Methylated Metal(loid) Species in Humans
- Biodisposition and Toxicity -

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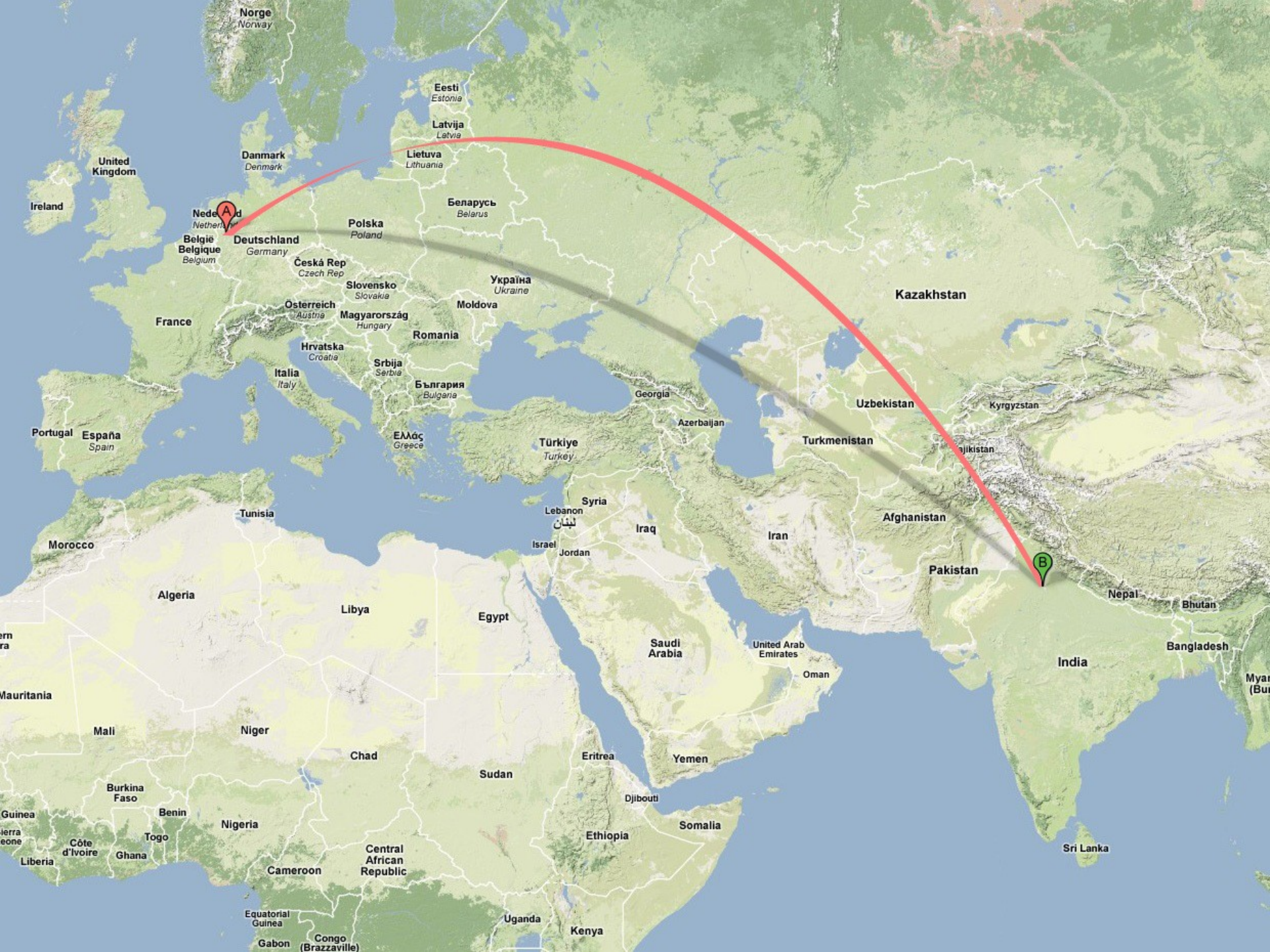
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Uganda
Kenya

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- Physical Examinations in Occupational and Environmental Medicine
- Routine Laboratory Investigations in Hygiene and Occupational Medicine
- Research Areas
  - Methods in Biological Monitoring of Industrial and Environm. Toxicants
  - In Vitro and Molecular Toxicology
  - Toxicoproteomics
  - MS Identification of Microorganisms

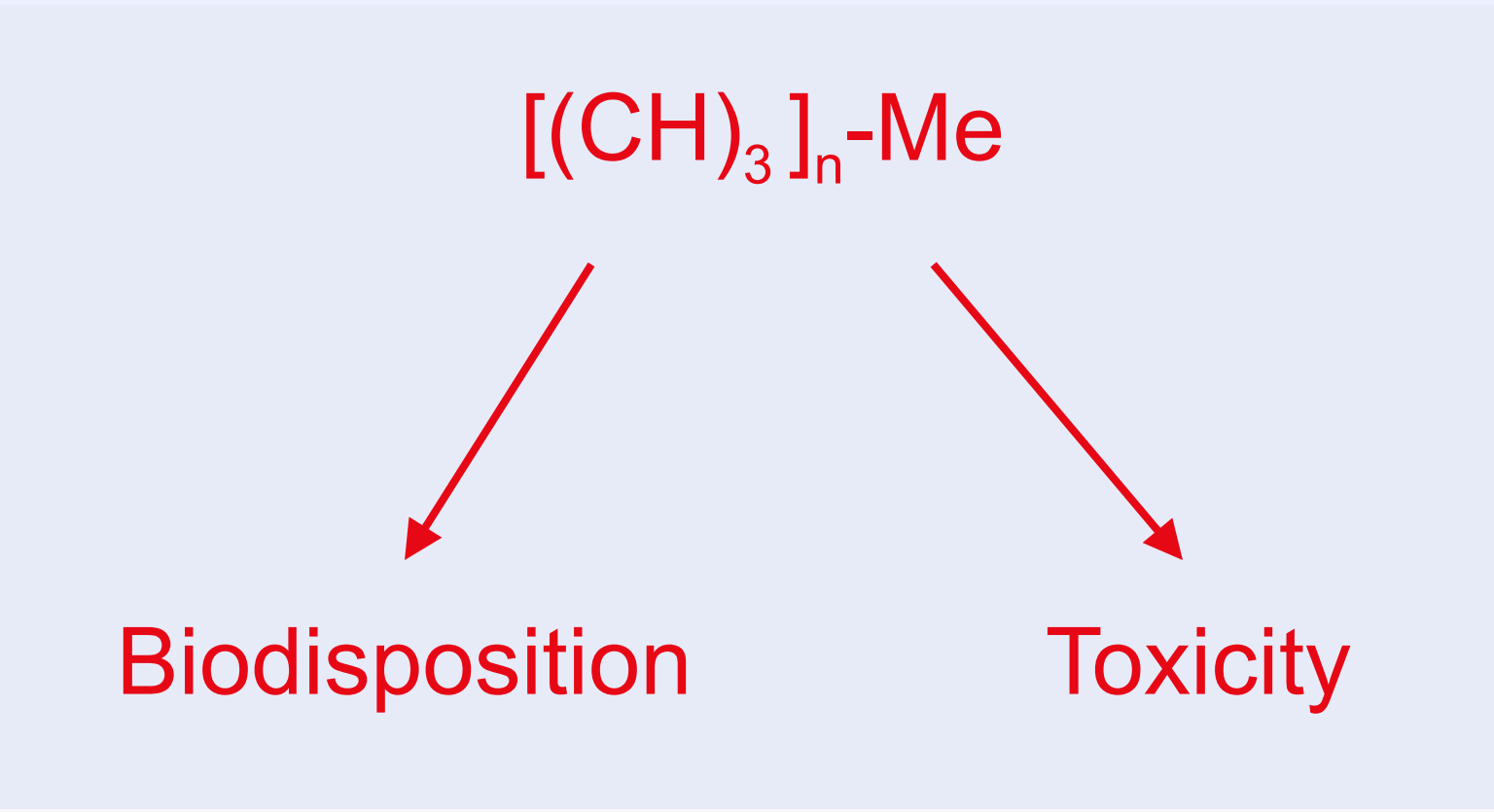
Indian-German Cooperation

Dr. Kunal Bhattacharya
Price winner at the Entox Meeting 2007
in Dortmund

Metal(loid)s - essentiality and toxicity

	Essentiality	Toxicity
Arsenic	trace element?, antibiotic, antiangioneogenetic properties	carcinogenicity
Bismuth	[drug for gastrointestinal disorders]	enzephalopathia
Calcium	trace element (e.g. bone metabolism)	hypercalcemia
Copper	trace element	Morbus Wilson
Iron	trace element (e.g. oxygen transport)	anemia, hemochromatosis, hemosiderosis
Magnesium	trace element	
Manganese	trace element (insulin production)	„Parkinsonism“
Mercury	[bactericidal effect]	neurotoxicity, nephrotoxicity
Selenium	trace element (glutathione peroxidase, thioredoxin reductase, deiodinases)	selenosis, diabestes type II, Keshan disease, Kaschin-Beck disease, hypothyroidism
Zinc	trace element (>100 enzymes)	hypozincemia

Specific aspect of metal(loid) toxicology:



„Cadet‘s liquid“

First synthesis by Louis Claude Cadet de Gassicourt (1760)

$As_2O_3 + CH_3COOK$ → $(CH_3)_4As_2$ + $(CH_3)_4As_2O$ + ...

cacodyl — cacodyloxide

Structur determination by

Robert Wilhelm Bunsen
(1811-1899)

„Gosio‘s gas“

since ~1780 Scheele‘s green (Cu arsenite)/Emerald green (Cu aceto arsenite) as pigments in wallpapers

Wallpaper of Napoleon‘s house in St. Helena

1839 Gmelin: Reports on intoxications in „Arsenic rooms“ (cacodyl oxide?)

1893 Gosio: Production of volatile alkylated arsenic species from As_2O_3 by moulds (e. g. *Scopulariopsis brevicaulis*)

1945 Challenger: „Gosio‘ gas“ = trimethylarsine (Me_3As) (concept of biological methylation)

„Minamata disease“

1953 First reports on cases of illnesses in Minamata

1956 Identification of MeHgSMe in *Hormomya mutabilis*

Causes: a) MeHgCl-containing industrial waste waters

b) Biomethylation of HgX_2 in sediments *(Jensen, Jernelöv, 1979)*

Additional cases: 1964 Japan 1973 Japan, Canada

>2250 cases of intoxication

- ataxia
- reduced visual field
- dysarthria
- tremor
- sensory disturbance
- dementia

>100 fatalities

Hg concentrations in hair up to 259 ppm

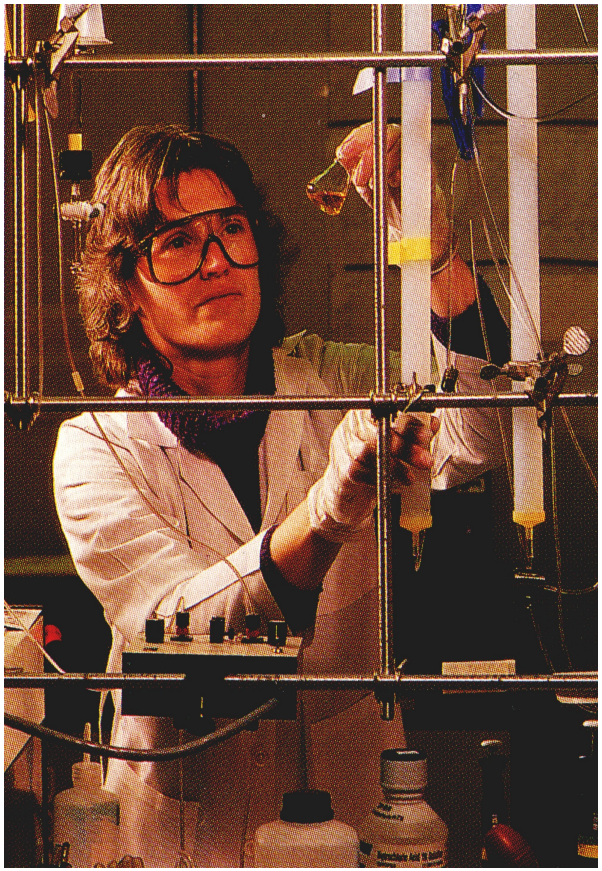
Congenital „Minamata disease“

Transport of MeHgX across the placenta

⇒
- microcephaly
- hyperreflexia
- motoric disorders
- amaurosis, deafness
- growth retardation
- mental retardation (IQ ⇓)
- intrauterine fetal death

Fetuses 4-10 times more susceptible to MeHg than adults

The Wetterhahn Case



Karen Wetterhahn

(Scientific American 45, 222, 1997)

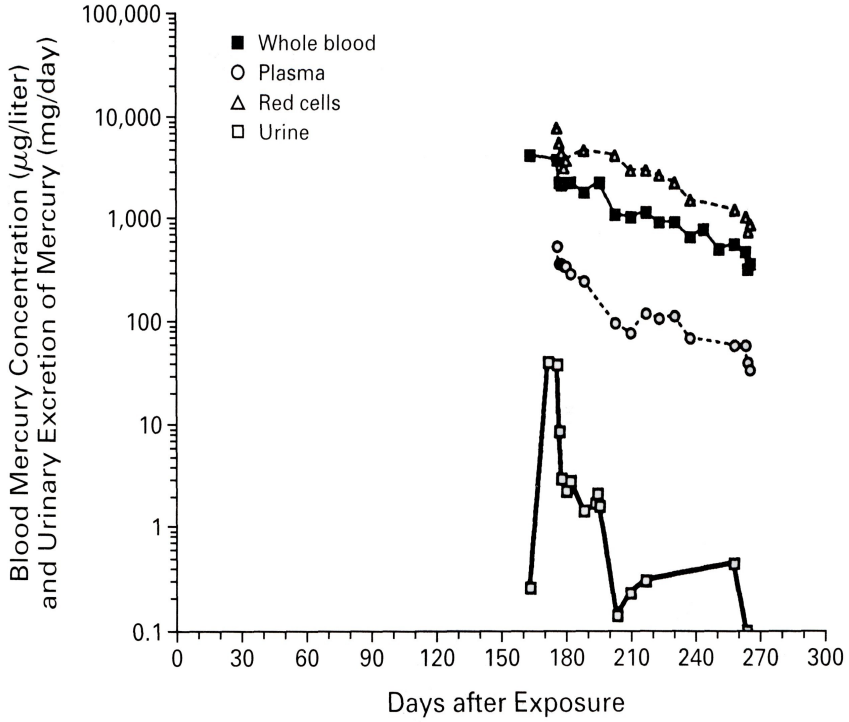
August 14th 1996 spillage of 0,1-0,5 ml Me_2Hg on the hand covered with latex gloves

November 1996 nausea, vomiting

January 1997 dysarthria, hearing disorder, impaired vision, paresthesias, impaired coordination

February 1997 coma

Exitus 298 days after the exposure



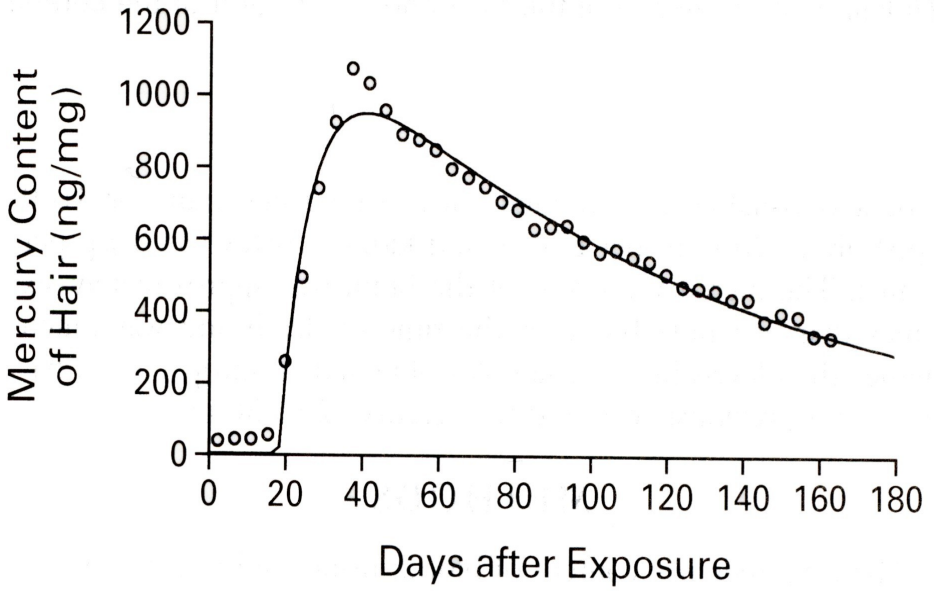
January 1997:

Hg_{blood}: 4 mg/l (normal <10 µg/l)

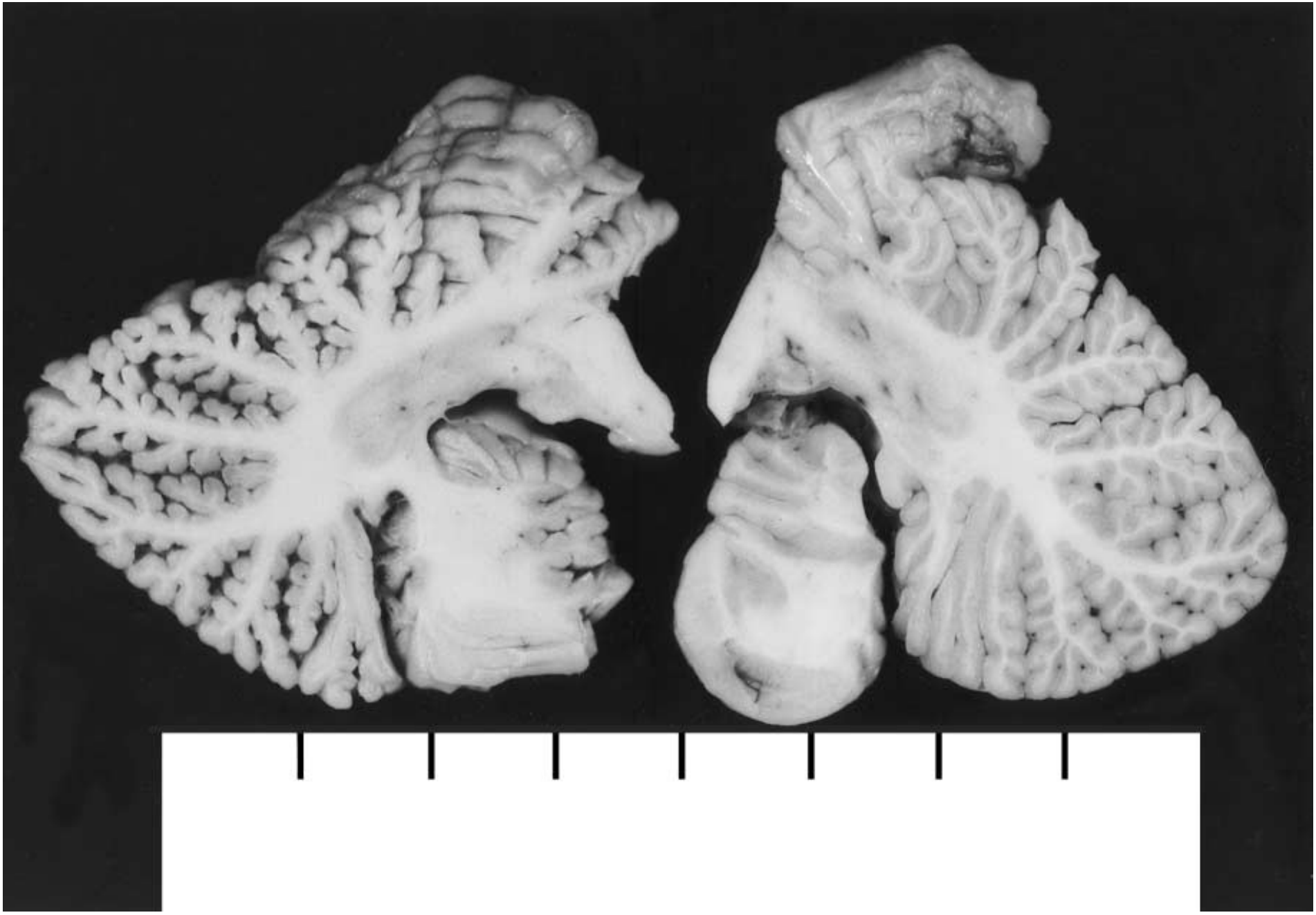
elimination after DMSA application:
400 mg Hg/day
(normally <0,01 mg Hg/day)

Hair analysis:
maximum value 1100 ng/mg

half life 75 days



(N. Engl. J. Med. 338, 1672, 1998)



(N. Engl. J. Med. 338, 1672, 1998)

- Some aspects of arsenic genotoxicity concerning methylated arsenic species

- Formation of methylated metal(loid) derivatives in humans

„Biomethylation of inorganic arsenic to monomethyl-arsonous acid (MMA^V) and dimethylarsinic acid (DMA^V) is a mechanism leading to detoxification“

Casarett & Doull, Toxicology, 1994

- DMA^V: LD_{50} (rat) = 2600 mg/kg
  Arsenite: LD_{50} (rat) = 41 mg/kg
- More rapid elimination of MMA^V und DMA^V compared to inorganic arsenic

  $\Rightarrow$ lower acute toxicity of MMA^V and DMA^V compared to inorganic arsenic compounds

Toxic effects of MMA^V and DMA^V

- Induction of genotoxic and clastogenic effects in mammalian cells by MMA^V und DMA^V

- Tumor promoting effect of DMA^V on bladder, kidney, liver, and thyroid carcinogenesis in rats

(Yamamoto et al., 1995)

- Carcinogenic or cocarcinogenic activity of DMA^V in skin and bladder of mice

(Huff et al., 2000; Kitchin, 2001)

Toxic effects of MMA^{III} and DMA^{III}

- Interaction of MMA^{III} and DMA^{III} with proteins and DNA

  (Kitchin, 2001)

- Cyto- and genotoxicity as well as inhibitory effects on enzymes: MMA^{III} and DMA^{III} > As_i^{III}

  (Petrick et al., 2000; Styblo et al., 2000; Thomas et al., 2001)

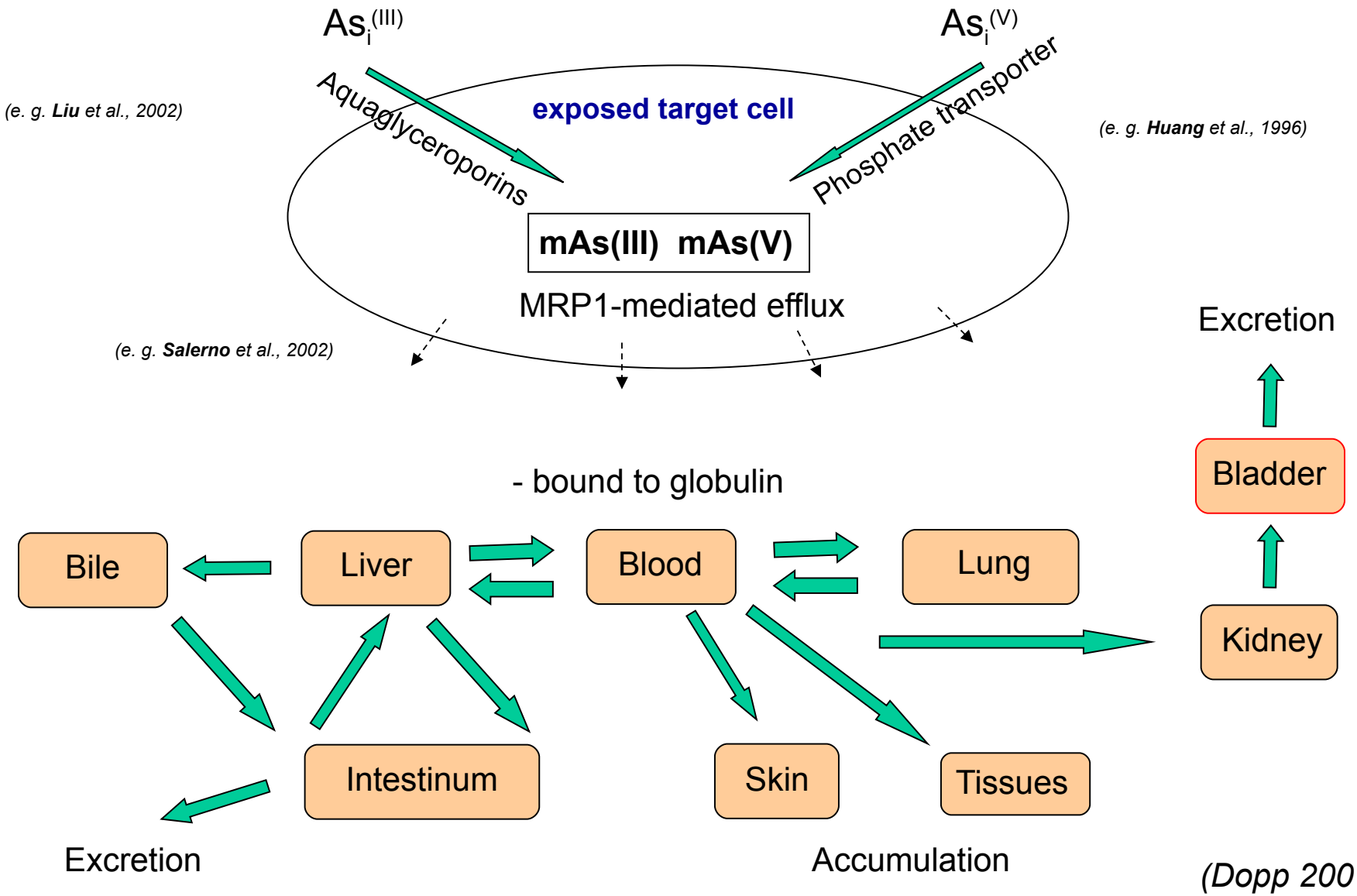
- Comet-assay (*in vitro*): DMA^{III} >>> MMA^{III} >> As_i^{III}

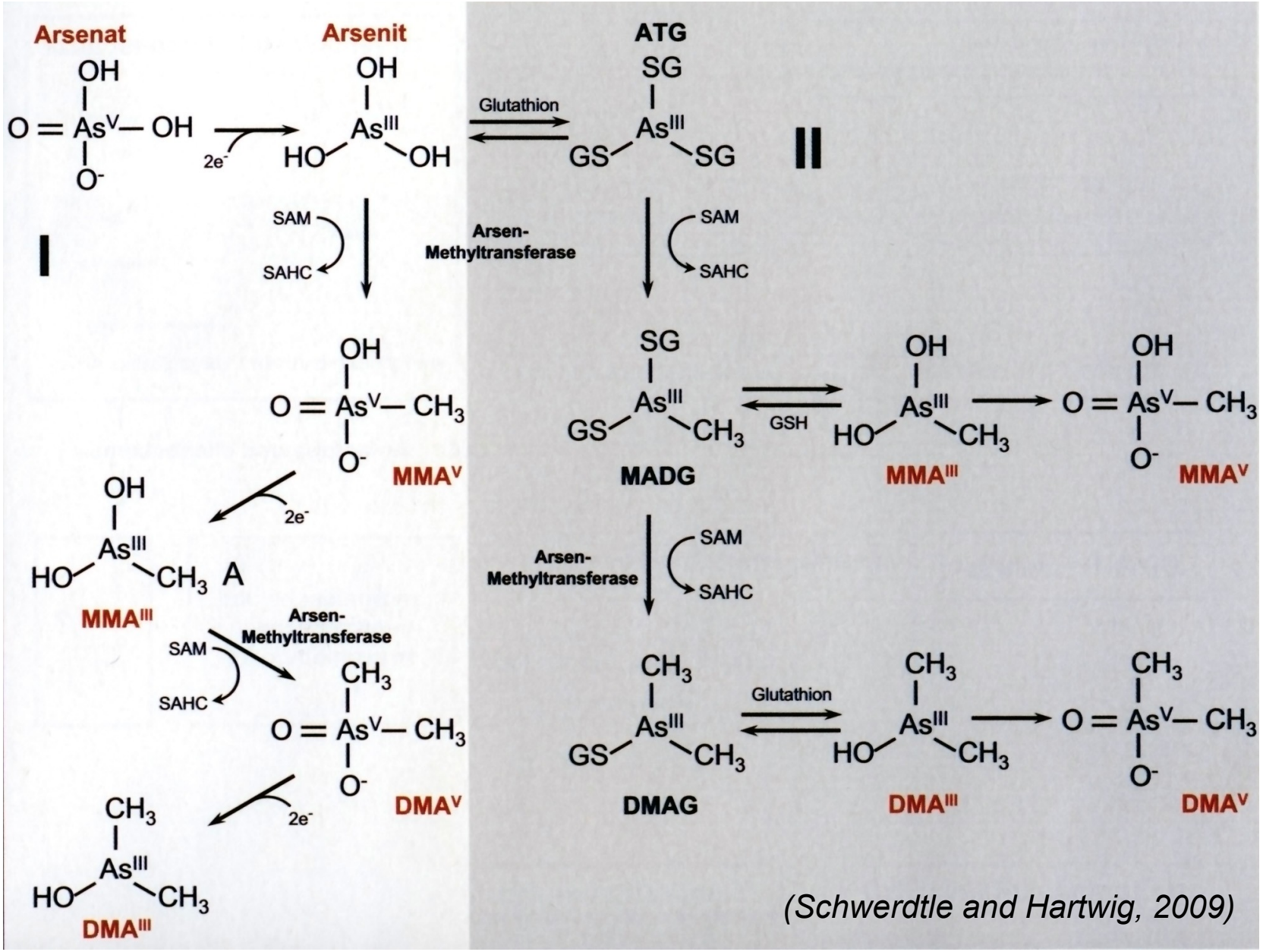
  (Mass et al., 2001)

- Induction of micronuclei in CHO cells

  (DMA^{III} > MMA^{III} > Asi^{III} > MMA^{V} > DMA^{V} > $TMAO^{V}$)

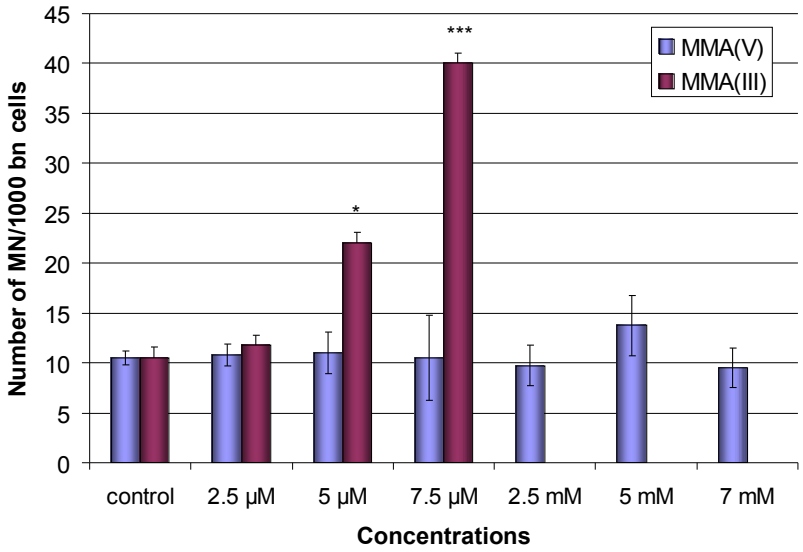
Biodisposition of arsenic

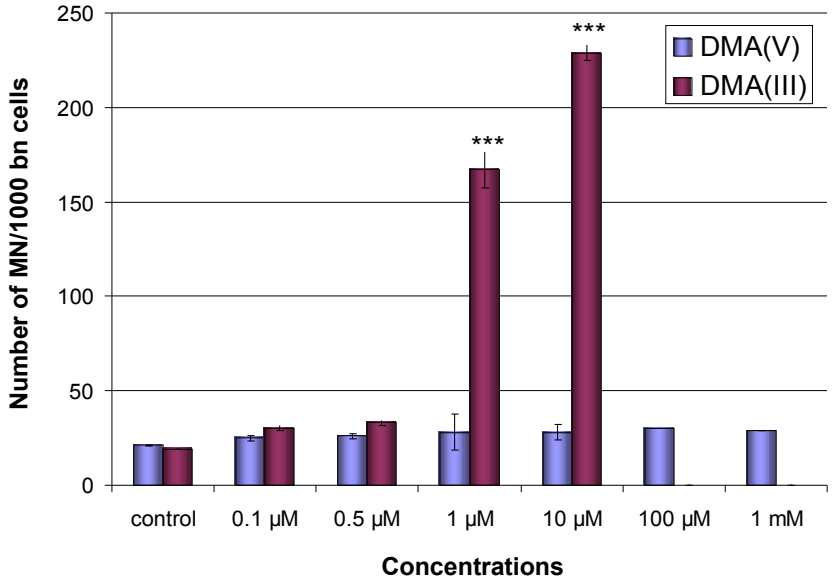


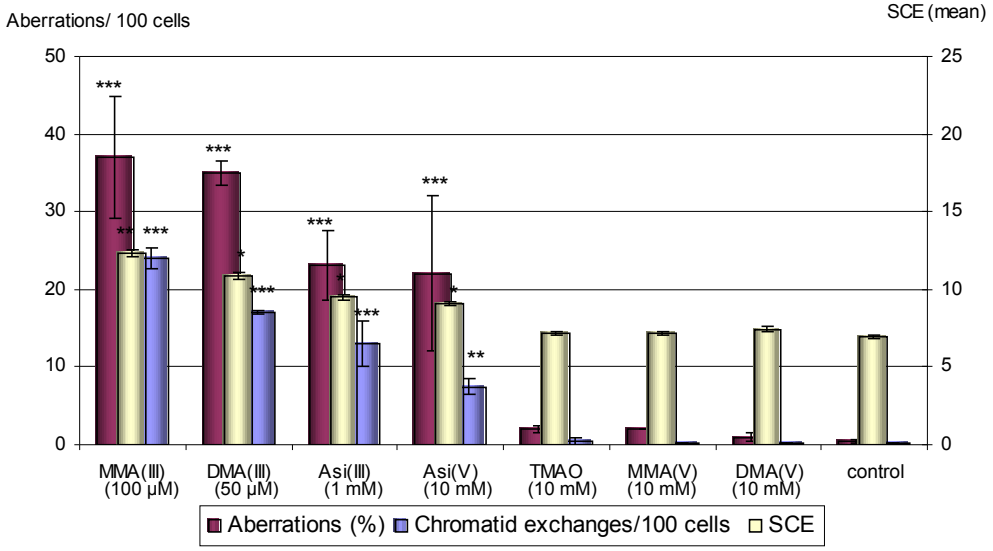


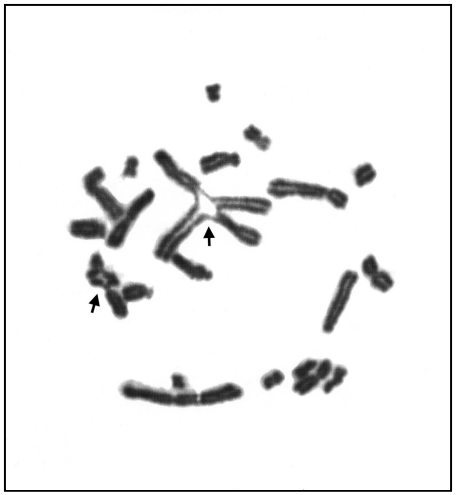
Metabolism of arsenic in humans. I: Pathway according to Challenger (1945)
II: Pathway accoridng to Hasegawa (2005)

Genotoxic effects in fibroblasts (CHO cells)





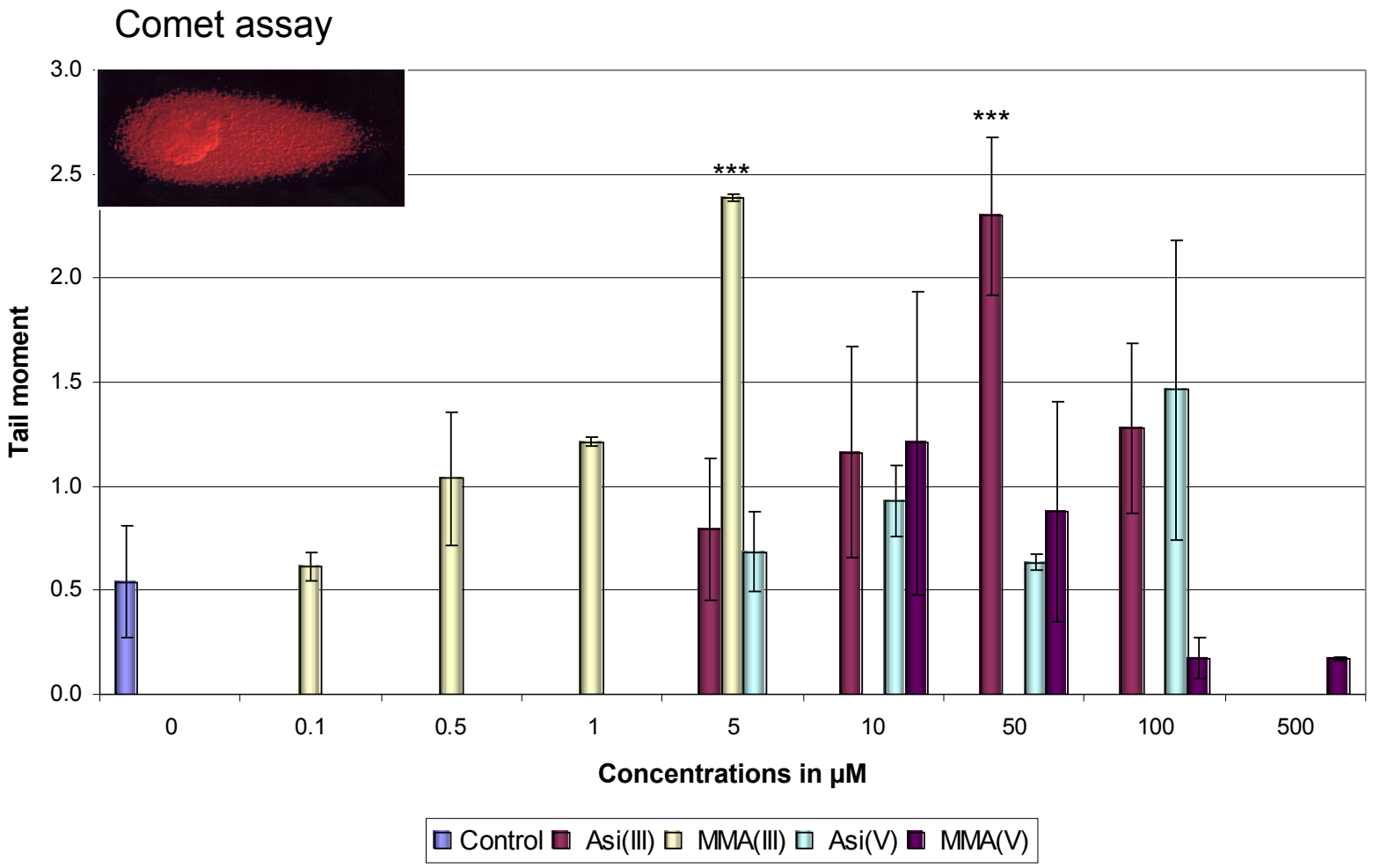




Chromatid translocation after exposure of CHO cells to MMA(III) (50 µM, 30 min)

(Dopp et al., 2004)

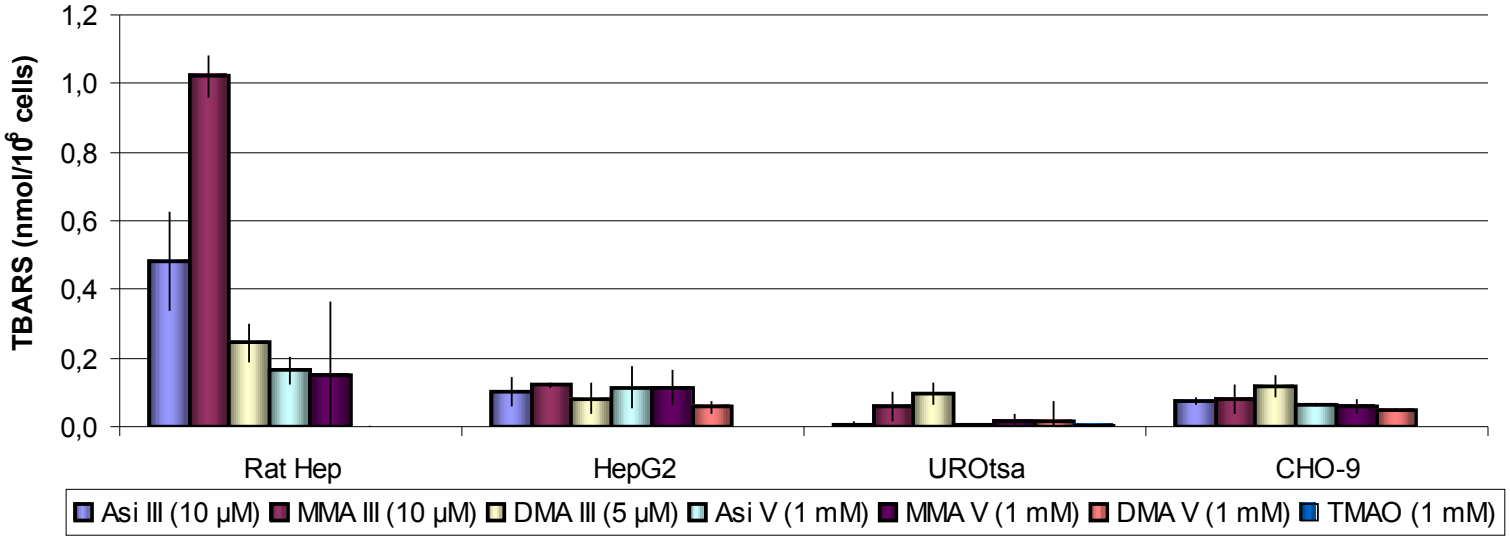
Genotoxic effects in primary human hepatocytes



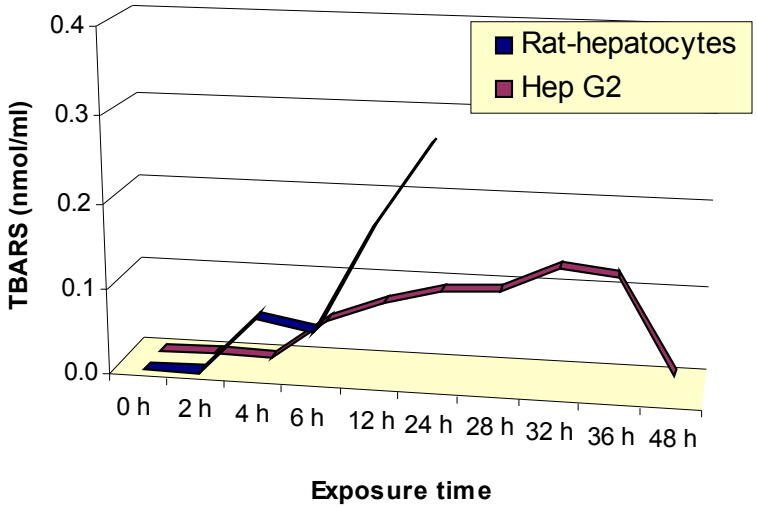
- significant induction of genotoxic effects by trivalent arsenic compounds

(Dopp et al., 2008)

Intracellular radical formation

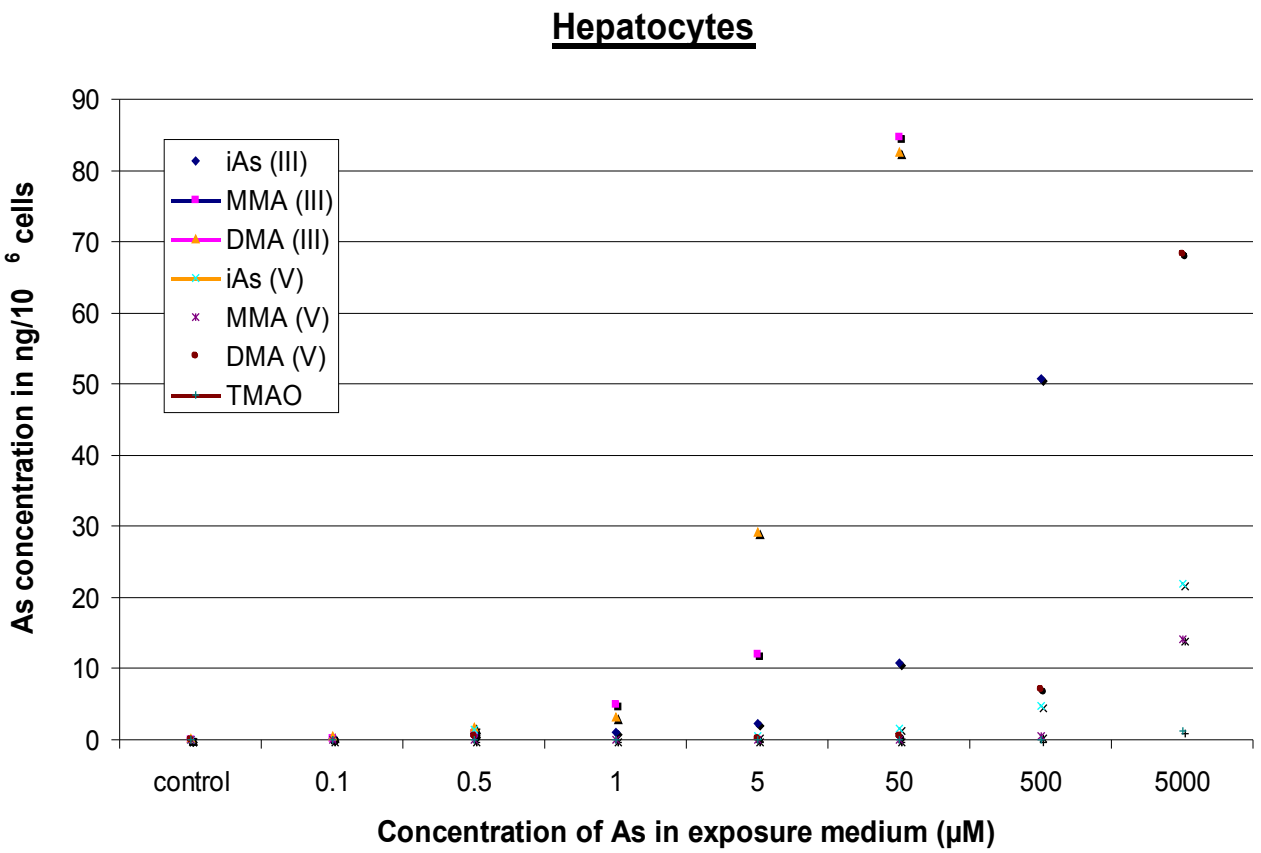


- Cell type-specific differences in radical formation [release of malon-dialdehyde (MDA)]
- Highest radical formation in primary hepatocytes
- Time-dependent formation of MDA [MMA(III)]



(Dopp, 2007)

Cellular uptake (absolute)



- Concentration-dependent uptake of arsenic compounds (exposure time: 1 h)
- Trivalent methylated compounds are better taken up than pentavalent arsenic compounds

(Dopp et al., 2008)

Cellular uptake (relative)

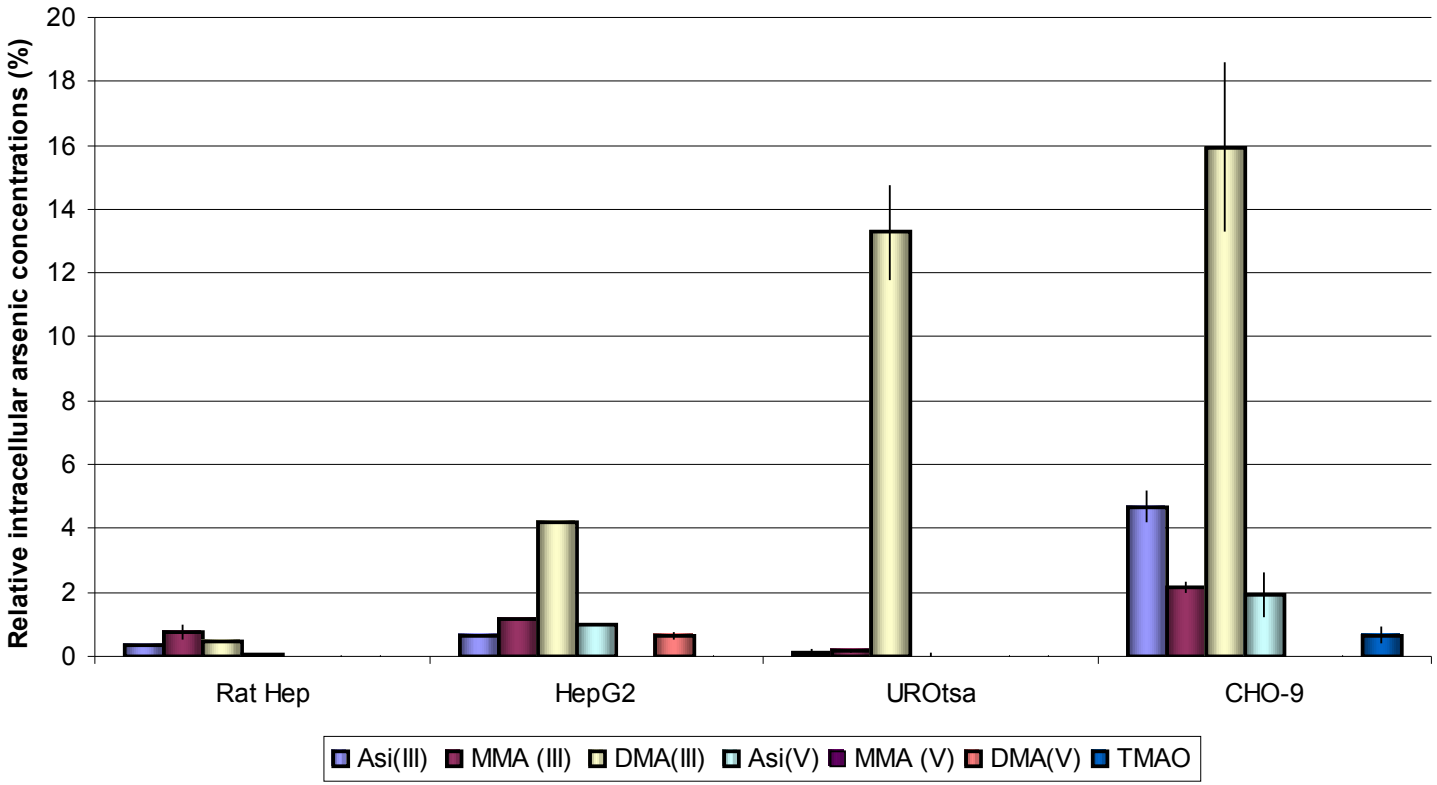
CHO cells

Concentration of arsenic in treatment solution (μM)	Intracellular concentration of arsenic expressed as % of dosed arsenic concentration						
	As_i (III)	As_i (V)	MMA(III)	MMA(V)	DMA(III)	DMA(V)	TMAO
0.1	-	-	-	-	0.80	-	-
0.5	1.20	1.17	0.37	-	9.98	n.d.	-
1	0.48	1.58	0.38	0.02	7.30	-	n.d.
5	-	-	-	-	6.67	-	-
10	0.78	0.41	1.10	n.d.	6.14	n.d.	0.13
25	-	-	2.19	-	-	-	-
50	-	-	1.58	-	-	-	-
100	0.41	0.28	-	0.03	-	0.01	0.01
500	0.19	0.14	-	0.01	-	0.02	0.01
1000	-	0.05	-	-	-	0.02	n.d.
10000	0.10	0.03	-	-	-	-	-

- is dependent upon the cell type (membrane permeability) and the arsenic species

(Dopp et al., 2004)

Uptake capabilities of methylating and non-methylating cells



Rat Hep: primary hepatocytes (rat)
HepG2: human hepatoma cells

methylating cells

UROtsa: human urothelial cells (virus-transformed)
CHO-9: fibroblasts (hamster)

non-methylating cells

Non-methylating cells are able to accumulate arsenic compounds to a higher extent than methylating cells (active extrusion)

(Dopp et al., 2005)

Summary / Conclusion (I)

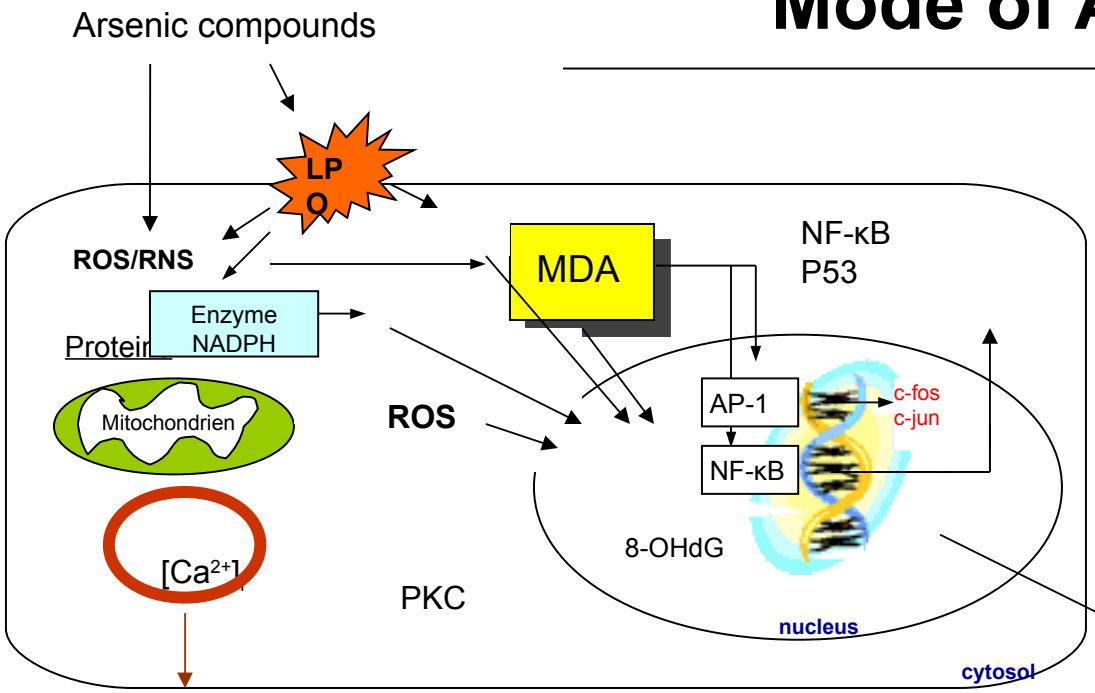
⇒ Cell type-specific differences in uptake and retention of arsenic compounds as well as in cytotoxic effects and intracellular radical formation

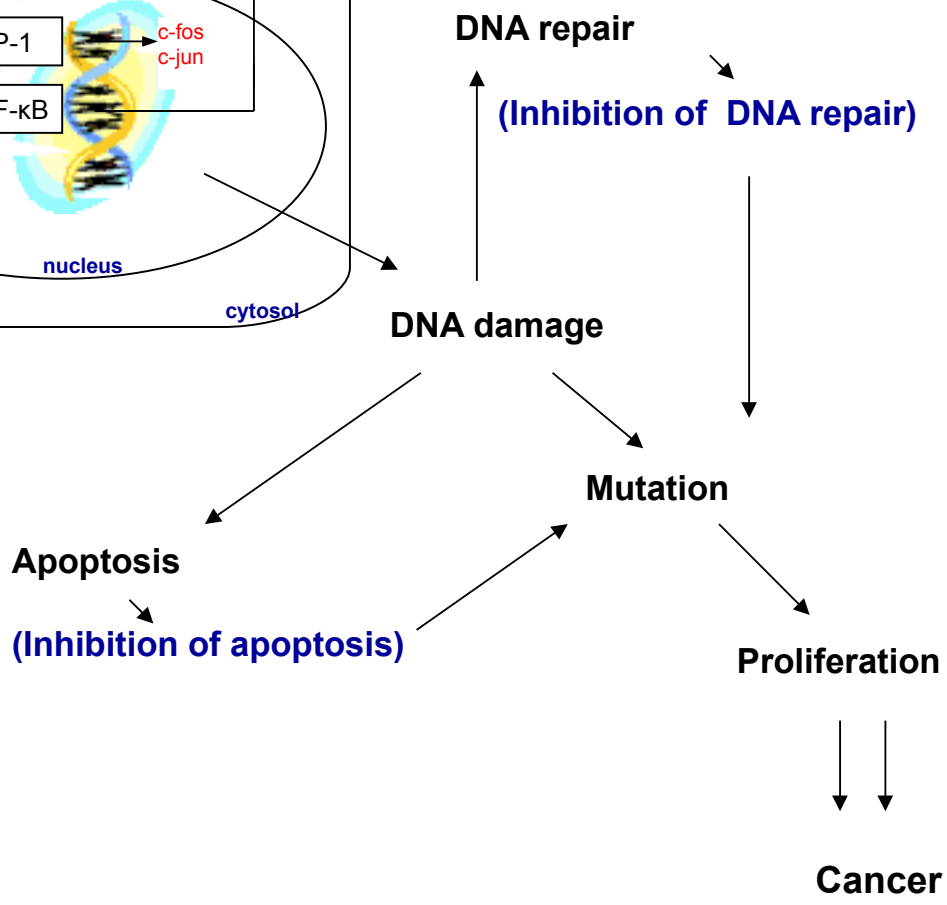
⇒ Trivalent methylated arsenic species were best membrane-permeable in all investigated cell types

⇒ Trivalent methylated arsenic compounds are the most genotoxic arsenic species in human hepatocytes

⇒ The high toxicity of trivalent methylated arsenic species appears to be – at least in part – a consequence of the high uptake

Mode of Action





Hypothesis:

- Oxidative damage of lipids, proteins, DNA
- Activation of transcription factors
- Influence of DNA methylation

Exposure to MMA^{III} and DMA^{III} *in vivo*?

- Detection of MMA^{III} (up to 240 µg/l) and DMA^{III} in urine samples after administration of DMPS to arsenic-exposed people in Inner Mongolia

  (Le et al., 2000; Aposhian et al., 2000)

- Detection of MMA^{III} in urine samples of arsenic-exposed people in Romania

  (Aposhian et al., 2000)

- Detection of DMA^{III} in urine samples of arsenic-exposed people in West Bengal

  (Mandal et al., 2001)

- Detection of MMA^{III} in urine samples of children in Brazil (multi-step analytical approach)

  (Hirner, 2006; Rabieh et al., 2008)

Concentration (µg/L) of metal(loid)s with proven methylation potential in the environment in blood of humans *(Goulle et al., 2005; Heitland and Köster, 2006)*

Metal(loid)			Biomethylation in Humans
	Germany	France	
Antimony	<0.01 – 0.1	0.05 – 0.13	(+)
Arsenic	0.1 - 4	3 - 18	++
Bismuth	<0.01 – 0.02	0.001 – 0.007	+
Cadmium	0.1 - 4	0.1 - 2	?
Germanium	-	11 - 20	?
Indium	<0.01 – 0.02	0.9 - 8	?
Lead	5 - 83	11 - 63	?
Mercury	0.02 - 16	0.9 - 8	?
Selenium	85 - 182	89 - 154	++
Tellurium	<0.14	0.11 – 0.45	(+)
Thallium	<0.01 – 0.05	0.01 – 0.04	?
Tin	0.02 – 0.8	0.1 – 1.8	?

Biotransformation of metall(oid)s by microorganisms in the environment (examples)

- Oxidation

  As(III) ➔ As(V) by *Bacillus spp.*, *Pseudomonas spp.*, *Alcaligenes faecalis*

  Fe(II) ➔ Fe(III) by *Thiobacillus ferrooxidans*

- Reduction

  Cr(VI) ➔ Cr(III) by *Enterobacter cloacae*, *E. coli*

  As(V) ➔ As(III) by *Micrococcus aerogenes*, *Alcaligenes spp.*, *Pseudomonas spp.*

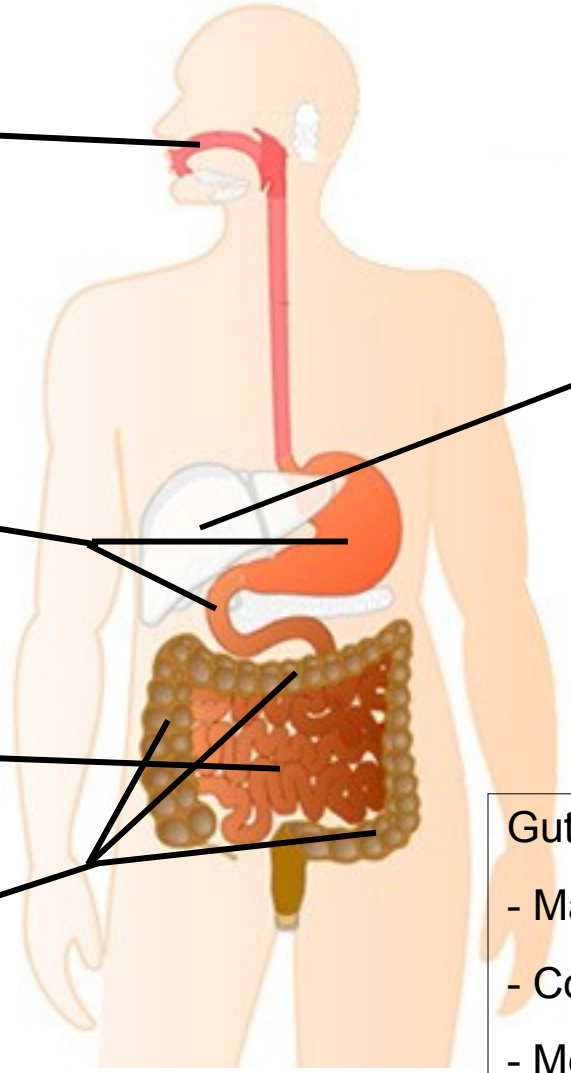
  Hg(II) ➔ Hg(0) by *Cryptococcus spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*

- Methylation

  As_i(V, III) ➔ $Me_{1\text{-}2}As$(V) and $Me_{1\text{-}3}As$(III) by *Aeromonas spp.*, *E. coli*, *Flavobacterium spp.*, *Methanobact. spp.*

  Hg^{2+} ➔ $MeHg^{+}$ by *Desulfovibrio desulfuricans*

Microbial production of methylated metal(loid)s in the human intestine?



Oral cavity
approx. 10^{11} germs
approx. 200 species

Stomach and duodenum
approx. 10^{1}-10^{3} germs
approx. 50 species

Small intestine
approx. 10^{4}-10^{8} germs
approx. 100 species

Colon
approx. 10^{14}-10^{15} germs
approx. 400-500 species

or

Methylation in the liver?

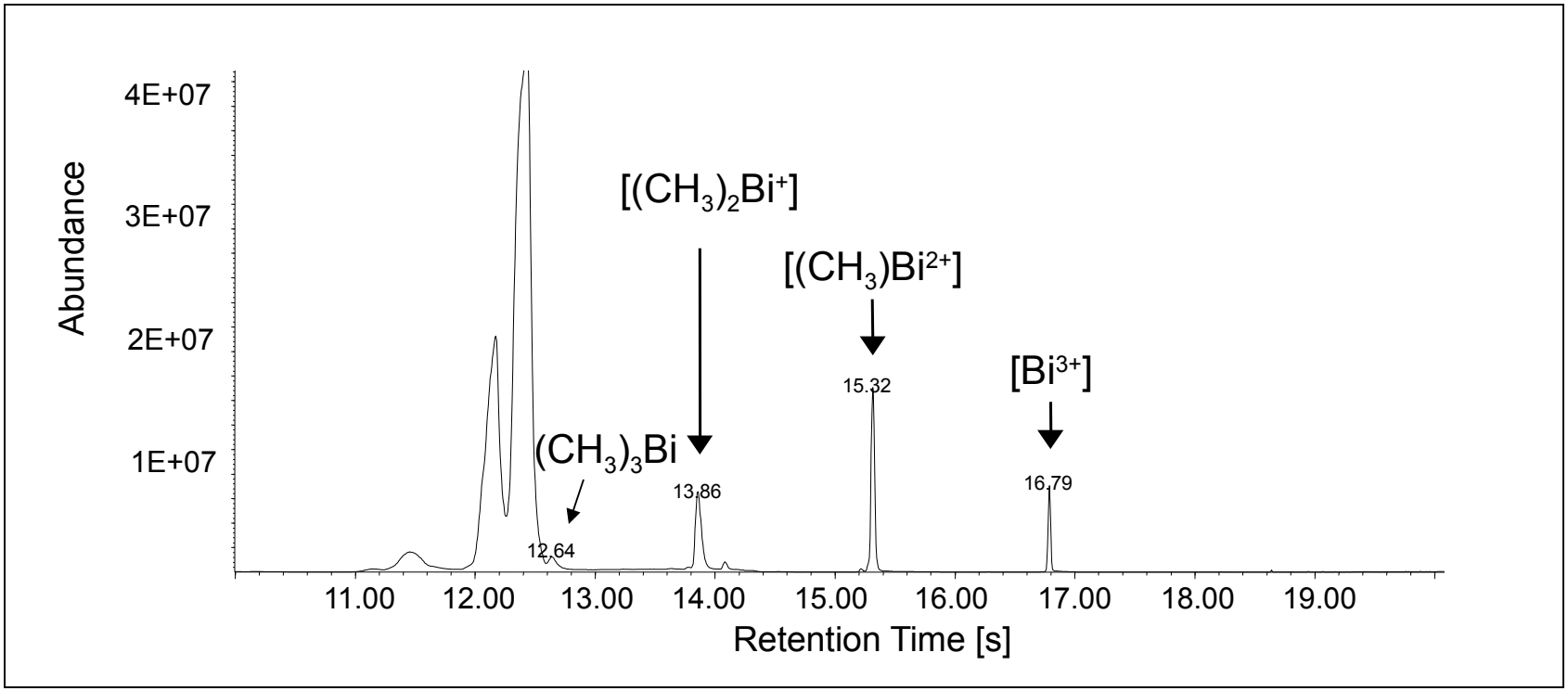
Gut flora

- Mass approx. 700 g
- Colonizing area approx. 400 m^2
- Metabolic potential comparable to liver

„Model Bismuth“

- Bismuth compounds used as drugs ⇒ „low“ toxicity
- Almost exclusively excreted *via* feces
- Methylation of bismuth by bacteria in the environment

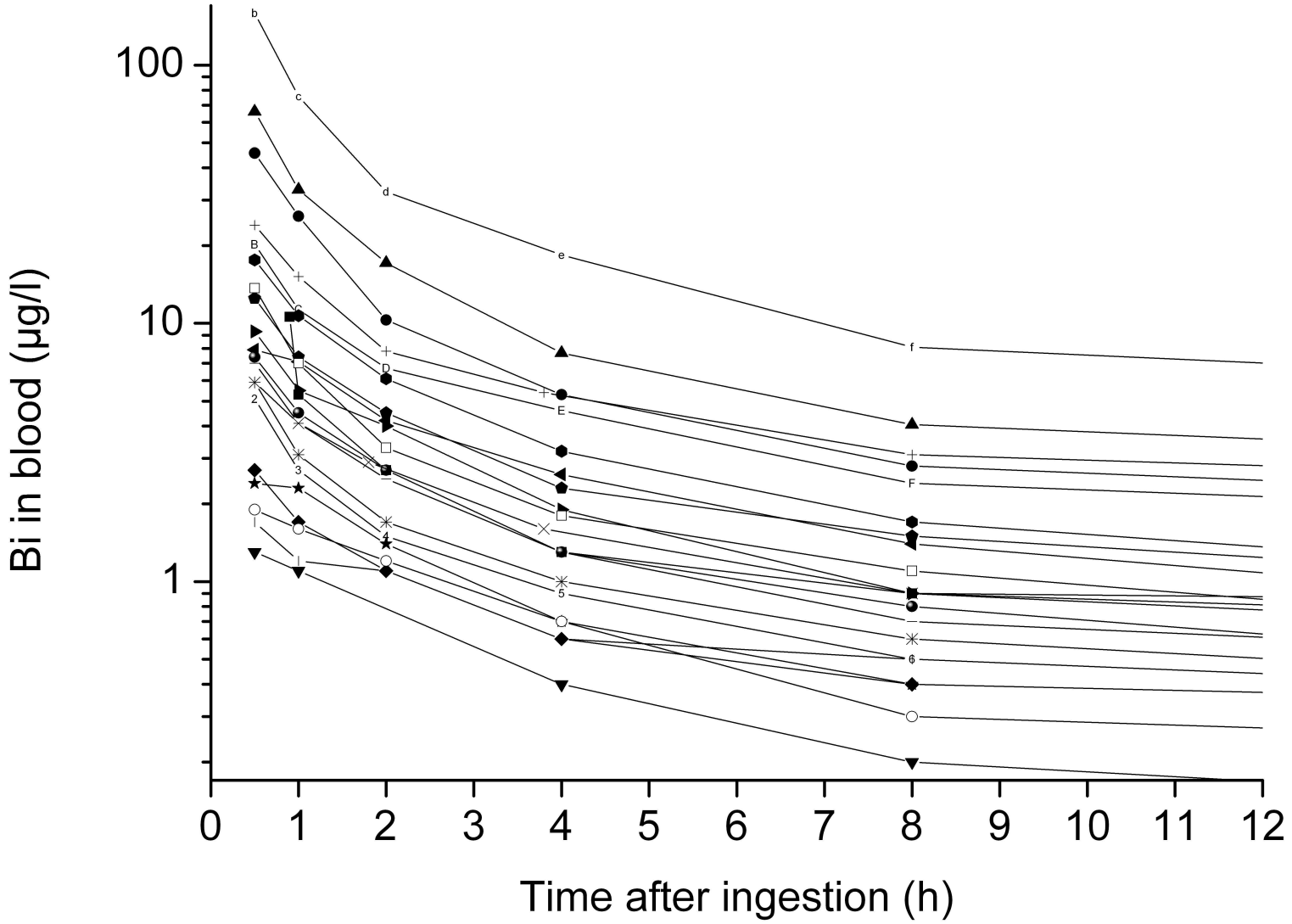
Example: Biotransformation of Bismuth by *Methanobacterium formicicum*



(Michalke u. Hensel, 2003)

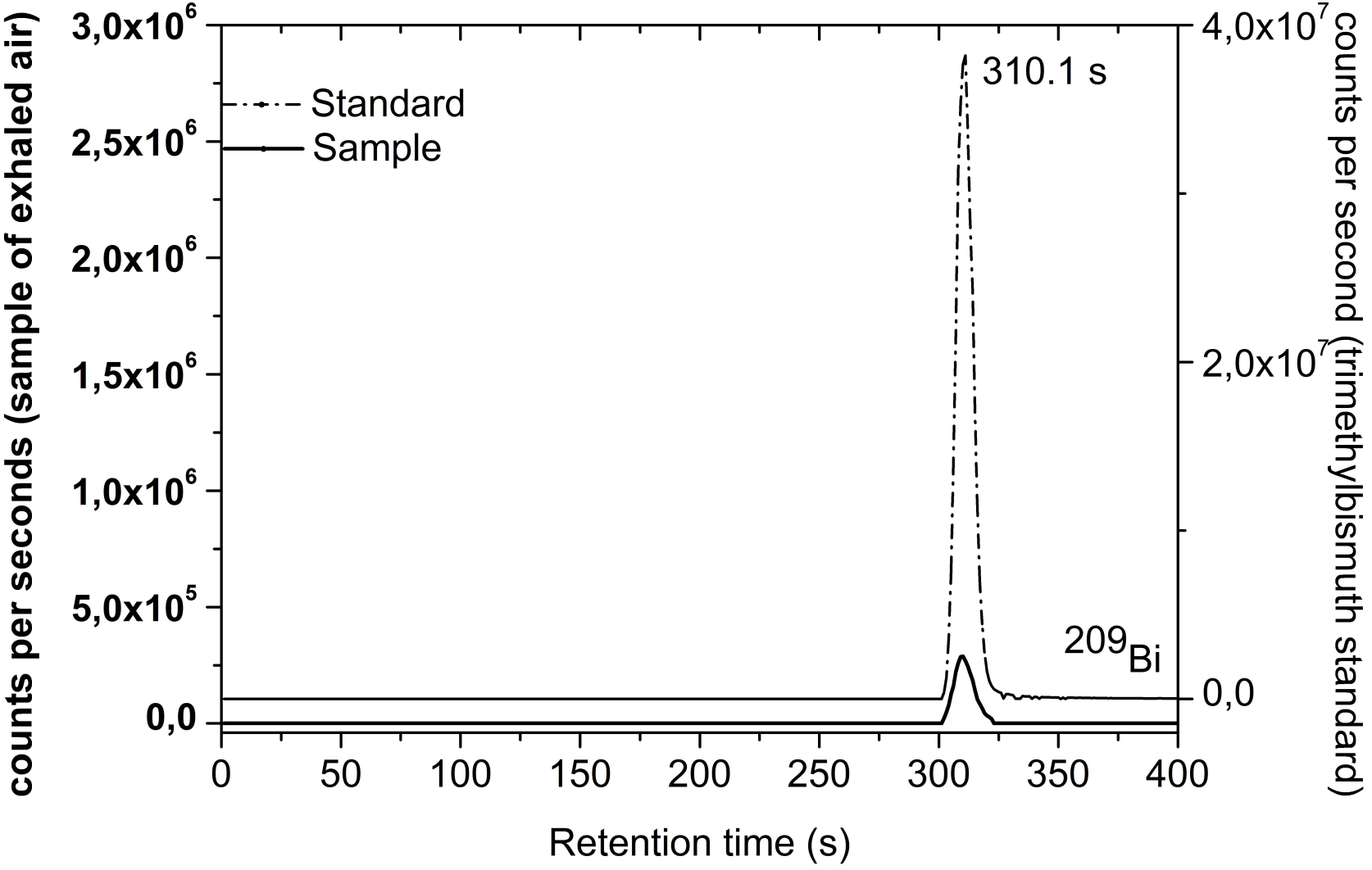
Volunteer study for the investigation of biomethylation of bismuth in the human intestine

Study protocol												
Day	- x	0							+ 1		+ 2	
Time		8:30	9:00	9:30	10:00	11:00	13:00	17:00	9:00	17:00	9:00	17:00
(hrs)		- 0,5	0	+ 0,5	+ 1	+ 2	+ 4	+ 8	+ 24	+ 32	+ 48	+ 56
	Screening	Exhaled air	Application of tablets (240 mg bismuth subcitrate = 215 mg Bi)	Exhaled air	Exhaled air	Exhaled air	Exhaled air	Exhaled air	Exhaled air		Exhaled air	
		Blood		Blood	Blood	Blood	Blood	Blood	Blood	Blood	Blood	Blood
		Urine sample before 9:00 h		---------- Urine samples ----------					First void urine; additional urine samples		First void urine; additional urine samples	
				Determination of urine volumes; notation of time of voiding and of urine volumes								
		Feces sample							Feces		Feces	
				--- Weighing of feces samples; notation of time of voiding and weights ---								



Total bismuth concentration in blood samples of volunteers (n = 20) taken in the first 12 h after ingestion of colloidal bismuth subcitrate containg 215 mg of bismuth

(Boertz et al., 2009)



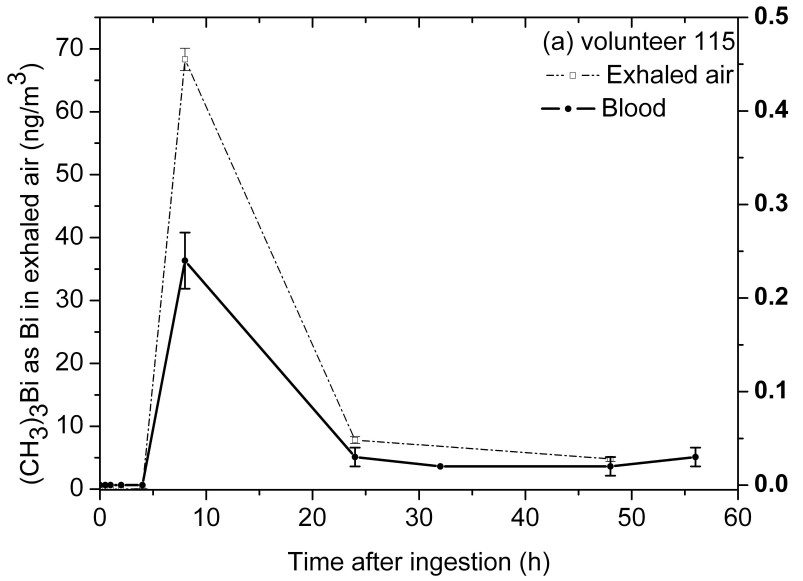
Identification of $(CH_3)_3Bi$ in exhaled air by GC/ICP-MS analysis

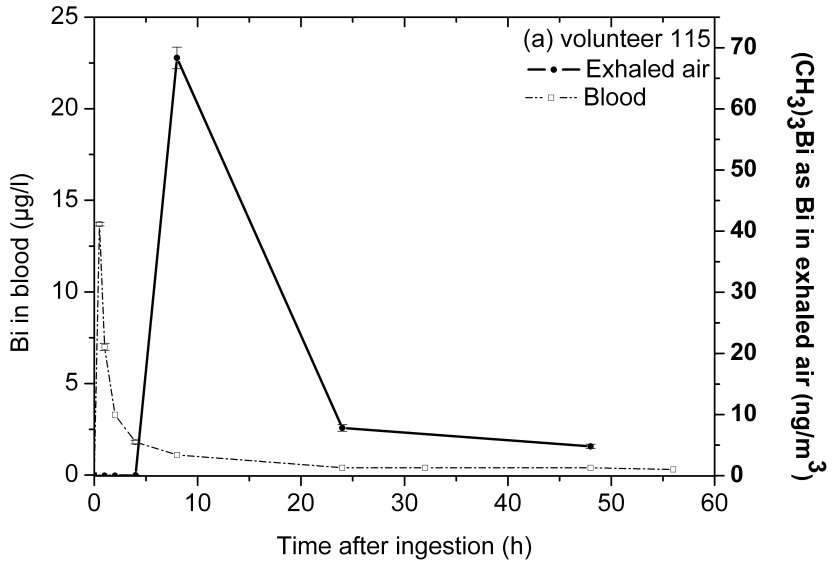
solid line: $(CH_3)_3Bi$ in a sample of exhaled air

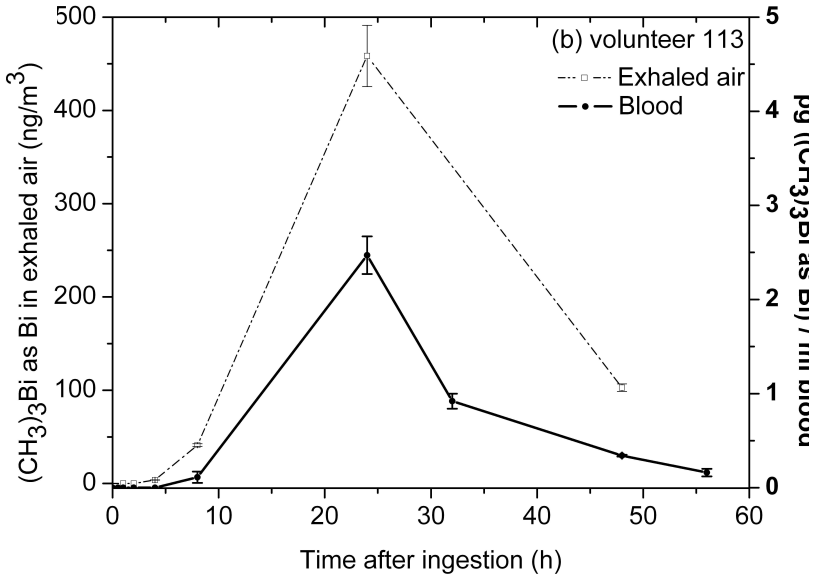
dotted line: $(CH_3)_3Bi$ standard

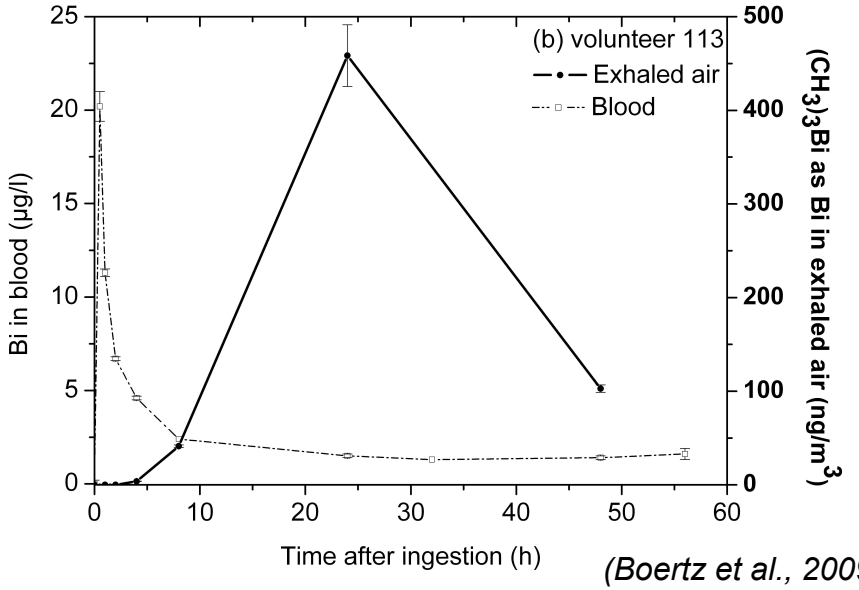
(Boertz et al., 2009)

Kinetics of $(CH_3)_3Bi$ in blood and exhaled air



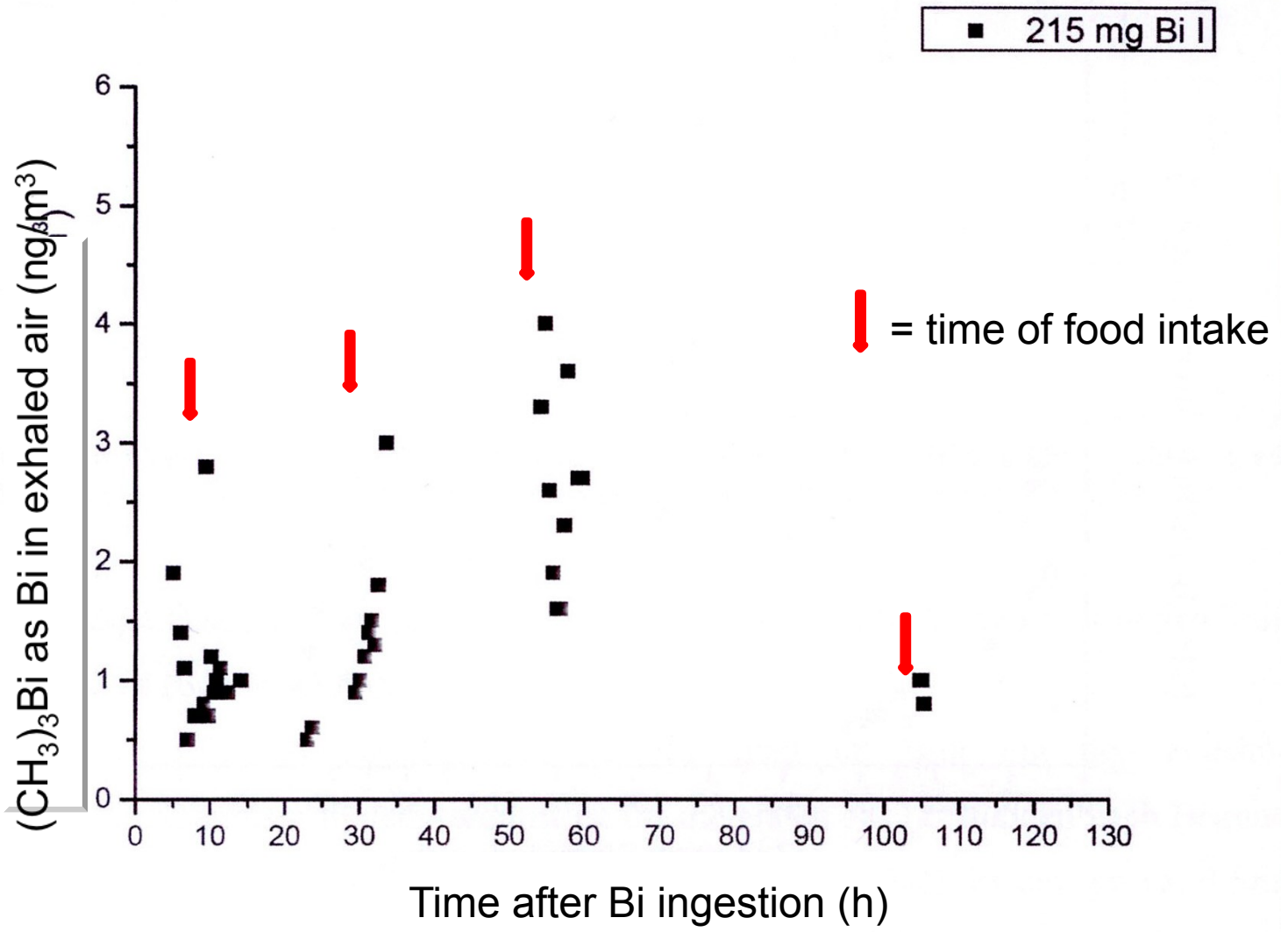






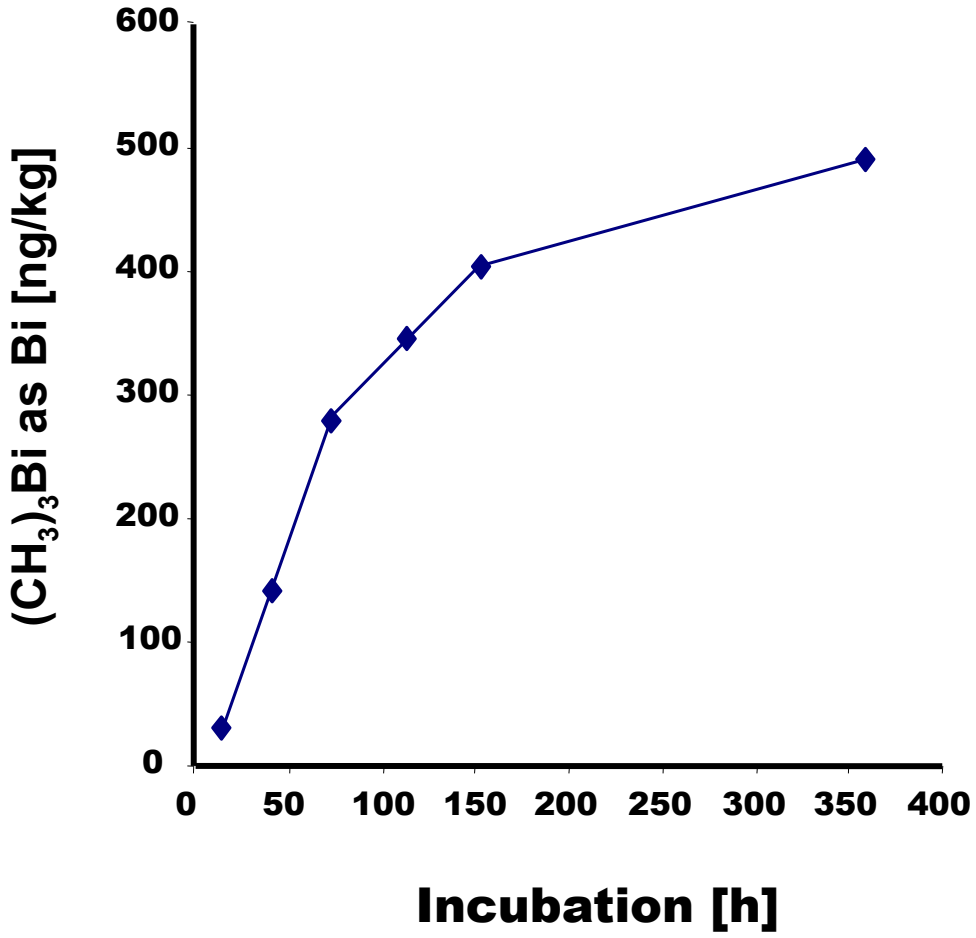
(Boertz et al., 2009)

Exhalation kinetics of $(CH_3)_3Bi$

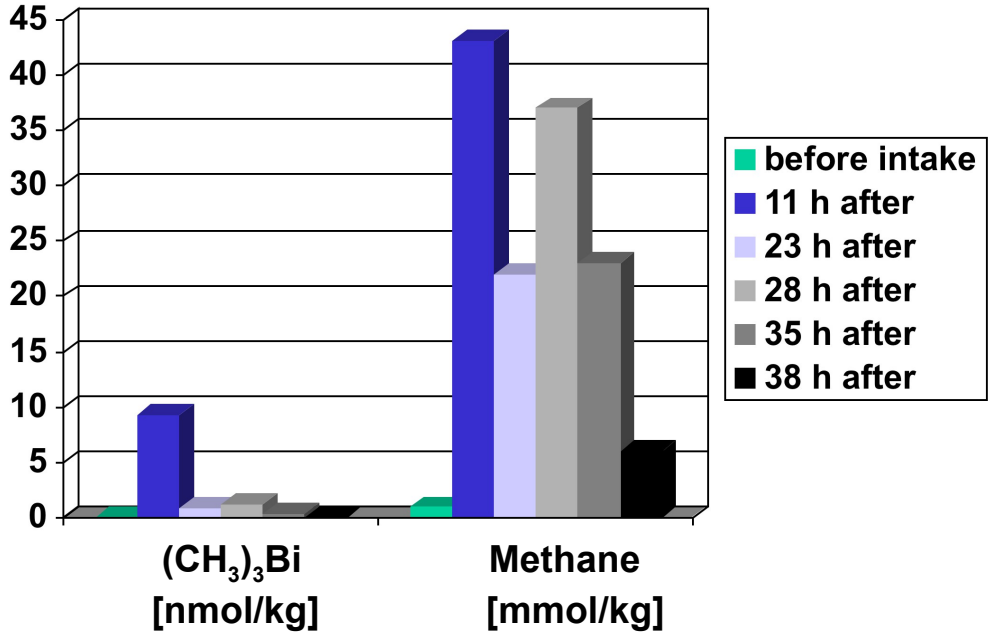


(Boertz et al., 2009)

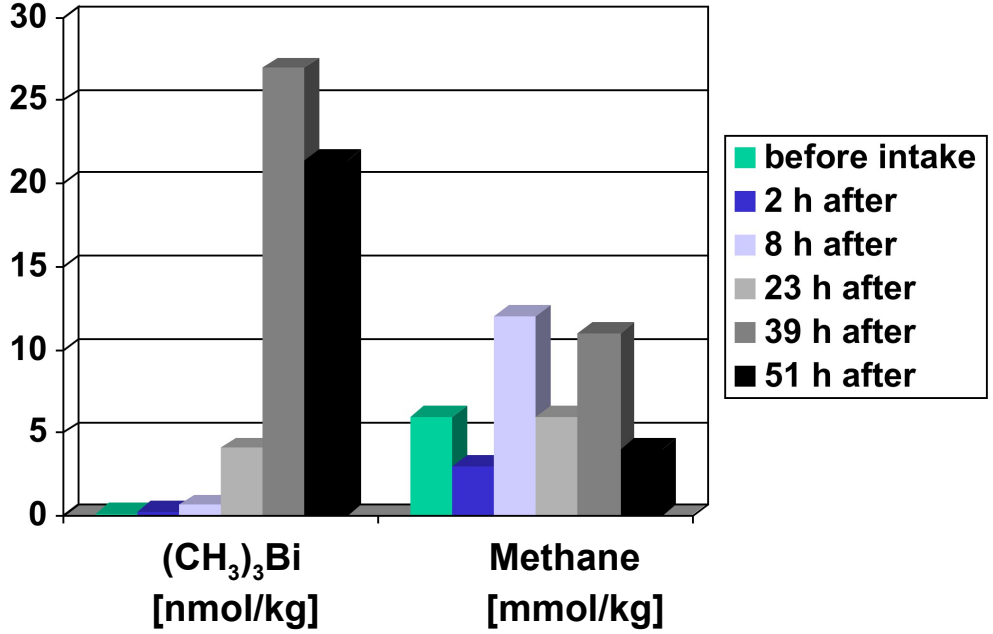
$(CH_3)_3Bi$ formation kinetics during fermentation of a feces sample obtained from a volunteer after oral intake of bismuth subcitrate



(Michalke et al., 2008)



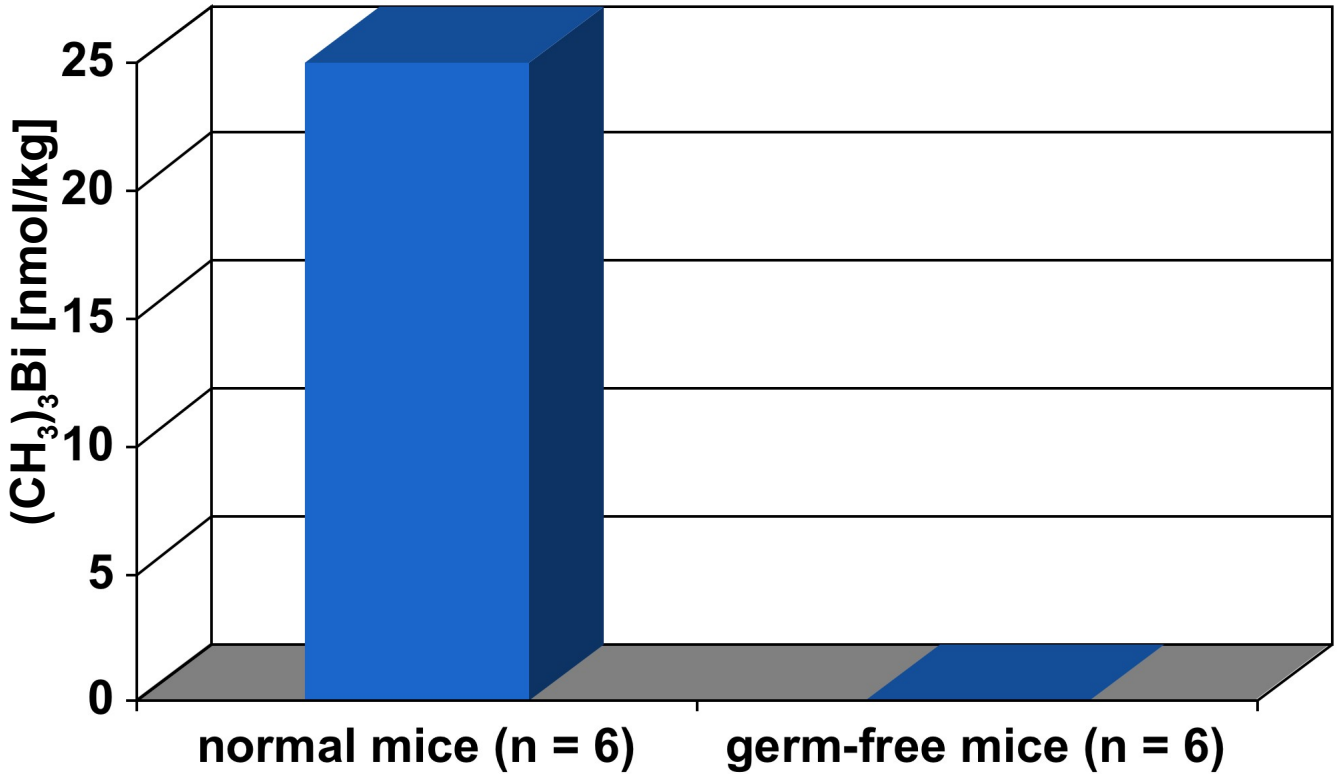
„weak methylator“



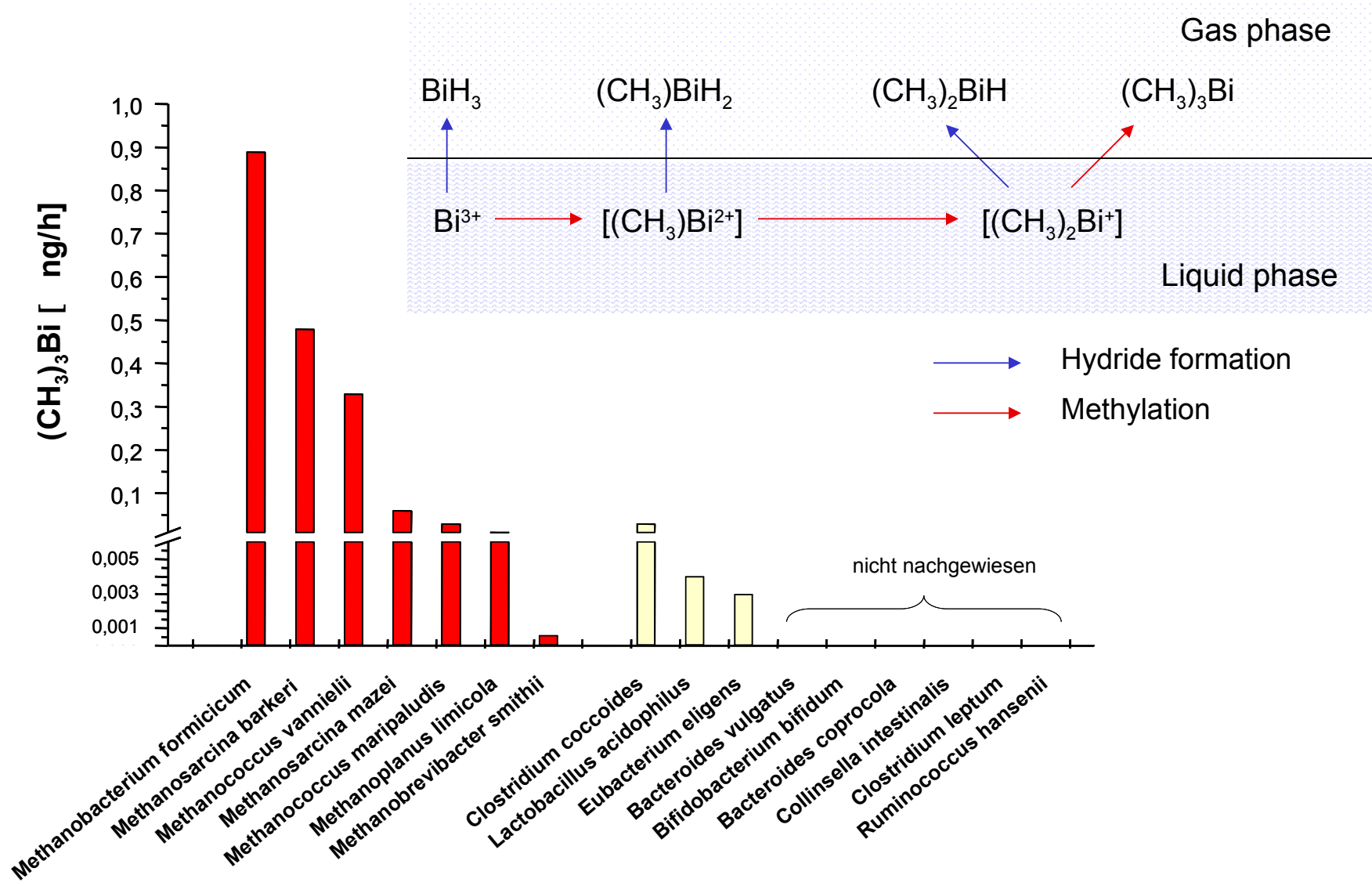
„strong methylator“

(Michalke et al.)

Ex situ production of $(CH_3)_3Bi$ during fermentation of feces samples of normal and germ-free mice following application of bismuth in the chow



(Michalke et al.)



Maximum rates of $(CH_3)_3Bi$ formation by pure cultures of methanoarchaea and bacteria exposed to 1 to 10 µM $Bi(NO_3)_3$

(Michalke et al., 2003)

Summary / Conclusions (II)

- Exhalation of the volatile metabolic product $(CH_3)_3Bi$ within few hours after oral intake of bismuth subcitrate
- Accumulation of $(CH_3)_3Bi$ during fermentation of feces samples after oral administration of bismuth
- No production of $(CH_3)_3Bi$ in germ-free mice in contrast to conventially raised mice

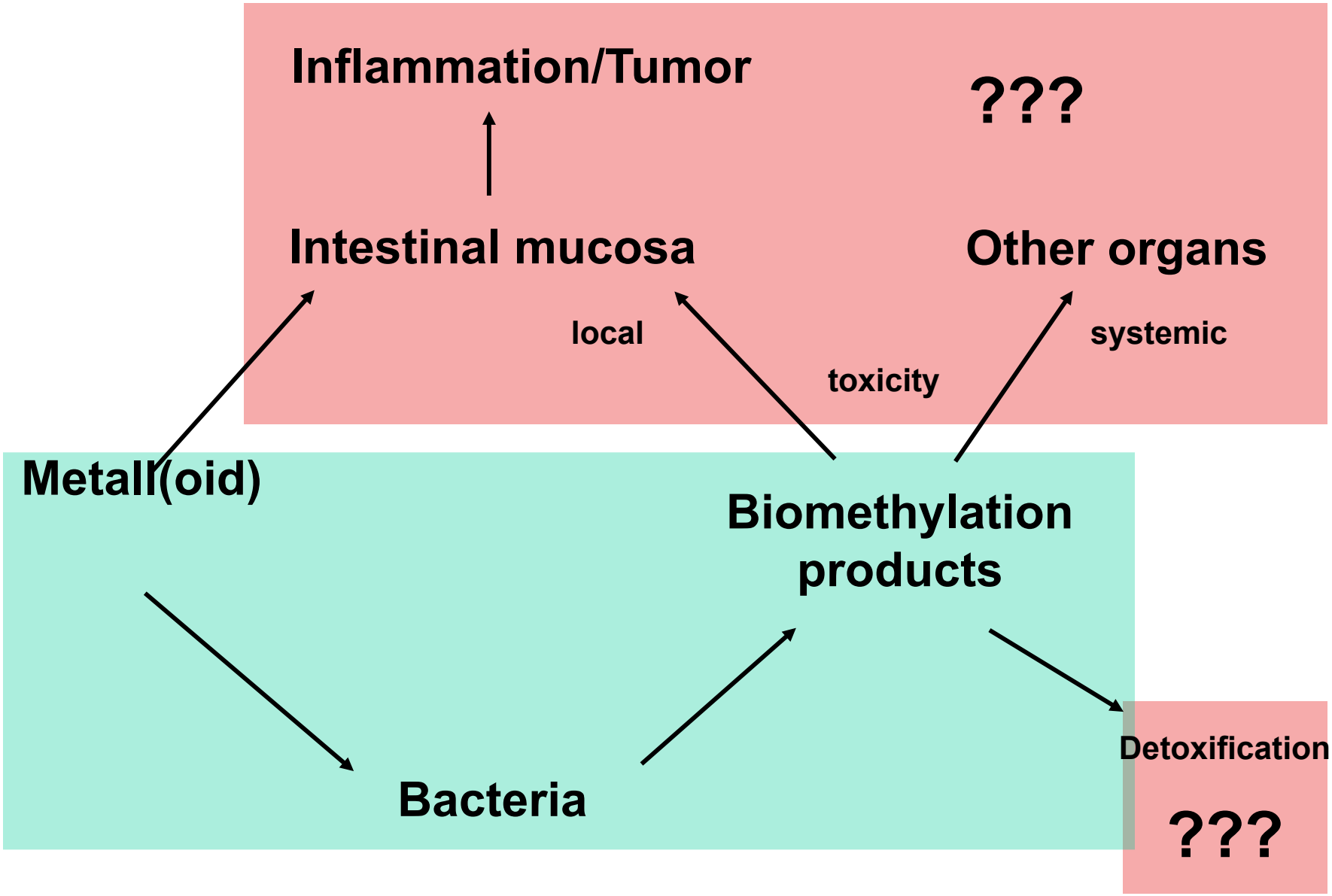
$\Rightarrow$ Human gut flora has the potential to methylate bismuth compounds

$\Rightarrow$ Methylation of bismuth in the liver cannot be excluded

Studies on uptake and cytotoxicity of bismuth species *in vitro*

- Concentration-dependent uptake $(CH_3)Bi^{2+}$ in lymphocytes and hepatocytes
- Higher membrane permeability of methylated bismuth species compared to bismuth subcitrate
- Higher cytotoxicity of $(CH_3)Bi^{2+}$ in hepatocytes compared to bismuth subcitrate

(von Recklinghausen et al., 2007)


Inflammation/Tumor
???
Intestinal mucosa
Other organs
local
systemic
toxicity
Metall(oid)
Biomethylation products
Bacteria
Detoxification
???

Indications of a bacterial methylation of mercury in the intestine

- Formation of MeHgX in incubations of inorganic mercury salts with germs of the mouth and gut flora

  (Heintze et al., 1983; Yannai und Berdicevsky, 1991)

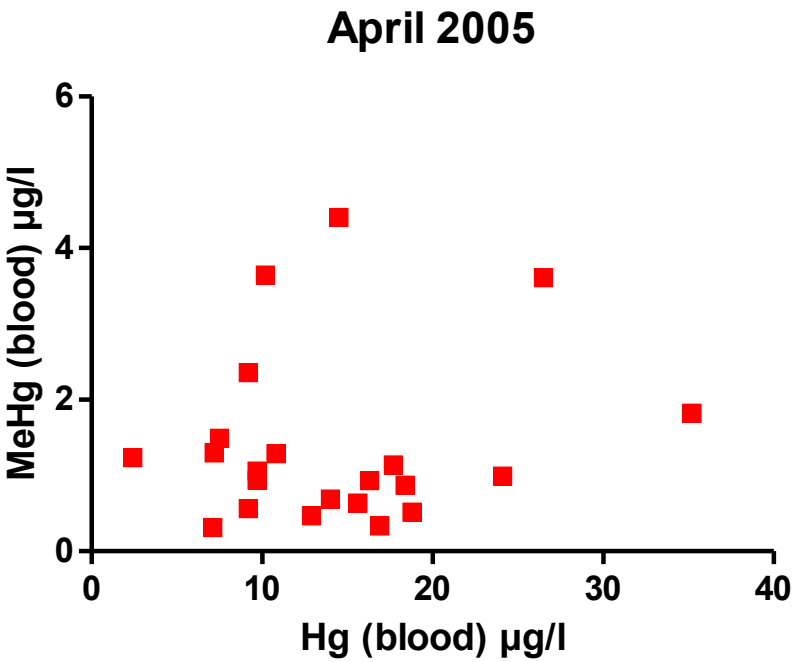
- Formation of MeHgX in incubations of intestinal loops of male rats with mercury chloride

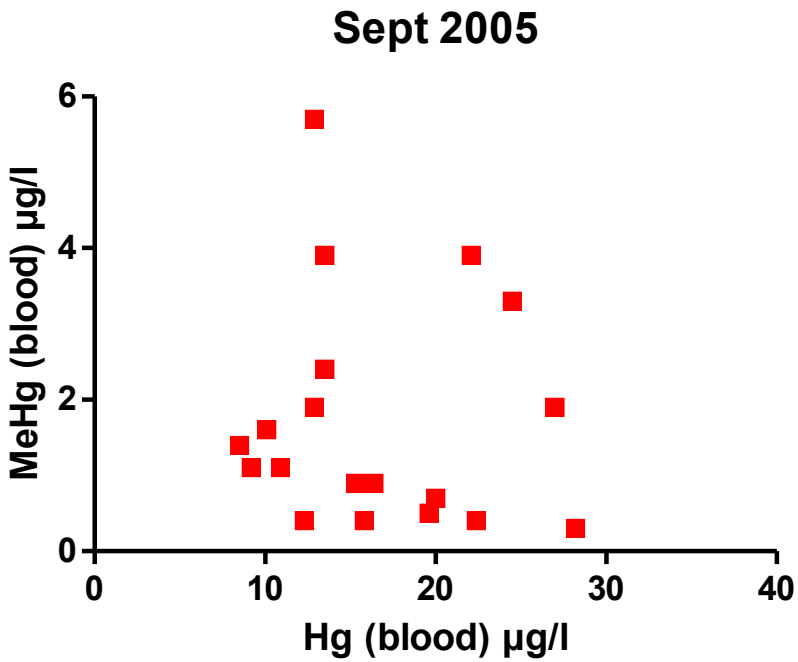
  (Ludwicki, 1989)

- Excretion of MeHgX and Me_2Hg in feces after removal of amalgam fillings and intake of the alga *Chlorella pyrenoidosa*

  (Kresimon, 2002)

Total Hg and MeHg concentrations in blood of workers exposed to metallic mercury in a mercury recycling plant



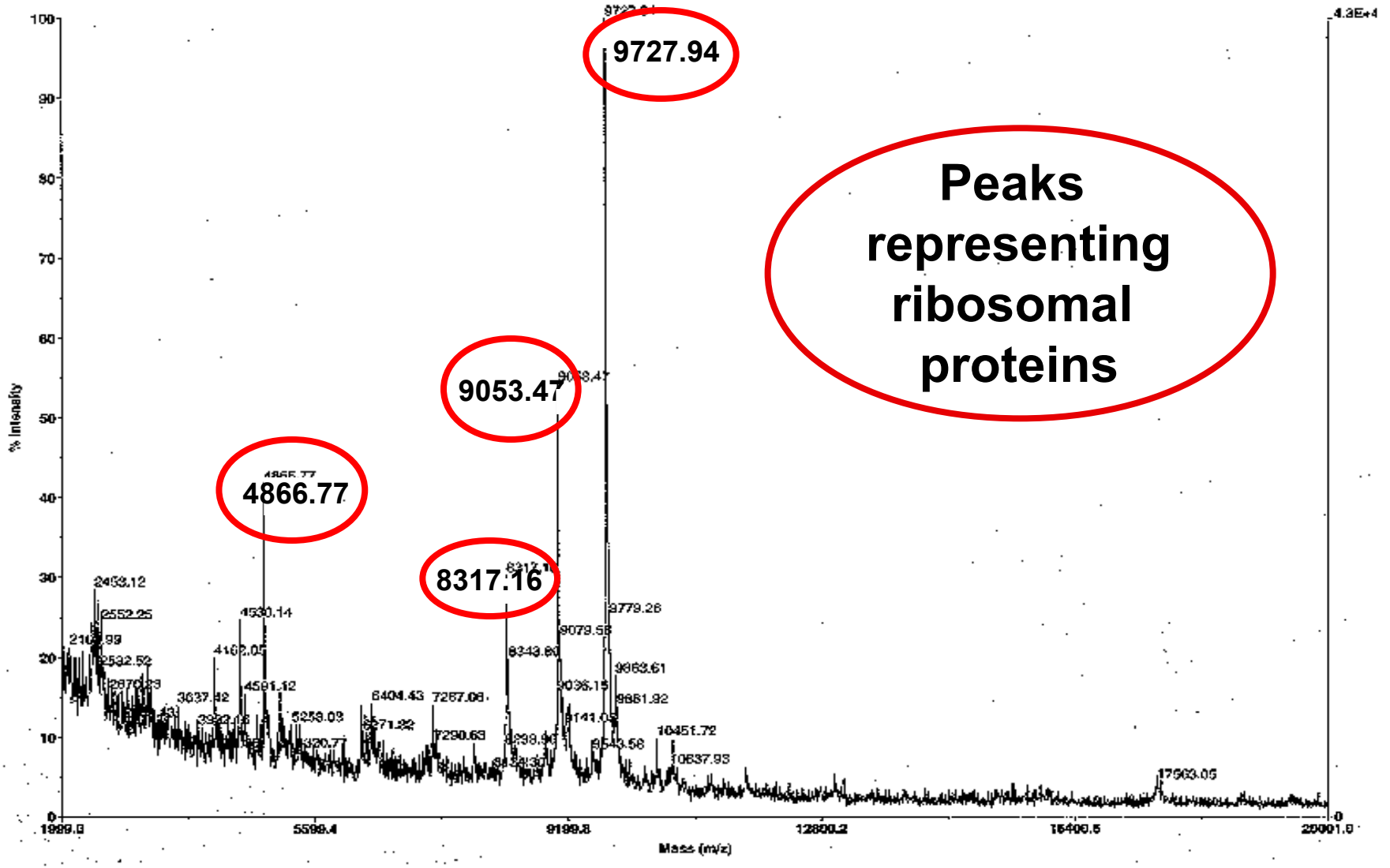


(Mosel et al. 2005)

Future directions

- Bacterial species?
- Fast and slow methylators of metal(loid)s?
- Influence of nutrition (*e. g.* vegetarians)?
- Local and systemic toxic effects of microbially formed organometal(loid) compounds?

Escherichia coli



(Mosel et al. 2009)

Summary (III)

- Occurrence of methylated metal(loid) species in the environment or internal formation $\Rightarrow$ human exposure
- Cases of poisoning $\Rightarrow$ high toxicity (neurotoxicity, genotoxicity) of some methylated metal(loid) species
- Amphiphilicity of metal(loid)s $\Rightarrow$ increased mobility
  $\Rightarrow$ increased toxicity
- Indications of microbial production of methylated metal(loid) species in the human intestinal tract

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