



# Exposure to arsenic compounds during the dismantling of chemical weapons: biomonitoring and genotoxic effects.

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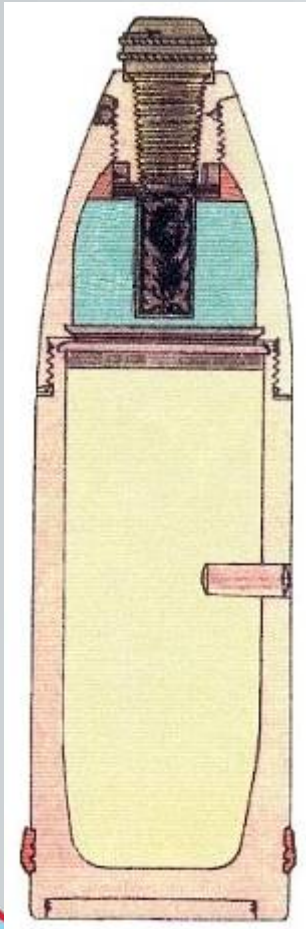
# Dismantling 2002- 2004

- AsCl<sub>3</sub>-Phosgene or AsCl<sub>3</sub>-HCN shells
- step-by-step dismantling
- ± 20 to 40 times per month
- 19 deminers daily exposed to arsenite
- Legal implications:
  - workplace exposure limit / PPE
  - biomonitoring of total arsenic in urine





# Shell QF 4,5" DD Type



- Calibre : 4,5" (114 mm)
- Length :  $\pm$  370 mm
- Wall thickness : 14 mm
- Explosive : 258 - 308 g TNT
- Toxic agent: max. 1400 g  
arsenic(III)chloride  
and  
hydrogen cyanide  
or phosgene





# Trivalent arsenite (As<sup>3+</sup>)

- Carcinogenic to humans (IARC Group 1)
- Workplace TLV-TWA (8 Hrs) = 10  $\mu\text{g}/\text{m}^3$

| Number of samples | Mean value ( $\mu\text{g}/\text{m}^3$ ) | 95 Pct ( $\mu\text{g}/\text{m}^3$ ) | UL <sub>0.95</sub> (X 0.95) ( $\mu\text{g}/\text{m}^3$ ) |
|-------------------|---|-------------------------------------|--|
| 122               | 158                                     | 1676                                | 3640   |



very strict workplace procedures  
+ personal protection





# Personal Protective Equipment

- CBRN suit (second generation)
- Active charcoal undergarment
- Boots and gloves
- Full face mask + compressed air supply
- Protection factor (laboratory, DSTL)
  - skin: at least 100
  - respiratory: at least 2500  
(APF > 2000, NIOSH and > 1000, OSHA)





# PPE for dismantling plant





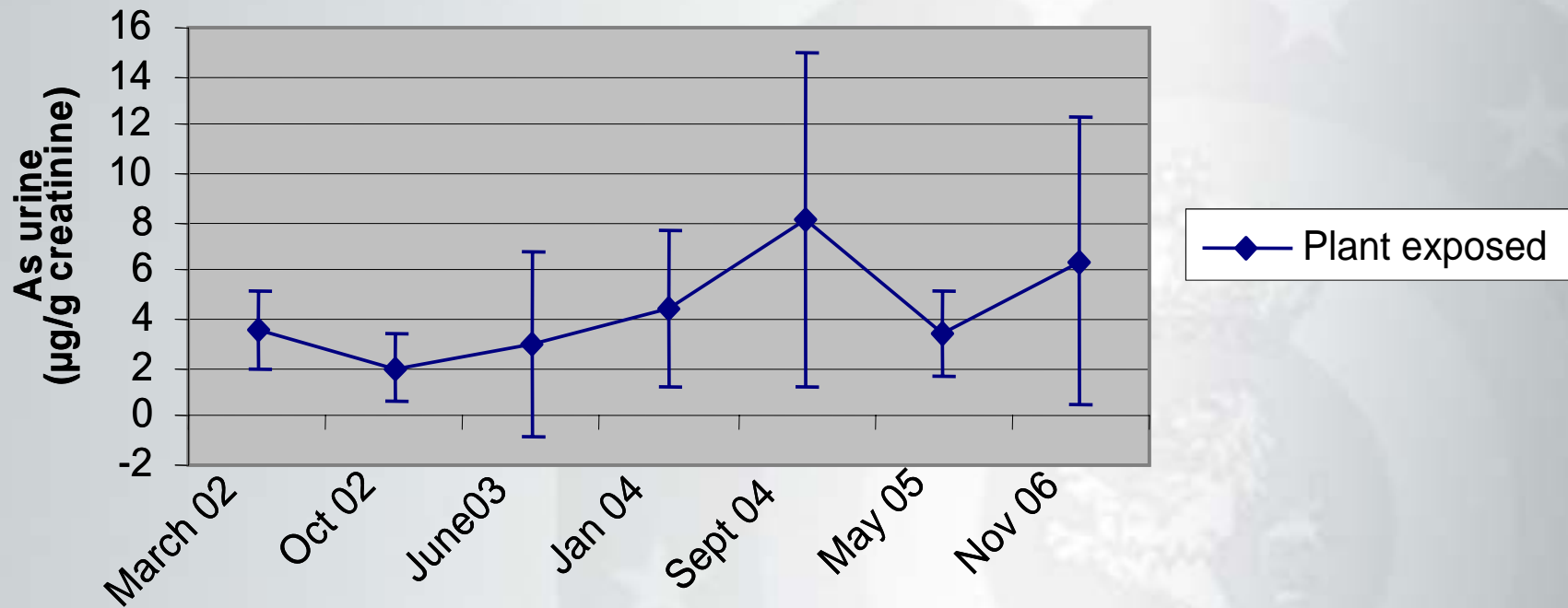
# Biomonitoring

- Total urinary arsenic:
  - Biological Exposure Index (BEI) = 35  $\mu\text{g}/\text{l}$
  - allowable biological value (VBA) = 30  $\mu\text{g}/\text{g creat.}$
- Total urinary arsenic:
  - free trivalent inorganic arsenic
  - monomethylarsonic acid (MMA)
  - dimethylarsinic acid (DMA)
- Individual result / mean value / evolution in time



# Total urinary arsenic

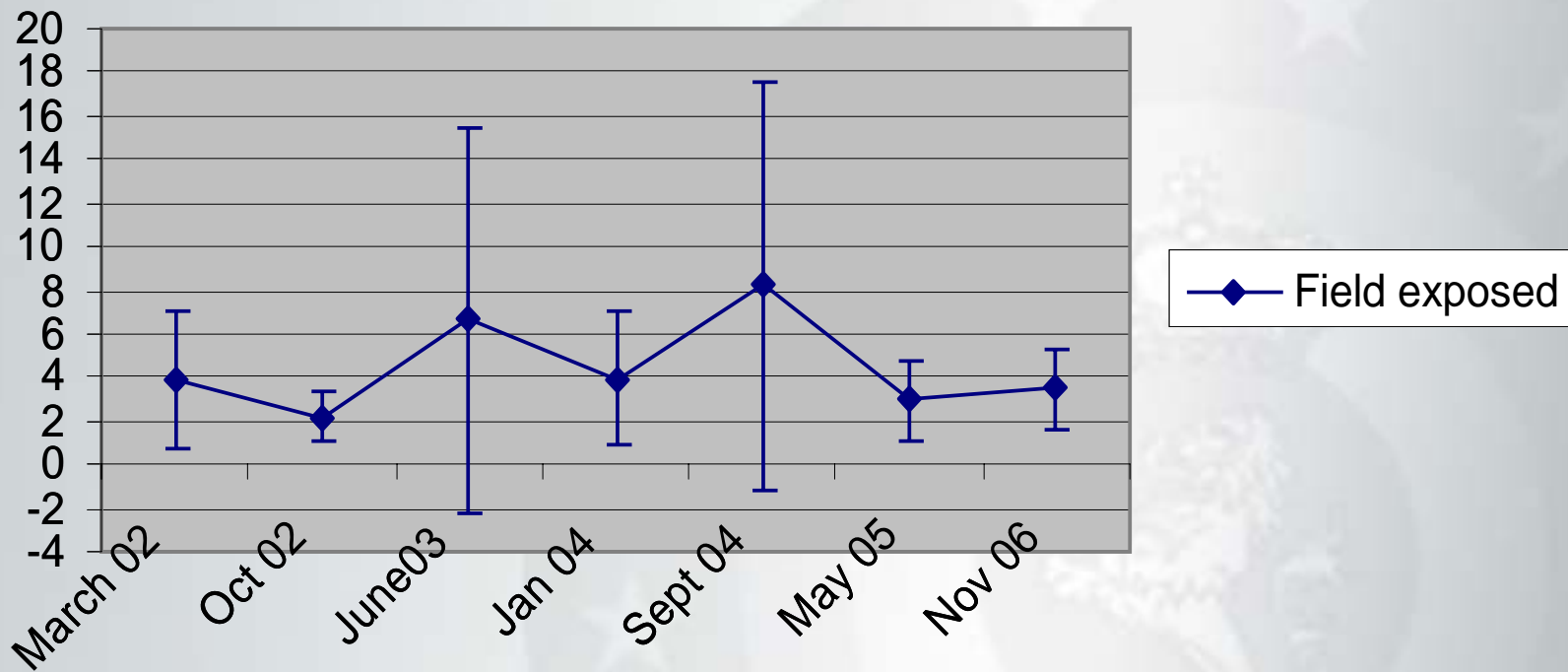
Fig. 1. As data Plant exposed 2002-2006





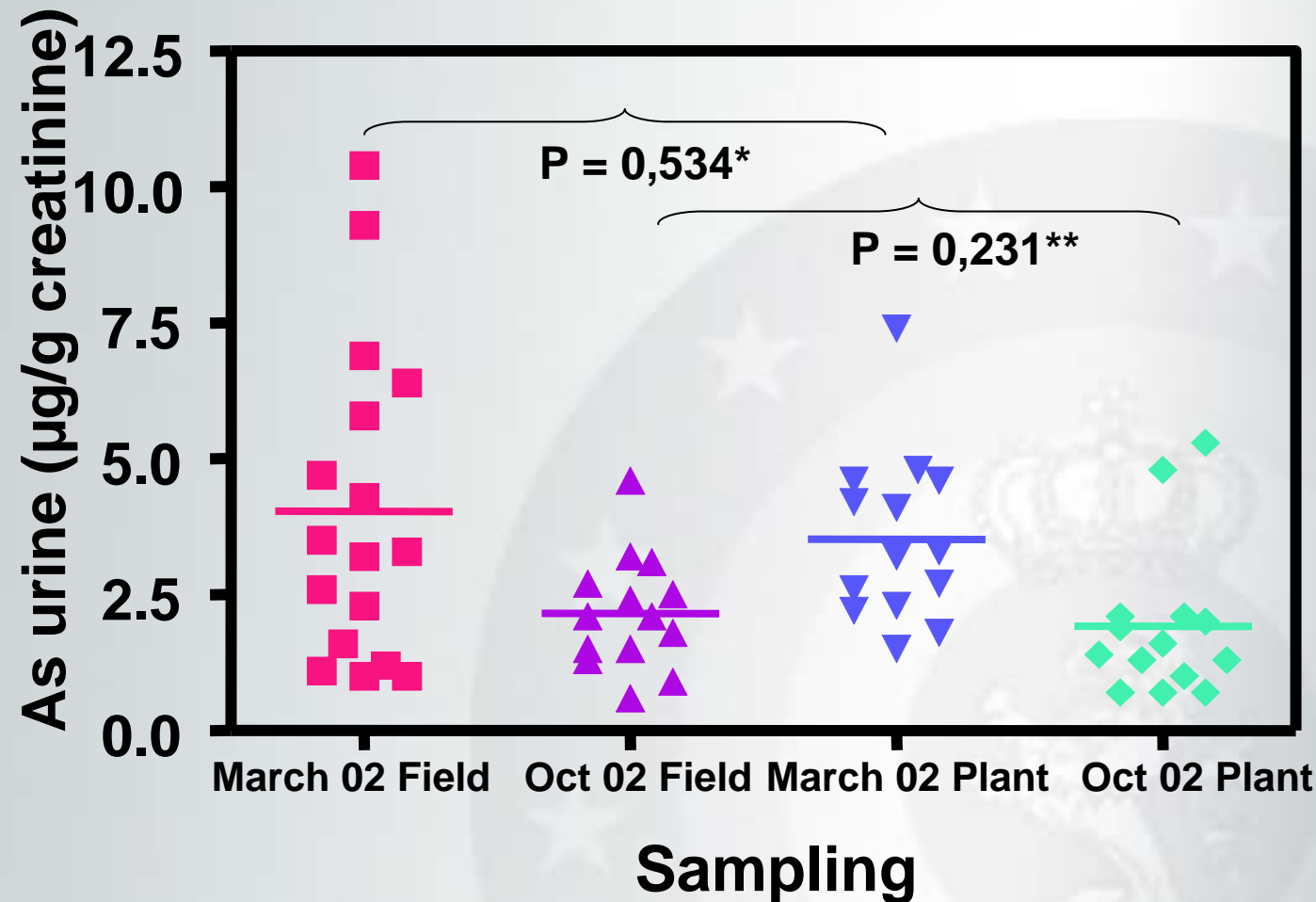
# Total urinary arsenic

Fig. 2 As data Field exposed 2002-2006



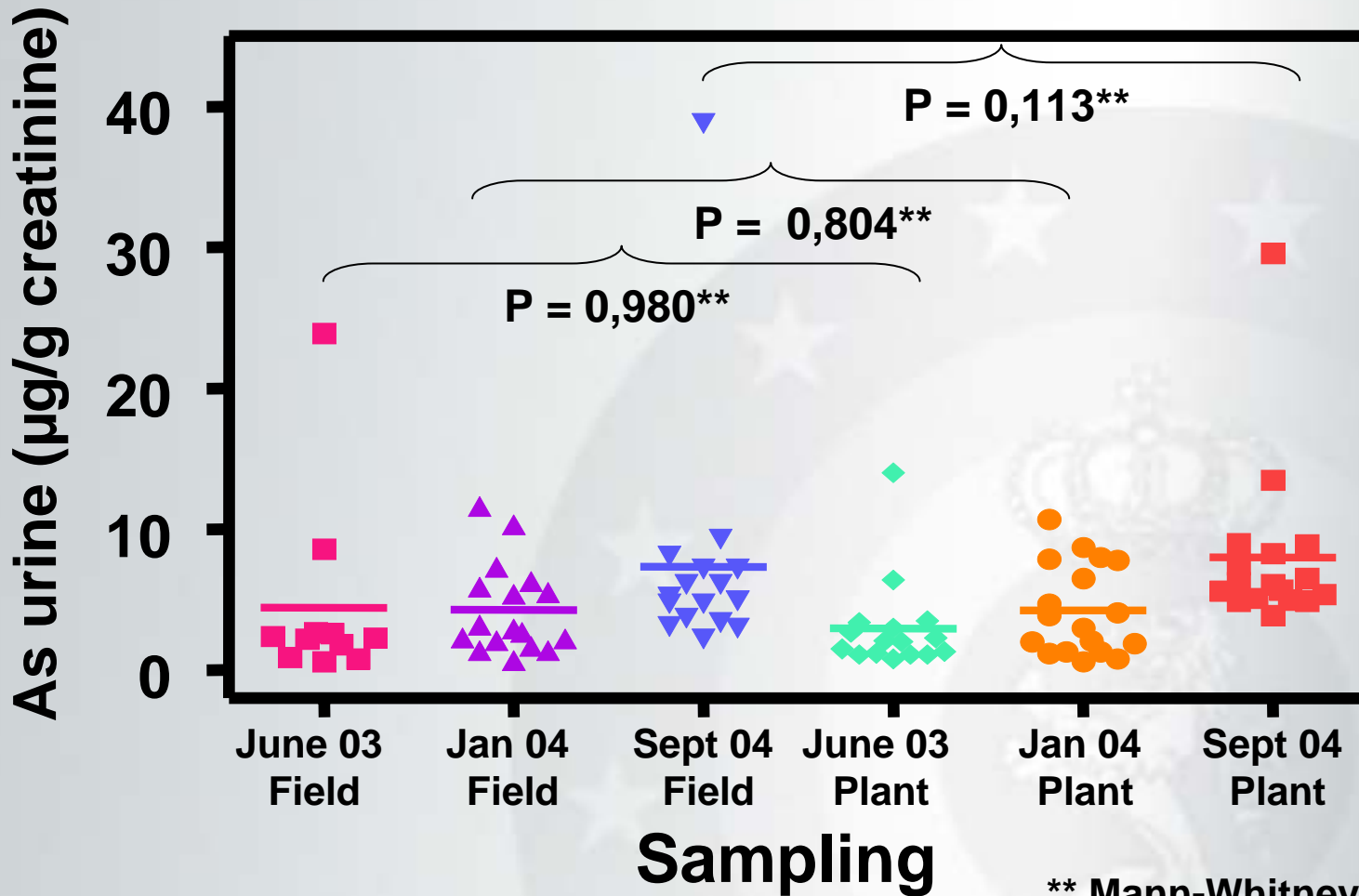


# Field ÷ Plant March-Oct 2002





# Field ÷ Plant 2003-2004



\*\* Mann-Whitney U test





# Biomonitoring

- No significant difference between plant and field
- Evolution in time from 2002 - 2004:
  - Plant exposed (n = 13):  
3,65 ± 1,65 to 8,04 ± 6,89 µg/g creat. (p = 0,006)
  - Field exposed (n = 16):  
4,22 ± 3,24 to 8,70 ± 10,16 µg/g creat. (p = 0,005)
- Carcinogenicity ?
  - arsenic causes cytogenetic damage
  - mechanism not fully understood





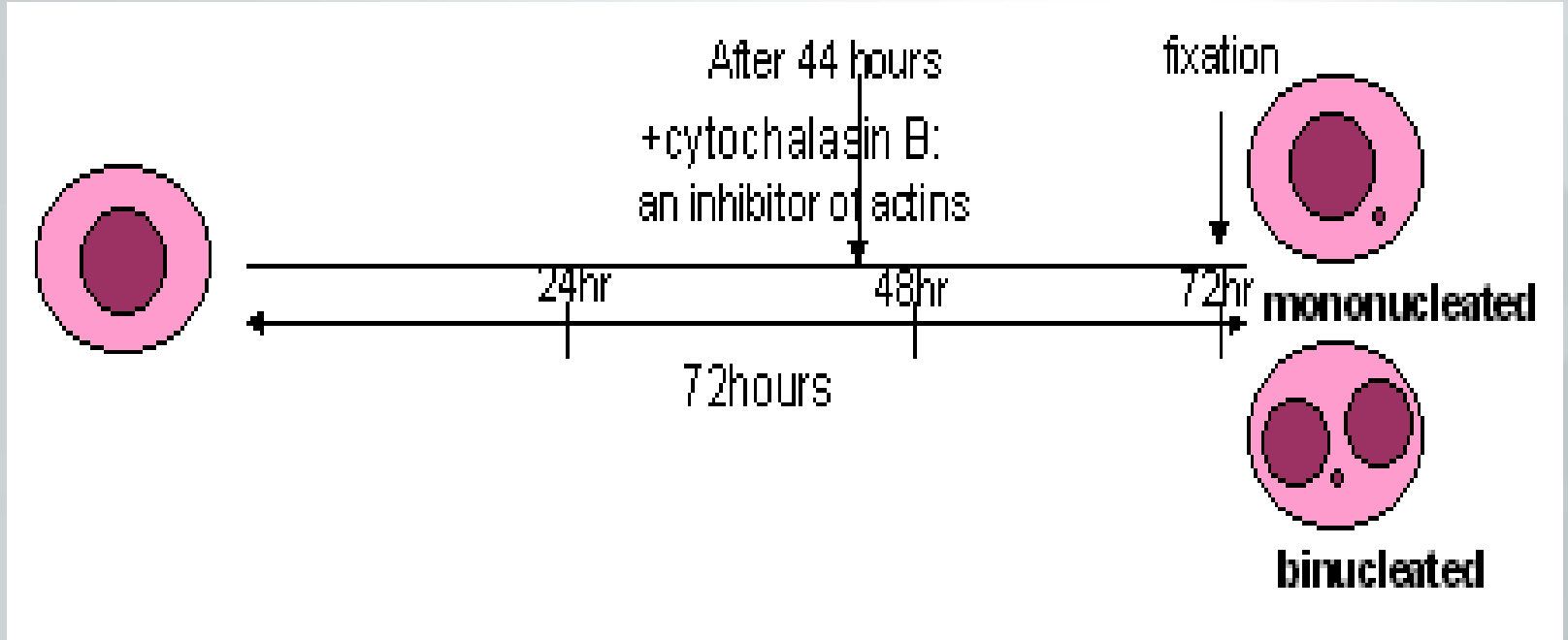
# Bio(effect)monitoring

- Objective: monitor early biological effects
- Increased risk of cancer, cytogenetic damage ?
  - sister chromatid exchange (SCE)
  - chromosomal aberration (CA)
  - cytokinesis-block micronucleus (CBMN)
- Confounding factors: age, smoking habits, medication, coffee and alcohol consumption
- No individual test, but comparison of populations





# Micronucleus (MN)

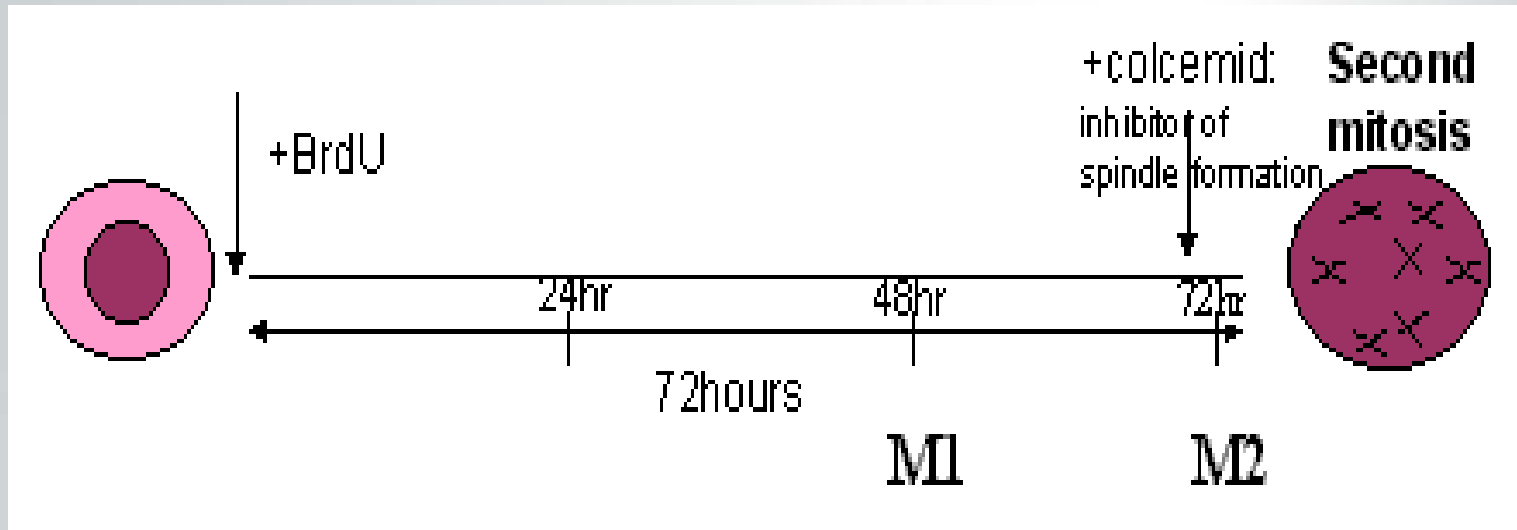


Predictive biomarker of cancer risk in a population of healthy subjects





# Sister Chromatid Exchange (SCE)



Biomarker of genotoxic effects in exposed subjects

No association with cancer risk





# Populations: plant / field / control

| Parameters                 | Controls (n = 21 ) |              |         | Field exposed (n = 21 ) |               |         | Plant exposed (n = 15 ) |              |         |
|----------------------------|--------------------|--------------|---------|-------------------------|---------------|---------|-------------------------|--------------|---------|
|                            | No.*               | Mean ± SD    | Range   | No.*                    | Mean ± SD     | Range   | No.*                    | Mean ± SD    | Range   |
| Age (years)                | 21                 | 38,29 ± 5,03 | 27 - 48 | 21                      | 40,52 ± 5,77  | 26 - 48 | 15                      | 37,67 ± 5,26 | 29 - 51 |
| Alcohol<br>(glass/week)    | 21                 | 5,67 ± 7,59  | 0 - 33  | 21                      | 11,05 ± 10,62 | 0 - 31  | 15                      | 10,40 ± 8,63 | 0 - 31  |
| Cigaretts/day<br>(smokers) | 6                  | 5,48 ± 9,07  | 0 - 25  | 6                       | 6,19 ± 12,34  | 0 - 50  | 2                       | 2,67 ± 7,29  | 0 - 25  |
| Coffee<br>(cups/day)       | 21                 | 3,81 ± 4,58  | 0 - 15  | 21                      | 5,64 ± 4,80   | 0 - 20  | 15                      | 3,07 ± 2,84  | 0 - 8   |

\* No.: number of workers with available values for a given parameter.





# Plant exposed 2002- 2004

| Parameter | Number workers | Plant exposed 02    | Plant exposed 04 | p-value        |
|-----------|----------------|---------------------|------------------|----------------|
|           |                | Mean ± SD           | Mean ± SD        |                |
| % CB      | 13             | <b>55,93 ± 5,47</b> | 51,72 ± 5,27     | <b>0,016*</b>  |
| ‰ MNCB    | 13             | 5,27 ± 3,11         | 3,65 ± 1,78      | 0,125*         |
| ‰ MNMC    | 13             | <b>2,98 ± 3,81</b>  | 0,55 ± 0,64      | <b>0,011**</b> |
| CBPI      | 13             | <b>1,88 ± 0,15</b>  | 1,53 ± 0,47      | <b>0,023**</b> |
| SCE       | 13             | 7,29 ± 0,81         | 7,02 ± 0,92      | 0,263**        |
| % HFC     | 13             | <b>15,36 ± 9,09</b> | 5,14 ± 5,88      | <b>0,001*</b>  |

\* paired t-test.\*\* Wilcoxon Signed Ranks test.

MN and HFC significantly lower in 2004 than in 2002





# Field exposed 2002- 2003

| Parameter | Number workers | Field exposed 02    | Field exposed 03   | p-value            |
|-----------|----------------|---------------------|--------------------|--------------------|
|           |                | Mean ± SD           | Mean ± SD          |                    |
| % CB      | 16             | <b>56,86 ± 3,25</b> | 50,93 ± 6,12       | <b>0,004*</b>      |
| % MNCB    | 16             | 3,31 ± 1,94         | <b>6,46 ± 2,16</b> | <b>0,001*</b>      |
| % MNMC    | 16             | 1,36 ± 1,48         | 0,78 ± 0,58        | 0,154*             |
| CBPI      | 16             | <b>1,96 ± 0,10</b>  | 1,58 ± 0,09        | <b>&lt; 0,001*</b> |
| SCE       | 16             | 5,83 ± 0,75         | <b>7,63 ± 0,76</b> | <b>&lt; 0,001*</b> |
| % HFC     | 16             | 5,57 ± 5,33         | 6,57 ± 4,33        | 0,552*             |

\* paired t-test

MNCB and SCE significantly higher in 2003 than in 2002





# 2002:

## Field/Control/Plant

| Parameter | Field exposed 02<br>(N = 21 ) | P-value       | Controls 01<br>(N = 21) | P-value             | Plant exposed 02<br>(N = 15) |
|-----------|-------------------------------|---------------|-------------------------|---------------------|------------------------------|
|           | Mean ± SD                     |               | Mean ± SD               |                     | Mean ± SD                    |
| % MNCB    | 3,43 ± 1,94                   | 0,980**       | 3,50 ± 1,67             | 0,114**             | 5,17 ± 3,04                  |
| % MNMC    | 1,12 ± 1,40                   | 0,683**       | 0,94 ± 0,51             | <b>0,053**</b>      | <b>2,93 ± 3,66</b>           |
| SCE       | <b>6,13 ± 1,06</b>            | <b>0,027*</b> | 5,40 ± 0,63             | <b>&lt; 0,001*</b>  | <b>7,29 ± 1,07</b>           |
| HFC       | 7,75 ± 7,89                   | 0,150**       | 4,05 ± 4,27             | <b>&lt; 0,001**</b> | <b>15,64 ± 10,18</b>         |

| Parameter | Field exposed 02<br>(N = 21) | Plant exposed 02<br>(N = 15) | P-value        |
|-----------|------------------------------|------------------------------|----------------|
|           | Mean ± SD                    | Mean ± SD                    |                |
| % MNCB    | 3,43 ± 1,94                  | <b>5,17 ± 3,04</b>           | <b>0,044*</b>  |
| % MNMC    | 1,12 ± 1,40                  | 2,93 ± 3,66                  | 0,062**        |
| SCE       | 6,13 ± 1,06                  | <b>7,29 ± 1,07</b>           | <b>0,003*</b>  |
| HFC       | 7,75 ± 7,89                  | <b>15,64 ± 10,18</b>         | <b>0,008**</b> |





# 2003- 04:

## Field/Control/Plant

| Parameter | Field exposed 03<br>(N = 22) | P-value | Controls 03<br>(N = 19 §) | P-value  | Plant exposed 04<br>(N = 19) |
|-----------|------------------------------|---------|---------------------------|----------|------------------------------|
|           | Mean ± SD                    |         | Mean ± SD                 |          | Mean ± SD                    |
| % MNCB    | 6,32 ± 2,56                  | 0,011*  | 4,43 ± 1,71               | 0,152*   | 3,63 ± 1,60                  |
| % MNMC    | 1,10 ± 1,25                  | 0,012** | 2,07 ± 1,40               | <0,001** | 0,61 ± 0,71                  |
| SCE       | 7,76 ± 0,91                  | <0,001* | 6,59 ± 0,80               | 0,035*   | 7,17 ± 0,83                  |
| % HFC     | 7,67 ± 5,91                  | 0,015*  | 3,59 ± 3,94               | 0,173*   | 5,69 ± 5,28                  |

| Parameter | Field exposed 03<br>(N = 22) | Plant exposed 04<br>(N = 19) | P-value |
|-----------|------------------------------|------------------------------|---------|
|           | Mean ± SD                    | Mean ± SD                    |         |
| % MNCB    | 6,32 ± 2,56                  | 3,63 ± 1,60                  | <0,001* |
| % MNMC    | 1,10 ± 1,25                  | 0,61 ± 0,71                  | 0,157** |
| SCE       | 7,76 ± 0,91                  | 7,17 ± 0,83                  | 0,038*  |
| % HFC     | 7,67 ± 5,91                  | 5,69 ± 5,28                  | 0,268*  |





# Conflicting results

## 2002-04

- Total urinary arsenic in 2004 significantly higher:
  - in the plant and the field exposed
- Cytogenetic parameters in 2004 significantly lower:
  - in the plant exposed compared with 2002 and compared with the field exposed in 2004
- Plant: evolution of arsenic  $>$  cytogenetic results
- Field: evolution of arsenic  $\div$  cytogenetic results
- Conclusion: arsenic is not the determining factor





# Confounding factors

- In the work environment 2002-2004:
  - plant: exclusively arsenic exposure  
always perfect personal protection
  - field: other cytotoxic exposures (e.g. HD)
    - « asymptomatic » exposures  
personal protection ÷ risk analysis  
(agent detection, evaluation of circumstances, experience,...)





# Regression analysis 2002

| Genotoxicity parameters | Predictors (controls 2001- exposed 2002) | Slope  | Partial $r^2$ | $R^2$ | p-value                      |
|-------------------------|--|--------|---------------|-------|------------------------------|
| % MNCB                  | 1. Plant exposed                         | 1,667  | 0,085         | 0,110 | <b>0,029</b>                 |
|                         | 2. Field exposed                         | -0,071 | 0,0002        |       | 0,916                        |
|                         |  |        |               |       | <b>0,043<sup>§</sup></b>     |
| % MNMC                  | 1. Plant exposed                         | 1,989  | 0,130         | 0,148 | <b>0,006</b>                 |
|                         | 2. Field exposed                         | 0,181  | 0,001         |       | 0,778                        |
|                         |  |        |               |       | <b>0,013<sup>§</sup></b>     |
| SCE                     | 1. Plant exposed                         | 1,898  | 0,373         | 0,381 | <b>&lt;0,001</b>             |
|                         | 2. Field exposed                         | 0,733  | 0,094         |       | <b>0,032</b>                 |
|                         |  |        |               |       | <b>&lt;0,001<sup>§</sup></b> |
| % HFC                   | 1. Plant exposed                         | 11,593 | 0,249         | 0,261 | <b>&lt;0,001</b>             |
|                         | 2. Field exposed                         | 3,696  | 0,038         |       | 0,181                        |
|                         |  |        |               |       | <b>0,001<sup>§</sup></b>     |

<sup>§</sup>Significance level in the model. Values in bold are significant p-values for each of the predictor variables





# Regression analysis

## 2004

| Genotoxicity parameters | Predictors (controls 2003- field 2003 - plant 2004) | Slope  | Partial $r^2$ | $R^2$ | p-value                       |
|-------------------------|---|--------|---------------|-------|-------------------------------|
| % MNCB                  | 1. Field exposed                                    | 1,850  | 0,134         | 0,301 | <b>0,005</b>                  |
|                         | 2. Age  | 0,093  | 0,064         |       | 0,057                         |
|                         | 3. Plant exposed                                    | -0,802 | 0,027         |       | 0,226                         |
| % MNMC                  | 1. Plant exposed                                    | -1,406 | 0,205         | 0,264 | <b>&lt;0,001</b>              |
|                         | 2. Field exposed                                    | -0,928 | 0,108         |       | <b>0,013</b>                  |
|                         | 3. Coffee   | -0,087 | 0,066         |       | 0,054                         |
| SCE                     | 1. Field exposed                                    | 1,160  | 0,298         | 0,417 | <b>&lt;0,001</b>              |
|                         | 2. Smoking status                                   | 0,880  | 0,220         |       | <b>&lt;0,001</b>              |
|                         | 3. Plant exposed                                    | 0,578  | 0,089         |       | <b>0,023</b>                  |
| % HFC                   | 1. Smoking status                                   | 5,175  | 0,208         | 0,288 | <b>&lt;0,001</b>              |
|                         | 2. Field exposed                                    | 4,027  | 0,121         |       | <b>0,007</b>                  |
|                         | 3. Plant exposed                                    | 2,099  | 0,034         |       | 0,167                         |
|                         |   |        |               |       | <b>&lt;0,001</b> <sup>§</sup> |

<sup>§</sup>Significance level in the model. Values in bold are significant p-values for each of the predictor variables





# Predictors of genotoxicity

2001-2002: plant exposure is determining

2003-2004: field exposure and smoking  
status

## Explanation:

Under controlled circumstances, with an optimal protection for a single agent less genotoxic effects are recorded (plant 2002-2004)

Varying circumstances, (sub)optimal protection

to multiple agents induces more genotoxic effects

(field, but e.g. also smoking controls)



# Comments

1. Management of toxic risk in plant is good !  
Cave: "Healthy worker effect"  
Comparison with any other group ?
2. Total urinary arsenic in controls ?  
In 2006:  $2,95 \pm 2,97 \mu\text{g} / \text{g creatinine}$
3. Statistical relevance ?  
small groups, longitudinal dropouts
4. Follow-up and re-evaluation of plant/field





# Field exposed

with potential toxic exposure:

- evaluation of circumstances (leaking munition?)

soil contamination? airborne toxic agent? ...

- earlier engagement of personal protection

- occupational hygiene: disposable protective

suits + strict separation

"contaminated / not

contaminated" + respect for "clean"

zone +...

Sustainable  
OPERATIONALITY

