cells were 2.21 (male: 3.08, female: 1.28) in oral mucosal epithelial cells and 2.22 (male: 4.18, female: 1.24) in the urothelial cells in exposure group. The mean micronucleus frequency in the exposure group was significantly higher than that in the control group where the frequency was 0.56 (male: 0.55, female: 0.57) in oral mucosal epithelial cell and 0.48 (male: 0.58, female: 0.43) in urothelial cells (IARC 2004).

Moore et al. (1997a) conducted a cross-sectional study of arsenic exposure only in the males who were in the targeted population of the above mentioned Biggs's study. Micronucleus in bladder cells was measured in 70 subjects with mean age of 42 (ranging 20-74) from the high exposure group who had been exposed to the drinking-water of mean arsenic concentration of 600 μg/L, and 55 subjects with mean age of 42 (ranging 19-75) in the low exposure group who had been exposed to the drinking-water of mean arsenic concentration of 15 μg/L for a long period of time. Mean duration of residence in the high exposure group and the low exposure group were 19.3 years ranging 0.4-61 years, and 28.3 years ranging 0.2-73 years, respectively. Age, smoking history, duration of residence, education, and race were matched. Micronucleus frequency was analyzed in 104 subjects from the relevant population except those with arsenic exposure of background level or below. As 104 subjects were classified into 5 groups by their urinary arsenic concentration, micronucleus frequency increased from the lowest group, group 1, to group 4 where the urinary arsenic concentration was 54-729 μg/L with a statistically significant dose-response relationship. However, the increase in the frequency was not significant in the highest exposure group, group 5, with urinary arsenic concentration of 729 μg/L and over. Centromere-positive micronucleus increased by 3.1 times (95%CI: 1.4-6.6) in group 4 and centromere-negative micronucleus increased by 7.5 times in group 3 (95%CI: 2.8-20.3), suggesting that the micronucleus was mainly attributable to chromosome breakage (IARC 2004).

Moreover, Moore et al. (1997b) conducted an intervention survey changing arsenic concentration in drinking-water of 600 μg/L to 45 μg/L in 34 targeted subjects of the survey. The micronucleus frequency in exfoliated bladder cells in all subjects decreased after 8 weeks. Micronucleus frequency per 1,000 cells was 2.63 before the intervention and 1.79 after the intervention. In terms of the smoking habit of the population, the micronucleus frequency in smokers was 4.55 before the intervention and decreased to 1.44 after the intervention, but no

change was observed in non-smokers where the frequency was 2.05 before intervention and 1.90 after intervention. The authors suggested from these data that the bladder cells of smokers are more sensitive to genotoxic damage by arsenic than the bladder cells of non-smokers (IARC 2004).

In a pilot survey conducted by Tian et al. (2001) in Inner Mongolia Autonomous Region in China, micronucleus frequency in various epithelial cells of people who had chronic arsenic exposure through drinking-water was investigated. The people were classified into two groups, one of which was the high exposure group of 19 subjects (male: 14, female: 5, mean age: 38) who drank drinking-water with arsenic concentration of 528 μg/L for over the mean 17 years. The other was the low exposure group of 13 subjects (male: 8, female: 5, mean age: 38) who drank drinking-water with arsenic concentration of 4.4 μg/L and being considered on smoking habit, occupation, diet, demographic factors, age, and health condition. In comparison with the low exposure group, micronucleus frequency in oral mucosal epithelial cells and cells in sputum collected from respiratory epithelium was 3.4 times higher in the high exposure group. The micronucleus in bladder urothelial cells were increased by 2.7 times in the high exposure group and by 2.4 times in non-smokers. When the smokers were excluded from the subjects, the effect of arsenic exposure increased in oral mucosal epithelial cells and in sputum and micronucleus frequency increased by 6 times (IARC 2004).

Basu et al. (2002) investigated the micronucleus formation in oral mucosal epithelial cells, urothelial cells and in peripheral blood lymphocyte by arsenic exposure through drinking-water in 45 subjects of 30 males and 15 females with mean age of 30 (ranging15-58) who had a skin disease associated with arsenic, and in 21 control subjects of 17 males and 4 females with mean age of 35 (range: 19-60) who lived in the 2 areas without arsenic contamination in West Bengal, India. The mean arsenic concentration in drinking-water of the exposure group was 368 μg/L (range: 15-800 μg/L) and that of the control group was 5.5 μg/L (range: 3-12 μg/L). The age composition and socio-economic status of the two groups were equivalent. The mean duration of arsenic exposure through drinking-water of the exposure group was 11.3 years (range: 2-22). The mean micronucleus frequency per 1,000 cells in the exposure group was 6.39 in peripheral blood lymphocyte, 5.15 in oral mucosal epithelial cells, and 5.74 in urothelial cells. While the mean frequency in the control group was 0.53 in peripheral blood lymphocytes, 0.77 in oral mucosal epithelial cells, and 0.56 in urothelial cells. Thus, the micronucleus frequency per 1,000 cells was significantly higher in the exposure group than that in the control group. In this report, the data were not adjusted for smoking habit (IARC 2004).

In addition, Basu et al. (2004) conducted the same type of survey as that mentioned above in 163 subjects (male: 86, female: 77, mean age: 35 (15-65)) in the exposure group who had been taking drinking-water with arsenic concentration of 214.7 μg/L and in 154 subjects

(male: 88, female: 66, mean age: 34 (15-60)) in the control group who had been taking drinking-water with the arsenic concentration of 9.2 μg/L. The age composition and socio-economic status of the exposure group and the control group were equivalent. The mean micronucleus frequency per 1,000 cells in the exposure group was 9.34 in peripheral blood lymphocyte, 5.94 in oral mucosal epithelial cells, and 6.65 in urothelial cells. While, the mean frequency in the control group was 1.66 in peripheral blood lymphocytes, 1.28 in oral mucosal epithelial cells, and 1.41 in urothelial cells. Thus, the micronucleus frequency per 1,000 cells was significantly higher in the exposure group than that in the control group. Martínez et al. (2004) investigated a potential of arsenic exposure through ground water to induce a remarkable increase of micronucleus frequency in peripheral blood lymphocyte in Chile. Arsenic concentration in drinking-water in the exposure group was 750 μg/L and 0.2 μg/L in the control group. The survey was conducted in 106 subjects of the exposure group which consisted of 24 males and 82 females with mean age: 40 and smoking rate: 19%, and in 111 subjects of the control group that consisted of 42 males and 69 females with mean age: 38, smoking rate: 32%. The mean micronucleus frequency in peripheral blood lymphocyte in the exposure group was 14.44 which was higher than 11.96 in the control group but it was statistically insignificant.

Moreover, Martínez et al. (2005) investigated micronucleus frequency in oral mucosal epithelial cells in 105 subjects in the exposure group (male: 24, female: 81, mean age: 40) and 102 subjects in the control group (male: 40, female: 62, mean age: 37.28) who lived in the same area. The mean micronucleus frequency per 1,000 cells in the exposure group and in the control group was 3.14 and 2.74, respectively. The mean micronucleus frequency per 1,000 cells was higher in the exposure group than in the control group, but the difference was not statistically significant.

The above mentioned Chakraborty et al. (2006) investigated the micronucleus in addition to chromosome aberration in oral mucosal epithelial cells. The frequency of micronucleus formation in oral mucosal epithelial cells was 1.0% in the exposure group and 0.3% in the control group of 0.3%.The frequency of micronucleus formation in the exposure group was significantly higher than that in the control group.(Chakraborty et al. 2006).

Above-mentioned Ghosh et al. (2006) investigated micronucleus in peripheral blood lymphocyte, oral mucosa and urothelial cell in addition to chromosome aberration in peripheral blood lymphocyte. The mean micronucleus frequency per 1,000 cells was 9.13, 5.62, and 6.01 in peripheral blood lymphocyte, oral mucosal epithelial cells, and in urothelial cell, respectively in the group with skin symptoms, and 6.30, 3.56, and 4.18, respectively in the group without skin symptoms. In the control group, the mean micronucleus frequency per 1,000 cells was 2.03, 1.67, and 1.70, in peripheral blood lymphocyte, oral mucosal epithelial

cells, and urothelial cells, respectively. A ninety-five percent confidence interval for the difference between the group with skin symptoms and the control group was 6.65-7.55 ($p < 0.000$), 3.50-4.40 ($p < 0.000$), 3.85-4.33 ($p < 0.000$) for peripheral blood lymphocyte, oral mucosal epithelial cells, and for urothelial cell, respectively, and 95%CI for the difference between the group without skin symptoms and the control group was 3.79-4.74 ($p = 0.001$), 1.53-2.25 ($p = 0.001$), 2.15-2.78 ($p = 0.001$), respectively. These CI in the exposure groups were significantly higher than that in control group.

Although it was the data on occupation exposure, Vuyyuri et al. (2006) investigated genotoxic damage in oral mucosal epithelial cells in 200 workers of arsenic-based glass manufacture (male: 144, female: 56, smoking rate: 60%, mean duration of exposure: 12.3 years) and 165 subjects in the control group (male: 107, female: 58, smoking rate: 62%) in South India. The mean arsenic concentrations in the blood were 56.8 μg/L and 11.7 μg/L in the exposure group and in the control group, respectively. Micronucleus frequency in oral mucosal epithelial cells in the exposure group was 1.52% while that in the control group was 0.21%. Also, micronucleus frequency was higher in smokers than in non-smokers both in the exposure group and in the control group (exposure group: smokers 1.51, nonsmokers 0.32; control group: smokers 0.25, nonsmokers 0.12), suggesting a significant effect of smoking.

(iii) Sister chromatid exchange (SCE)

Lerda (1994) investigated SCE in human peripheral blood lymphocyte in the population who had been exposed to arsenic through drinking-water for at least 20 years in Argentina. The mean arsenic concentration in drinking-water for 282 subjects, the exposure group, with mean age of 57 (ranging 27-82) was 130 μg/L (ranging 10-660 μg/L), and that for 155 subjects with mean age of 39 (ranging 29-51) in the control group was 20 μg/L (ranging 0-70 μg/L). The estimated exposure to genotoxic substances other than arsenic was also considered. SCE frequency per cell in peripheral blood lymphocyte was 10.46 (7.23-14.90) in the exposure group and 7.49 (5.17-10.87) in the control group. The SCE frequency in the exposure group was significantly higher than that in the control group ($p < 0.001$). When the subjects aged 50 years and over in the exposure group were excluded from the survey to homogenize the age in the exposure group and control group, no significant correlation was found between SCE and sex, and between SCE and age in the population under 50. Moreover, arsenic concentration in drinking-water was associated with SCE both in males and females, and no significant difference was observed between sexes. The details of the statistic evaluation were not reported in this document and assay of arsenic in urine was a colorimetric analysis with low sensitivity. Therefore, the working group in IARC considered the value of this study limited (IARC 2004).

Above-mentioned Mahata et al. (2003) investigated SCE along with chromosome aberration. SCE frequency per cell in peripheral blood lymphocyte was 7.26 in the exposure group and was 5.95 in the control group, indicating that arsenic exposure increased SCE frequency.

(iv) DNA damage

Although it was the data on occupation exposure, the above-mentioned Vuyyuri et al. (2006) investigated genotoxic damage in leukocyte along with micronucleus in oral mucosal epithelial cells. Comet assay of DNA damage showed that the tail length detected with DNA of the exposure group was significantly longer than that of the control group, as were 14.95 μm in the exposure group and 8.29 μm in the control group. No significant difference of DNA damage was observed between males and females in either group, while significant increase of DNA damage was observed in elderly population and in smoker group.

(v) Summary of genotoxicity studied in epidemiological research

Only a few surveys of gene mutation induced by arsenic are available in epidemiological studies, and no significant increase in frequency of gene mutation has been observed in these surveys. However, statistical insignificancy of the observed increase in the gene mutation frequency could be due to the very small number of targeted subjects having huge individual variance in each survey. Therefore, further reports will be necessary for conclusion. Regarding chromosome aberration and SCE, although some negative data have been reported, significantly positive relationship between arsenic exposure and frequency of gene mutation in urothelial cells, in oral mucosal epithelial cells and in peripheral blood lymphocyte cells have been suggested from comparison of the arsenic exposure level in drinking-water in many reports. Moreover, dose-response relationship with arsenic concentration in drinking-water has been observed in micronucleus frequency in human urothelial cells, oral mucosal epithelial cells, in peripheral blood lymphocytes. It was also observed between chromosome aberration in human peripheral blood lymphocytes and arsenic concentration in drinking-water, and between SCE in human peripheral blood lymphocytes and arsenic concentration in drinking-water. Some reports suggest that the sensitivity of arsenic exposure is modified by smoking, whereas some reports contradict this suggestion.

**(2) Effect on experimental animals**

(i) *In vitro* studies (including human cells)

Table 10 shows the result of *in vitro* studies of arsenic compounds.

a. Gene mutation

(a) Inorganic arsenic compounds

○ As(III)

Data on the effects of sodium arsenite were negative in a reverse mutation test (each test was with the dose of 1,873,000 μg/L and 144 μg As/plate) using *Escherichia coli* and *Salmonella typhimurium* and in a gene mutation test (dose unknown) using yeast (*Saccharomyces cerevisiae)* (Rossman et al. 1980, Singh 1983, Kligerman et al. 2003). As per a study using cultured mammalian cell, sodium arsenite was all negative in the following gene mutation studies: A study ,) in Syrian hamster embryo cells using ouabain resistant (with the dose of 750μg As/L) or using 6-thioguanine tolerance (with the dose of 750μg As/L ) as an index; a study (with the dose of 749 μg As/L) in Chinese hamster ovary (CHO) cells using 6-thioguanine tolerance as an index; a study in CHO cells using ouabain resistant as an index (with the dose of 375 and 750μg As/L), or using 6-thioguanine resistance as an index (with the dose of 7,492μg As/L) (Rossman et al. 1980, Lee et al. 1985a, b, Yang et al. 1992). Meanwhile, sodium arsenite was positive in a study with the dose of 3,746 μg As/L at which cell viability significantly decreased for CO-AS52 cells (Meng and Hsie 1996). Also, sodium arsenite was positive in a study (with 577, 865 μg As/L) in mice lymphoma cells (L5178Y/TK$^{+/-}$). Sodium arsenite was also positive in a gene mutation study (577μg As/L) in Human-Hamster Hybrid Cells (S1), although it was not a standard study (Oberly et al. 1996, Moore et al. 1997, Hei et al. 1998).

○ As(V)

Disodium arsenate was negative in a reverse mutation test (1,200 μg As/plate) using *S. typhimurium* and in a gene mutation study (7,492 μg As/L for both indexes) in Syrian hamster embryo cells which used ouabain resistant or 6-thioguanine resistance as an index (Lee et al. 1985a, Kligerman et al. 2003). However, it was positive in a study (4,571 μg As/L) in mice lymphoma cells (L5178Y/TK$^{+/-}$) (Moore et al. 1997).

(b) Organic arsenic compounds

Organic arsenic compounds such as MMA(III) (7.07 μg As/plate), DMA(III) (161 μg As/plate), MMA(V) (1,156 μg As/plate), and DMA(V) (1,170 μg As/plate) were all negative in a reverse mutation test using *S. typhimurium,* but DMA(V) was positive in a reverse mutation test using *E. coli* at the dose of 749,200 μg As/L even though it was highly concentrated (Yamanaka et al. 1989b, Kligerman et al. 2003). Yamanaka et al. (1989b) attributed mutagenicity of DMA(V) in *E. coli* to the involvement of a reaction product from dimethylarsine, a metabolite of DMA(V), and oxygen molecules.

In a gene mutation test using mouse lymphoma cells (L5178Y/TK$^{+/-}$), MMA(III), DMA(III), MMA(V) and DMA(V) were positive at the concentration of 21, 96.6,

1,156,530 and 2,341,543 μg As/L, respectively, with which the cell viability was relatively low (Kligerman et al. 2003, Moore et al. 1997c). In CHO derived cells (G12), MMA(III) induced mutagenesis of *gpt* locus at 45μg As/L and over with which cell viability was low (survival rate: 43%) (Klein et al. 2007).

(c) Synthetized organic arsenic compounds

Roxarsone was negative in a gene mutation test (2,848 μg As/plate) in *S. typhimurium*, but was positive in mouse lymphoma cells (L5178Y/TK$^{+/-}$) (284,824 μg As/L) (NTP 1989b).

(d) Summary of gene mutation

Arsenic compounds are considered to be negative in a mutation test using bacteria. Although some arsenic compounds were positive in mutation tests using cultured animal cells with a relatively high concentration that resulted a low cell viability, most of them were positive in a test using mouse lymphoma cells (L5178Y/TK$^{+/-}$). Even though arsenic compounds may induce large deletion mutation, the potential to induce point mutation is considered to be low.

b. Chromosome Aberration

(a) Chromosome aberration test

○ Inorganic arsenic compounds

<u>As(III)</u>

Studies in cultured mammalian cells demonstrated that sodium arsenite increased chromosome aberration at the concentration of 865μg As/L in mouse lymphoma cells (L5178Y/TK$^{+}$/-) and at 465 μg As/L in Syrian hamster embryo cells (Moore et al. 1997c, Lee et al. 1985a). Sodium arsenite has been reported to induce chromosome aberration in CHO Cells at the concentration of 75, 749 and 2,997 μg As/L (Wan et al. 1982, Lin and Tseng 1992, Haung et al. 1993, Kochhar et al. 1996). However, tetraploid formation was not observed with at the dose of 285 μg As/L in Chinese Hamster Lung Fibroblast Cell Line (V79) (cell proliferation was inhibited by 50% with 476 μg As/L) (Eguchi et al. 1997).

In human peripheral blood lymphocytes, sodium arsenite increased aneuploidy due to impairment of microtubules at the concentration of $0.75 \times 10^{-8}$ μg As/L (Vega et al. 1995) and 0.07 μg As/L (Ramirez et al. 1997), and induced chromosome aberration at 58 μg As/L (Nordenson et al. 1981) and 187 μg As/L (Kligerman et al. 2003). Also, in human leucocyte, sodium arsenite, arsenic trichloride, and arsenic trioxide induced

chromosome aberration at 1,800μg As/L, 450μg As/L, and at 1,800 μg As/L, respectively (Nakamuro and Sayato 1981). Sodium arsenite induced chromosome aberration in human umbilical cord fibroblast at 285 μg As/L (Oya-Ohta et al. 1996). Chromosome aberration was observed at 375 μg As/L (cell viability 13%) in human dermal fibroblast (Yih et al. 1997). In human epithelial carcinoma cells, sodium arsenite at 225μg As/L and over increased aneuploidy by inhibitory action of sodium arsenite on perturbation of spindle apparatus (Huang and Lee 1998).

As(V)

A study in cultured mammalian cells has shown that disodium arsenate at 4,571μg As/L induced chromosome aberration in a mouse lymphoma cells (L5178Y/TK$^{+/-}$) (Moore et al. 1997c). Disodium arsenate has been reported also to significantly increase polyploid in Syrian hamster embryo cells at 2,397 μg As/L and to increase chromosome aberration at 4,795 μg As/L (Lee et al. 1985a). Disodium arsenate has been reported not to induce tetraploid formation at 13,486 μg As/L in V79 cells where the 50% inhibition dose of cell proliferation was 21,104 μg As/L (Eguchi et al. 1997), whereas, chromatid exchange and chromosome aberration have been reported to be significantly increased by disodium arsenate at 749 μg As/L in CHO Cells (Kochhar et al. 1996).

In human peripheral blood lymphocytes, disodium arsenate did not induce chromosome aberration at 232 μg As/L (Nordenson et al. 1981), but induced it at 749 μg As/L (Kligerman et al. 2003). It has been reported that chromosome aberration was induced by arsenic acid and by arsenic pentoxide at 5,400 μg As/L in human leucocytes (Nakamuro and Sayato 1981), and by disodium arsenate at 1,199 μg As/L in human umbilical cord fibroblast (Oya-Ohta et al. 1996).

○ Organic arsenic compounds

In a study in cultured mammalian cells, chromosome aberration were induced by MMA(V), DMA(V) at 1,850,448 As/L and 3,746,468μg As/L, respectively, in mouse lymphoma cells (L5178Y/TK$^{+}$/-). However, the data, as were insufficient, did not allow the authors to conclude that MMA(V) and DMA(V) induced chromosome aberration (Moore et al. 1997c). In Syrian hamster embryo cells, chromosome aberration was induced by DMA(III) at 75 μg As/L (Ochi et al. 2004). In V79 cells, MMA(V) at 104,888 μg As/L, arsenobetaine at 5,244,400 μg As/L and arsenocholine at 457,012 μg As/L did not induce tetraploid formation, whereas DMA(V), TMAO induced it at 52,444 μg As/L and 524,440 μg As/L, respectively (Eguchi et al. 1997). In addition, DMA(V) has been reported to induce tetraploid formation at 33,931 μg As/L in V79 cells (Endo et al. 1992).

It has been reported that, in human peripheral blood lymphocyte, MMA(III), DMA(III), MMA(V), and DMA(V) induced chromosome aberration at the concentration of 45.0, 101, 224,760 and 224,760 μg As/L, respectively (Kligerman et al. 2003). On the other hand, another study reported that DMA(V) at 8,469 μg As/L failed to induce chromosome aberration in human peripheral blood lymphocytes (Endo et al. 1992).

In human umbilical cord fibroblast, chromosome aberration were induced by MMA(V), DMA(V), TMAO, arsenobetaine, arsenocholine, tetramethyl arsonium iodide, and arsenosugar (2',3'-Dihydroxypropyl-5-deoxy-5-dimethylarsinoyl-b-D-riboside) at the concentration of 104,888, 52,444, 277,204, 824,120, 2,247,600, 1,423,480 and 1,123,800 μg As/L, respectively (no data of cell viability available for all arsenic compounds) (Oya-Ohta et al. 1996).

(b) Micronucleus test

○ As(III) and As(V)

Sodium arsenate and disodium arsenate were reported to enhance micronucleus formation in mouse lymphoma cells (L5178Y/TK$^{+/-}$) at 865 μg As/Land 4,571 μg As/L, respectively (Moore et al. 1997c). Micronucleus formation, in CHO-K1 cells and in CHO-XRS-5 cells, was increased by sodium arsenite at 375 μg As/L and at 749 μg As/L, respectively (Fan et al. 1996, Wang and Huang 1994). Wang and Huang (1994) reported that arsenous acid probably induce micronucleus through excessive production of hydrogen peroxide. In V79 cells, micronucleus formation was significantly enhanced by diarsenic trioxide at 75 μg As/L (Gebel 1998). Meanwhile, gallium arsenide in Syrian hamster embryo cells was negative in a micronucleus test at 5,180 μg As/L.

In human peripheral blood lymphocyte, micronucleus formation by sodium arsenite was observed at the concentration of 37.5 μg As/L (Schaumloffel and Gebel 1998). In human fibroblast cells, sodium arsenite formed micronucleus with centromere acting as aneugen by interfering spindle function at low concentration (375 μg As/L). In contrast, sodium arsenite at high concentration (2,248 μg As/L) formed micronucleus without centromere by acting as clastogen (Yih and Lee 1999).

○ Organic arsenic compounds

In a mouse lymphoma cell (L5178Y/TK$^{+/-}$), it has been reported that MMA(V) increased frequency of micronucleus formation at 1,850,448 μg As/L. While, DMA(V) failed to increase frequency of micronucleus formation even at 4,683,085 μg As/L (Moore et al. 1997c).

(c) Summary of chromosome aberration

The chromosome aberration both in functional abnormality and in numerical abnormality was induced by arsenic compounds in mammal cells and in several cultured human cells. It is considered that clastogenicity of trivalent arsenic was higher than that of pentavalent arsenic, and that clastogenicity of inorganic arsenic compounds is higher than that of organic arsenic compounds.

c. Sister Chromatid Exchange (SCE)

(a) As(III) and As(V)

In CHO Cells, the frequency of SCE was increased by sodium arsenite at 1, 75, 375, 1,498 μg As/L and t by disodium arsenate at 0.75 μg As/L to a level higher than that in the control group (Wan et al. 1982, Lee et al. 1985b, Fan et al. 1996, Kochhar et al. 1996). In Syrian hamster embryo cells, SCE was induced by sodium arsenite and by disodium arsenate at 60 μg As/L and 749 μg As/L, respectively (Lee et al. 1985a).

Studies in human peripheral blood lymphocytes have reported that sodium arsenite at the concentration of 60, 292 and 375 μg As/L induced SCE (Nordenson et al. 1981, Beckman and Nordenson 1986, Jha et al. 1992, Hartmann and Speit 1994), whereas another study reported that no SCE was induced by sodium arsenite at 749 μg As/L (Kligerman et al. 2003). Disodium arsenate was negative even at the high concentration of 11,238 μg As/L (Kligerman et al. 2003). In a study using human lymphoblastoid, sodium arsenite was positive at 37.5 μg As/L and arsenic acid was negative at 749 μg As/L (Rasmussen and Menzel 1997).

(b) Organic arsenic compounds

A study in human peripheral blood lymphocytes has reported that SCE was induced by DMA(III) at 230 μg As/L and weak abnormality was induced by MMA(V) and DMA(V) at 224,760 μg As/L and 749,200 μg As/L, respectively. However, MMA(III) was negative at 135 μg As/L (Kligerman et al. 2003). Also, DMA(V) was negative at 749 μg As/L in a study using human lymphoblastoid, (Rasmussen and Menzel 1997).

(c) Summary of sister chromatid exchange

When SCE is induced by arsenic compounds, the toxicity of the compound depends on its chemical form. In cultured animal cells, As(III) and As(V) induced SCE, but no report was found for organic arsenic compounds. As(III) induced SCE in human peripheral blood lymphocyte and in human lymphocyte, but As(V), MMA(III) and DMA(V) did not.

d. DNA damage

(a) DNA damage

○ Tests using bacteria

Inorganic arsenic compounds

It has been reported that sodium arsenite did not induce SOS gene expression in *E. coli* ($WP2_s$ (λ), PQ37) at 60,386 μg As/L and at 239,749 μg As/L (Rossman et al. 1984, Lantzsch and Gebel 1997). Also, in rec assay using *Bacillus subtilis,* sodium arsenite, arsenic trichloride, and disodium arsenate were positive at a dose as high as 3,746,000 μg As/L (Nishioka 1975).

Organic arsenic compounds

In an induction test of MMA(III) and DMA(III) in *E. coli* ($WP2_s$(λ) using prophage inducing activity as an index, MMA(III) and DMA(III) were both negative at 0-749 μg As/L (Kligerman et al. 2003).

○ Alkaline elution procedure

As(III)

A study in which diarsenic trioxide was dissolved with alkali and used as arsenous acid, DNA strand break was observed at 75 μg As/L in human fetal lung fibroblasts (Dong and Luo 1993).

Organic arsenic compounds

DNA strand break induced by DMA(V) was observed at the concentration of 370-749,200μg As/L in human type II alveolar epithelial cells (Tezuka et al. 1993, Rin et al. 1995, Kato et al. 1994, Kawaguchi et al. 1996, Yamanaka et al. 1990, 1995, 1997).

Synthetized organic arsenic compounds

DNA strand break by alkaline elution procedure was detected in a mouse lymphoma cell ($L5178Y/TK^{+/-}$) with roxarsone at the cytotoxic dose of 299,680 μg As/L (Storer et al. 1996).

○ Unscheduled DNA synthesis test

In an unscheduled DNA synthesis test in which diarsenic trioxide was dissolved with alkali and used as arsenous acid, diarsenic trioxide was positive at 75 μg As/L in human fetal lung fibroblasts (Dong and Luo 1994).

○DNA nicking assay

Inorganic arsenic compounds

Sodium arsenite (22,476,000 μg As/L) and disodium arsenate (74,920,000 μg As/L) were both negative in a DNA nicking assay using bacteriophageφX174 DNA (Mass et al. 2001).

Organic arsenic compounds

MMA(III) (2,247,600 μg As/L) and DMA(III) (11,238 μg As/L) were positive and MMA(V) (224,760,000 μg As/L) and DMA(V) (22,476,000 μg As/L) were negative in a DNA nicking assay using bacteriophageφX174 DNA (Mass et al. 2001).

(b) Comet assay

○ As(III) and As(V)

Sodium arsenite was positive at 749μg As/L in a comet assay using CHO Cells, and at 375 μg As/L in the assay using bovine arterial endothelial cells (Lynn et al. 1997, 1998, Liu and Jan 2000).

A slight and dose-dependent increase of DNA strand break was induced by sodium arsenite and disodium arsenate at 74,902 μg As/L in the assay using human leucocyte (Mass et al. 2001). In the assay using human leukemia cell, sodium arsenite was positive at 14,984μg As/L (Hartmann and Speit 1994) and at 7.49 μg As/L (Wang et al. 2002).

○ Organic arsenic compounds

In a comet assay using human leucocyte, MMA(III) at 1,498 μg As/L and DMA(III) at 394 μg As/L were positive , , while MMA(V) at 65,555 μg As/L and DMA(V) at 74,920 μg As/L were negative and (Mass et al. 2001). MMA(III), MMA(V) and DMA(V) were positive at 7.49, 7.49 and 74.9 μg As/L, respectively, in a study using human leukemia cells (Wang et al. 2002).

(c) Summary of DNA damage

Arsenic compounds have been reported to be either positive or negative in a DNA damage test using bacteria. Although only a few reports are available regarding the study of DNA damage in cultured animal cells, there are several studies on DNA damage in cultured human cells that reported DNA strand break induced by both inorganic and organic arsenic compounds. Regarding a result of comet assay, it should be noted that the effect of apoptosis is also observed together with a positive reaction as a characteristic of the method.

e. Others

(a) Cell transformation assay

○ As(III) and As(V)

A cell transformation assay in mouse fibroblast cells indicated that sodium arsenite increased colony-forming ability at 749 μg/L (Sabbioni et al. 1991). In Syrian hamster embryo cells, a dose-dependent increase in colony formation was induced by sodium arsenite at the concentration of0-375 μg/L, and by disodium arsenate at 599-8,990 μg/L (Lee et al. 1985a). Also, the significant increase in frequency of transformation was induced by gallium arsenide at 129 μg/L (Kerckaert et al. 1996).

○ Organic arsenic compounds

Arsenobetaine was negative in cell transformation assay using mouse fibroblast cells even at 37,460 μg/L (Sabbioni et al. 1991).

f. Summary of *in vitro* studies

Arsenic compounds are considered to induce DNA damage and chromosome aberration in cultured cells including human cells. The toxicity of As(III) is considered to be higher than that of As(V), and inorganic arsenic compounds are considered to be more toxic than organic arsenic compounds in terms of DNA damage and chromosome aberration.

(ii) *In vivo* study

Table 11 shows the result of *in vivo* studies of arsenic compounds.

a. Gene mutation study

Intraperitoneal administration of diarsenic trioxide or DMA(V) with the dose of 5,756 μg As/kg body weight for 5 times into Muta$^{TM}$ mice did not induce *LacZ* gene mutation in the lungs, kidneys, bladder, and bone marrow (Noda et al. 2002).

b. Chromosome aberration

(a) Chromosome aberration test

○ As(III)

Subcutaneous administration of 58 μg As/kg body weight of sodium arsenite for 4 times or a single oral gavage of 1,442 μg As/kg body weight to Swiss mice has been reported to induce chromosome aberration in bone marrow cells (Das et al. 1993, Roy Choudhury et al. 1996, Biswas et al. 1999, Poddar et al. 2000). However, diarsenic trioxide was reported to induce no chromosomal virulence in bone marrow cells or in spermatogonium when administered 250,000 μg As/L through drinking-water for 2 to

8 weeks or administered 12,000 μg As/kg body weight intraperitoneally, (Poma et al. 1981, 1987).

○ Organic arsenic compounds

Intraperitoneal administration of DMA(V) of 162,870 μg As/kg body weight into ICR mice induced aneuploidy in bone marrow cells, but not chromosome aberration (Kashiwada et al. 1998).

(b) Micronucleus test

○ As(III)

Oral administration of sodium arsenite with the dose of 2,884 μg As/kg body weight to B6C3F1 mice for 4 times, or intraperitoneal administration of a dose of 288 As/kg body weight to BALB/c mice was reported to increase micronucleus formation in bone marrow cells (Tice et al. 1997, Deknudt et al. 1986, Tinwell et al. 1991). Significant increase of micronucleus formation in reticulocyte comparing to the control group was observed after intraperitoneally administered (5 times) of diarsenic trioxide into Muta™ mice at a dose of 5,756 μg As/kg body weight (Noda et al. 2002).

○ Organic arsenic compounds

Micronucleus formation was not observed in reticulocyte by intraperitoneal administration of DMA(V) to Muta™ mice with the dose of 5,755 μg As/kg body weight for 5 times (Noda et al. 2002).

c. DNA Damage

(a) DNA Damage

In a study using alkaline elution procedure, single oral administration of high dose of DMA(V) (702,463 μg As/kg body weight) to ICR mice induced DNA strand break in the lungs 12 hours after the administration, but the same effect was not observed in the liver, kidneys, and spleen (Yamanaka et al. 1989, 1993, Yamanaka and Okada 1994).

(b) Comet assay

The increase of DNA strand break in leukocyte was observed 24 hours after the oral administration of diarsenic trioxide (98-1,629 μg As/kg body weight) to Swiss albino male mice (Sleha et al. 2001).

d. Others

(a) Dominant lethal test

○As(III)

No significant difference was detected in the effects of intraperitoneal administration of sodium arsenite in a dominant lethal test with the dose of 2,884 μg As/kg body weight to BALB/c mice, indicating that sodium arsenite was not genotoxic in reproductive cells (Deknudt et al. 1986).

(b) Sex-linked recessive lethal test

○ Synthetized organic arsenic compounds

No increase in sex-linked recessive lethal mutation rate was observed after infusion of roxarsone at 1,953,890 μg As/L or oral administration of roxarsone at 1,988,638 μg As/L to drosophila. (NTP 1989b).

e. Summary of *in vivo* study

Although only a few reports of *in vivo* study about arsenic compounds are available, oral, intraperitoneal and subcutaneous administration of As(III) in mice have been reported to induce chromosome aberration, increase of micronucleus formation and DNA damage. While DMA(V) administration was reported to induce DNA damage in the lungs or aneuploidy in bone marrow, induction of gene mutation or micronucleus were not observed.

Table 10 Results of *in vitro* genotoxicity study of arsenic

| Test | Compounds | Target | Dose (μg As/L) | Result | References |
|---|---|---|---|---|---|
| a. Gene mutation | | | | | |
| (Prokaryote) | | | | | |
| Reverse mutation test | Sodium arsenite | *Escherichia coli* WP2 | 1,873,000 | − | Rossman et al. 1980 |
| | | *Salmonella typhimurium* TA98, TA100, TA104 | 144 μg As/plate | − | Kligerman et al. 2003 |
| | Disodium arsenate | *S. typhimurium* TA98, TA100, TA104 | 1,200 μg As/plate | − | Kligerman et al. 2003 |
| | MMA(III) | *S. typhimurium* TA98, TA100, TA104 | 7.07 μg As/plate | − | Kligerman et al. 2003 |
| | DMA(III) ($C_2H_6AsI$) | *S. typhimurium* TA98, TA100, TA104 | 161 μg As/plate | − | Kligerman et al. 2003 |
| | MMA(V) | *S. typhimurium* TA98, TA100, TA104 | 1,156 μg As/plate | − | Kligerman et al. 2003 |
| | DMA(V) | *E. coli* | 749,200 | + | Yamanaka et al. 1989b |
| | | *S. typhimurium* TA98, TA100, TA104 | 1,170 μg As/plate | − | Kligerman et al. 2003 |

| | | | | | |
|---|---|---|---|---|---|
| Gene mutation test | Roxarsone | *S. typhimurium* TA100, TA1535, TA1537, TA98 | 2,848 μg As/plate | − | NTP 1989b |
| (Eukaryote) | | | | | |
| Gene mutation test | Sodium arsenite | *Saccharomyces cerevisiae* | 749,200 | − | Singh 1983 |
| (Mammal cells) | | | | | |
| Gene mutation test | Sodium arsenite | Mouse L5178Y ($Tk^{+/-}$) | 577 (cell viability: 50% or 44%) | + | Moore et al. 1997c |
| | | | 865 | + | Oberly et al. 1996 |
| | | Syrian hamster embryo cells (ouabain resistant) | 750 | − | Lee et al. 1985a |
| | | Syrian hamster embryo cells (6-thioguanine tolerant) | 750 | − | Lee et al. 1985a |
| | | CHO Cells (ouabain resistant) | 375 | − | Lee et al. 1985b |
| | | CHO Cells (6-thioguanine tolerant) | 750 | − | Lee et al. 1985b |
| | | CHO Cells (CHO-AS52) | 3,746 (cell viability: 24.04%) | + | Meng and Hsie 1996 |
| | | CHO Cells (ouabain resistant) | 375 | − | Rossman et al. 1980 |
| | | CHO Cells (6-thioguanine tolerant) | 7,492 | − | Rossman et al. 1980 |
| | | CHO Cells | 749 | − | Yang et al. 1992 |
| | | Human-Hamster Hybrid Cells (S1) | 577 (cell viability: 60%) | + | Hei et al. 1998 |
| | Disodium arsenate | Mouse L5178Y (trifluorothymidine-tolerant) | 32,651 | − | Amacher and Paillet 1980 |
| | | Mouse L5178Y ($Tk^{+/-}$) | 4,571 (cell viability: 6% or 35%) | + | Moore et al. 1997 |
| | | Syrian hamster embryo cells (ouabain resistant) | 7,492 | − | Lee et al. 1985a |
| | | Syrian hamster embryo cells (6-thioguanine tolerant) | 7,492 | − | Lee et al. 1985a |
| | MMA(III) | Mouse L5178Y ($Tk^{+/-}$) | 21.0 (cell viability: 53%) | + | Kligerman et al. 2003 |
| | | | 21.0 (cell viability: 43%) | + | Kligerman et al. 2003 |
| | | Chinese hamster ovary cells (*gpt* gene) | 45.0 (cell viability: 43%) | + | Klein et al. 2007 |

| | | | | | |
|---|---|---|---|---|---|
| | DMA(III) ($C_2H_6AsI$) | Mouse L5178Y ($Tk^{+/-}$) | 96.6 (cell viability: 43%) | + | Kligerman et al. 2003 |
| | | | 176 (cell viability: 16%) | + | Kligerman et al. 2003 |
| | MMA(V) | Mouse L5178Y ($Tk^{+/-}$) | 1,156,530 (cell viability: 55 or 66%) | + | Moore et al. 1997 |
| | DMA(V) | Mouse L5178Y ($Tk^{+/-}$) | 2,341,543 (cell viability: 38, 64 or 70%) | + | Moore et al. 1997 |
| | Roxarsone | Mouse L5178 (trifluorothymidine-tolerant) | 284,824 (cytotoxicity: 341,840) | + | NTP 1989b |
| b. Chromosome aberration<br>(a) Chromosome aberration test<br>(Cultured animal cells) | | | | | |
| Chromosome aberration test | Sodium arsenite | Mouse L5178Y ($Tk^{+/-}$) | 865 | + | Moore et al. 1997 |
| | | Syrian hamster embryo cells | 465 | + | Lee et al. 1985a |
| | | Syrian hamster embryo cells | 225 | + | Barrett et al. 1989 |
| | | CHO Cells | 75 | + | Wan et al. 1982 |
| | | | 749 | + | Lin and Tseng 1992 |
| | | CHO Cells(CHO-K1) | 2,997 | + | Huang et al. 1993 |
| | | | 75 | + | Kochhar et al. 1996 |
| | | Chinese hamster V79 cells | 285 (50% inhibition of cell proliferation: 476) | – | Eguchi et al. 1997 |
| | Diarsenic trioxide | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | Arsenic trichloride | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | Disodium arsenate | Mouse L5178Y(TK+/-) | 4,571 | + | Moore et al. 1997 |
| | | Syrian hamster embryo cells | 4,795 | + | Lee et al. 1985a |
| | | | 4,795 | + | Barrett et al. 1989 |
| | | CHO Cells | 3,746 | + | Wan et al. 1982 |
| | | | 749 | + | Kochhar et al. 1996 |
| | | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | | | 13,486 (50% inhibition of | – | Eguchi et al. 1997 |

| | | | | |
|---|---|---|---|---|
| | | cell proliferation: 21,104) | | |
| DMA(III) ($C_2H_6AsI$) | Syrian hamster embryo cells | 75 | + | Ochi et al. 2004 |
| MMA(V) | Mouse L5178Y ($Tk^{+/-}$) | 1,850,448 | + | Moore et al. 1997 |
| | Chinese hamster V79 cells | 104,880 (50% inhibition of cell proliferation: 374,600) | − | Eguchi et al. 1997 |
| DMA(V) | Mouse L5178Y($TK^{+}$/-) | 3,746,468 | + | Moore et al. 1997 |
| | Chinese hamster V79 cells | 33,931 | + | Endo et al. 1992 |
| | | 52,444 (50% inhibition of cell proliferation: 1,791,565) | + | Eguchi et al. 1997 |
| TMAO | Chinese hamster V79 cells | 524,440 (50% inhibition of cell proliferation: > 5,508,823) | + | Eguchi et al. 1997 |
| AsBe | Chinese hamster V79 cells | 5,244,400 (50% inhibition of cell proliferation: > 4,208,989) | – | Eguchi et al. 1997 |
| AsC | Chinese hamster V79 cells | 457,012 (50% inhibition of cell proliferation: > 4,540,606) | – | Eguchi et al. 1997 |
| p-Arsenosobenzoic acid, sodium salt | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| Methylthioarsine | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| Oxophenylarsine | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| (2-Diphenylarsino ethyl)diphenyl phosphine | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |

| | | | | | |
|---|---|---|---|---|---|
| | 4-Amino-phenyl arsonic acid (p-Arsanilic acid) | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | Tetraphenyl arsonium chloride, hydrochloride | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | 4-((2-Arsonophenyl)azo)-3-hydroxy-2,7-naphthalen disulfonic acid, disodium salt | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| | 2-Amonobenzene arsonic acid | Chinese hamster V79 cells | Not listed | – | Endo et al. 1992 |
| (Cultured human cells) | | | | | |
| Chromosome aberration test | Sodium arsenite | Human peripheral blood lymphocyte | 58 | + | Nordenson et al. 1981 |
| | | | 37 | + | Wan et al. 1982 |
| | | | 172 | + | Sweins 1983 |
| | | | 60 | + | Beckman and Nordenson 1986 |
| | | | 225 | + | Eastmond and Tucker 1989 |
| | | | 375 | + | Jha et al. 1992 |
| | | | $0.75\times10^{-8}$ | + | Vega et al. 1995 |
| | | | 0.07 | + | Ramírez et al. 1997 |
| | | | 674 | (+) | Rupa et al. 1997 |
| | | | 187 | + | Kligerman et al. 2003 |
| | | Human leucocyte | 1,800 | + | Nakamuro and Sayato 1981 |
| | | Human dermal fibroblast | 375 (cell viability: 13%) | + | Yih et al. 1997 |
| | | Human umbilical cord derived fibroblast | 285 | + | Oya-Ohta et al. 1996 |
| | | Human cervical carcinoma cells (HelaS3) | 375 | + | Huang and Lee 1998 |
| | | Human cervical carcinoma cells (Hela CCL2) | 225 | + | Huang and Lee 1998 |
| | | Human cervical carcinoma cells (KB) | 225 | + | Huang and Lee 1998 |

| | | | | |
|---|---|---|---|---|
| | Human cervical carcinoma cells (HFW) | 375 | + | Huang and Lee 1998 |
| | Human cervical carcinoma cells (HFLF) | 375 | + | Huang and Lee 1998 |
| | Human cervical carcinoma cells (C33A) | 375 | + | Huang and Lee 1998 |
| | Human cervical carcinoma cells (293) | 375 | + | Huang and Lee 1998 |
| Arsenic trichloride | Human leucocyte | 450 | + | Nakamuro and Sayato 1981 |
| Arsenic trioxide | Human leucocyte | 180 | + | Nakamuro and Sayato 1981 |
| Disodium arsenate | Human peripheral blood lymphocyte | 232 | - | Nordenson et al. 1981 |
| | | 749 | + | Kligerman et al. 2003 |
| | Human umbilical cord derived fibroblast | 1,199 | + | Oya-Ohta et al. 1996 |
| Arsenic acid | Human leucocyte | 5,400 | (+) | Nakamuro and Sayato 1981 |
| Arsenic pentoxide | Human leucocyte | 5,400 | (+) | Nakamuro and Sayato 1981 |
| MMA(III) | Human peripheral blood lymphocyte | 45.0 | + | Kligerman et al. 2003 |
| DMA(III) ($C_2H_6AsI$) | Human peripheral blood lymphocyte | 101 | + | Kligerman et al. 2003 |
| MMA(V) | Human peripheral blood lymphocyte | 224,760 | + | Kligerman et al. 2003 |
| | Human umbilical cord derived fibroblast | 104,888 | + | Oya-Ohta et al. 1996 |
| DMA(V) | Human peripheral blood lymphocyte | 224,760 | + | Kligerman et al. 2003 |
| | Human lymphocyte | 8,469 | − | Endo et al. 1992 |
| | Human umbilical cord derived fibroblast | 52,444 | + | Oya-Ohta et al. 1996 |
| TMAO | Human umbilical cord derived fibroblast | 277,204 | + | Oya-Ohta et al. 1996 |
| AsBe | Human umbilical cord fibroblast | 824,120 | + | Oya-Ohta et al. 1996 |
| AsC | Human umbilical cord derived fibroblast | 2,247,600 | + | Oya-Ohta et al. 1996 |
| Tetramethyl arsonium iodide $(CH_3)_4As^+I^-$ | Human umbilical cord derived fibroblast | 1,423,480 | + | Oya-Ohta et al. 1996 |

| | | | | | |
|---|---|---|---|---|---|
| | Arsenosugar (2',3'-Dihydroxypropyl-5-deoxy-5-dimethylarsinoyl-b-D-riboside) | Human umbilical cord derived fibroblast | 1,123,800 | + | Oya-Ohta et al. 1996 |
| (b) Micronucleus test<br>(Cultured animal cells) | | | | | |
| Micronucleus test | Sodium arsenite | Mouse L5178Y ($Tk^{+/-}$) | 865 | + | Moore et al. 1997 |
| | | CHO Cells (CHO-K1) | 749 | + | Wang and Huang 1994 |
| | | CHO Cells (CHO-XRS-5) | 375 | + | Fan et al. 1996 |
| | | CHO Cells | 2,997 | + | Liu and Huang 1996 |
| | | | 2,997 | + | Liu and Huang 1997 |
| | | | 2,997 | + | Wang et al. 1997 |
| | | | 749 | + | Lynn et al. 1998 |
| | Diarsenic trioxide | Chinese hamster V79 cells | 75 | + | Gebel 1998 |
| | Gallium Arsenide | Syrian hamster embryo cells | 5,180 | − | Gibson et al. 1997 |
| | Disodium arsenate | Mouse L5178Y ($Tk^{+/-}$) | 4,571 | + | Moore et al. 1997 |
| | MMA(V) | Mouse L5178Y ($Tk^{+/-}$) | 1,850,448 | + | Moore et al. 1997 |
| | DMA(V) | Mouse L5178Y ($Tk^{+/-}$) | 4,683,085 | − | Moore et al. 1997 |
| (Cultured human cells) | | | | | |
| Micronucleus test | Sodium arsenite | Human peripheral blood lymphocyte | 37.5 | + | Schaumloffel and Gebel 1998 |
| | | Human fibroblast | 375 | + | Yih and Lee 1999 |
| c. SCE test<br>(Cultured animal cells) | | | | | |
| SCE test | Sodium arsenite | Syrian hamster embryo cells | 60 | + | Lee et al. 1985a |
| | | CHO Cells | 75 | + | Wan et al. 1982 |
| | | | 375 | + | Lee et al. 1985b |
| | | | 1,498 | + | Fan et al. 1996 |
| | | CHO Cells (CHO-K1) | 1 | + | Kochhar et al. 1996 |
| | Disodium arsenate | CHO Cells | 0.75 | + | Kochhar et al. 1996 |
| | | Syrian hamster embryo cells. | 749 | + | Lee et al. 1985a |
| (Cultured human cells) | | | | | |
| SCE test | Sodium arsenite | Human peripheral blood lymphocyte | 292 | + | Nordenson et al. 1981 |

| | | | | | |
|---|---|---|---|---|---|
| | | | 60 | + | Beckman and Nordenson 1986 |
| | | | 375 | + | Jha et al. 1992 |
| | | | 375 | + | Hartmann and Speit 1994 |
| | | | 749 | − | Kligerman et al. 2003 |
| | | Human lymphoblastoid | 37.5 | + | Rasmussen and Menzel 1997 |
| | Arsenic acid | Human lymphoblastoid | 749 | − | Rasmussen and Menzel 1997 |
| | Disodium arsenate | Human peripheral blood lymphocyte | 11,238 | − | Kligerman et al. 2003 |
| | MMA(III) | Human peripheral blood lymphocyte | 135 | − | Kligerman et al. 2003 |
| | DMA(III) ($C_2H_6AsI$) | Human peripheral blood lymphocyte | 230 | (+) | Kligerman et al. 2003 |
| | MMA(V) | Human peripheral blood lymphocyte | 224,760 | (+) | Kligerman et al. 2003 |
| | DMA(V) | Human peripheral blood lymphocyte | 749,200 | (+) | Kligerman et al. 2003 |
| | | Human lymphoblastoid | 749 | − | Rasmussen and Menzel 1997 |
| d. DNA damage<br>(a) DNA damage<br>(Prokaryote) | | | | | |
| SOS test | Sodium arsenite | *E. coli* PQ37 | 60,386 (cytotoxic dose: 30,193) | − | Lantzsch and Gebel 1997 |
| | | *E. coli* | 239,749 | − | Rossman et al. 1984 |
| rec-assay | Sodium arsenite | *Bacillus subtilis* | 3,746,000 | + | Nishioka 1975 |
| | Arsenic trichloride | *Bacillus subtilis* | 3,746,000 | + | Nishioka 1975 |
| | Disodium arsenate | *Bacillus subtilis* | 3,746,000 | + | Nishioka 1975 |
| Prophage induction method | MMA(III) | *E. coli* WP2s(λ) | 0-749 | − | Kligerman et al. 2003 |
| | DMA(III) ($C_2H_6AsI$) | *E. coli* WP2s(λ) | 0-749 | − | Kligerman et al. 2003 |
| (In vitro) | | | | | |
| DNA nicking assay | Sodium arsenite | bacteriophageφX174 DNA | 22,476,000 | − | Mass et al. 2001 |
| | disodium arsenate | bacteriophageφX174 DNA | 74,920,000 | − | Mass et al. 2001 |

| | | | | | |
|---|---|---|---|---|---|
| | MMA(III) | bacteriophageφX174 DNA | 2,247,600 | + | Mass et al. 2001 |
| | DMA(III) ($C_2H_6AsI$) | bacteriophageφX174 DNA | 11,238 | + | Mass et al. 2001 |
| | MMA(V) | bacteriophageφX174 DNA | 224,760,000 | − | Mass et al. 2001 |
| | DMA(V) | bacteriophageφX174 DNA | 22,476,000 | − | Mass et al. 2001 |
| (Cultured animal cells) | | | | | |
| Alkaline elution procedure | Roxarsone | Mouse L5178Y | 299,680 (Cytotoxicity: 599,360) | + | Storer et al. 1996 |
| (Cultured human cells) | | | | | |
| Alkaline elution procedure | Sodium arsenite | Human fetal lung fibroblasts | 75 | + | Dong and Luo 1993 |
| | DMA(Ⅴ) | Human pulmonary alveolar epithelial cells | Unknown | + | Yamanaka et al. 1990 |
| | DMA(V) | Human type II alveolar epithelial cells | 374,600 | + | Kato et al. 1994 |
| | | | 749,200 | + | Yamanaka et al. 1995 |
| | DMA(V) ($C_2H_6AsO_2Na$) | Human type II alveolar epithelial cells | 37,460 | + | Tezuka et al. 1993 |
| | | | 749,200 | + | Rin et al. 1995 |
| | | | 749,200 | + | Kawaguchi et al. 1996 |
| | DMA(V) ($C_2H_6AsO_2Na\cdot 3H_2O$) | Human type II alveolar epithelial cells | 375 | + | Yamanaka et al. 1997 |
| Unscheduled DNA synthesis test | Sodium arsenite | Human fetal lung fibroblasts (2BS cells) | 75 | + | Dong and Luo 1994 |
| Filter binding method | DMA(V) | Human type II alveolar epithelial cells | 749,200 | + | Kato et al. 1994 |
| (b) Comet assay<br>(Cultured animal cells) | | | | | |
| Comet assay | Sodium arsenite | CHO Cells (CHO-K1) | 749 | + | Lynn et al. 1997 |
| | | CHO Cells | 5,994 | + | Lynn et al. 1998 |
| | | Bovine aortic endothelial cells | 375 | + | Lynn et al. 1998 |
| | | Bovine arterial endothelial cells | 375 | + | Liu and Jan 2000 |
| (Cultured human cells) | | | | | |
| Comet assay | Sodium arsenite | Human leucocyte | 0-74,920 | (+) | Mass et al. 2001 |
| | Sodium arsenite | Human leucocyte cells | 14,984 | + | Hartmann and Speit 1994 |
| | | | 7.49 | + | Wang et al. 2002 |

| | | | | | |
|---|---|---|---|---|---|
| | Disodium arsenate | Human leucocyte | 0-74,920 | (+) | Mass et al. 2001 |
| | MMA(III) | Human leucocyte | 1,498 | + | Mass et al. 2001 |
| | | Human leukemia cells | 7.49 | + | Wang et al. 2002 |
| | DMA(III) ($C_2H_6AsI$) | Human leucocyte | 394 | + | Mass et al. 2001 |
| | MMA(V) | Human leucocyte | 65,555 | − | Mass et al. 2001 |
| | | Human leukemia cells | 7.49 | + | Wang et al. 2002 |
| | DMA(V) | Human leucocyte | 74,920 | − | Mass et al. 2001 |
| | DMA(V) ($C_2H_6AsO_2Na \cdot 3H_2O$) | Human leukemia cells | 74.9 | + | Wang et al. 2002 |
| e. Others | | | | | |
| Cell transformation assay | Sodium arsenite | Mouse fibroblast (BALB/3T3 C1 A 31-1-1) | 749 | + | Sabbioni et al. 1991 |
| | | Syrian hamster embryo cells | 0-375 | + | Lee et al. 1985a |
| | Gallium Arsenide | Syrian hamster embryo cells | 129 | + | Kerckaert et al. 1996 |
| | Disodium arsenate | Syrian hamster embryo cells | 1,008 | + | DiPaolo and Casto, 1979 |
| | | | 599-8,990 | + | Lee et al. 1985a |
| | carboxymethylene trimethylarsonium bromide: $C_5H_{12}AsBrO_2$ | Mouse fibroblast (BALB/3T3 C1 A 31-1-1) | 37,460 | − | Sabbioni et al. 1991 |

+: Positive, (+) : Slightly positive, −: Negative

Table 11 Results of *in vivo* genotoxicity study of arsenic

| Test | Compounds | Target | Dose[1)] (μg As/kg body weight) | Result | Reference |
|---|---|---|---|---|---|
| a. Gene mutation | | | | | |
| Mutagenicity test | Diarsenic trioxide | Muta™ mouse lung, kidney, bladder, bone marrow (intra-peritoneal admin-istration: 5 times) | 5,756 | − | Noda et al. 2002 |
| | DMA(V) | Muta™ mouse lung, kidney, bladder, bone marrow (intra-peritoneal admin-istration: 5 times) | 5,755 | − | Noda et al. 2002 |
| b. Chromosome aberration | | | | | |
| (a) Chromosome aberration test | | | | | |
| Chromosome aberration test | Sodium arsenite | Swiss mouse bone marrow cells (oral administration) | 1,442 | + | Das et al. 1993 |
| | | Swiss mouse bone marrow cells (oral administration) | 1,442 | + | Biswas et al. 1999 |
| | | Swiss mouse bone marrow cells (oral administration) | 1,442 | + | Poddar et al. 2000 |
| | | Swiss mouse bone marrow cells (sub-cutaneous admin-istration: 4 times) | 58 | + | Roy Choudhury et al. 1996 |
| | Diarsenic trioxide | Swiss mouse bone marrow cells (ad-ministration in drinking-water: 2-8 weeks) | 250,000 μg As/L | − | Poma et al. 1987 |

| | | | | | |
|---|---|---|---|---|---|
| | | Swiss mouse spermatogonia (administration in drinking-water: 2-8 weeks) | 250,000 μg As/L | − | Poma et al. 1987 |
| | | Swiss mouse bone marrow cells (intraperitoneal administration) | 12,000 | − | Poma et al. 1981 |
| | | Swiss mouse spermatogonium (intraperitoneal administration) | 12,000 | − | Poma et al. 1981 |
| | | CFLP mouse (inhalation exposure in pregnant mouse, 4 hours each day during gestation period of 9 to 12 days) | 21,589 μg As/$m^3$ | (+) | Nagymajtenyi et al. 1985 |
| | DMA(V) | ICR mouse bone marrow (intraperitoneal administration) | 162,870 | + | Kashiwada et al. 1998 |
| (b) Micronucleus test | | | | | |
| Micronucleus test | Sodium arsenite | B6C3F1 mouse bone marrow (oral administration: 4 times) | 2,884 | + | Tice et al. 1997 |
| | | BALB/c mouse bone marrow (intraperitoneal administration) | 288 | + | DeKnudt et al. 1986 |
| | | BALB/c mouse bone marrow (intraperitoneal administration) | 5,768 | + | DeKnudt et al. 1986 |

| | | | | | |
|---|---|---|---|---|---|
| | | BALB/c mouse bone marrow (intraperitoneal administration) | 2,884 | + | Tinwell et al. 1991 |
| | Diarsenic trioxide | Muta™ mouse peripheral blood reticulocytes (intraperitoneal administration: 5 times) | 5,756 | + | Noda et al. 2002 |
| | DMA(V) | Muta™ mouse peripheral blood reticulocytes (intraperitoneal administration: 5 times) | 5,755 | − | Noda et al. 2002 |
| c. DNA damage | | | | | |
| Alkaline elution procedure | DMA(V) (Dimethylarsinic acid sodium salt : $C_2H_6AsO_2Na$) | ICR mouse lung (oral administration) | 702,463 | + | Yamanaka et al. 1989 |
| | | ICR mouse lung (oral administration) | 702,463 | + | Yamanaka et al. 1993 |
| | | ICR mouse lung (oral administration) | 702,463 | + | Yamanaka and Okada 1994 |
| | | ICR mouse liver, kidney, spleen (oral administration) | 702,463 | − | Yamanaka and Okada 1994 |
| | | ICR mouse liver, kidney, spleen(oral administration) | 702,463 | − | Yamanaka et al. 1989 |
| Comet assay | Diarsenic trioxide | Swiss albino mouse leukocyte (oral administration) | 98 | + | Saleha et al. 2001 |
| d. Others | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Dominant lethal test | Sodium arsenite | BALB/c mouse (intraperitoneal administration) | 2,884 | − | Deknudt et al. 1986 |
| Sex-linked recessive lethal test | Roxarsone | Drosophila (oral administration) | 1,988,638 μg As/L | − | NTP 1989b. |
| | | Drosophila (infusion) | 1,953,890 μg As/L | − | NTP 1989b. |

+: positive, (+) : slightly positive, −: negative

[1)] If the result is + or (+), the dose denotes the minimum concentration at which the effect is confirmed and if the result is −, it denotes the maximum concentration at which no effect was confirmed, unless specified.

## 6. Mechanism of toxicity

Mechanism of action of arsenic has been discussed from several points of view in many reports. Despite investigations previously conducted by overseas organizations such as IARC, EFSA and ATSDR, the mechanism of action of arsenic has not been fully understood. In this section, scientific findings on the mechanism of carcinogenesis by arsenic compounds were summarized mainly based on the reports from IARC (2004, 2012) and ATSDR (2007).

(1) Gene mutation, chromosome aberration, DNA damage

After due consideration, FSCJ arrived at the conclusion regarding genotoxicity of arsenic as is summarized hereunder.

In epidemiologic studies, there is no report of arsenic induced significant increase in frequency of gene mutation. However, it should be noted that some of data on the increase by arsenic might be insignificant due to the large dispersion of the data between each subject. Meanwhile, it has been reported that the frequency of chromosome aberration and sister chromatid exchange (SCE) in urothelial cells, oral mucosal epithelial cells and peripheral blood lymphocyte cells were significantly increased by arsenic exposure through drinking-water. Moreover, dose-response relationship with the concentration of arsenic has been reported for arsenic-induced increase in the frequency of micronucleus formation in urothelial cells, oral mucosal epithelial cells, and in peripheral blood lymphocyte cells in humans. Dose-response relationship with arsenic exposure has been also reported for chromosome aberration and sister chromatid exchange in human peripheral blood lymphocyte. In some reports, however, arsenic was reported not to induce SCE. Effect of smoking on the sensitivity to arsenic exposure is also controversial.

Arsenic compounds are considered to induce chromosome aberration and DNA damage in cultured cells including human cells. It is generally considered that trivalent compounds are more toxic than pentavalent compounds, and organic arsenic compounds are more toxic than organic arsenic compounds among pentavalent compounds.

Although reports of *in vivo* study of arsenic compounds are only a few, oral, intraperitoneal, and subcutaneous administration of As(III) have been reported to induce chromosome aberration, increase of micronucleus formation and DNA damage in mice. While DMA (V) administration has been reported to induce DNA damage in the lungs or aneuploidy in bone marrow, induction of gene mutation and micronucleus are not detected.

According to IARC (2004), this induction of genotoxicity may contribute to gene instabilizing effect of arsenic exposure. Also, it has been suggested that MMA(III) and DMA(III), intermediate metabolites in arsenic methylation, were molecules which induce DNA damage in cells *in vitro* leading to DNA strand break by reactive oxygen species, and that these metabolites and reactive oxygen played a major role in an induction of bladder cancer in rats (IARC 2004).

IARC has also suggested that reactive oxygen is formed both *in vitro* and *in vivo* by exposure to arsenic compounds and may be associated with DNA damage induced by As(III), MMA(III) and DMA(III) or with stress response which changes gene expression (IARC2004, 2012). Oxidative DNA damage induced by arsenic was summarized as follows:

The cell which was exposed to low-concentration of trivalent arsenic compounds showed oxidative DNA damage (Wang et al. 2002, IARC 2012). Induction potency of oxidative DNA damage and toxicity of As(III) and MMA(III) were equivalent in human bladder epithelial cells (IARC 2012).

For example, As(III) increased large deletion mutation in human-hamster hybrid cells through generation of reactive oxygenate cytotoxic concentration (Hei et al. 1998, IARC 2012). Ferritin is released from iron by DMA(III) and this free iron causes reactive oxygen through Fenton reaction or through Harber-Weiss reaction. Also, DNA strand break through generation of reactive oxygen was detected in φX174 DNA when MMA(III) or DMA(III) was added *in vitro* (IARC 2004).

Despite the fact that low-concentration of trivalent arsenic compounds cause oxidative DNA damage that generate 8-OhdG which was considered to cause G/*T* base substitution, not only As(III) and MMA(III) but also DMA(III) is considered not to be a definite point mutation substance (Klein et al. 2007, IARC 2012). This is probably due to the effective repair and elimination of oxidative DNA damage (IARC 2012).

Meanwhile, there are reports about oxidative DNA damage caused by DMA(V).

In mice, reductively metabolized DMA(V) causes lung-specific DNA damage by the dimethylarsinic oxide peroxide radical ($(CH_3)_2AsOO\cdot$) and may induce DNA break or DNA-protein cross-linking in cultured cells (Tezuka et al. 1993, Yamanaka and Okada 1994, IARC 2012).

DMA(III) is contained in the urine of rats to which DMA(V) was administered, and reactive oxygen generated in the urine induces gene damage which may play an important role in induction of bladder cancer by arsenic in rats. For example, it was reported that the formation of 8-OHdG,the most commonly used marker of oxidative DNA damage, increased in bladder cancer which was induced by DMA(III) administration in rats (IARC 2004).

Despite the reports such as mentioned above, it should be noted that the data of gene mutation or DNA damage includes the data obtained from the test system which was not necessarily suitable for detection of indirect effect of formation of oxidative DNA adduct.

(2) Changes in DNA repair

It has been considered that arsenic enhanced mutagenicity by affecting both nucleotide excision repair and base excision repair. IARC (2012) reported that the inhibition of nucleotide excision repair in human dermal fibroblast was the most pronounced with MMA(III) and then with DMA(III) and As(III) in order.

(i) Inorganic arsenic compounds

According to the report of Hartwig et al. (1997) referred in IARC (2004), As(III) inhibits nucleotide excision repair of DNA damage induced by ultraviolet rays (UVC) in human fibroblast by inhibiting the DNA break process at 2.5 μM and inhibiting DNA binding process at 20 μM and 50 μM. Also, according to IARC (2012), while As(III) suppresses the expression of certain DNA repair genes in addition to inhibiting specific protein, As(III) may have an opposite effect that increases DNA repair along with cellular antioxidant defense system at a very low concentration.

IARC (2004, 2012) summarized mechanism for the inhibition of DNA repair by inorganic arsenic as follows:

As(III) is not a specific inhibitor of DNA repair enzyme, but is a substance that affects DNA damage signaling system which controls DNA repair. *Poly* (ADP-ribose) polymerase (PARP) -1 associated with signal transduction is involved in base excision repair by interacting with X-ray complementing group 1 gene (XRCC1) which is a DNA repair protein, DNA polymerase β and DNA ligase III. This involvement may suppress the binding process in the base excision repair process (IARC 2012).

As(III) inhibits several DNA repair enzymes including DNA ligase I and II, and zinc finger protein associated with disulfide covalent bond. It was shown that PARP activity, one of zinc finger DNA repair enzyme system, was inhibited by As(III) of low concentration such as 5 μM and 10 nM in Molt-3 cell derived from human T cell lymphoma cells and in HeLa cells. However, other zinc finger DNA repair proteins such as xeroderma pigmentosum group A protein

or bacteria-derived DNA-formamidopyrimidine glycosylase in mammals are not inhibited by As(III) (IARC 2004).

(ii) Organic arsenic compounds

According to IARC (2012), MMA(III) and DMA(III) are more potent as PARP inhibitory substance than As(III). The inhibition of PARP is caused by permutation of zinc in zinc finger protein (IARC 2012).

(3) Changes in DNA methylation

Currently, the number of reports that indicate the epigenetic effect of arsenic compounds in cultured cells and *in vivo* is increasing. For example, the effect of arsenic and its metabolites on variety of DNA methylation and gene specific DNA methylation were reported. Histone modification by arsenic exposure and effects of exposure to arsenic compounds on chromatin structure and micro-RNA by were also reported (Rossman and Klein 2011).

Arsenic induced changes in DNA methylation may be involved in cancer progress, as has been suggested by *in vitro* and *in vivo* studies that carcinogenesis induced by arsenic was also induced by changes in DNA methylation condition, excessive methylation or low methylation (IARC 2004). Gene amplification induced by arsenic compounds or changes in gene expression by DNA methylation changes were also reported (Klein et al. 2007, IARC 2012). The reports on DNA methylation induced by arsenic compounds referred in IARC (2012) are as follows:

- Change in DNA methylation, accompanied by histone modification, was observed to be induced by As(III) and MMA(III) (Jensen et al. 2008, Zhou et al. 2008).
- In Chinese hamster V79-13 cells where DNA methylation changes and aneuploidy were induced by the exposure to As(III) of low-concentration, gene instability was observed in the earlier stage (Sciandrello et al. 2004).
- Low methylation of total DNA was observed along with excessive methylation of specific gene in several cells transformed by As(III) (Bendbrahim-Tallaa et al. 2005a, Liu and Waalkes 2008).
- Oxidative damage of DNA by As(III) changed the DNA methylation pattern (Cerda and Weitzman 1997).
- The change in DNA methylation pattern may be attributable to the changes in *S*-adenosylmethionine (SAM) accumulation in the cells or to a decrease in DNA methylation transferase activity (Hamadeh et al. 2002, Benbrahim-Tallaa et al. 2005a, Reichard et al. 2007, Liu and Waalkes 2008).
- The changes in DNA methylation were also observed in humans who were exposed to arsenic (Chanda et al. 2006, Marsit et al. 2006).

(4) Cell transformation

It has been reported that cell transformation is induced by arsenic exposure.

Arsenic induces cell transformation in Syrian hamster embryo cells, BALB/3T3 cells, and in rat liver cells, TRL1215. Formation of fibrosarcoma which indicates metastasis to lungs was confirmed by injecting the latter cells to nude mice (Lee et al. 1985a, IARC 2004). Although a long-term exposure of human osteosarcoma cells to As(III) of a low concentration was reported to induce the mutation and transformation as the secondary effect of gene instability, these changes were not induced by an exposure to MMA(III) (Mure et al. 2003, IARC 2012).

(5) Changes of cell proliferation

An increase in cell proliferation by arsenic exposure has been reported in several experimental systems directly or indirectly. According to IARC (2004), cell proliferation was induced by arsenic *in vitro* in normal human skin keratinocytes, and bladder hyperplasia was induced in rats by DMA(V) exposure. The increase of ornithine decarboxylase activity, a biomarker of cell proliferation, was observed in the kidneys or in the liver in arsenic administered rats (IARC 2004).

(6) Changes in cellular signal transduction

One of the important signaling pathways affected by As(III) is the pathway mediated by an antioncogene *p53* (IARC 2012). Inorganic arsenic compounds have been reported to modify several gene expressions which relate to cell proliferation and cytophylaxis including *p53* (ATSDR 2007). Trivalent arsenic compounds affect the activation of PARP and p53 protein possibly through the effect on the DNA repair system or through aneuploidy induction. While PARP is necessary for DNA repair or the formation and function of normal spindle apparatus, trivalent arsenic compounds inhibit PARP activation. Trivalent arsenic compounds inhibit signal transmission mediated by p53 protein which plays an important role in DNA damage response as a tumor suppressor gene product to maintain genome stability and to function as a cell cycle checkpoint (Rossman and Klein 2011).

As(III) suppresses activation of the p53 protein or decrease of the *p21* gene expression after DNA being damaged. Since this suppression affects the arrest of the cell cycle (to obtain an opportunity for normal DNA repair before DNA replication) from G1 term to S term in DNA damaged cells, it may explain a part of mechanisms that enhances mutagenicity. The *p53* protein is also important for nucleotide excision repair. The inhibition of thioredoxin reductase by As(III), MMA(III) and DMA(III) may possibly result *p53* dysfunction through accumulation of oxidized form thioredoxin. The increase in the expression of cell proliferation regulator gene such as cyclin D by As(III) may lead to the failure of regulatory mechanism for cell cycle (IARC 2012).

Arsenic compounds stimulate Jun kinase which belongs to the family of cell mitogenic factor activating protein kinase, and increase AP-1 which is a DNA-binding transcription factor. Arsenic also induces proto-oncogene such as *c-jun*, *c-fos*, *c-myc*, and tumor necrosis factor (TNF) -α. The decrease of the *p53* protein accompanying the increase of Mdm2 protein has been observed in arsenic-added human epidermal keratinocyte cells (HaCaT cells). The arsenic induces skin cancer possibly by inhibiting the formation of *p53-MDM2* feedback control loop whereby disabling the control of normal proliferation, according to a model of arsenic-induced skin cancer (IARC 2004).

(7) Changes in binding to steroid receptors and gene expression

Inorganic arsenic inhibits binding of steroids to glucocorticoid receptors, but it does not affect binding of ligands to receptors for androgen, estrogen, mineralocorticoids, or progesterone. In other words, arsenic may be used as a selective inhibitor for glucocorticoid receptor for the assessment of progesterone receptor contained in breast cancer tissue. However, another report showed that arsenic inhibited binding of estradiol to estrogen receptor-α (ER-α) in MCF-7 cells. Moreover, arsenic inhibited expression of ER-α in the breast cancer cell system while did not affect the expression of ER-β, thus it was suggested that arsenic could be a new therapeutic method for ER-α positive breast cancer (IARC 2004).

(8) Gene amplification

Gene amplification has been considered as one of the mechanisms involved in arsenic carcinogenicity (IARC 2004). It has been reported that inorganic arsenic increased amplification of dihydrofolate reductase (*DHFR*) gene in Mouse 3T6 cells, and this effect of As(V) was stronger than that of As(III) (IARC 2004, ATSDR 2007).

(9) Acceleration of mutation and genotoxicity

It has been suggested that oncogenic action of arsenic is attributable to acceleration of carcinogenesis. An arsenic exposure alone does not cause skin tumors in mice. However, it was reported that combined exposure of arsenic and ultraviolet rays induced the skin tumors that were larger in number and in size than the skin tumors caused by ultraviolet exposure alone (ATSDR 2007). Arsenic has been considered as a co-mutagen which synergistically enhances genotoxicity in the combination with many genotoxic agents including ultraviolet rays (IARC 2004).

Ⅴ. Evaluation in international organizations

## 1. International Agency for Research on Cancer (IARC)

Carcinogenicity assessment by IARC categorized arsenic and arsenic compound (IARC 1987), arsenic (IARC 2004) and inorganic arsenic (IARC 2012) in drinking-water into group 1 (Carcinogenic to humans).

According to the assessment by IARC in 2004, the sufficient evidence has been reported that arsenic in drinking-water induces cancers of the urinary bladder, lung and skin dose-dependently. Regarding urinary bladder cancer, IARC conducted the risk assessment based on the results from ecological studies in Taiwan, China (Chen et al. 1985; Chen et al. 1988a; Wu et al. 1989; Chen and Wang 1990; Chiang et al. 1993; Guo et al. 1997; Tsai et al. 1999), in Chile (Rivara et al. 1997; Smith et al. 1998), and from other cohort studies. Dose dependent effect of arsenic to induce lung cancer was confirmed by ecological studies in Taiwan (Chen et al. 1988; Wu et al. 1989) and in Argentina (Hopenhayn-Rich et al. 1998). In addition to ecological studies in Taiwan (Tseng et al. 1968; Guo et al. 2001), the study on increased incidence in Mexico (Cebrian et al. 1983) and increased mortality in Chile (Smith et al. 1998) were used for the assessment of skin cancer. In addition, the evidence for carcinogenicity of inorganic arsenic in the experimental animals was considered to be limited (IARC 2004).

By the reassessment conducted in 2012, IARC retained the categorization of inorganic arsenic into group 1, considering that carcinogenicity in human by exposure to inorganic arsenic such as arsenic trioxide, arsenite, and arsenate was sufficiently proved. The carcinogenicity of inorganic arsenic in experimental animals was also considered to be sufficiently proved based on the carcinogenicity test results including increased incident rate of lung tumour (poorly differentiated adenocarcinoma) in mice by disodium arsenate (Cui et al. 2006) and increase of renal tumors in rats by sodium arsenite (Soffritti et al. 2006).

Furthermore, IARC reassessed the carcinogenicity of organic arsenic in 2012, and categorized DMA(V) and MMA(V) into group 2B (possibly carcinogenic to humans). This categorization was made on the basis of the fact that; oral administration of DMA(V) increased incidence of lung adenoma or lung cancer in A/J mic (Hayashi et al. 1998), due to dose-dependent induction of bladder transitional cell carcinoma was reported in F344 rats (Wei et al. 1999), and that oral administration of MMA(V) in rats or mice resulted the body weight suppression and decreased survival rate without the dose-dependent relationship in carcinogenicity. Based on these data, IARC concluded that there was sufficient evidence for carcinogenicity of DMA(V) in experimental animals but the data for MMA(V) was insufficient. Therefore, IARC categorized DMA(V) and MMA(V) into group 2B. Also, AsBe and other organic arsenic compounds which are not metabolized in human are categorized into group 3 (not classifiable as to its carcinogenicity to humans).

In addition, IARC (2012) conducted reassessment of genotoxicity of arsenic, and concluded that arsenic did not directly react with DNA but oxidative DNA damage could be observed in the cell treated with As(III) at low concentration. Also, IARC reported that As(III) at cytotoxic concentration induced DNA strand break and chromosome aberration.

## 2. FAO/WHO Joint Expert Committee on Food Additives (JECFA)

JECFA specified provisional tolerable inorganic arsenic daily intake to be 0.002 mg As/kg bw/day based on the available data in the 27th conference in 1983. However, JECFA deemed that the data was not sufficient to recommend acceptable intake of arsenic in foods, therefore concluded that it was necessary to collect the information about (i) arsenic accumulation in human who were exposed to several chemical forms of arsenic in foods and drinking-water, (ii) identification, absorption, excretion, and toxicity of arsenic compounds in foods (especially in fish), (iii) contribution of arsenic in fish to body burden, (iv) epidemiological survey of the population who exposed to the known form of arsenic (JECFA 1984).

JECFA considered evaluation of the importance of organic arsenic in fish in the 33rd conference in 1988. At the same time, JECFA reconfirmed the Provisional Tolerable Weekly Intake (PTWI) of inorganic arsenic. Based on the data by Grantham and Jones (1977), JECFA specified PTWI to be 15 μg As/kg bw/week assuming the water intake to be 1.5L/day and body weight to be 70 kg, considering that the sign of toxicity is possibly increased if arsenic concentration in drinking-water exceeds 100 μg As/L . JECFA (1989) explained that JECFA retained the maximum weekly intake amount as the provisional value because the purpose of specifying PTWI is to reduce the arsenic intake in the people who were exposed to the high inorganic arsenic concentration through drinking-water, although it is evident that the margin of intake with which toxic effect is observed in the actual epidemiological survey and the margin of PTWI are small.. Also, JECFA stated that it is necessary to conduct an additional epidemiological survey on human health effect of naturally-derived organic arsenic in marine animals and algae, although organic arsenic intake of the ethnic group in the area where they consume a lot of fish was estimated as approximately 50 μg/kg bw/day and no disease by arsenic has been reported in this group.

Subsequently, JECFA reevaluated PTWI in the 72th conference in 2010 and conducted a quantitative assessment on carcinogenicity risk of inorganic arsenic. Using the epidemiological data on inorganic arsenic concentration in drinking-water and lung cancer by Chen el al (2010), JECFA calculated the 95% lower confidence limit of benchmark dose ($BMDL_{0.5}$) to be 3.0 μg/kg bw/day (2-7 μg/kg bw/day based on the range of estimated value of the total oral exposure from food and drinking-water) specifying bench mark response (BMR) associated with onset of lung cancer at 0.5%. According to JECFA, uncertainty of this $BMDL_{0.5}$ value is attributable to the assumption of total exposure, and to extrapolation of

$BMDL_{0.5}$ to the other populations due to nutrition condition such as low-protein intake other life factors in the targeted population. JECFA considered the previous PTWI of 15μg As/kg bw/week (2.1 μg/kg bw/day) not appropriate any longer since it was within the range of above mentioned $BMDL_{05}$, 2-7 μg As/kg bw/day, therefore withdrew it. Thus JECFA also deemed that more accurate information about inorganic arsenic actually contained in foods at the time of consumption and reliable analytical method for iAs in foods are necessary to improve assessment of oral exposure to inorganic arsenic. In addition, JECFA considered adverse effects that could occur as a result of exposure to inorganic arsenic from water and food as follows. In areas where arsenic concentration in water are elevated (e.g. above the WHO guideline value of 10 μg/l) but less than 50 μg/l, adverse effects of arsenic could possibly occur. But these would be at a low incidence that would be difficult to detect in epidemiological studies (JECFA 2011).

**3. World Health Organization (WHO) Guidelines for drinking-water quality**

In Guidelines for drinking-water quality, 1996, WHO estimated the lifetime skin cancer risk associated with the ingestion of arsenic in drinking-water using a multistage model that is both linear and quadratic in dose. With this model and data on males, the concentration of arsenic in drinking-water associated with estimated excess lifetime skin cancer risk of 10-5 is 0.17 μg/L. However, WHO noted that these values may overestimate the actual risk of skin cancer because of possible simultaneous exposure to other compounds in the water and possible dose-dependent variations in metabolism that could not be taken into consideration. Consequently, WHO recommended a provisional guideline value of 0.01 mg/L (10 μg/L) with a view to reducing the concentration of arsenic in drinking-water. The excess lifetime risk of skin cancer associated with exposure to this concentration was estimated to be $6x10^{-4}$ (WHO 1996).

In the guidelines for drinking-water quality of 2004 (3rd edition, WHO 2004), WHO retained provisional guideline value of 0.01 mg/L (10 μg/L) considering the scientific uncertainty. In the guideline of 2011 (4th edition, WHO 2011), WHO retained the guideline value of 0.01 mg/L (10 μg/L) as provisional on the basis of treatment performance and analytical achievability.

In the 4th edition of guideline, WHO explained the basis of guideline value derivation as follows. There remains considerable uncertainty over the actual risks at low concentrations, and available data on the mechanism do not provide a biological basis for using either linear or non-linear extrapolation. In view of the practical difficulties in removing arsenic from drinking-water, as well as the practical lower limit of quantitative analysis is 1-10 μg/L, WHO retained the guideline value of 10 μg/L as provisional. As was described in the guideline, it is technically feasible to achieve arsenic concentration of 5 μg/L or lower. However, WHO suggested that it is more reasonable to expect that 10 μg/L should be achieve by conventional treatment (e.g.

coagulation), since careful optimization and control of process are required to achieve 5 μg/L or lower (WHO 2011).

## 4. United States Environmental Protection Agency (EPA)

(1) Inorganic arsenic

EPA's Integrated Risk Information System (EPA/IRIS) conducts an assessment of chemical substances providing information about chronic non-carcinogenicity as oral reference dose (oral RfD) which is equivalent to TDI. On the other hand, regarding the carcinogenic effect, EPA/IRIS provides the information about carcinogenicity categorization, and also the risk information of oral exposure as required.

(i) Chronic non-carcinogenic effect

As an assessment of chronic non-carcinogenic effect of arsenic or inorganic arsenic, EPA/IRIS calculated NOAEL as 9 μg/L (reduced value: 0.8 μg /kg bw/day), using hyperpigmentation and increase in keratosis revealed from the cross-sectional study (Tseng et al. (1968) of arsenic contaminated well-water in the residents in Taiwan, as index. In the same manner, EPA/IRIS calculated LOAEL to be 170 μg/L (an equivalent: 14 μg /kg bw/day) based on the study by Tseng (1977) on dose-dependent increase of Blackfoot disease caused by arsenic in Taiwan. The arsenic intake through food in the targeted population was unknown when the well-water concentration of arsenic (μg/L) was converted to the intake amount (μg /kg bw/day). Therefore, EPA/IRIS calculated NOAEL and LOAEL estimating the arsenic intake from daily diet, rice and sweet potato, in Taiwan as 2 μg/day. Uncertainty factor of 3, due to lack of data for eliminating potential of reproductive toxicity and uncertainty of whether this NOAEL is regarded as effect on all humans who have high sensitivity, was applied to NOAEL 0.8 μg /kg bw/day and oral RfD was specified as 0.3 μg /kg bw/day.

(ii) Carcinogenicity

a. Classification of carcinogenicity

Regarding carcinogenicity of arsenic and inorganic arsenic, increase in mortality due to lung cancer in several populations who were exposed by inhalation was observed, increase in mortality due to cancers of the liver, renal, lung and urinary bladder, and increase in the incidence of skin cancer in the population who had been exposed to drinking water with high concentrated inorganic arsenic are known. EPA/IRIS considered that these human data provide sufficient evidence of human carcinogenicity, therefore classified arsenic and inorganic arsenic into Group A (human carcinogen)

b. Carcinogenicity assessment by oral exposure

EPA/IRIS calculated excessive carcinogenicity risk of oral exposure to inorganic arsenic, using linearized multistage-model of low-dose extrapolation, based on the data of remarkable increase in incidence of skin cancer from arsenic exposure through well-water observed in cross-sectional study by Tseng et al. (1968) and Tseng (1977). As a result, the risk of developing cancer related to the oral exposure in life time at the rate of 1 mg dose of relevant substance per 1kg of bw (oral slope factor) was specified to be 1.5 per mg/kg/day. Based on this value, the carcinogenic unit risk (excessing carcinogenic risk when a person intakes drinking-water contained 1 μg of this substance per liter for life time) by drinking-water was calculated to be $5x10^{-5}$ per μg/L, assuming the body weight of adult to be 70 kg and the amount of drinking-water per day to be 2 L. Based on this value, the concentrations in drinking-water which reach a certain level of carcinogenic risk when the water is consumed, were calculated and shown in Table 12.

Table 12 Concentration of arsenic in drinking-water by certain risk levels

| Risk level | Concentration |
|---|---|
| $10^{-4}$ | 2 μg/L |
| $10^{-5}$ | 0.2 μg/L |
| $10^{-6}$ | 0.02 μg/L |

Many mechanisms are involved in carcinogenic action of inorganic arsenic in human (US EPA 2007). Science Advisory Board of EPA stated that As(III) and its metabolites do not directly interact with DNA and they have no direct genotoxicity. However, the same report suggested that As(III) and its metabolites may induce indirect genotoxicity such as aneuploidy and increase in frequency of micronucleus formation due to the effect on DNA methylation (US EPA 2007).

(2) Organic arsenic

EPA conducted a review on MSMA, disodium methanearsonate (DSMA), calcium acid methanearsonate (CAMA), DMA(V) (cacodylic acid), and on sodium cacodylate) for reregistration of pesticide in 2006. In the diet risk assessment, EPA specified the Acute Reference Dose (ARfD) and Chronic Reference Dose (cRfD) for each MMA group (MSMA, DSMA, and CAMA) and DMA group (DMA and Sodium Cacodylate).

For MMA group, the ARfD of 100 μg/kg bw was calculated by applying uncertainty factor 100 to NOAEL of 10 mg/kg body weight/day. The index of the NOAEL were diarrhea and vomiting observed 2 to 5 hours after the particular administration on the day 1 week after the start of the administration for chronic toxicity test in dogs. Chronic RfD of 30 μg/kg bw/day was calculated by applying uncertainty factor of 100 to NOAEL 3.2 mg/kg bw/day. The index

of the NOAEL were decrease in body weight, diarrhea, body weight gain, food consumption, pathological findings in gastrointestinal tract and thyroid gland observed in chronic toxicity test in rats.

For DMA group (DMA and sodium cacodylate), EPA specified ARfD as 0.12 mg/kg bw/day by applying uncertainty factor 100 to NOAEL of 12 mg/kg bw/day which is derived from the NOAEL and LOAEL with following index: for NOAEL; decrease in fetus body weight, crown-rump length shortening, delayed ossification or lack of ossification observed in developmental toxicity study in rats, for LOAEL; mortality, abortion, decrease in body weight, and decrease in food consumption observed in developmental toxicity study in rabbits. Also, EPA specified cRfD as 0.014 mg/kg bw/day by applying uncertainty factor of 30 to $BMDL_{10}$ 430 μg/kg bw/day, for which regenerative proliferation of bladder epithelium in rats was used as index (US EPA 2006).

EPA classified MMA into "no evidence for carcinogenicity" due to insufficient evidence of carcinogenicity in rat and mouse in the assessment for reregistration of pesticide (US EPA 2006). EPA used to classify DMA into category D "not classifiable as to human carcinogenicity" because of no human data and of insufficient animal data for carcinogenicity in EPA/IRIS (1996). However, EPA designated DMA as "not carcinogenic up to doses resulting in regenerative proliferation" in the assessment for reregistration of pesticide (US EPA 2006).

## 5. European Food Safety Authority (EFSA)

The EFSA scientific panel (CONTAM panel) on contaminant in food chain released a scientific opinion on arsenic in foods in 2009 and conducted an assessment of inorganic arsenic based on the epidemiological data for cancers of the urinary bladder, lung and skin, and dermal lesions caused by oral exposure to inorganic arsenic in humans. The COMTAM panel applied the arsenic concentration in drinking-water as the exposure metric for the assessment, since total dietary exposure to inorganic arsenic was not measured in all of the available studies. . The panel applied Bench-mark approach to the data on dermal lesions by Ahsan et al (2006), Rahman et al (2006a), and Xia et al (2009). For the assessment of skin cancer, the panel used change point calculated by Karagas et al. (2002). On applying Bench-mark approach, CONTAM panel selected a 1% extra risk which was within the range of observed data as BMR. A 95% lower confidence limit of the benchmark concentration (BMCL) calculated by NRC from the data of Chiou et al.(2001) and change point calculated by Karagas et al. (2004) were used for assessment of urinary bladder cancer. The BMCL calculated by NRC from the data of Ferreccio et al. (2000) was used for the assessment of lung cancer. Table 13 shows the calculated results of data and BMDL for dose-response assessment of inorganic arsenic by CONTAM panel

Table 13 Data for dose-response assessment of inorganic arsenic by EFSA

| Endpoint | Population | Reference point of arsenic in drinking-water (μg/L water) | Reference point of arsenic intake (μg/kg bw/day) |
|---|---|---|---|
| Dermal lesion | Bangladesh (Ahsan et al. 2006) | $BMCL_{01}$: 23 [(a)] | $BMDL_{01}$: 2.2-5.7 [(b)] |
| Dermal lesion | Bangladesh (Rahman et al. 2006a) | $BMCL_{01}$: 5 [(a)] | $BMDL_{01}$: 1.2-4.1 [(b)] |
| Dermal lesion | Mongolia (Xia et al. 2009) | $BMCL_{01}$: 0.3 [(a)] | $BMDL_{01}$: 0.93-3.7 [(b)] |
| Lung cancer | Chile (Ferreccio et al. 2000) | $BMCL_{01}$: 14 (NRC, 2001) | $BMDL_{01}$: 0.34-0.69 [(c)] |
| Bladder cancer | North east part of Taiwan (Chiou et al. 2001) | $BMCL_{01}$: 42 (NRC, 2001) | $BMDL_{01}$: 3.2-7.5 [(b)] |
| Skin cancer | U.S. A.(New Hampshire) (Karagas et al. 2002) | Change point [(d)]: 1-2 | Change point: 0.16-0.31 [(c)] |
| Bladder cancer | U.S. A. (New Hampshire) (Karagas et al. 2004) | Change point: Approximately 50 | Change point: 0.9-1.7 [(c)] |

$BMCL_{01}$: 95% lower confidence limit of the benchmark concentration of 1% extra risk

$BMDL_{01}$: 95% lower confidence limit of the benchmark dose of 1% extra risk

(a): Calculated by CONTAM panel for this report

(b): Extrapolated from the $BMCL_{01}$ assuming 3-5 L water and 50-200μg/day inorganic arsenic in food per day, 55 kg bw.

(c): Extrapolated from $BMCL_{01}$ assuming 1-2 L water and 10-20 μg/day inorganic arsenic in food consumed per day, 70 kg bw.

(d): The maximum likelihood change point before the trend becomes significant, which provides an indication of a no effect level rather than a BMDL.

(Quoted from Table 43 of EFSA (2009))

The lowest calculated $BMDL_{01}$ was 0.34 μg/kg bw/day which was estimated from the data of lung cancer in the targeted population in Chile by Ferreccio et al. (2000) and the highest calculated $BMDL_{01}$ was 7.5 μg/kg bw/day which was estimated from the data of urinary bladder cancer in north-east part of Taiwan by Chiou et al (2001). However, CONTAM panel used the whole range of $BMDL_{01}$, 0.3-8 μg/kg bw/day, instead of single reference value since there was uncertainty in exposure levels in these major epidemiological surveys.

Since inorganic arsenic is not directly DNA-reactive, many carcinogenic mechanisms such as oxidative damage, epigenetic effect, and interference with DNA repair have been suggested. And CONTAM panel considered that threshold mechanism may be assumed for all these carcinogenic mechanisms. However, considering the uncertainty with respect to the shapes of the dose-response relationships, CONTAM panel concluded that specifying TDI or TWI was not appropriate. Thus, CONTAM panel used the margin of exposure (MOE) approach in the assessment. However, estimated mean daily inorganic arsenic food exposure was 0.13-0.56 μg/kg bw/day in Europe and 0.37-1.22 μg/kg bw/day in high exposure adult group (95 percentile). These values were within the range of $BMDL_{01}$. Also, estimated daily inorganic arsenic food exposure in the population who ate huge amount of rice and in the population who ate huge amount of algae-based products in Europe were approximately 1 μg/kg bw/day and 4 μg/kg

bw/day, respectively. Since there was almost no MOE, or no MOE at all, the risk in some consumers could not be excluded. Based on this assessment, CONTAM panel recommended to reduce the exposure to inorganic arsenic in food. In addition, toward more accurate risk assessment of inorganic arsenic, CONTAM panel mentioned the necessity for accumulating data on each chemical species in each food products to support exposure assessment and dose-response data.

CONTAM panel did not consider organic arsenic species including arsenosugar and arsenolipid which are mainly metabolized to DMA in human and AsBe which was found largely in seafood in the risk assessment, because AsBe is known to have no adverse effects and because no toxicological data were available for other organic arsenic compounds (EFSA 2009).

## 6. Japan

(1) The Revision of Drinking-water Quality Standards, the Health Science Council, Ministry of Health, Labour and Welfare

In the "Ministerial Ordinance Concerning Water Quality Standards" (Ministry of Health, Labour and Welfare Ordinance No. 101, May 30, 2003), for the standards of arsenic and its compound, it is provided that arsenic should be 0.01 mg/L or less. In the assessment in revision of drinking-water quality standards, it was considered that “it is not possible to identify the TDI or the virtual safety dose (VSD) of arsenic based on the carcinogenicity and also not possible to calculate reliable health-based guideline for arsenic concentration in drinking-water based on these TDI and VSD at this stage”, and concluded that “ therefore provisional standards of 10 μg/L should be retained considering high uncertainty of risk assessment on carcinogenicity of arsenic and practical difficulty to eliminate arsenic from drinking-water” (Ministry of Health, Labour and Welfare 2003).

(2) New Energy and Industrial Technology Development Organization (NEDO)

In initial risk assessment of chemical substances, NEDO assessed the chronic effects of oral exposure of arsenic and inorganic arsenic compounds on human health by calculating MOE as follows: NEDO used the converted NOAEL of 0.8 μg/kg bw/day which was calculated by EPA (2005) with an index of increase in pigmentation and hyperkeratosis obtained from cross-sectional study (Tseng et al. 1968; Tseng 1977) in residents who had taken arsenic contaminated well-water in Taiwan. NEDO divided this converted NOAEL by estimated daily oral intake (from food and drinking-water) per 1 kg body weight, 0.73 μg As/kg bw/day. As a result, MOE in oral pathway was calculated to be 1.1 which was smaller than the product of uncertainty factors of 10 obtained in NEDO assessment. Hence NEDO concluded that arsenic is suggested to be harmful on human health at present (NEDO 2008).

(3) Health Risk Subcommittee, Atmospheric Environment Committee, the Central Environment Council, Ministry of the Environment (reference)

The Central Environment Council conducted an assessment on health risk of arsenic and its compounds in the atmospheric environment in the Health Risk Subcommittee of Atmospheric Environment Committee, though it is not an assessment for oral exposure.

The committee considered it difficult to clearly conclude the presence or absence of threshold in carcinogenicity of arsenic compounds in atmospheric environment, since there was evidence of genotoxicity in inorganic arsenic compounds, while other evidences suggested that there were carcinogenicity mechanisms not associating with gene mutation. However, the Central Environment Council concluded that it was appropriate to conduct a risk assessment assuming that there was no threshold, since arsenic and inorganic arsenic compounds have been evidently shown to be carcinogenic to human lung with inhalation exposure and its genotoxicity was scientifically proven.

Regarding carcinogenicity, the Central Environment Council calculated the unit risk to be $1.7\times10^{-3}/(\mu g/m^3)$ using lung cancer excess death as endpoint, based on the 3 cohort studies in copper smelters in Tacoma, Washington, U.S.A. (Enterline et al. 1995), Anaconda, Montana, U.S.A. (Lubin et al. 2000), and Ronnskar, Sweden (Sandstrom & Wall 1993) where sufficient data of dose-response relationship were available. The Central Environment Council specified the evaluation value for carcinogenicity of arsenic and inorganic arsenic compounds as 6 ng $As/m^3$, which is the atmospheric concentration corresponding to excess life time cancer risk of $10^{-5}$.

Since sufficient quantitative data of toxicity except the data for carcinogenicity was not available, the Central Environment Council did not calculate the evaluation value for toxicity other than carcinogenicity (Ministry of the Environment, 2010).

(4) Committee for Recommendation of Occupational Exposure Limits, Japan Society for Occupational Health (reference)

Committee for Recommendation of Occupational Exposure Limits, Japan Society for Occupational Health (1997) evaluated carcinogenicity of arsenic and categorized it into Group 1 (Carcinogenic to humans). In the assessment of arsenic and arsenic compounds in 2000, the committee determined the excess mortality risk as $10^{-3}$ or $10^{-4}$ .Considering that a linear model without threshold dose was appropriate for the risk assessment, the committee suggested the excess respiratory cancer mortality risk as 3 μg $As/m^3$ for $10^{-3}$ and 0.3 μg $As/m^3$ for $10^{-4}$ in terms of the cumulative exposure for 40 years of work from the data in epidemiological survey (Enterline et al. 1995) on 2,802 workers in copper smelter in Washington, U.S.A. (Japan Society for Occupational Health, 2000) .

## Ⅵ. **Risk assessment** (extracted from Part III of the original risk assessment report)

Arsenic is a metalloid that occurs as various inorganic and organic compounds in environment. Arsenic in environment is derived from weathering of mineral, volcanic activity , and human intentional activities. Arsenic compounds are mainly taken through food and drinking-water. Foods contain both inorganic and organic arsenic compounds, and drinking-water contains mainly inorganic arsenic. Especially, large amount of arsenic compounds are contained in marine animals and algae. Since Japanese have a dietary habit of eating seaweed or seafood traditionally, people intake larger amount of arsenic from food compare to other countries. Concerning arsenic intake from agricultural products, the intake from rice is relatively high. Taking these conditions into consideration, FSCJ conducted a risk assessment of arsenic in foods based on the data from various studies, epidemiological surveys and others. The data used in the assessment are on pharmacokinetics, acute toxicity, repeated dose toxicity, carcinogenicity, reproduction and developmental toxicity, genotoxicity of inorganic and organic arsenic compounds and synthetized organic arsenic compounds.

### 1. Pharmacokinetics

As(III) and As(V) administered orally in experimental animals are rapidly absorbed from gastrointestinal tract. Particularly, As(III) and As(V) contained in drinking-water are absorbed completely. However, the absorption rate decreases depending on solubility of arsenic compound and on other food ingredients and nutrient present in gastrointestinal tract. In human, absorbed inorganic arsenic compound are methylated *in vivo*, and mainly arsenic acid, arsenite as well as MMA(V), DMA(V) are excreted in urine. Methylation activities are different in species. Excretion of MMA(V) and DMA(V) in urine are not observed in marmoset, chimpanzee and guinea pig, due to the deficiency of arsenic methyltransferase in liver. However, mouse, rat, hamster, rabbit, and rhesus monkey have arsenic methyltransferase in liver and have arsenic methylating activity. Also, in these animals, methylation from MMA(V) to DMA(V) occurred effectively and the excretion rate of MMA(V) in urine is significantly lower than that of human. In addition, methylation has been suggested to largely contribute to the toxicity of inorganic arsenic.

Although only limited data on pharmacokinetics of organic arsenic are available, most of the MMA(V), DMA(V) and arsenosugar which are orally taken are rapidly absorbed from gastrointestinal tract and mainly excreted into urine in human. Arsenosugar is mainly metabolized to DMA(V) in human but most of AsBe are not metabolized and rapidly excreted into urine.

2. Toxicity

In human, neurotoxicity caused by acute and subacute exposure to inorganic arsenic through oral intake in an accident and others has been observed. Also, potential of long-term oral intake of inorganic arsenic to induce dermal lesions, cancer, developmental effect, neuro developmental effect, and cardiovascular disease, has been reported. Of them, regarding cancer, an epidemiological survey on exposure to inorganic arsenic through drinking-water in arsenic contaminated area revealed dose-dependency between inorganic arsenic intake and incidence of cancers of the skin, lung and urinary bladder... Recently, it was reported that a dose-dependent tendency between inorganic arsenic intake estimated by FFQ and lung cancer risk in Japanese male current smokers was reported. On the other hand, oral administration of inorganic arsenic to experimental animals has been reported to effect on cardiovascular system, respiratory organ system, digestive organ system, hematologic system, immune system, neuro system, and neuro development. However, carcinogenicity was found only in some studies with administration through drinking-water in rodents, and in trans-placental carcinogenicity studies in mice.

Regarding effects of organic arsenic on human, available data is only few. In experimental animals, except a report of urinary bladder cancer observed in rats with oral administration of DMA(V), data concerning the effects of organic arsenic including synthetized compounds are limited and data which contributes to risk assessment is not available.

Genotoxicity of arsenic compound such as DNA damage and chromosomal aberration has been known in human cells and cultures cells. In general, the toxicity of As(III) is considered to be higher than that of As(V) both in inorganic and organic arsenic compound. It is also considered that the toxicity of inorganic arsenic compound is higher than that of organic arsenic compounds. Although reports of *in vivo* experiments on genotoxicity of arsenic compound are not many, a chromosome aberration, increase of micronucleus formation, and DNA damage have been observed after oral, intraperitoneal and subcutaneous administration of As(III) in mice. Concerning DMA(V) administration, induction of gene mutation or micronucleus formation were not observed、although DNA damage in lung and induction of chromosome mutagenicity in marrow and others are reported. Arsenic-induced chromosome aberration, SCE and micronucleus formation have been reported in epidemiological studies, while there is no epidemiological data of a significant increase in arsenic-induced gene mutation.

In addition, it is unlikely that gene mutation is the main cause of arsenic-induced carcinogenesis. Though chromosome aberration, DNA damage, changes in DNA repair, changes in DNA methylation, cell transformation, changes in cell proliferation, changes in cellular signal transduction, changes in binding to steroid receptors and following gene expression, gene amplification, acceleration in induction of mutation/genotoxicity are suggested as mechanisms of the carcinogenesis, how much these mechanisms are involved in arsenic-carcinogenicity is unknown.

From the above, FSCJ concluded that it was appropriate to conduct a dose-response assessment using reliable epidemiological data of inorganic arsenic instead of the data of experimental animals which contained a large species difference in pharmacokinetics or development of toxicity. After examining epidemiological data about effects of oral exposure to inorganic arsenic, FSCJ selected the data for dose-dependent assessment as follows. Data concerning non-carcinogenic effect were the data of dermal lesions (Haque et al.2003; Ahsan et al.2006; Chen et al.2006; Guo et al.2006; Rahman et al.2006; Xia et al.2009), the effects on nervous system (decreased IQ) (Wasserman et al. 2004) and the effects on reproduction and development (Milton et al. 2005; von Ehrenstein et al. 2006; Rahman et al. 2007; Cherry et al. 2008). Data concerning carcinogenic effects were the data of lung cancer (Ferreccio et al. 2000; Chen et al. 20004; Chen et al. 2010b) and of urinary bladder cancer (Chen et al. 2010a). Regarding dermal lesions, it could be taken as precancerous lesions because skin cancer was observed in the epidemiological study of inorganic arsenic exposure through drinking-water. However, FSCJ decided to evaluate dermal lesions as a chronic non-carcinogenic effect of arsenic, since evaluation of dermal lesions was not an evaluation of carcinogenicity itself. Organic arsenic was not evaluated in a dose-dependent assessment because few data of toxicity of organic arsenic was available.

3. Exposure level

Duplicate diet studies in adults in Japan on inorganic arsenic intake have reported following mean value: 10.3μg/day (1.8-22.6 μg/day) (mean per body weight: 0.206 μg/kg bw/day) by Mohri et al. (1990), 33.7 μg/day (8.34-101 μg/day) (mean per body weight: 0.674 μg/kg bw/day) by Yamauchi et al. (1992), 6.52 μg/day (2.0-57 μg/day) (mean per body weight: 0.130 μg/kg bw/day) by Oguri et al.(2012), 18.6 μg/day (2.18-161 μg/day) (mean per body weight: 0.315 μg/kg bw/day) by FSCJ (2013). Also FSCJ (2013) reported that 95 percentile value to be 0.754 μg/kg bw/day. Although, the number of target subjects was not necessarily sufficient in the study by Mohri et al. (1990), Yamauchi et al. (1992) and by Oguri et al. (2012) , and some of the studies by Oguri et al. (2012) and by FSCJ (2013) included intake amount only for 1 day. Nonetheless, FSCJ indicates these values as reference values since the values in these studies were within the certain range despite the time and place of investigation being different.

4. Dose-dependent assessment

(1) Applying the NOAEL/LOAEL method and BMD method

(i) Method for selecting a point of departure (POD)

Generally, in the risk assessment of chemical substances in foods, the following methods are used for the substance which shows no carcinogenicity. If quantitative epidemiological data is

not available NOAEL or LOAEL obtained from animal studies are taken as the point of departure (POD)[2] for calculating TDI, and uncertainty factor for species difference is applied. Even if it is not clear that the genotoxicity of the substance is the main cause of its carcinogenicity, TDI may be calculated using NOAEL or LOAEL in the same manner. On the other hand, if the epidemiological data applicable to quantitative assessment is available, it is not necessary to consider the uncertainty of species difference. However, if NOAEL/LOAEL method is applied to epidemiological data, the following problems will arise: i.e. it will be difficult to specify the cut-off value of dose group based on the objective evaluation criteria, availability of significance in risk ratio will be changed depend on the number of targeted subjects by dose group, LOAEL will be specified regardless of how much is the risk ratio, LOAEL will differ because the statistical power will differ depend on whether the several studies are combined or not. In such a situation, recently BMD method is used to specify POD also in dose-dependent assessment using epidemiological data. EFSA (2009a) stated that BMD method can be applied for all chemical substances in foods, and recommended to use BMD method especially in the case where only LOAEL not NOAEL is available, or in the case in which POD is required for calculating the amount of exposure margin for carcinogenic substance or for dose-response assessment of epidemiological data. However, even if the BMD method is applied, there are still some problems such as ambiguous criteria in selection of model as premise, large dependency on model to be used in some cases, and difficulty of adjusting confounding factor.

As a result of the investigation by FSCJ, the problems for applying BMD method for this assessment are clarified as follows:

- Reference literatures which are applicable to the analysis are limited
- BMCL will vary greatly with models to which the same data is applied
- It is not possible to adjust confounding in the analysis where Benchmark Dose Software (BMDS) of EPA is used.

In contrast, when BMD method is used for supplementing NOAEL/LOAEL, some effectiveness for covering the defects of NOAEL/LOAEL method were confirmed as follows:

- The range of the confidence limit becomes wider and BMDL becomes lower if the sample size is small or dispersion of data is large
- It is not limited to the dose setting used in the investigation
- It is possible to consider the shape of dose-response curve

Therefore, taking these problems into consideration, FSCJ concluded to calculate BMCL using BMD method for supplementing NOAEL/LOAEL in the arsenic risk assessment.

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[2] Value at the starting point which is a standard of toxicity response curve at specifying the standard value of health effect assessment in normal intake range for human in the result of dose-response assessment from the animal studies or from epidemiologic survey in human. Generally, NOAEL, LOAEL, BMDL are used. (ILSI JAPAN 2011).

(i) Applying BMD method

In the calculation of BMCL by BMD method, each model of Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage-Cancer, Probit, Weibull, and Quantal-Linear were used for the analysis of the data in 8 literatures (Table 14, 15) in which BMCL can be calculated by BMDS ver 2.1.2 of EPA.

a. Selection of BMR

Regarding selection of BMR, EFSA (2009a) specified the standard of BMR as 5% for the case where the endpoint is continuous data in animal studies, and as 10% for quantal data. Also, EFSA considered that it is possible to use BMR of 1% if the number of targeted population is large in human studies. In addition, EFSA stated that BMR of 10% was appropriate for the substance which showed genotoxicity and carcinogenicity. However, when BMD method is applied to epidemiological data, BMR should be specified based on the upper limit of socially tolerable risk increase and not by type of subjects or by the consistency with NOAEL/LOAEL. That is, BMR should be specified severely (e.g. 1% or 5%) for the incident rate of grave diseases such as cancer, and not so severely for non-fatal disease (5% or 10%). Since the test value such as IQ is a surrogate marker, BMR should be specified only for the case which the relationship with disease or health is clear and should be specified according to the minimal clinical important difference. However, the level of 1% increase may be practically meaningless even though the increase rate can be calculated mathematically, since background data seem to vary

In addition, BMR largely depends on the model used when extrapolation is required because of no 0 exposure and other reasons. When the contribution of confounding factor is large, this should be taken into account, too. Thus, in these cases, a careful calculation is required unless the dose-response relationship is theoretically established clearly in the relevant data.

Therefore, FSCJ specified BMR in this assessment as follows: 1% for carcinogenic effect, 5% for dermal lesions since most of the cases are not fatal as cancer, and 5% for reproductive and developmental effects considering that infant death and fetus death observed in the case of arsenic exposure through drinking-water are possibly caused by the factors other than arsenic exposure.

b. Model selection

For the selection of model, we analyzed the data in table 14 and 15 using BMDS. We decided basically to select the model which provided the lowest BMCL, from among models that the p-value was not less than 0.10, fitted well around BMCL, and with low Akaike's Information Criterion (AIC). However, considering that the models which result large deviation between calculated BMC and BMCL are of less reliable, the model with BMC/BMCL ratio of 10 or less

was selected. In addition, we excluded the model that provided the extrapolation value exceeding the range of the measured value for BMCL calculated in the relevant analysis.

Table 14 Data from reports used in BMD-analysis (non-carcinogenic effect)

| Literature | Endpoint | Exposure concentration (μg/L) | Exposure concentration used in BMD-analysis*(μg/L) | Number of targeted individuals | Number of cases with endpoint |
|---|---|---|---|---|---|
| Haque et al. 2003 | Dermal lesions | ≥300 | 404 | 34 | 26 |
| | | 200-299 | 242 | 54 | 40 |
| | | 100-199 | 147 | 115 | 66 |
| | | 50-99 | 74 | 70 | 32 |
| | | <50 | 12 | 132 | 28 |
| Ahsan et al. 2006 | Dermal lesions | 175.1-864.0 | 255 | 2,183 | 242 |
| | | 91.1-175.0 | 125 | 2,185 | 162 |
| | | 40.1-91.0 | 62 | 2,202 | 144 |
| | | 8.1-40.0 | 23 | 2,122 | 90 |
| | | 0.1-8.0 | 1.8 | 2,259 | 57 |
| Rahman et al. 2006 | Dermal lesions | ≥300 | 400 | 345 | 108 |
| | | 150-299 | 224.5 | 745 | 194 |
| | | 50-149 | 99.5 | 675 | 124 |
| | | 10-49 | 29.5 | 314 | 53 |
| | | <10 | 5 | 255 | 25 |
| Xia et al. 2009 | Dermal lesions | >300 | 400 | 95 | 9 |
| | | 100.1-300 | 200.05 | 1,072 | 107 |
| | | 50.1-100 | 75.05 | 1,624 | 128 |
| | | 20.1-50 | 35.05 | 3,670 | 235 |
| | | 10.1-20 | 15.05 | 1,336 | 53 |
| | | 5.1-10 | 7.55 | 900 | 32 |
| | | 0-5 | 2.5 | 3,467 | 58 |
| Rahman et al. 2007 | Reproduction and development (fetus death) | ≥409 | 515 | 5,607 | 511 |
| | | 277-408 | 340 | 5,606 | 528 |
| | | 167-276 | 225 | 5,611 | 488 |
| | | 10-166 | 77 | 5,602 | 453 |
| | | <10 | 0.5 | 5,612 | 464 |
| | Reproduction and development (infant death) | ≥409 | 515 | 5,109 | 285 |
| | | 276-408 | 339 | 5,131 | 308 |
| | | 164-275 | 224 | 5,122 | 282 |
| | | 10-163 | 74 | 5,113 | 269 |
| | | <10 | 0.5 | 5,119 | 229 |

* Median exposure concentration calculated from the value in each report. However, the exposure concentration of Haque et al. 2003 and Rahman et al. 2007 are the values originally indicated in respective reports.

Table 15 Data from reports used in BMD analysis (carcinogenic effect)

| Literature | Endpoint | Exposure concentration(μg/L) | Exposure concentration used for BMD-analysis[*1](μg/L) | Number of targeted individuals | Number of cases with endpoint |
|---|---|---|---|---|---|
| Ferreccio et al. 2000 | Lung cancer | 200-400 | 300 | 208 | 79 |
| | | 50-199 | 124.5 | 174 | 50 |
| | | 30-49 | 39.5 | 31 | 8 |
| | | 10-29 | 19.5 | 44 | 5 |
| | | 0-10 | 5 | 113 | 9 |
| Chen et al. 2010b | Lung cancer (cumulated exposure in 40 years) | ≥10,000 | 12500 | 632 | 29 |
| | | 5,000-<10,000 | 7500 | 524 | 23 |
| | | 1000-<5,000 | 3000 | 2,078 | 51 |
| | | <1000 | 500 | 2,583 | 43 |
| | | 0 | 0 | 1,071 | 32 |
| | Lung cancer | ≥300 | 400 | 7,809[*2] | 31 |
| | | 100-299.9 | 199.95 | 10,409[*2] | 28 |
| | | 50-99.9 | 74.95 | 10,309[*2] | 20 |
| | | 10-49.9 | 29.95 | 24,171[*2] | 51 |
| | | <10 | 5 | 26,519[*2] | 48 |
| Chen et al. 2010a | Bladder cancer (cumulated exposure in 40 years) | ≥10,000 | 12500 | 632 | 11 |
| | | 5,000-<10,000 | 7500 | 524 | 5 |
| | | 1,000-<5,000 | 3000 | 2,078 | 12 |
| | | 400-<1,000 | 700 | 1,120 | 3 |
| | | <400 | 300 | 2,534 | 6 |
| | Bladder cancer | ≥300 | 400 | 691 | 11 |
| | | 100-299.9 | 199.95 | 909 | 8 |
| | | 50-99.9 | 74.95 | 907 | 5 |
| | | 10-49.9 | 29.95 | 2,093 | 8 |
| | | <10 | 5 | 2,288 | 5 |

[*1] Median exposure concentration calculated from the value in each report.

[*2] Number of targeted individuals are calculated from the incident per 100,000 persons and number of cases with each endpoint.

(2) Non-carcinogenic effects

In addition to identifying NOAEL/LOAEL for arsenic in drinking-water in each epidemiological survey, FSCJ attempted to calculate BMCL using BMD method to complement NOAEL/LOAEL on the data of non-carcinogenic effects which can be used for dose-dependent assessment. The data used for this examination were on: dermal lesions (Haque et al.2003; Ahsan et al.2006; Chen et al.2006; Guo et al.2006; Rahman et al.2006; Xia et al.2009), effects on nervous system (decreased IQ) (Wasserman et al. 2004) and effects on reproduction and development (Milton et al. 2005; von Ehrenstein et al. 2006; Rahman et al. 2007; Cherry et al. 2008). The NOAEL and LOAEL identified for arsenic in drinking-water, and the results of BMD -analysis were shown in Table 16.

For dermal lesions, though the NOAEL could not be identified, the LOAEL, $BMCL_{05}$, $BMC_{05}$ were 7.6-124.5 μg/L, 19.5-54.1 μg/L, and 23.2-84.4 μg/L, respectively. The NOAEL and LOAEL for effects on nervous system (decreased IQ) were 27.8 μg/L and 113 μg/L, respectively. The BMCL could not be calculated since appropriate data which could be applied in BMD-analysis were not available. The NOAEL and LOAEL for effects on reproduction and development were 30-221.5 μg/L and 70-342 μg/L, respectively. The $BMCL_{05}$ could not be calculated since there was no model where BMR 5% could be applied in BMD-analysis.

Based on these results, FSCJ concluded that the most sensitive index among non-carcinogenic effects of inorganic arsenic exposure was dermal lesions.

(3) Carcinogenic effects

Regarding the data on carcinogenic effects which can be used for dose-dependent assessment (lung cancer: Ferreccio et al. 2000; Chen et al. 2004; Chen et al. 2010b, urinary bladder cancer: Chen et al. 2010a), FSCJ attempted to identify NOAEL/LOAEL for arsenic in drinking-water in each epidemiological survey, and to calculate BMCL by BMD method. Table 17 shows NOAEL and LOAEL of arsenic in drinking water and results of BMD-analysis.

The NOAEL and LOAEL for lung cancer were 19.5-200 μg/L and 39.5-400 μg/L, respectively. The $BMCL_{01}$ could not be calculated since no model was applicable in BMD analysis with BMR 1%. The NOAEL, LOAEL, $BMCL_{01}$, and $BMC_{05}$ for urinary bladder cancer were 75 μg/L, 187.5-200 μg/L, 140-186 μg/L, and 230-288 μg/L, respectively.

Table 16 NOAEL/LOAEL and BMCL of inorganic arsenic in drinking-water for non-carcinogenic effect

| Population | Endpoint | NOAEL[*1,*2] (μg/L) | LOAEL[*2] (μg/L) | BMD model | BMC05 (μg/L) | $BMCL_{05}$ (μg/L) |
|---|---|---|---|---|---|---|
| India (Haque et al. 2003) | Dermal lesions | <50 (25.0) | 50-99 (74.5) | LogProbit | 32.0 | 26.2 |
| Bangladesh (Ahsan et al. 2006) | Dermal lesions | 0.1-8.0 (4.05) | 8.1-40 (24.05) | Gamma | 84.4 | 38.8 |
| Bangladesh (Chen et al. 2006) | Dermal lesions | 0.1-28.0 (14.05) | 28.1-113 (70.55) | No applicable data for BMDS | | |
| Bangladesh (Rahman et al. 2006) | Dermal lesions | < 10.0 (5.0) | 10-49 (29.5) | LogLogistic | 68.3 | 54.1 |
| The Inner Mongolia Autonomous Region of China (Guo et al. 2006) | Dermal lesions | <50 (25.0) | 50-199 (124.5) | No applicable data for BMDS | | |
| The Inner Mongolia Autonomous Region of China (Xia et al. 2009) | Dermal lesions | 0-5 (2.5) | 5.1-10 (7.55) | LogProbit | 23.2 | 19.5 |
| Bangladesh (Wasserman et al. 2004) | Nervous system (decreased IQ) | 5.6-50 (27.8) | 50.1-176 (113.05) | No applicable data for BMDS | | |
| Bangladesh (Milton et al. 2005) | Reproduction and development (Spontaneous abortion) | - | 51-100 (75.5) | No applicable data for BMDS | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| India<br>(von Ehrenstein et al. 2006) | Reproduction and development<br>(Stillbirth) | 50-199<br>(124.5) | ≥200<br>(274.5) | No applicable data for BMDS | | |
| Bangladesh<br>(Rahman et al. 2007) | Reproduction and development<br>(Fetus death) | 167-276<br>(221.5) | 277-408<br>(342.5) | No applicable model | - | - |
| | Reproduction and development<br>(Infant death) | 164-275<br>(219.5) | 276-408<br>(342) | No applicable model | - | - |
| Bangladesh<br>(Cherry et al. 2008) | Reproduction and development<br>(Stillbirth) | 10-50<br>(30) | ≥50<br>(70) | No applicable data for BMDS | | |

- BMCL was not calculated in BMD analysis

[*1] Since NOAEL was not identified for dermal lesions, reference dose was shown

[*2] Median of each concentration group are in brackets in the NOAEL and LOAEL columns

Table 17 NOAEL/LOAEL and BMCL of inorganic arsenic in drinking-water for carcinogenic effect

| Population | Endpoint | NOAEL* (μg/L) | *(μg/L) | BMD model | BMC01 (μg/L) | $BMCL_{01}$ (μg/L) |
|---|---|---|---|---|---|---|
| Chile (Ferreccio et al. 2000) | Lung cancer | 10-29 (19.5) | 30-49 (39.5) | No applicable model | - | - |
| Taiwan (Chen et al. 2004) | Lung cancer | 10-99 (54.5) | 100-299 (200) | No applicable data for BMDS | | |
| Taiwan (Chen et al. 2010b) | Lung cancer | 25-125 (75) | 125-250 (187.5) | No applicable model | - | - |
| | | ※Accumulated exposure in 40 years | | | | |
| | Lung cancer | 100-299.9 (200) | ≥300 (400) | No applicable model | - | - |
| Taiwan (Chen et al. 2010a) | Bladder cancer | 25-125 (75) | 125-250 (187.5) | Multistage Cancer Quantal-Linear | 210 | 140 |
| | | ※Accumulated exposure in 40 years | | | | |
| | Bladder cancer | 50-99.9 (74.95) | 100-299.9 (200) | LogLogistic | 288 | 186 |

- BMCL was not calculated in BMD analysis

* Median of each concentration population group are in brackets in the NOAEL and LOAEL columns

(4) Estimation of daily intake of inorganic arsenic in the area with contaminated drinking-water

In the report of epidemiological survey which was used for dose-dependent assessment, the total exposure of inorganic arsenic including arsenic derived from meal was not revealed, although inorganic arsenic concentration in drinking-water was used as an exposure index. Therefore, the conversion from inorganic arsenic concentration in drinking-water to the daily total intake including the exposure derived from meal was required. FSCJ summarized the information about inorganic arsenic intake derived from meal and intake of drinking-water in the targeted areas of the dose-response assessment in the report that are West Bengal in India, Bangladesh, the Inner Mongolia Autonomous Region of China, Taiwan, and Chile, and estimated the daily inorganic arsenic intake in the area with contaminated water.

(i) Inorganic arsenic intake from meal and intake of drinking-water in the areas with contaminated water

a. West Bengal in India

There were several reports on arsenic intake from meal in West Bengal in India. Roychowdhury et al. (2002) reported that the total arsenic intake in adult from cooked food was 171 and 189 μg/day based on the daily intake of grains, bread products, vegetables, spices and others which had been collected from each household in 2 different areas in West Bengal. They reported also that the mean arsenic concentration in drinking-water was 133 μg/L, and the amount of drinking-water for male and female in the entire area were 4 L/day and 3 L/day, respectively.

From the inorganic arsenic concentration in rice cooked with water at 50, 250, 500 μg/L, Signes et al. (2008) estimated inorganic arsenic intake through the cooked rice to be 104, 399, 750 μg/day, respectively. The assumed amount of drinking-water used in this estimation was 2.5 L/day.

Signes-Pastor et al. (2008) reported the mean inorganic arsenic intake from meal and from drinking-water to be 63 μg/day and 88 μg/day based on the analysis of As(III) and As(V) in rice, vegetables, and in drinking-water collected in diet survey.

Pal et al. (2009) calculated the mean inorganic arsenic intake from cooked rice prepared with two different groups of rice which were grown in two different seasons in an arsenic contaminated area. They calculated the inorganic arsenic concentration in two groups of cooked rice, both of which were cooked with water with inorganic arsenic concentration lower than 3 μg/L, to be 34 and 97 μg /day respectively.

b. Bangladesh

There were several reports on the total arsenic intake in Bangladesh. Watanabe et al. (2004) estimated the total arsenic intake from meal (rice, bread, potato) to be 214 μg/day for male and 120 μg/day for female based on the results of FFQ and from total arsenic concentration contained in foods. This estimation did not calculate the intake from water used in cooking, but estimated separately the volume of the water used in cooking　rice, bread and curry to be 1.6

L/day for male and 0.95 L/day for female. Also, the daily intake of drinking-water was reported to be 3 L/day for male and for female based on the measured data.

Smith et al. (2006) reported the mean intake of inorganic arsenic from different groups of cooked rice prepared with water containing 200, 300, 400, 500 μg/L of total arsenic. According to the report, the mean intake of inorganic arsenic from cooked rice of each group was 19, 153, 232, and 53 μg/day. The daily intake of drinking-water was reported to be 2-4 L/day in their report

Kile et al. (2007) reported the median value of the total arsenic intake derived food and drinking-water to be 48.4 μg/day based on the 6-day duplicate diet study in 47 females. Kile et al. specified the median value of arsenic concentration in the relevant drinking-water to be 1.6 μg/L (range<1-450 μg/L), and the mean daily intake of drinking-water to be 2.7 L/day.

Rahman et al. (2008) reported that the total arsenic intake from rice was 200-350 μg/day based on the average rice consumption of 400-650 g/day and the total arsenic concentration in rice of 0.5 mg/kg in Bangladesh.

JFCFA (2011) conducted a dose-response assessment based on the data of total arsenic intake including above mentioned data. This assessment was on the assumptions that 70% of the total arsenic was inorganic, that inorganic arsenic intake from meal tanged from 50 to 400 μg/day, and that the mean exposure to inorganic arsenic from meal was 75 μg/day. In addition, total intake of water in Bangladesh was estimated to be 4 L/day including water for cooking water, based on the above mentioned data of water intake, and the value was used for dose-response assessment.

c. Taiwan

Schoof et al. (1998) have reported the mean intake of inorganic arsenic by an adult to be 50 μg/day (range: 15-200 μg/day) for Taiwan, based on the consumption of rice (225 g/day) and yam (500 g/day) and on the inorganic arsenic concentration. However, intake of inorganic arsenic from water used for cooking was not included.

JECFA (2011) conducted a dose-response assessment of the survey in Taiwan. As the data to use for the dose-response assessment, they estimated the intake of inorganic arsenic from meal to be 50-200 μg/day and mean exposure level to be 75 μg/day, based on the available data. The daily intake of water was estimated to be 3 L/day including cooking water that was required for cooking yum, and the value was used for the dose-response assessment.

d. The Inner Mongolia Autonomous Region of China

The data on inorganic arsenic intake from meal and intake of water in the Inner Mongolia Autonomous Region of China could not be obtained.

e. Asia

EPA (2007) recommended to use the inorganic arsenic intake from meal within the range from the lowest 50μg/day to the highest 200 μg/day in the sensitive analysis of a population in

Asia, based on the arsenic exposure data in each Asian region. , EFSA (2009) uses the value of 50-200 μg/day as the inorganic arsenic intake from foods for dose-response assessment in a survey of Asian region, based on the above mentioned information of intake.

Based on the information of water intake in each region in Asia, EFSA (2009) adopted a value of 3-5 L/day as the daily water intake including the amount of cooking water in rural area in Asia for the dose-response assessment.

f. Chile

Concerning the inorganic arsenic intake in Chile, Diaz et al. (2004) estimated the daily intake from the analysis of inorganic arsenic in raw vegetables, cooked vegetables, bread and drinking-water of 2 different seasons in which arsenic concentrations in drinking-water are different. Accordingly, a mean daily intake of inorganic arsenic for the season when drinking-water contains inorganic arsenic at 572 μg/L was reported to be 55 μg/day from meal only and to be 1,389 μg/day from meal and drinking-water. While, a mean daily intake of inorganic arsenic for the season when drinking-water contains inorganic arsenic at 41 μg/L was reported to be 31 μg/day from meal only and to be 125 μg/day from meal and drinking-water. (Diaz et al. 2004). Based on this report, JECFA (2011) considered that the mean intake of inorganic arsenic in adults who eat moderately was 0.52-0.92 μg/kg bw/day from food only and 2.08-21.48 μg/kg bw/day from food and drinking-water (JECFA 2011).

EFSA (2009) assumed the total intake of water including water used for cooking in the population of North and South America to be 1-2 L/day based on the fact that the intake of water in the U.S. reported by NRC (1999, 2001) was 1-2 L/day and that approximately 10% of the total intake of water in the U.S. and Canada was water used for cooking.

(ii) Estimation of total daily intake based on the concentration of inorganic arsenic in drinking-water

Available data on daily intake of arsenic from meal are limited even the data on total arsenic is included. Moreover, in the targeted groups of food seemed to be biased by survey, and data on intake for the same country and region vary from survey to survey. In addition, in arsenic contaminated areas where the exposure to inorganic arsenic is considered to be due largely to drinking-water, intake of inorganic arsenic from meal is not necessarily high even that the data included the water used for cooking. Therefore, in selection of data for conversion, FSCJ attempted to collect data which contain a set of information on arsenic concentration in drinking-water, intake of water, intake of total arsenic or of inorganic arsenic from food as far as possible, and estimated the total daily intake of arsenic based on the information from the literatures listed in Table 18. The Inner Mongolia Autonomous Region of China was excluded from the target for estimation of total daily intake since the data of arsenic intake from meal was not available.

A total daily intake of inorganic arsenic was estimated from inorganic arsenic concentration in drinking-water combining the intake from drinking-water and from meal following the equation below. The total intake was estimated based on the survey results in the relevant region using the data on cooked food as much as possible and considering information obtained. Two methods are applied for calculation. Method 1 calculated the total intake using the estimated value of inorganic arsenic intake in each region as fixed value. Method 2 calculated the total intake expressing intake from food as a function of inorganic arsenic concentration in water used for cooking. In method 2, the estimated value of inorganic arsenic intake from food was calculated as a function of arsenic concentration in drinking-water.

$$\frac{\left(\text{Inorganic arsenic concentration in drinking-water (μg/L)} \times \text{Daily intake of water (L/day)}\right) + \text{Oral inorganic arsenic exposure from food (μg/day)}}{\text{Body weight (kg)}} = \text{Estimated daily intake (μg/kg bw/day)}$$

To estimate daily intake of inorganic arsenic from meal based on the reports which provided information on the intake of total arsenic but not on the intake of inorganic arsenic from meal, FSCJ assumed the proportion of inorganic arsenic in total arsenic in rice to be 0.7 (Rahman & Hasegawa 2011), 1 for vegetables and 0 for fishes. Regarding the intake of inorganic arsenic from food in which water used for cooking was not counted, FSCJ estimated the intake from cooked food assuming that 1.3 L/day of water was used for cooking in Asian region according to Watanabe et al (2004). Daily intake of water used for the estimation did not include water used for cooking. To estimate intake of water in Taiwan and Chile where relevant information was not available, FSCJ employed the value of 3 L/day for Taiwan and 1.5 L/day for Chile according to JECFA (2011) and EFSA (2009) respectively although the data include the amount of water for cooking. As for body weight used for estimation, the values that have been used for conversion by EFSA (2009) and JECFA (2011) were used. The values were 55 kg for West Bengal in India, Taiwan, Bangladesh and 60 kg for Chile. Table 19-22 shows the results of the calculation.

Regarding non-carcinogenic effects, LOAEL and $BMDL_{05}$ for dermal lesions were calculated to be 4.3-5.2 μg/kg bw/day and 4.0-4.2 μg/kg bw/day, respectively. For the effect on nervous system (decreased IQ), NOAEL and LOAEL were 3.0-4.1 μg/kg bw/day and 7.7-10.7 μg/kg bw/day, respectively. For the effect on reproduction and development, NOAEL and LOAEL were determined to be 8.8-11.1 μg/kg bw/day and 11.9-15.7 μg/kg bw/day, respectively.

Regarding carcinogenic effects, NOAEL and LOAEL for lung cancer were 4.1-4.9 μg/kg bw/day and 8.5-10.6 μg/kg bw/day, respectively. For urinary bladder cancer, NOAEL, LOAEL,

and $BMDL_{01}$ were 5.0-12.1 μg/kg bw/day, 11.5-16.0 μg/kg bw/day, and 9.7-13.5 μg/kg bw/day, respectively.

However, the above mentioned NOAEL, LOAEL, and BMDL are values calculated from the total intake which was estimated on the basis of insufficient data and many assumptions. In addition, there is a case where the value was calculated from the data on rice only, and the intake may have been underestimated throughout whole calculation. Furthermore, in the calculation for dermal lesions, many cases were with the contribution of drinking-water less than 50% of daily total intake. Various epidemiological surveys, where a dose-response relationship is considered between dermal lesions and the arsenic concentration in drinking-water, are assuming a high contribution of drinking-water on the other hand. This discrepancy seems to suggest that the calculation results may diverge from the actual status of exposure in the contaminated area.

Table18 Water intake used for estimating daily intake of inorganic arsenic in the area with contaminated water and data related to inorganic exposure from meal

| Country/region | Literature | Concentration in drinking-water (μg/L) | Water intake[*1] (L) | Total arsenic intake from meal (μg/day) | Inorganic arsenic intake from meal (μg/day) | Foods used for the calculation of intake | Cooked/uncooked | Arsenic intake from meal used for the estimation (μg/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Method 1 | | Method 2 |
| Bangladesh | Watanabe et al. 2004 | - | Male 3/Female 3 (Actual value) | Male 214/Female 120 | 127/83 (Converted from the total arsenic [*2]) | Rice, bread, potato | Uncooked 1.6L (man) and 0.95L (woman)(mean: 1.3L) of cooking water | - | | Wc×1.3(L) + (127+ 83)/2 |
| | Smith et al. 2006 | 200-500 | 3(2-4) (Actual value) | - | 114 | Only rice | Cooked | 114 | 82 (Mean) | - |
| | Kile et al. 2007 | 1.6 (Median) | Female 2.7 (Actual value) | Female 48 (Median) | 39 Converted from the total arsenic based on actual percentage of inorganic arsenic, 82%) | All (meal set for duplicate diet study) | Cooked | (39×127/83 + 39)/2 = 49[*3] | | - |
| Taiwan | Schoof et al. 1998 | - | - | - | 50 (15-200) | Yam, rice | Uncooked | 50 | | Wc×1.3(L)[*4] + 50 |
| Chile | Diaz et al. 2004 | 41/572 | - | - | 31/55 | Corn, vegetables, potato, bread | Cooked | (31+55)/2 = 43 | | 0.0452×Wc +29.1[*5] |
| West Bengal in India | Roychowdhury et al. 2002 | 133 (Mean of overall area) | (Male 4/Female 3) | 171/189 (2 villages) | 123/137 (Converted from the total arsenic [*2]) | Rice, vegetables, spices | Cooked | (123+137)/2 = 130 | | - |
| | Signes et al. 2008 | 50/250/500 (Hypothetical concentration) | (Assumed as 2.5) | - | 104/399/750 | Rice only | Arsenic intake was estimated from inorganic arsenic concentration in cooked rice. Rice is cooked with water with arsenic concentration of 50, 250, 500 μg/L. | - | | 1.434×Wc + 35[*6] |

Wc Inorganic arsenic concentration in drinking-water used in the thesis of dose-dependent assessment (μg/L)

*1 Cooking water is not included
*2 Assuming the proportion of inorganic arsenic in the total arsenic in rice, in vegetables, in fish as 0.7(Rahman & Hasegawa 2011), 1, 0, respectively
*3 Intake amount of male was calculated based on the rate of male and female intake (Watanabe et al. 2004) and male and female mixed mean was calculated
*4 The amount of cooking water in Bangladesh(male 1.6L,female 0.9L; mean 1.3L) was used since cooking water data in Taiwan could not be obtained
*5 Derive regression equation using 31, 55 μg/day as inorganic arsenic intake from meal where the drinking-water concentrations were 41,572 μg/L
*6 Derive regression equation using 104, 399, 750 μg/day as inorganic arsenic intake from meal where the drinking-water concentrations were 50,250,500 μg/L

Table 19 Summary of NOAEL, LOAEL, and BMDL (Non-carcinogenic effect: Method 1)

| | Literatures used for dose-dependent assessment | Country/region | Concentration in drinking-water (μg/L) | Water intake (L/day) | Daily intake from drinking-water(μg/day) | Daily intake from food (μg/day) | Daily intake (μg/day) | bw (kg) | Daily intake (μg/kg bw/day) | Geometric mean value (μg/kg bw/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| Dermal lesions | LOAEL | | | | | | | | | |
| | Haque et al. 2003 | India | 74.5 | 3.5 | 261 | 130 | 391 | 55.0 | 7.1 | 4.3 |
| | Ahsan et al. 2006 | Bangladesh | 24.1 | 3.0 | 72.3 | 82.0 | 154 | 55.0 | 2.8 | |
| | Chen et al. 2006 | Bangladesh | 70.6 | 3.0 | 212 | 82.0 | 294 | 55.0 | 5.3 | |
| | Rahman et al. 2006 | Bangladesh | 29.5 | 3.0 | 88.5 | 82.0 | 171 | 55.0 | 3.1 | |
| | $BMDL_{05}$ | | | | | | | | | |
| | Rahman et al. 2006 | Bangladesh | 54.1 | 3.0 | 162 | 82.0 | 244 | 55.0 | 4.4 | 4.0 |
| | Ahsan et al. 2006 | Bangladesh | 38.8 | 3.0 | 116 | 82.0 | 198 | 55.0 | 3.6 | |
| | Haque et al. 2003 | India | 26.2 | 3.5 | 91.7 | 130 | 222 | 55.0 | 4.0 | |
| Nervous system | NOAEL | | | | | | | | | |
| | Wasserman et al. 2004 | Bangladesh | 27.8 | 3.0 | 83.4 | 82.0 | 165 | 55.0 | 3.0 | 3.0 |
| | LOAEL | | | | | | | | | |
| | Wasserman et al. 2004 | Bangladesh | 113.1 | 3.0 | 339 | 82.0 | 421 | 55.0 | 7.7 | 7.7 |
| Reproduction and development | NOAEL | | | | | | | | | |
| | von Ehrenstein et al. 2006 (Stillbirth) | India | 125 | 3.5 | 436 | 130 | 566 | 55.0 | 10.3 | 8.8 |
| | Rahman et al. 2007 (Fetus death) | Bangladesh | 222 | 3.0 | 665 | 82.0 | 747 | 55.0 | 13.6 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Rahman et al. 2007 (Infant death) | | 220 | 3.0 | 660 | 82.0 | 741 | 55.0 | 13.5 | |
| Cherry et al. 2008 (Stillbirth) | Bangladesh | 30 | 3.0 | 90.0 | 82.0 | 172 | 55.0 | 3.1 | |
| LOAEL | | | | | | | | | |
| Milton et al. 2005 (Spontaneous abortion) | Bangladesh | 75.5 | 3.0 | 227 | 82.0 | 309 | 55.0 | 5.6 | 11.9 |
| von Ehrenstein et al. 2006 (Stillbirth) | India | 275 | 3.5 | 961 | 130 | 1093 | 55.0 | 19.8 | |
| Rahman et al. 2007 (Fetus death) | Bangladesh | 343 | 3.0 | 1029 | 82.0 | 1111 | 55.0 | 20.2 | |
| Rahman et al. 2007 (Infant death) | | 342 | 3.0 | 1026 | 82.0 | 1108 | 55.0 | 20.1 | |
| Cherry et al. 2008 (Stillbirth) | Bangladesh | 70 | 3.0 | 210 | 82.0 | 292 | 55.0 | 5.3 | |

Table 20 Summary of NOAEL, LOAEL, and BMDL (Non-carcinogenic effect: Method 2)

| | Literatures used in dose-dependent assessment | Country/region | Concentration in drinking-water (μg/L) | Water intake (L/day) | Daily intake from drinking-water (μg/day) | Intake from meal considering inorganic arsenic concentration in cooking water (μg/day) | Daily intake (μg/day) | bw (kg) | Daily intake (μg/kg bw/day) | Geometric mean value (μg/kg bw/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| Dermal lesions | LOAEL | | | | | | | | | |
| | Haque et al. 2003 | India | 74.5 | 2.5 | 186 | 142 | 328 | 55.0 | 6.0 | 5.2 |
| | Ahsan et al. 2006 | Bangladesh | 24.1 | 3.0 | 72.2 | 136 | 208 | 55.0 | 3.8 | |
| | Chen et al. 2006 | Bangladesh | 70.6 | 3.0 | 212 | 197 | 408 | 55.0 | 7.4 | |
| | Rahman et al. 2006 | Bangladesh | 29.5 | 3.0 | 88.5 | 143 | 232 | 55.0 | 4.2 | |
| | $BMDL_{05}$ | | | | | | | | | |
| | Haque et al. 2003 | India | 26.2 | 2.5 | 65.5 | 72.6 | 138 | 55.0 | 2.5 | 4.2 |
| | Ahsan et al. 2006 | Bangladesh | 38.8 | 3.0 | 116 | 155 | 272 | 55.0 | 4.9 | |
| | Rahman et al. 2006 | Bangladesh | 54.1 | 3.0 | 162 | 175 | 338 | 55.0 | 6.1 | |
| Nervous system | NOAEL | | | | | | | | | |
| | Wasserman et al. 2004 | Bangladesh | 27.8 | 3.0 | 83.4 | 141 | 225 | 55.0 | 4.1 | 4.1 |
| | LOAEL | | | | | | | | | |
| | Wasserman et al. 2004 | Bangladesh | 113 | 3.0 | 339 | 252 | 591 | 55.0 | 10.7 | 10.7 |
| Reproduction and development | NOAEL | | | | | | | | | |
| | von Ehrenstein et al. 2006 (Stillbirth) | India | 125 | 2.5 | 311 | 214 | 525 | 55.0 | 9.6 | 11.1 |
| | Rahman et al. 2007 (Fetus death) | Bangladesh | 222 | 3.0 | 665 | 393 | 1058 | 55.0 | 19.3 | |
| | Rahman et al. 2007 (Infant death) | | 220 | 3.0 | 659 | 390 | 1049 | 55.0 | 19.1 | |
| | Cherry et al. 2008 (Stillbirth) | Bangladesh | 30 | 3.0 | 90.0 | 144 | 234 | 55.0 | 4.3 | |
| | LOAEL | | | | | | | | | |
| | Milton et al. 2005 (Spontaneous abortion) | Bangladesh | 75.5 | 3.0 | 227 | 203 | 430 | 55.0 | 7.8 | 15.7 |
| | von Ehrenstein et al. 2006 (Stillbirth) | India | 275 | 2.5 | 686 | 429 | 1115 | 55.0 | 20.3 | |
| | Rahman et al. 2007 (Fetus death) | Bangladesh | 343 | 3.0 | 1028 | 550 | 1578 | 55.0 | 28.7 | |
| | Rahman et al. 2007 (Infant death) | | 342 | 3.0 | 1026 | 550 | 1576 | 55.0 | 28.6 | |
| | Cherry et al. 2008 (Stillbirth) | Bangladesh | 70 | 3.0 | 210 | 196 | 406 | 55.0 | 7.4 | |

Table 21 Summary of NOAEL, LOAEL, and BMDL (Carcinogenic effect: Method 1)

| | Literatures used in dose-dependent assessment | Country/region | Concentration in drinking-water (μg/L) | Water intake (L/day) | Daily intake from drinking-water (μg/day) | Daily intake from food (μg/day) | Daily intake (μg/day) | Body weight (kg) | Daily intake (μg/kg bw/day) | Geometric mean value (μg/kg bw/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lung cancer | NOAEL | | | | | | | | | |
| | Ferreccio et al. 2000 | Chile | 19.5 | 1.5 | 29.3 | 43.0 | 72.3 | 60.0 | 1.2 | 4.1 |
| | Chen et al. 2004 | Taiwan | 54.5 | 3.0 | 164 | 50.0 | 214 | 55.0 | 3.9 | |
| | Chen et al. 2010b (Accumulated mean in 40 years) | Taiwan | 75 | 3.0 | 225 | 50.0 | 275 | 55.0 | 5.0 | |
| | Chen et al. 2010b | | 200 | 3.0 | 600 | 50.0 | 650 | 55.0 | 11.8 | |
| | LOAEL | | | | | | | | | |
| | Ferreccio et al. 2000 | Chile | 39.5 | 1.5 | 59.3 | 43.0 | 102 | 60.0 | 1.7 | 8.5 |
| | Chen et al. 2004 | Taiwan | 200 | 3.0 | 600 | 50.0 | 650 | 55.0 | 11.8 | |
| | Chen et al. 2010b (Accumulated mean in 40 years) | Taiwan | 188 | 3.0 | 564 | 50.0 | 614 | 55.0 | 11.2 | |
| | Chen et al. 2010b | | 400 | 3.0 | 1200 | 50.0 | 1250 | 55.0 | 22.7 | |
| Bladder cancer | NOAEL | | | | | | | | | |
| | Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 75 | 3.0 | 225 | 50.0 | 275 | 55.0 | 5.0 | 5.0 |
| | Chen et al. 2010a | | 75 | 3.0 | 225 | 50.0 | 275 | 55.0 | 5.0 | |
| | LOAEL | | | | | | | | | |
| | Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 188 | 3.0 | 563 | 50.0 | 613 | 55.0 | 11.2 | 11.5 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chen et al. 2010a | | 200 | 3.0 | 600 | 50.0 | 650 | 55.0 | 11.8 | |
| $BMDL_{01}$ | | | | | | | | | |
| Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 140 | 3.0 | 420 | 50.0 | 470 | 55.0 | 8.5 | 9.7 |
| Chen et al. 2010a | | 186 | 3.0 | 558 | 50.0 | 608 | 55.0 | 11.1 | |

Table 22 Summary of NOAEL, LOAEL, and BMDL (Carcinogenic effect: Method 2)

| | Literatures used in dose-dependent assessment | Country/region | Concentration in drinking-water (μg/L) | Water intake (L/day) | Daily intake from drinking-water (μg/day) | Intake from meal considering inorganic arsenic concentration in cooking water(μg/day) | Daily intake (μg/day) | Body weigh t (kg) | Daily intake (μg/kg bw/day) | Geometric mean value (μg/kg bw/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lung cancer | NOAEL | | | | | | | | | |
| | Ferreccio et al. 2000 | Chile | 19.5 | 1.5 | 29.3 | 30.0 | 59.2 | 60.0 | 1.0 | 4.9 |
| | Chen et al. 2004 | Taiwan | 54.5 | 3.0 | 164 | 121 | 284 | 55.0 | 5.2 | |
| | Chen et al. 2010b (Accumulated mean in 40 years) | Taiwan | 75 | 3.0 | 225 | 148 | 373 | 55.0 | 6.8 | |
| | Chen et al. 2010b | | 200 | 3.0 | 600 | 310 | 910 | 55.0 | 16.5 | |
| | LOAEL | | | | | | | | | |
| | Ferreccio et al. 2000 | Chile | 39.5 | 1.5 | 59.3 | 30.9 | 90.1 | 60.0 | 1.5 | 10.6 |
| | Chen et al. 2004 | Taiwan | 200 | 3.0 | 599 | 309 | 908 | 55.0 | 16.5 | |
| | Chen et al. 2010b (Accumulated mean in 40 years) | Taiwan | 188 | 3.0 | 563 | 294 | 856 | 55.0 | 15.6 | |
| | Chen et al. 2010b | | 400 | 3.0 | 1200 | 570 | 1770 | 55.0 | 32.2 | |
| Bladder cancer | NOAEL | | | | | | | | | |
| | Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 75 | 3.0 | 225 | 440 | 665 | 55.0 | 12.1 | 12.1 |
| | Chen et al. 2010a | | 75.95 | 3.0 | 225 | 440 | 665 | 55.0 | 12.1 | |
| | LOAEL | | | | | | | | | |
| | Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 188 | 3.0 | 563 | 294 | 856 | 55.0 | 15.6 | 16.0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chen et al. 2010a | | 200 | 3.0 | 600 | 310 | 910 | 55.0 | 16.5 | |
| $BMDL_{01}$ | | | | | | | | | |
| Chen et al. 2010a (Accumulated mean in 40 years) | Taiwan | 140 | 3.0 | 420 | 232 | 652 | 55.0 | 11.9 | 13.5 |
| Chen et al. 2010a | | 186 | 3.0 | 558 | 292 | 850 | 55.0 | 15.5 | |

5. Summary and issues to be solved

According to the epidemiological survey in the area where the people drink arsenic contaminated water for a long period of time, non-carcinogenic effects of inorganic arsenic exposure such as dermal lesions, neurodevelopmental effect, and reproductive and developmental effects depending on the inorganic arsenic concentration in drinking-water were observed. Among these effects, the effect observed with the lowest concentration of inorganic arsenic was dermal lesions. The LOAEL and $BMCL_{05}$ were 7.6-124.5 μg/L and 19.5-54.1 μg/L, respectively.

Also, carcinogenesis (cancers of the lung, urinary bladder and others) and genotoxicity such as chromosomal aberration due to inorganic arsenic exposure was observed in human. The risk assessment of these substances has been conducted assuming that there was no threshold in carcinogenesis. However, in these years, the approach of recognizing threshold in carcinogenesis by indirect genotoxic substances which shows interaction such as chromosomal numerical abnormalities, oxidative stress, DNA-Synthesis inhibition, has been introduced. Also, it was suggested that even it was a direct genotoxic substance, actual threshold of carcinogenicity by direct genotoxic carcinogen could be specified if the strength of genotoxicity and involvement of mechanism of secondary interaction in carcinogenesis (producing an adduct by binding to DNA) was taken into account (Morita et al. 2005).

Since chromosome aberration is induced by arsenic in human, genotoxicity is suggested to be involved in the mechanisms for carcinogenesis caused by inorganic arsenic exposure. However, potential direct effects of arsenic on DNA cannot be confirmed from the currently available data. The data from animal studies in rodents suggest that, in the mechanism of carcinogenesis by arsenic exposure, DNA damage is caused by indirect interaction of arsenic with DNA rather than by direct interaction with DNA such as mutagenesis which produces DNA adduct. From this point of view, threshold mechanism may be assumed. However, the data is insufficient at this particular stage.

Taken together, FSCJ concluded that the situation does not allow us to discuss whether a threshold dose exists for carcinogenic exposure level.

In this assessment, FSCJ calculated the NOAEL (or LOAEL) or BMDL of inorganic arsenic by adding intake amount from meal to the concentration in drinking-water based on the above mentioned epidemiological survey of long-term intake of arsenic contaminated water. The result obtained were: LOAEL 4.3-5.2 μg/kg bw/day and $BMDL_{05}$ 4.0-4.2 μg/kg bw/day for dermal lesions; NOAEL 3.0-4.1 μg/kg bw/day for effect on nervous system (decreased IQ); NOAEL 8.8-11.1 μg/kg bw/day for effect on reproduction and development; NOAEL 4.1-4.9 μg/kg bw/day for lung cancer; NOAEL 5.0-12.1 μg/kg bw/day and $BMDL_{01}$ 9.7-13.5 μg/kg bw/day for cancer of the urinary bladder. However, in calculation of NOAEL or BMDL from the epidemiological survey based on the concentration in drinking-water, the intake may have been underestimated overall when the daily intake of inorganic arsenic was calculated from the concentration in drinking-water. Also, non-negligible deviation was considered to be contained in

the results since the exposure from food was relatively high even in some cases where exposure from drinking-water was also high, as shown in Table 19-22.

In contrast, the intake of inorganic arsenic of people living a normal life without any excessive exposure to arsenic by accident or contaminations were estimated from several duplicate diet studies in Japan. The mean intake thus estimated was 0.130-0.674 μg/kg bw/day. Of them, the data from the survey conducted by FSCJ (2013) provided the mean value of 0.315 μg/kg bw/day and 95 percentile value of 0.754 μg /kg bw/day.

The NOAEL and BMDL calculated in this assessment and the estimated intake of inorganic arsenic are considered to be with each uncertainty. However, the difference between them is not so big. Thus, some highly exposed Japanese may have taken inorganic arsenic higher than NOAEL or BMDL calculated in this assessment.

It is necessary to consider adequacy for estimating the total intake of inorganic arsenic from the arsenic concentration in drinking-water in the area contaminated with arsenic. It is also necessary to consider adequacy for applying the result of dose-dependent assessment based on this estimated value to the assessment of arsenic exposure conditions in Japan where the people hardly intake arsenic from drinking-water. In addition, there are still many issues to be solved for conducting the detailed risk assessment in which TDI and unit risk are evaluated by calculating NOAEL or BMDL. For example, the difference in dietary habit, environment, sanitary conditions, medical care system, and lifestyles, between Japan and areas with contaminated drinking-water are likely associated with several factors. And such factors may have implications for the difference in health effect outcomes of inorganic arsenic exposure. The Implications of smoking or other compounds in health effects observed in inorganic arsenic exposure are mostly unknown.

There are yet many factors which cannot be revealed by a risk assessment with the latest scientific knowledge, if findings on carcinogenic mechanisms and others that are required for a hazard assessment, or if the uncertainty of exposure assessment is high. Then, an estrangement between the estimation and reality shall be considered to be the consequence. If there is discrepancy between the result of hazard assessment and the current situation in Japan, the real situation shall be taken into consideration.

Therefore, it is necessary to reveal the actual status of exposure and the arsenic exposure from food in Japan to support the exposure assessment and dose-response data in future. Then, an epidemiological survey in the population who are exposed to a normal level in life and study of toxic mechanism need to be conducted. In addition, it is necessary to accumulate additional data on the toxicological effects of organic arsenic because the relevant data which contributes to risk assessment are yet insufficient.

<Appendix: Abbreviation etc.>

| | |
|---|---|
| AAS | atomic absorption spectrometry |
| ACE | angiotensin-converting enzyme |
| AFS | atomic fluorescence spectrometry |
| AES | atomic emission spectrometry |
| As(III) | trivalent arsenic |
| As(V) | pentavalent arsenic |
| AS3MT | trivalent arsenic methyltransferase |
| AsBe | arsenobetaine |
| AsC | arsenocholine |
| ATSDR | The Agency for Toxic Substances and Disease Registry (USA) |
| BBN | N-Butyl-N-butan-4-ol-nitrosamine |
| BMCL | 95% confidence limit of benchmark concentration |
| BMD | benchmark dose |
| BMDL | 95% confidence limit of benchmark dose |
| BMDS | bench mark dose software |
| BMI | body mass index |
| BMR | bench mark response |
| BUN | blood urea nitrogen |
| CAMA | calcium acid methanearsonate |
| Camk4 | $Ca^{2+}$/ calmodulin-dependent protein kinase IV |
| CHO Cell | Chinese hamster ovary cells |
| CI | confidence interval |
| CONTAM panel | The EFSA Panel on Contaminants in the Food Chain |
| CpG | cytosine phosphate guanine |
| DEN | N-Nitrosodiethylamine |
| DES | diethylstilbestrol |
| DMA(III) | dimethyl arsinous acid |
| DMA(V) | dimethyl arsenic acid (Cacodylic acid) |
| DMAE | thio-dimethyl arseno ethanol |
| DMBA | 9,10-Dimethyl-1,2-benzanthracene |
| DMBDD assay | multiorgan initiation assay |
| DPAA | diphenylarsinic acid |
| DSMA | disodium methanearsonate |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EPA | U.S. Environmental Protection Agency |
| EPA/IRIS | U.S. Environmental Protection Agency/Integrated Risk Information System |

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|---|---|
| ER-α | estrogen receptor-α |
| ETAAS | electrothermal atomic absorption spectrometry |
| EU | European Union |
| FAO | Food and Agriculture Organization of the United Nations |
| FFQ | Food Frequency Questionnaire |
| GC | gas-chromatography |
| HEALS | Health Effects of Arsenic Longitudinal Study |
| HEPO | high efficiency photo-oxidation |
| HGAAS | hydride generation atomic absorption spectrometry |
| HR | hazard ratio |
| HSD | hydroxysteroid dehydrogenase |
| IARC | International Agency for Research on Cancer |
| $IC_{50}$ | 50% inhibitory concentration |
| ICP | inductively coupled plasma |
| ICSC | International Safety Chemical Cards |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LC | liquid chromatography |
| $LD_{50}$ | median lethal dose |
| LE rat | Long Evans rat |
| LTP | long-term potentiation |
| LOAEL | lowest observed adverse effect level |
| LOD | limit of detection |
| LOEL | lowest observed effect level |
| LOQ | limit of quantitation |
| LPS | lipopolysaccharide |
| MAP | microtubule-associated protein |
| MMA(III) | methyl arsonous acid |
| MMA(V) | monomethyl arsonic acid |
| MOE | margin of exposure |
| MOS | margin of safety |
| MS | mass spectrometry |
| MSMA | sodium methylarsonate |
| MT | metallothionein |
| MT-null | metallothionein-I/II-knock out |
| NA | Not applicable |
| ND | Not detected |
| NEDO | New Energy and Industrial Technology Development Organization |
| NF-H | neurofilament protein heavy subunits |

| | |
|---|---|
| NF-L | neurofilament protein light subunits |
| NF-M | neurofilament protein medium subunits |
| NHANES | National Health and Nutrition Examination Survey |
| NOAEL | no observed adverse effect level |
| NOEL | no-observable-effect level |
| NTP | National Toxicology Program |
| OR | odds ratio |
| PAD | peripheral arterial disease |
| PO | photooxidation |
| PTWI | provisional tolerable weekly intake |
| RfD | reference dose |
| RR | relative risk |
| SAM | *S*-adenosyl-L-methionine |
| SCE | sister chromatid exchange |
| SD | standard deviation |
| SD rat | Sprague-Dawley rat |
| SE | standard error |
| SMR | standardized mortality ratio |
| TAM | tamoxifen |
| TDI | tolerable daily intake |
| TeMA | tetramethyl arsonium |
| TMAO | trimethyl arsine oxide |
| TPA | 12-*O*-tetradecanoylphorbol-13-acetate |
| TWI | tolerable weekly intake |
| UVR | ultraviolet rays |
| V79 cell | Chinese hamster lung fibroblast cell line |
| WHO | World Health Organization |

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