SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

'Omic Biomarkers for Assessing Cellular Toxicity: Integration of *In Vivo* and *In Vitro* Data— Why It Is Important

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Introduction

- There is a pressing need for new toxicity test systems which can provide useful risk assessment information in a rapid, cost effective manner with minimal use of animal tests

- "'Omic" based tests offer one mechanism–based set of approaches which are capable of addressing the complex issue of sensitive sub-populations

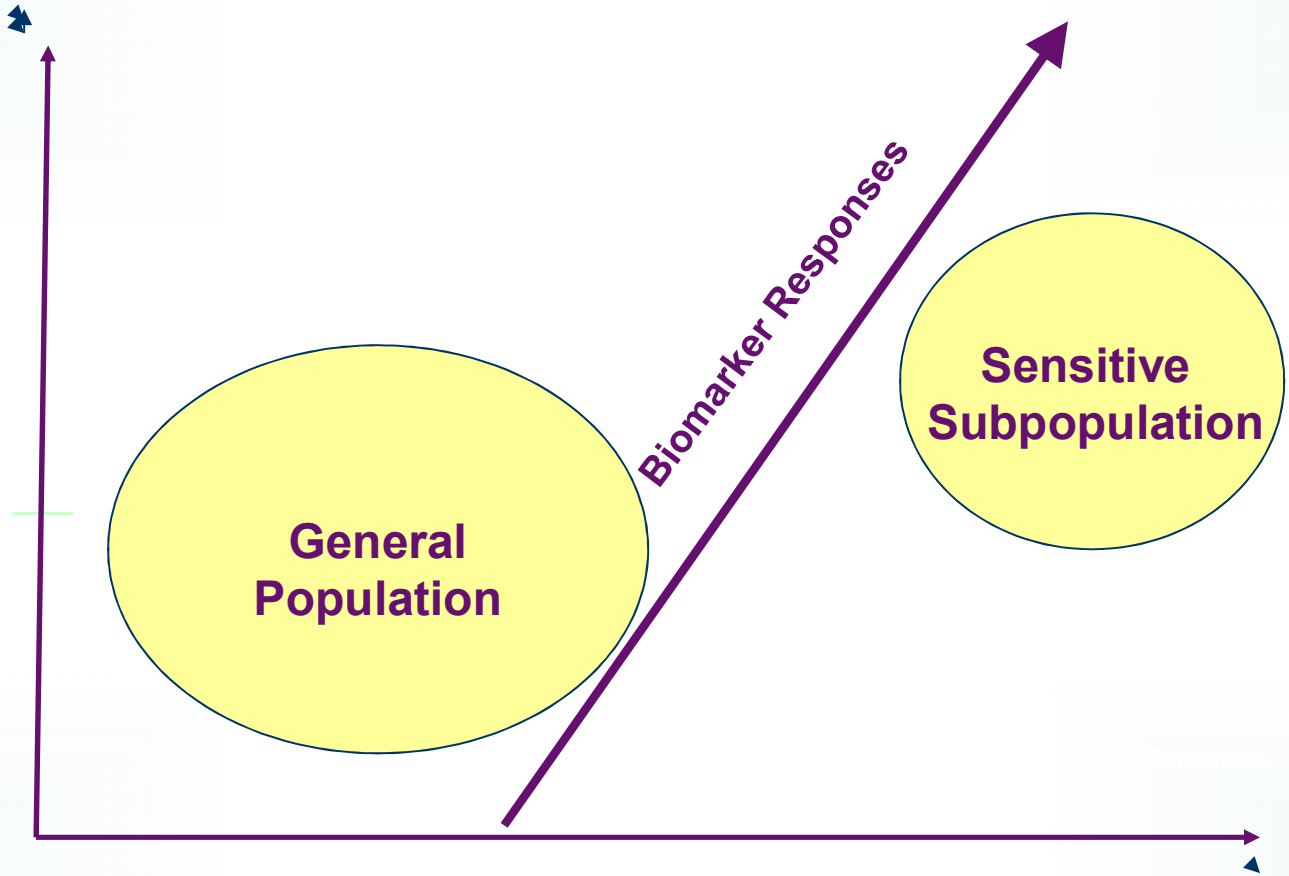
- In order for "'omic" biomarkers to be fully accepted as valuable risk assessment tools, they must be validated by ancillary data and identification of modifying factors which affect dose response relationships.

Introduction *cont'd*

- Integration of *in vivo* and *in vitro* data via computational techniques is an essential central component in both the development and validation of such biomarker tests.

- This presentation will attempt to briefly review some *in vivo* and *in vitro* studies focused on identification of gender differences in responses to binary mixtures of metallic semiconductor elements in target cell populations.

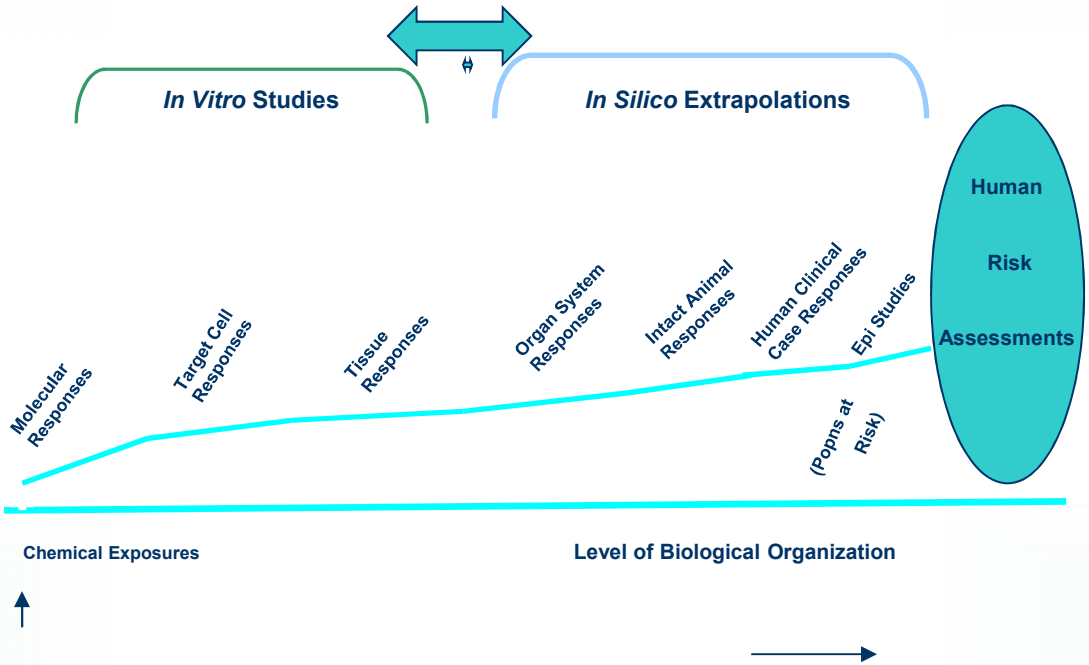
- The overall goal of these studies is to provide new "mode of action"- based tests for identification of sensitive subpopulations at special risk for toxicity.


Biomarker Responses
General Population
Sensitive Subpopulation

Biomarkers

- Need for objective measures (biomarkers) of cell injury from toxic chemicals that more precisely link exposure (dose) and biochemical effects that occur prior to clinical disease
- Need for improved risk assessments for multiple chemical exposures (e.g., Mixtures) and sensitive sub-populations
- To be of maximal value, it is essential to establish mechanistic linkages between biomarkers and other parameters of toxicity
- Ideally biomarker responses should be conserved or consistent across species

Predictability of Biomarker Responses (Biomarker Validation Needs)

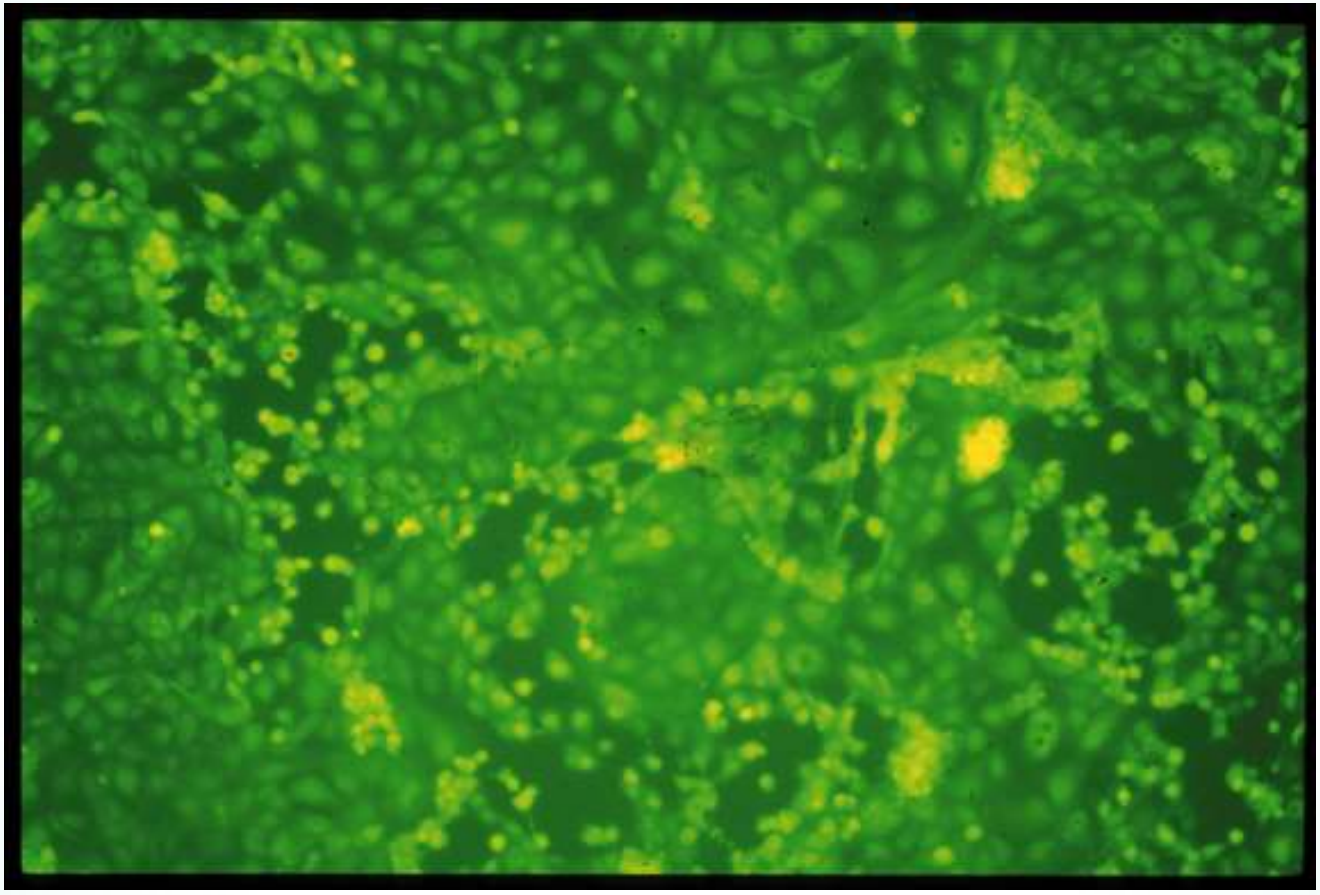


Biomarkers

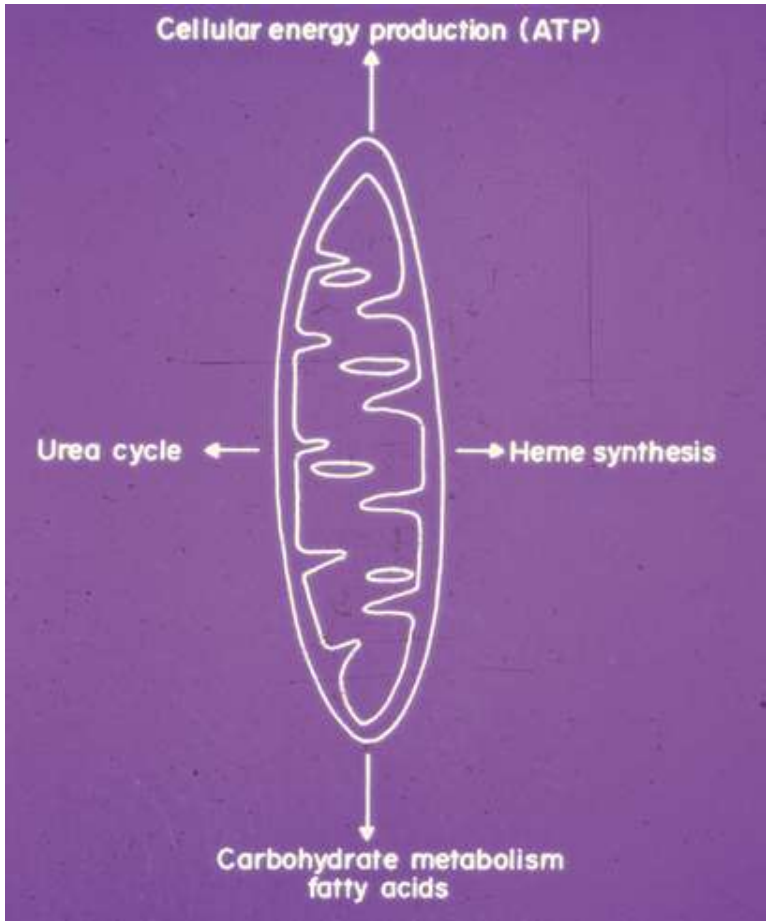
- **GENOMICS:** Chemical-specific alterations in gene expression patterns in target cell populations
- **PROTEOMICS:** Chemical-specific alterations in the actual expression of gene products (proteins)
- **METABOLOMICS/METABONOMICS:** Chemical-induced alterations in essential biological pathways with measurement of metabolic products/precursors in accessible body fluids (e.g., urine)

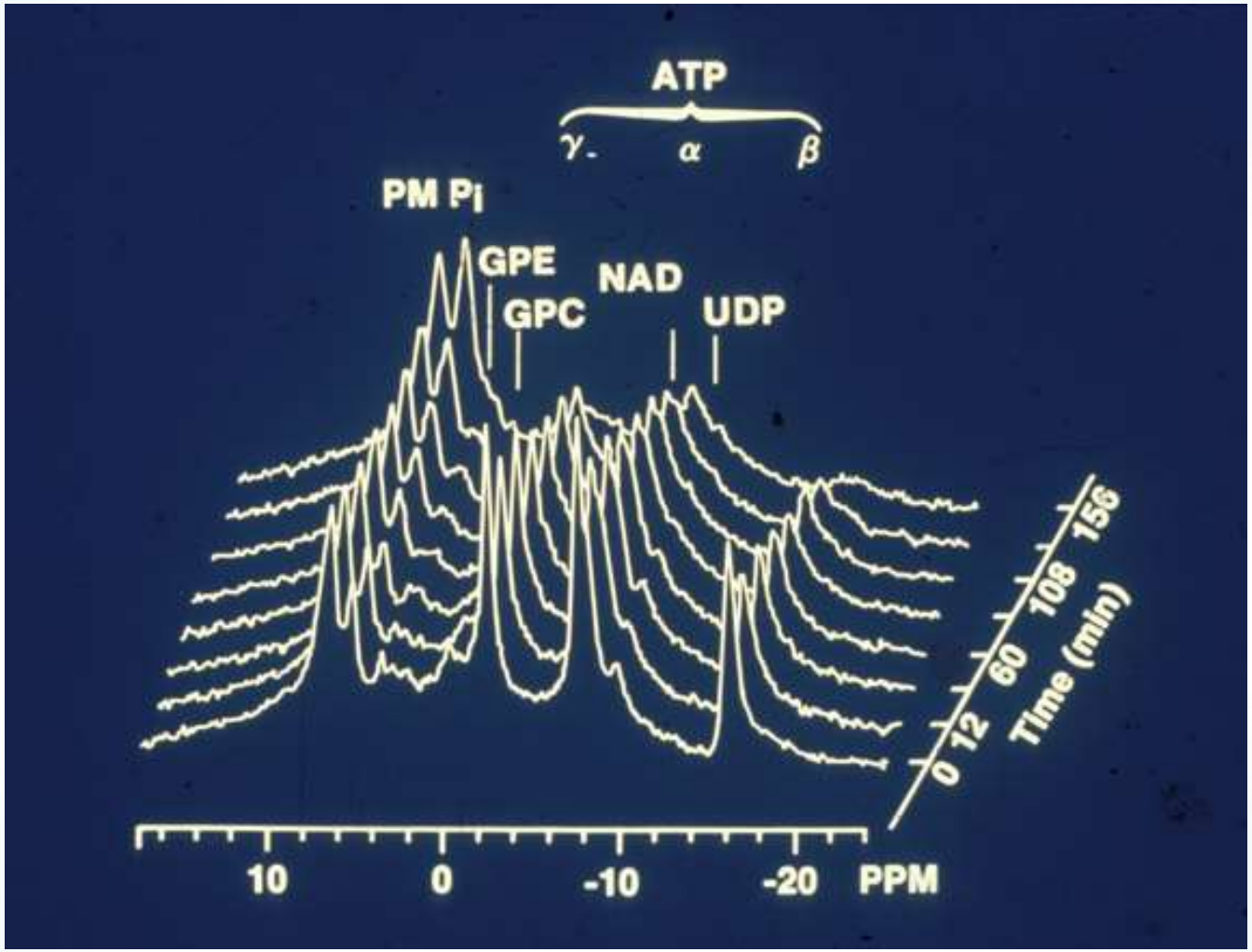
Biomarker Modifying Factors

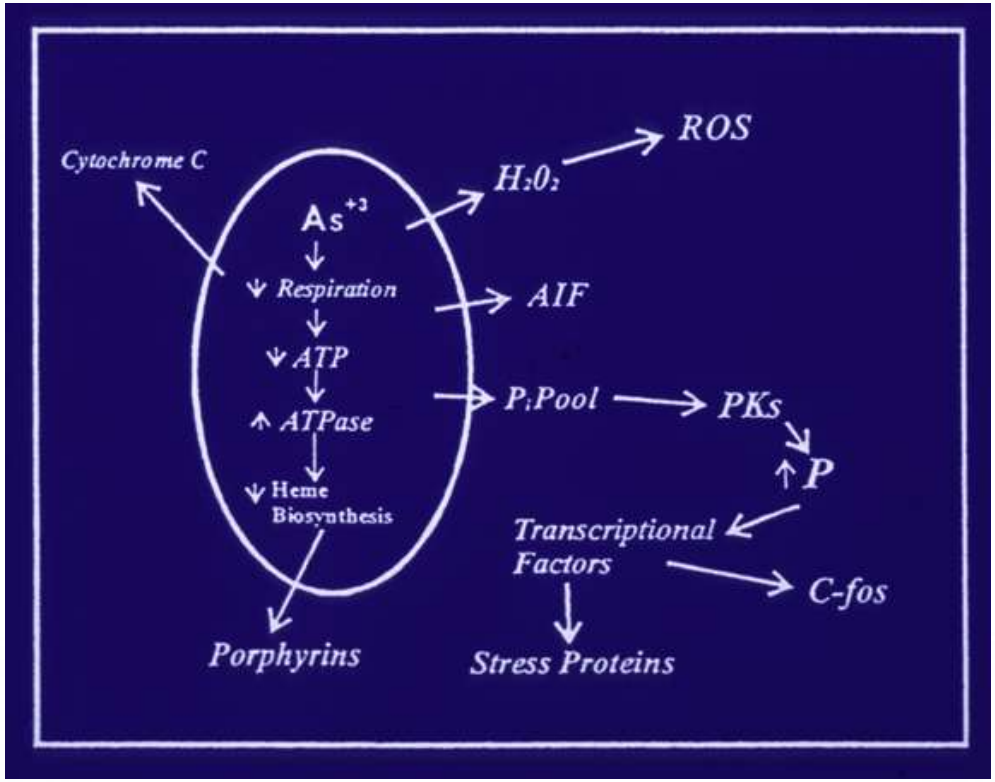
- **Dose Response/Time Course**
- **Exposed Population**
  (Age, ****Gender***, Nutritional Status, Genetic Susceptibility)
- **Compensatory Mechanisms**
  (Inducible Enzyme Systems, Metal-Binding Proteins, Stress Proteins)
- **Antioxidant Systems**
  (e.g., Glutathion)



Mitochondrial Functions




ATP
γ
α
β
PM Pi
GPE
GPC
NAD
UDP
0 12 60 108 156
Time (min)
10
0
-10
-20
PPM


Cytochrome C
As+3
Respiration
ATP
ATPase
Heme Biosynthesis
Porphyrins
H2O2
ROS
AIF
PiPool
PKs
P
Transcriptional Factors
C-fos
Stress Proteins

Experimental Design

Syrian Golden Hamsters

↓

Injected s.c. with 100 mg/kg InAs or GaAs Particles

↓

Urine collection 24 hr prior to sacrifice

↓

Sacrifice after 10 or 30 days

↓

SDS gel electrophoresis of liver, kidney, urine proteins

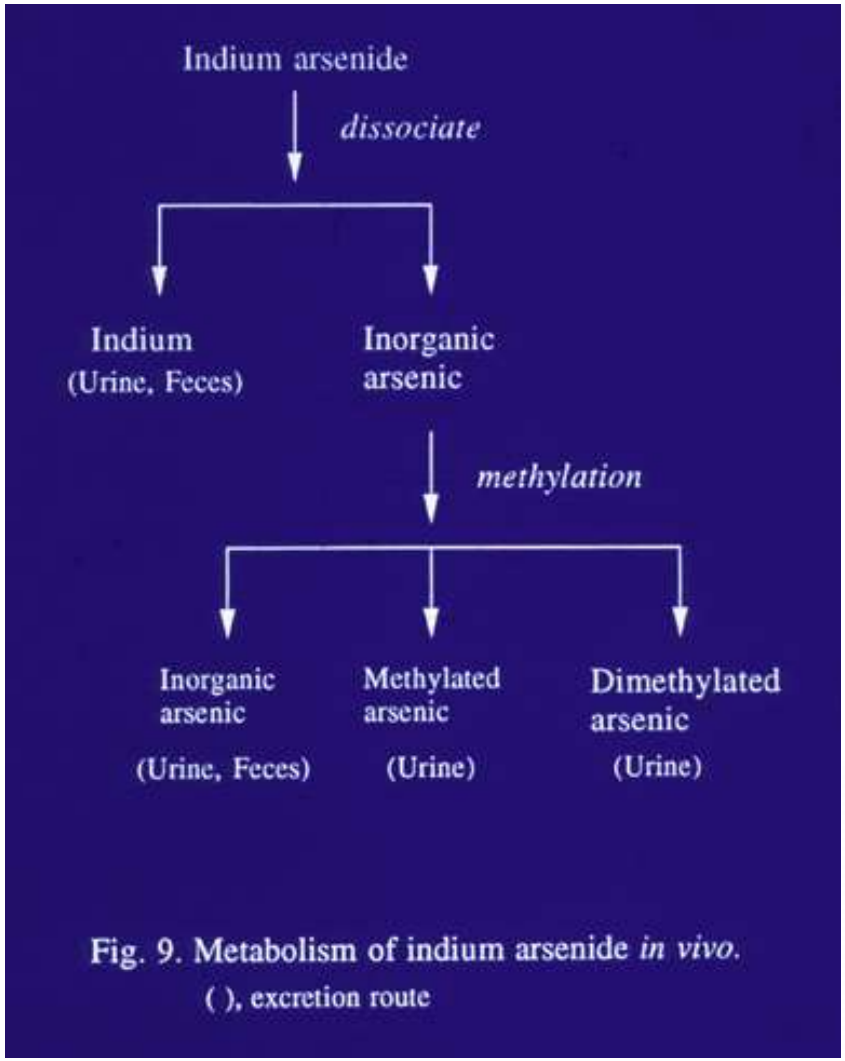
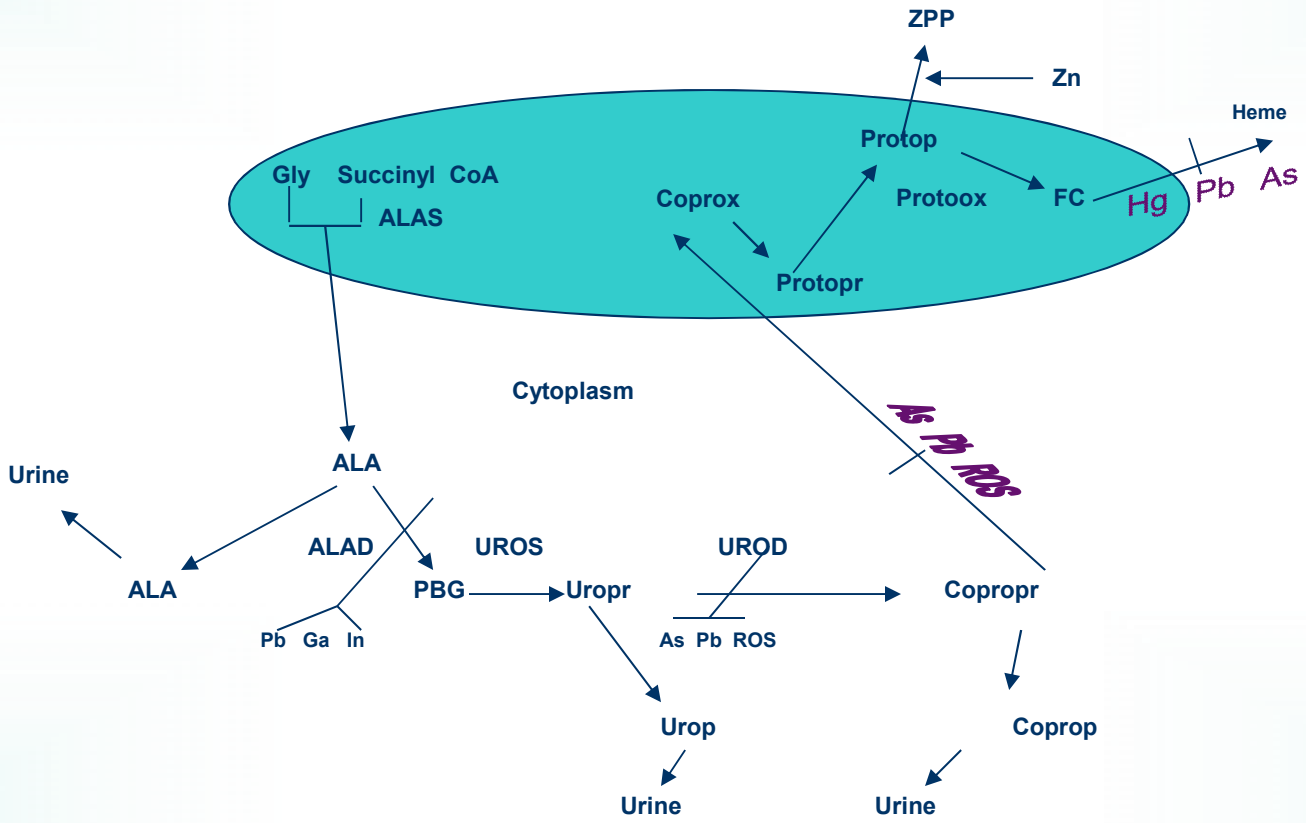
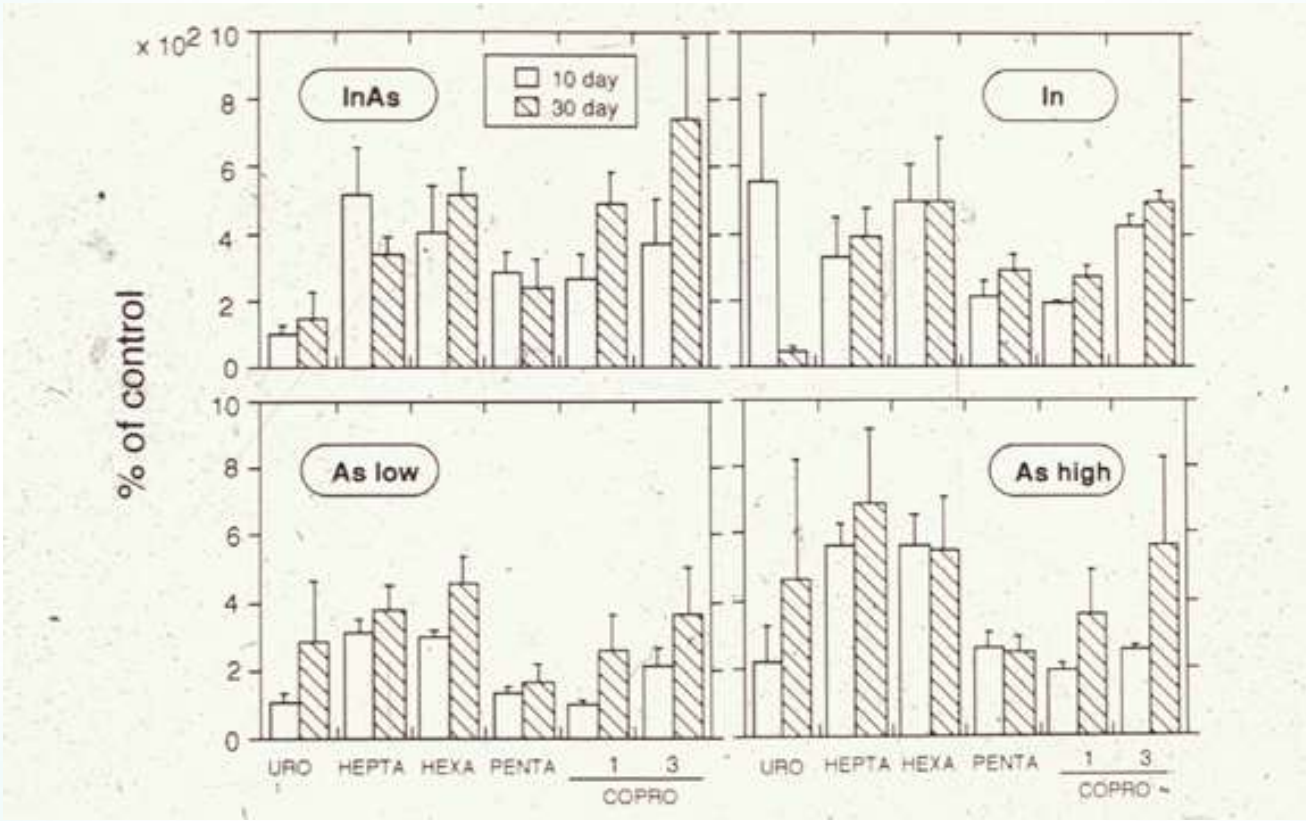


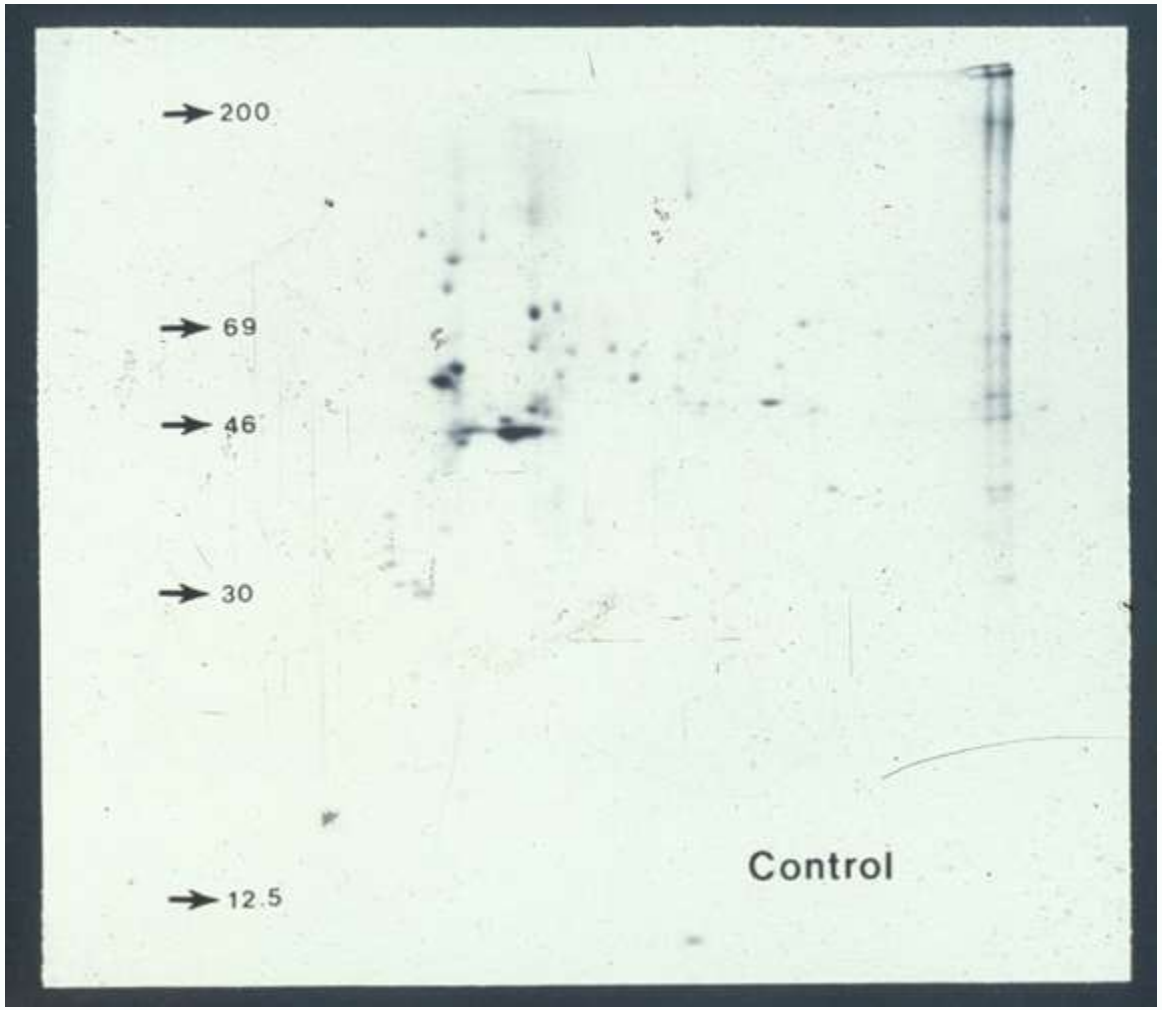
Fig. 9. Metabolism of indium arsenide *in vivo*.
( ), excretion route

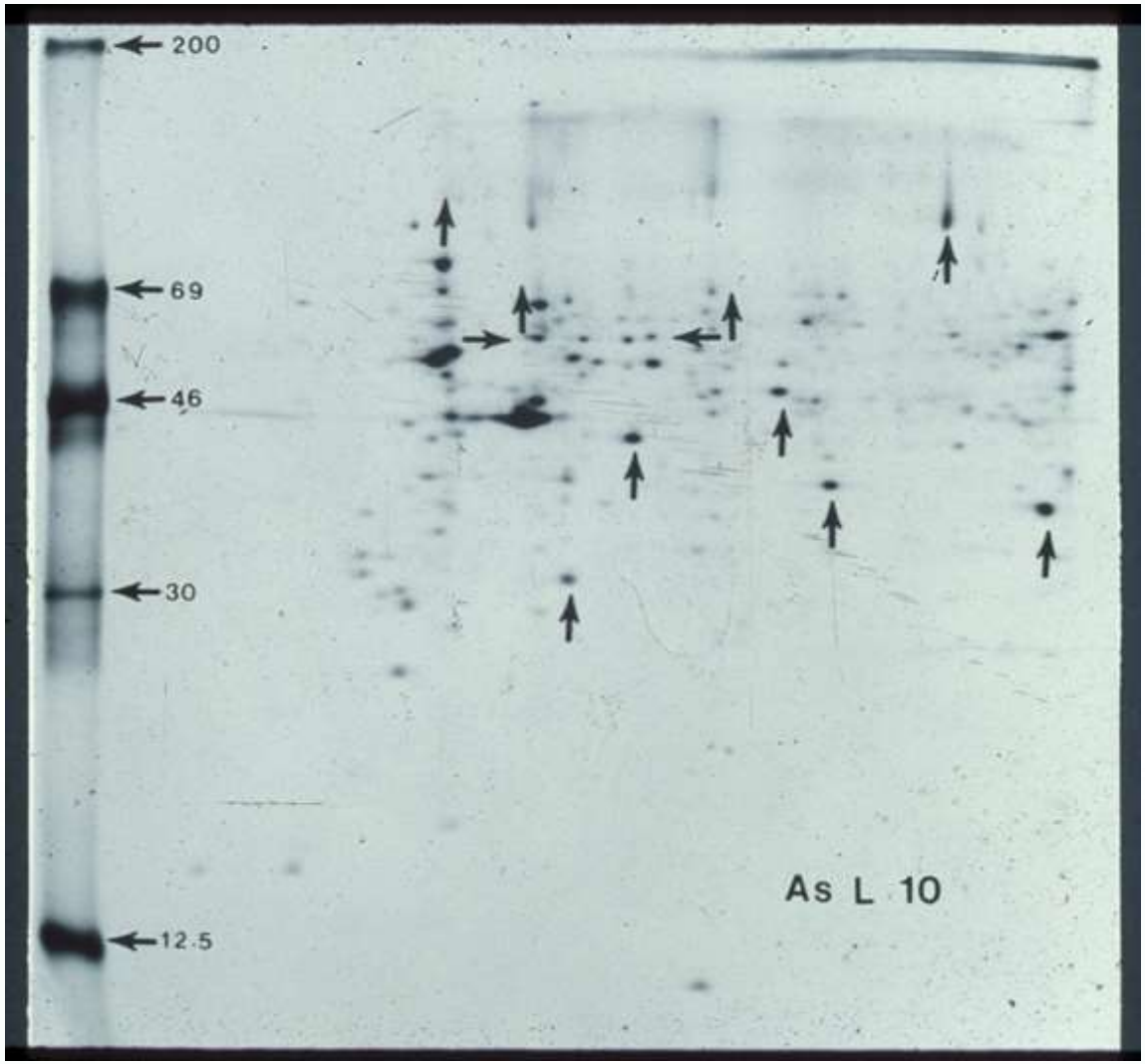
Heme Pathway

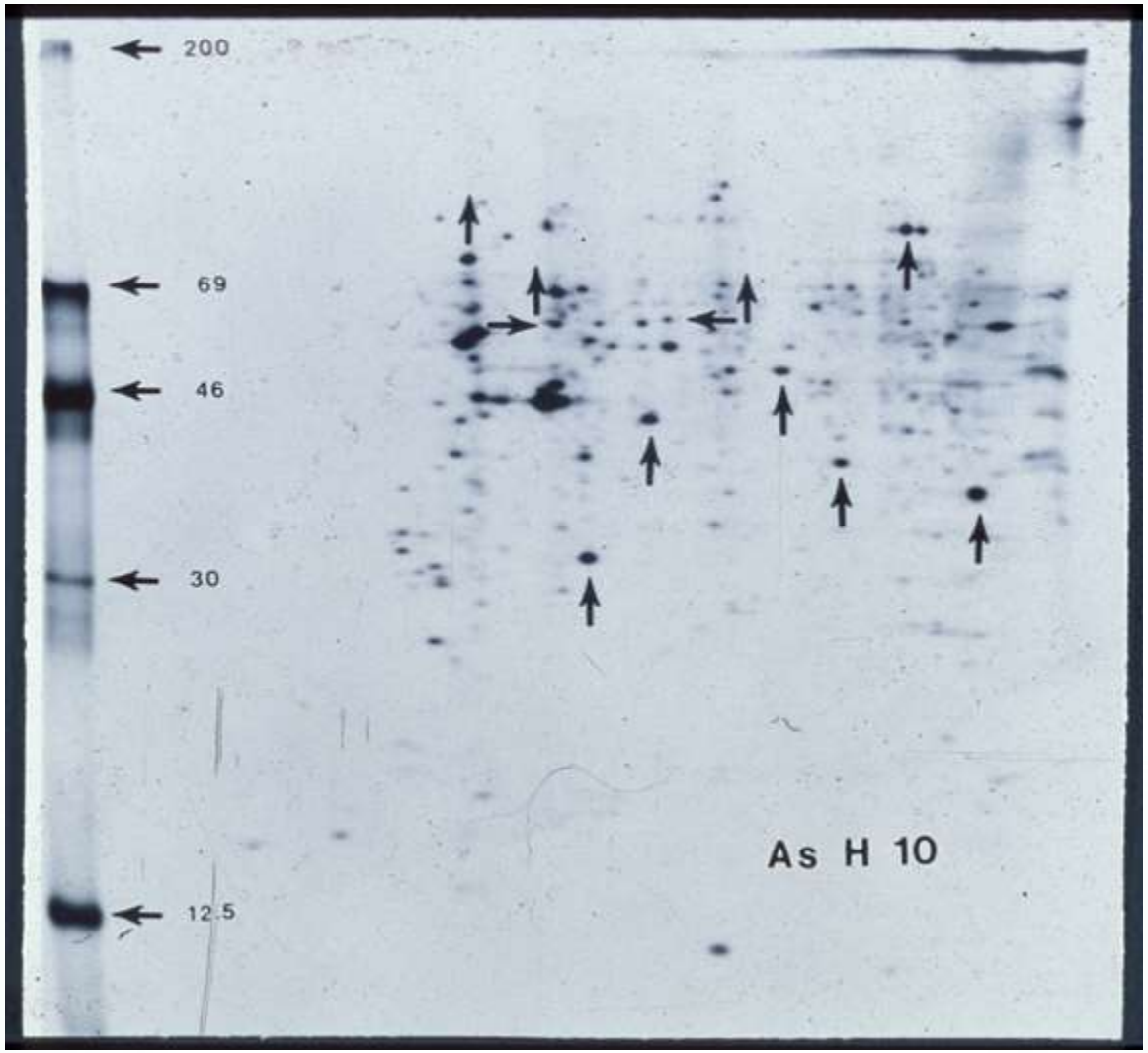


Urinary Porphyrins




200
69
46
30
12.5
Control


200
69
46
30
12.5
As L 10


200
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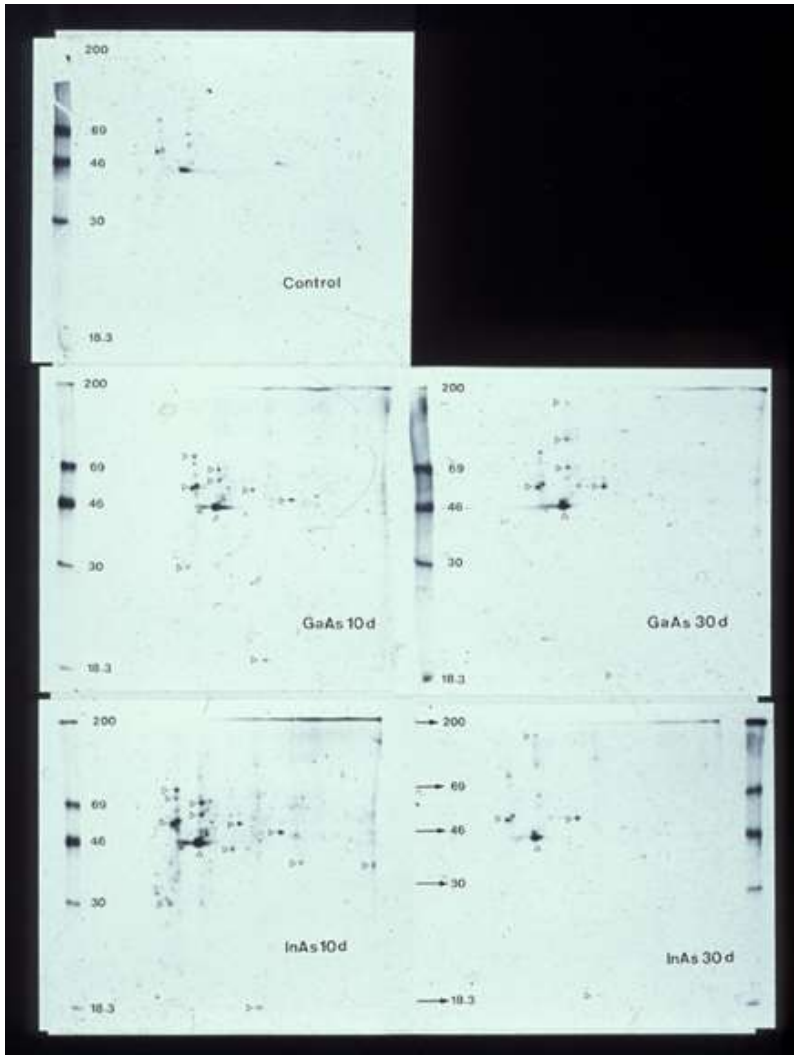
As H 10


200
69
46
30
18.3
Control
GaAs 10 d
GaAs 30 d
InAs 10 d
InAs 30 d

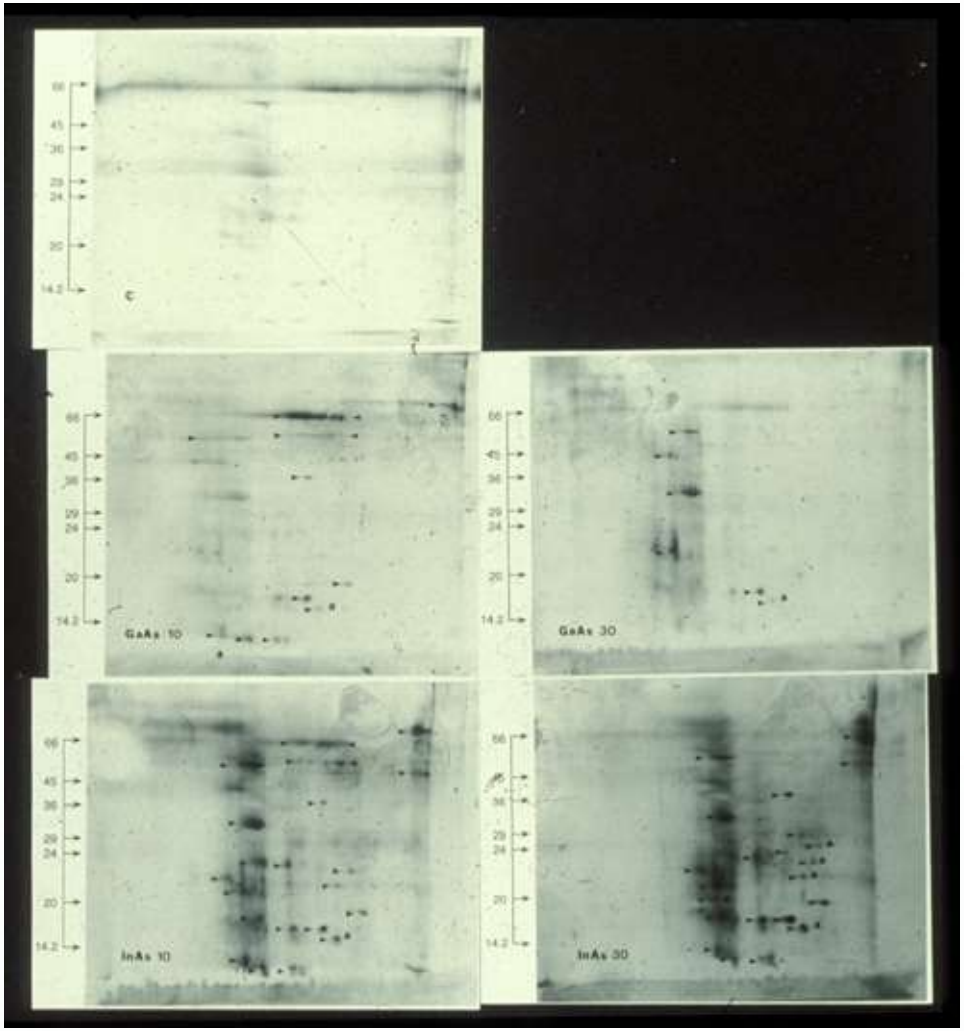
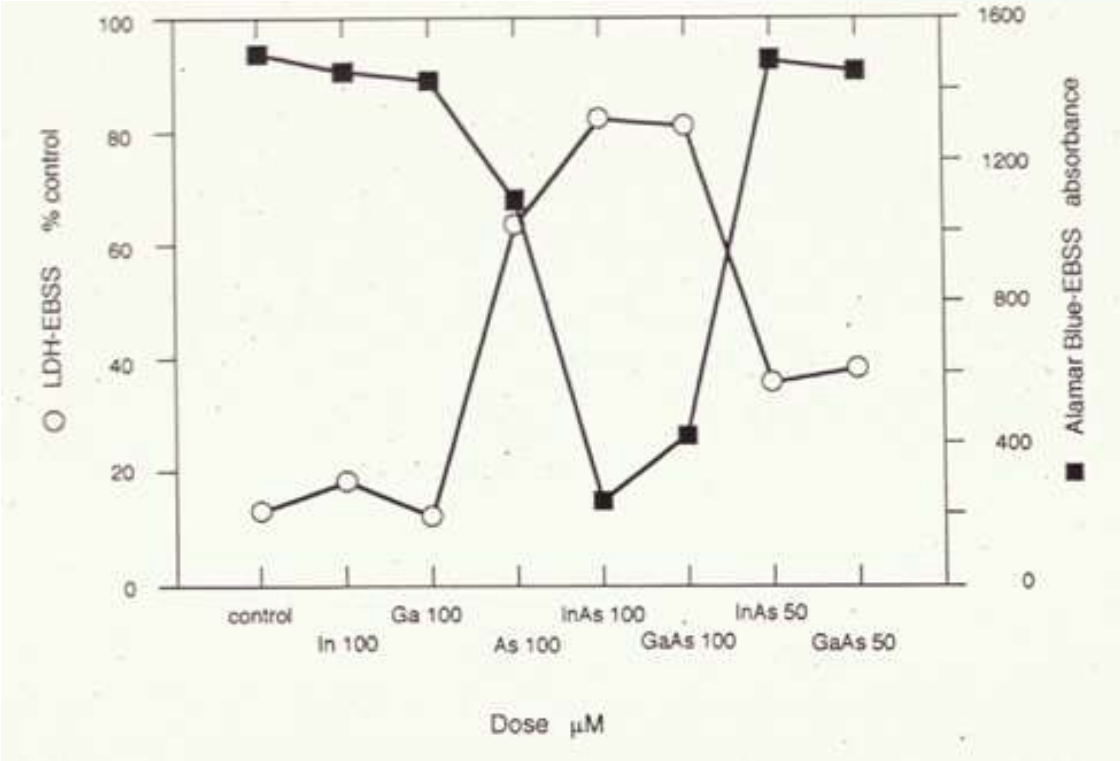

66
45
36
29
24
20
14.2
C
GaAs 10
GaAs 30
InAs 10
InAs 30

Figure 3.
Cellular toxicity assay using LDH release or Alamar Blue for hamster kidney epithelial cells exposed to In, Ga, or As at 100 μM concentrations, In + As or Ga + As at 100 μM or 50 μM concentrations for each elements showing that the combination of In + As and Ga + As at 100 μM concentrations were the most toxic. The data also Indicate a correlation between LDH release and Inhibition of Alamar Blue metabolism.

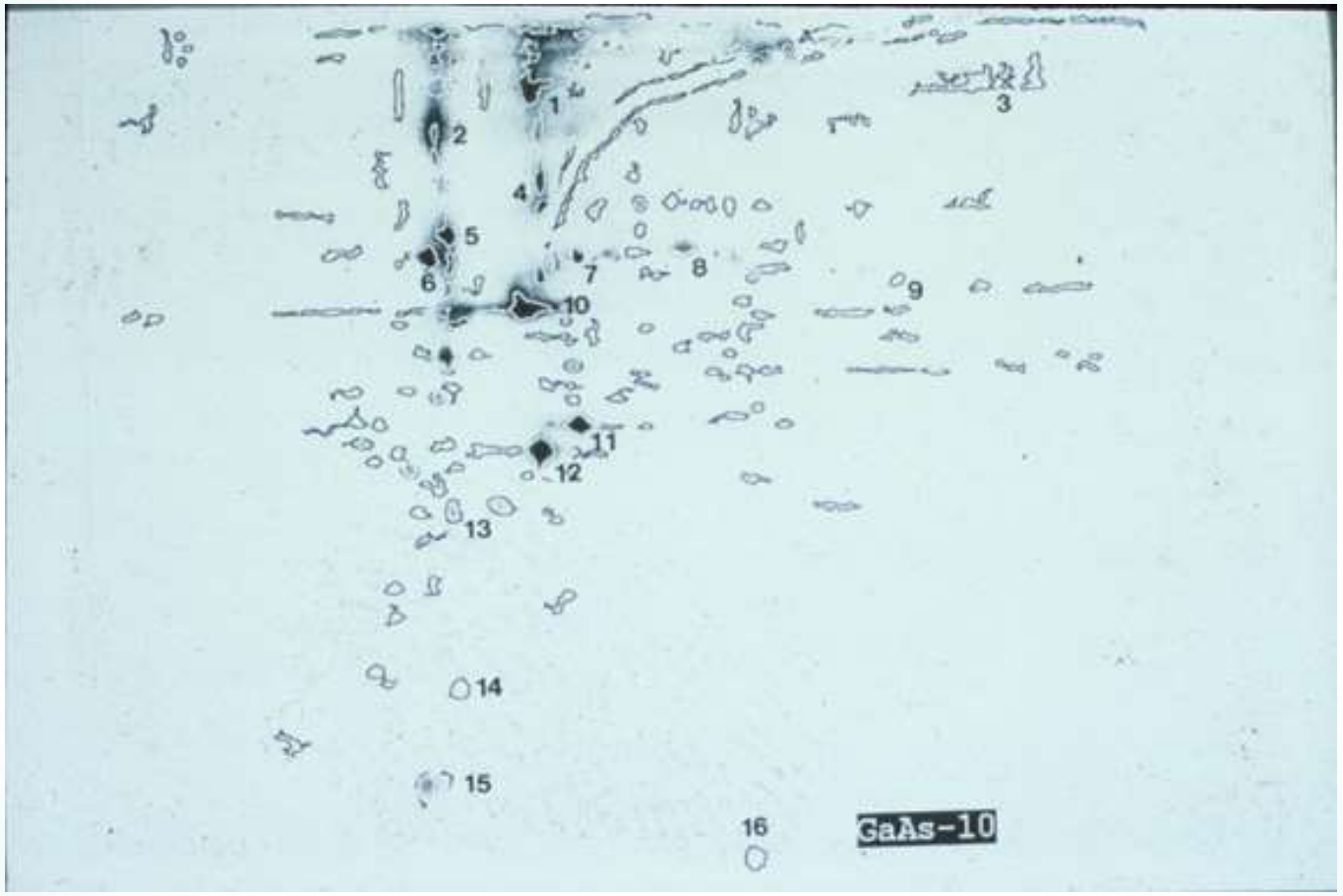


Conclusions

- InAs and GaAse each produce compound-specific proteinuria patterns as determined by 2-D gel electrophoresis and silver staining. The intensity of these patterns appeared to increase with duration of exposure.

- Western blot studies of the urine showed no evidence of the 32, 70, or 90 kD stress proteins despite evidence of their induction in the liver and kidney indicating that these proteins are not translocated or appear in the urine as a result or cell death.

- Overall, the results of these studies indicate that the observed proteinuria patterns maybe of potential use as biomarkers for assessing nephrotoxicity from these agents.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
GaAs-10

Changes in Polypeptides at 30 Days After Exposure to InAs or GaAs in Male Hamster Kidney Cells

MW Range	Common Spot #[1]	InAs[1]	GaAs[2]
100–90	1	1.00	2.1*
	18	1.00	.7
89–70	2	–	–
	12	–	1.1
69–50	3	.90	4.0*
	11	–	1.2
	22	–	–
	23	–	–
	25	–	–
	32	–	–
49–40	10	.85	1.8
	16	1.20	1.6
	26	–	–
	28	–	–
39–30	4	.79	2.0*
	5	1.80	.5*
	6	–	.1*
	7	–	.3*
	8	1.50	3.6*
	13	.90	.1*
	14	.72	.6
	15	1.20	1.7
	17	.74	2.4*
	19	–	–
	20	–	–
29–20	30	–	–
	31	–	–

1) Spot number denoted on gel

2) Spot intensity (ratio of tx/control)

*a ≥ 2 fold increase/decrease in polypeptides

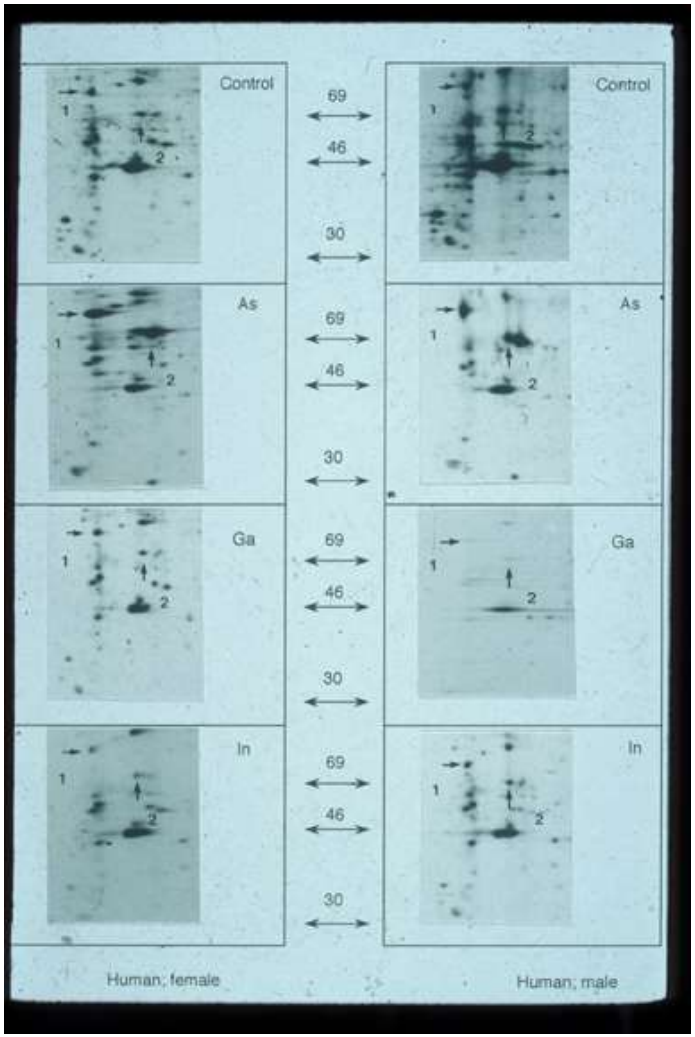
Changes in Polypeptides at 30 Days After Exposure to InAs or GaAs in Female Hamster Kidney Cells

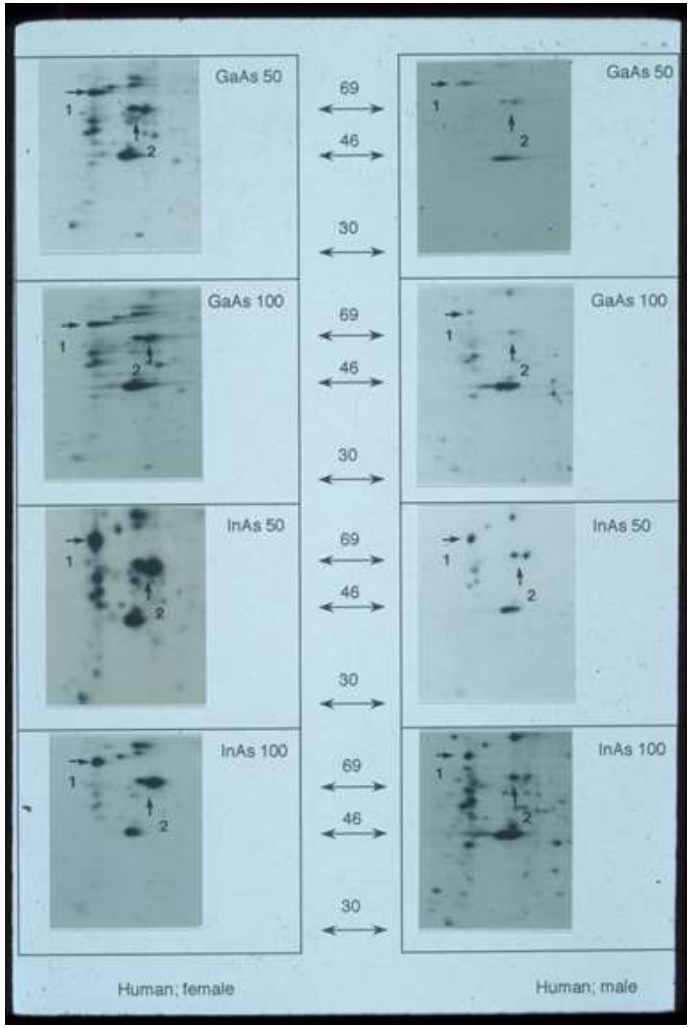
MW Range	Common Spot #[1]	InAs[1]	GaAs[2]
100–90	1	.92	.96
	18	.56	.17*
	29	.71	.37*
89–70	2	6.60*	5.50*
	12	.47*	1.20
69–50	3	.68	1.10
	11	.51	.96
	22	.32*	.78
	23	.31*	.83
	24	.26*	.64
	25	.26*	1.26
	32	.90	.96
49–40	10	.43*	.90
	16	2.50*	.57
	26	.58	.45*
	27	–	1.60
	28	–	10.00*
	33	–	1.40
	34	.43*	.57
39–30	4	–	–
	5	2.00*	1.20
	6	.14*	.70
	7	.32*	.61
	8	.39*	.50
	13	.47*	1.20
	14	.37*	1.00
	15	.76	1.25
	17	1.00	.96
	19	.20*	1.10
	20	–	.50
≤29	30	.84	.76
	31	.54	.60

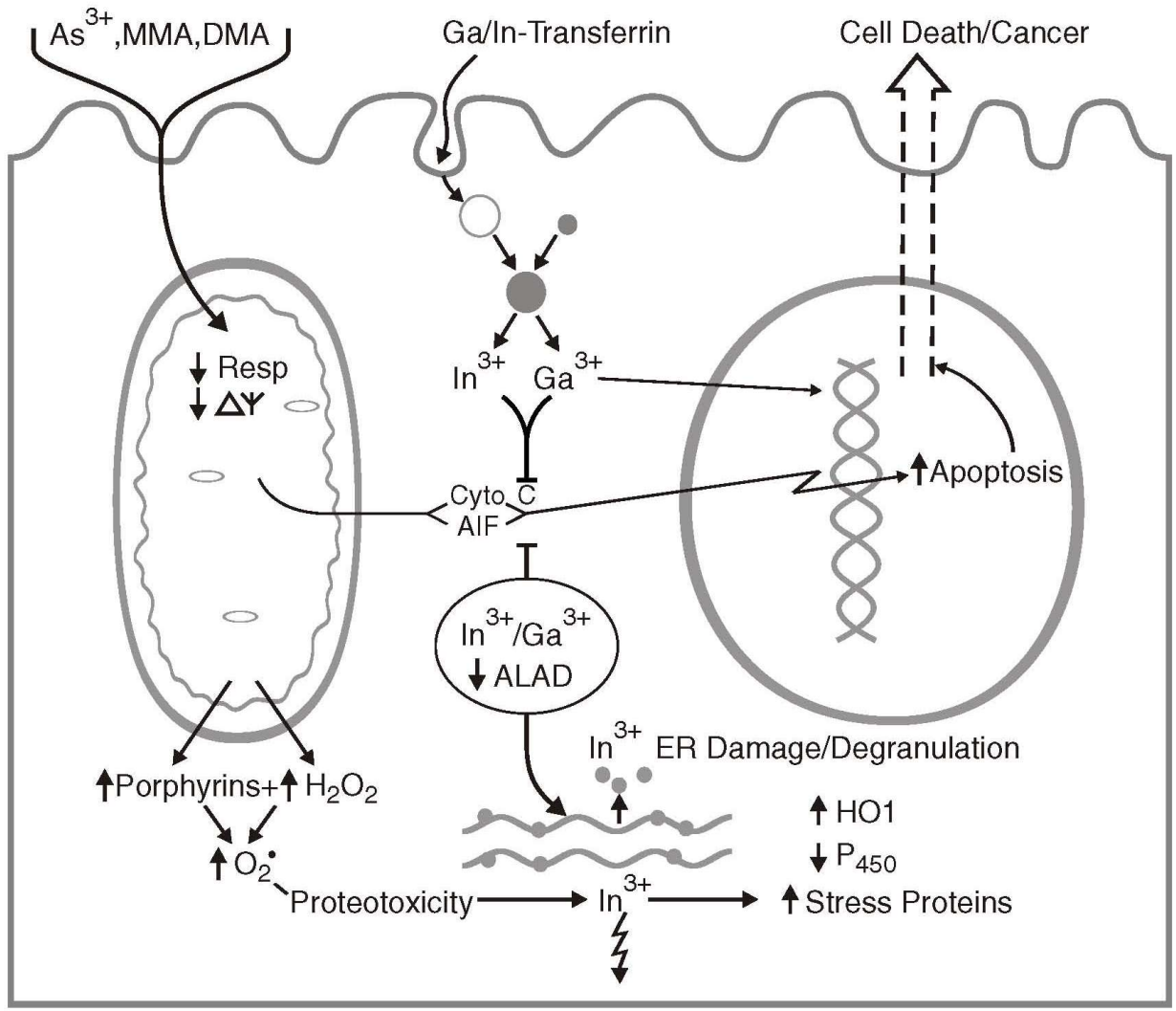
1) Spot number denoted on gel

2) Spot intensity (ratio of tx/control)

*a ≥ 2 fold increase/decrease in polypeptides


Control
As
Ga
In
69
46
30
1
2
Human; female
Human; male


GaAs 50
GaAs 100
InAs 50
InAs 100
69
46
30
1
2
Human; female
Human; male


As3+,MMA,DMA
Ga/In-Transferrin
Cell Death/Cancer
↓ Resp
↓ ΔΨ
In3+ Ga3+
Cyto C
AIF
↑Apoptosis
In3+/Ga3+
↓ ALAD
In3+ ER Damage/Degranulation
↑Porphyrins+↑ H2O2
↑ HO1
↓ P450
↑ O2•
Proteotoxicity
In3+
↑ Stress Proteins

Conclusions

- There are a number of established and evolving biomarkers for assessing the effects of toxic agents, such as Ga, In, or As alone or in mixtures and delineating the "biologically active" fraction of these agents prior to the onset of clinical disease

- Validation of biomarkers for risk assessment studies on human populations requires a validation process that includes consideration of modifying factors such as GENDER that may alter relationships between exposure and effect

- Integration of data from *in vivo* and *in vitro* studies and extrapolation to human populations via *in silico* approaches is essential for improving risk assessments and identifying sensitive sub-populations

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