Minne Heringa, October 29 2007

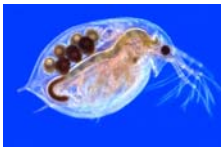
kiwa
Partner for progress

Measurement of genotoxicity in (drinking) water

3d NORMAN workshop

Monitoring of (drinking) water

- Pollution of ground water and surface water → drinking water quality?
- Current monitoring
  - Chemical monitoring
  - Biological monitoring
- New
  - Bio-assays: exposure of cells *in vitro*



Which bioassays?

- Human health
- Low concentrations → no acute effects, sensitivity issue
- Life-time exposure → chronic toxicity most relevant

- Genotoxicity
- Endocrine disruption
- Other chronic toxicity?
  - Reproductive tox, carcinogenicity, neurological disorders, liver toxicity, kidney toxicity, etc.

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Which bioassays?

- Low concentrations → no acute effects, sensitivity issue
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- Genotoxicity
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- Other chronic toxicity?
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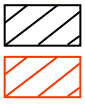
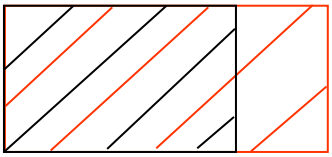
Genotoxicity and carcinogenicity

- Genotoxic = causes DNA damage
- Carcinogenic = causes cancer
  - Genotoxic carcinogens
  - Non-genotoxic carcinogens: e.g. growth promotors



- Test for genotoxicity
- Test for non-genotoxic carcinogenicity?



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Why test for genotoxicity?

Compound	Log K_{ow}	Genotoxicity/ Carcinogenicity	Detected in*
Phenol	1.8	Genotoxic in mammalian cells	S
2,4-D	2.8	Genotoxic in mammalian cells	S
atrazine	2.5	Suspected genotoxicant	S, D
simazine	2.5	Genotoxic in mammalian cells	S, D
chlorotoluron	2.3	Genotoxic in mammalian and bacterial cells	S
diuron	2.8	Genotoxic in mammalian and bacterial cells	S, D
trichloromethane	2.0	IARC 2B**	S, D
romodichloromethane	1.88	Suspected genotoxicant	S, D
ibromochloromethane	2.2	Suspected genotoxicant	S, D
tribromomethane	2.38	Suspected genotoxicant	S, D
tetrachloroethene	1.7	IARC 2B**	S, D
dimethylamine	-0.38	Genotoxic in mammalian cells	S
urotropine	-2.3	Genotoxic in mammalian cells	S
NDMA	-0.57	Carcinogenic (IARC 1)	S

Abstracted from van Genderen et al., *Inventory and toxicological evaluation of organic micropollutants*, report RIWA 2000

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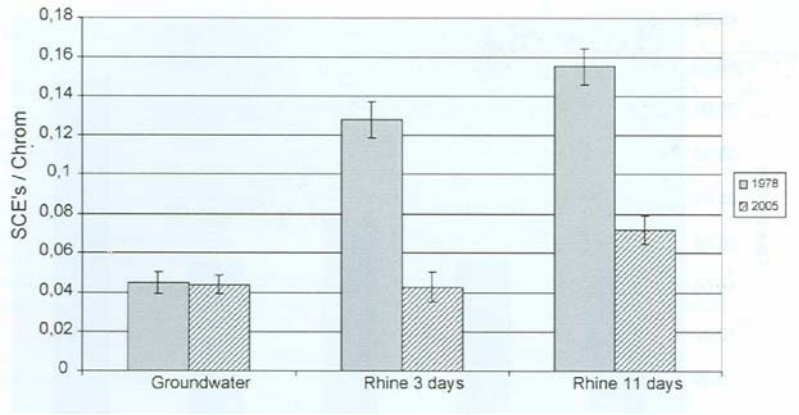
Why test for genotoxicity?

- **Alink et al. *Mut Res* 631 (2007), p.93-100:**
  - **Eastern mudminnow exposed to Lekkanaal surface water**
  - **After 11 days significant genotoxic effects**



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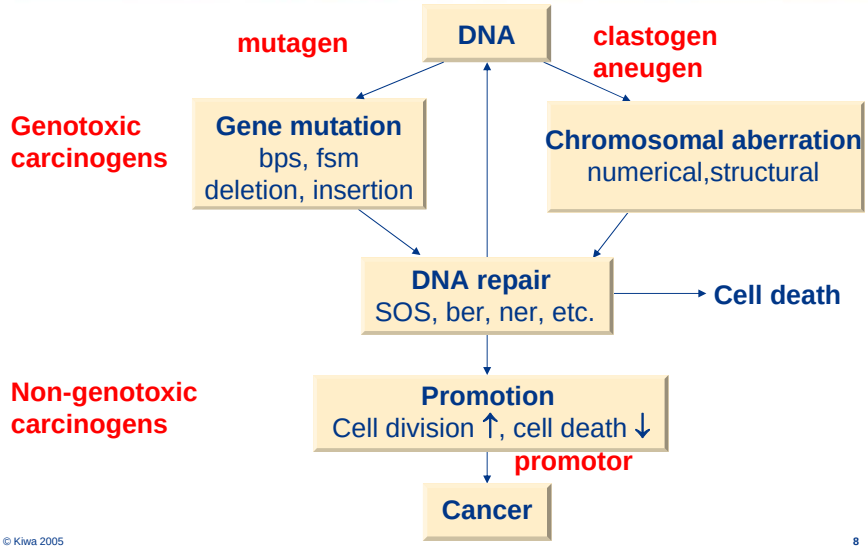
Mechanism of carcinogenicity → types of genotoxicity



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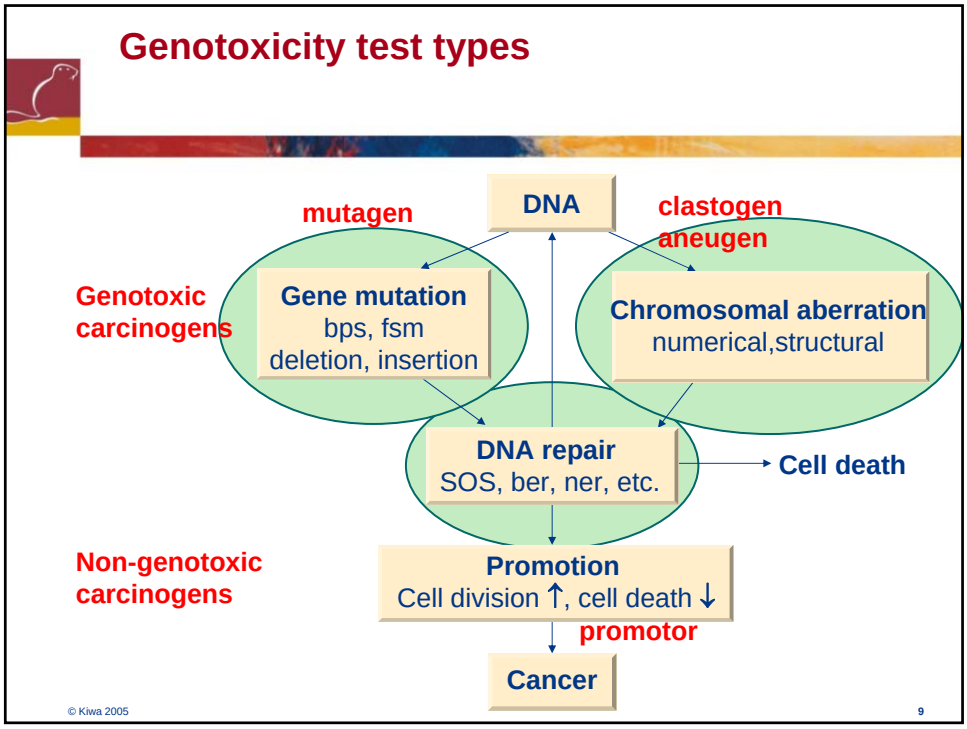
Genotoxicity test types
mutagen
DNA
clastogen
aneugen
Genotoxic
carcinogens
Gene mutation
bps, fsm
deletion, insertion
Chromosomal aberration
numerical,structural
DNA repair
SOS, ber, ner, etc.
Cell death
Non-genotoxic
carcinogens
Promotion
Cell division ↑, cell death ↓
promotor
Cancer
© Kiwa 2005
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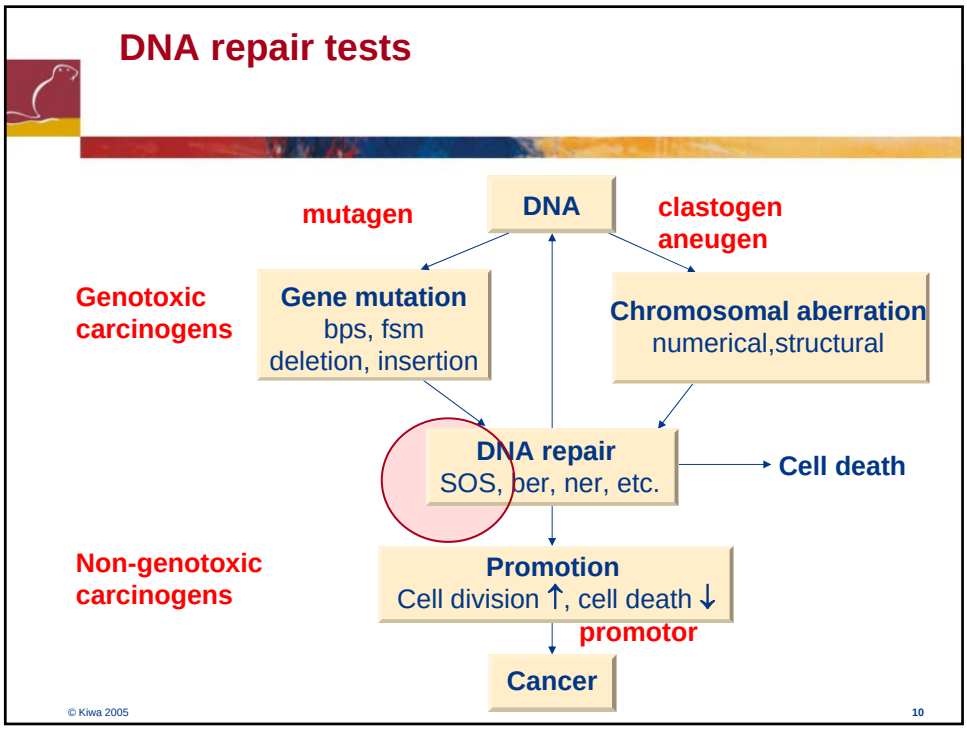
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Overview of DNA repair tests

- **Well known repair assays**
  - **Unscheduled DNA synthesis (UDS)**
  - **Umu**
  - **SOS-Chromo**
  - **Vitotox®**
  - **Mutatox™**
  - **GreenScreen® (yeast and human)**
  - **RadarScreen**

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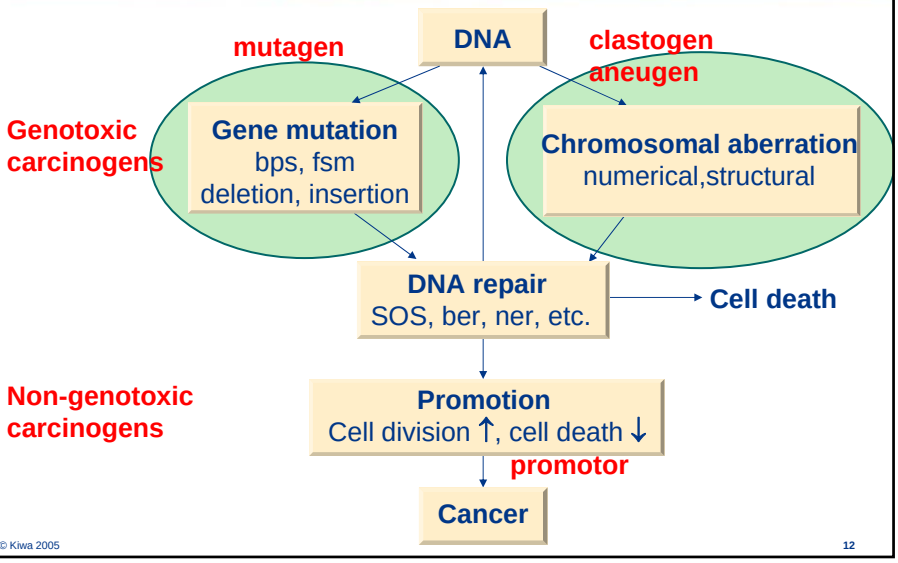
Genotoxicity test types



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Mutagenicity assays

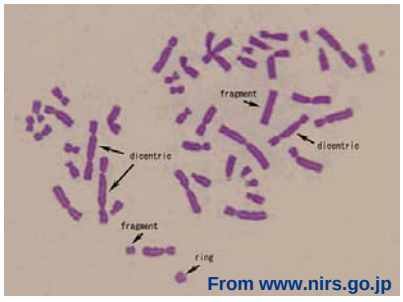
- **Ames test / Ames II**
  pathogenic bacteria, 3-4 days, colony counting
- ***Vibrio harveyi* neomycin resistance**
  safe bacteria, 3-4 days, colony counting, not well known

From www.hydrotox.de

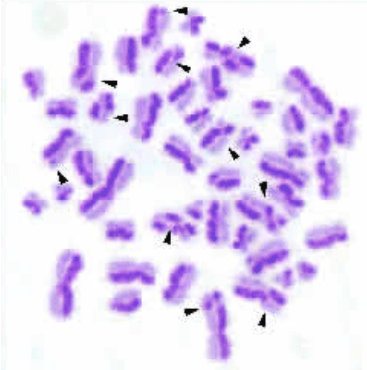
- **HGPRT**
  mammalian cell line, ± 2 weeks, colony counting
- **TK / mouse lymphoma**
  special mammalian cell line, min. 2 weeks, colony counting

Chromosome aberration assays

- **Sister chromatid exchange (SCE)**
  Colouring, microscope evaluation of every chromosome
- **Chromosome aberration (CA)**
  Colouring, microscope evaluation of every chromosome



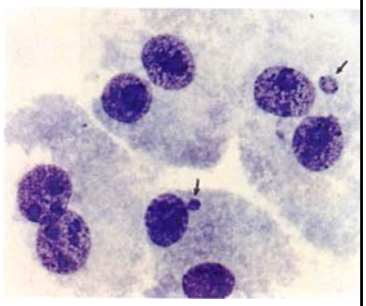
From www.nirs.go.jp



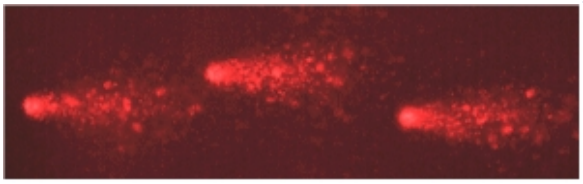
Sister Chromatid Exchange
Illustration produced in the laboratory of Dr Al Rowland, Massey University

Chromosome aberration assays

- **Micronucleus**
  Cell division arrest, colouring, microscope ev. of binucleated cells (software)
- **Comet**
  Lysis, electroforesis, microscope ev. of cell (software)



Source: Darroudi *et al., 1996*



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Strategy *in vitro* genotoxicity assays

- **Kiwa WR:**
  stage 1: bacterial mutagenicity + chrom. aberr.
  stage 2: mammalian mutagenicity / other chrom. aberr
- **Gezondheidsraad (Health Council, NL; 1988):**
  bacterial mutagenicity + mammalian mutagenicity + chromosomal aberrations
- **German working group GUM (2007):**
  stage I: bacterial mutagenicity +*in vitro* micronucleus
  stage II: *in vivo*

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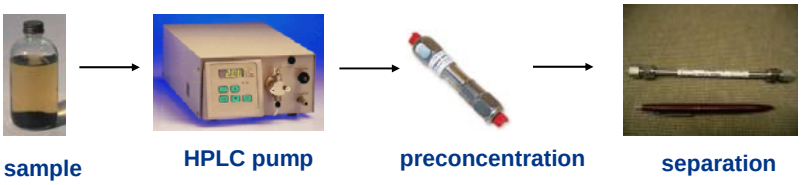
Which tests?
Able to use in HPLC-ToxPrint®



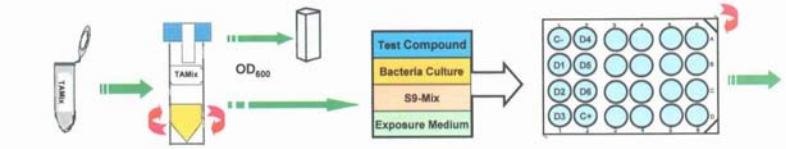
sample → HPLC pump → preconcentration → separation

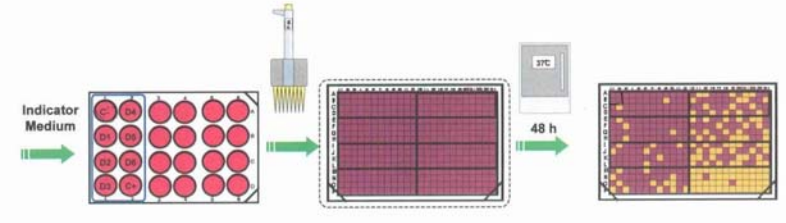
separation → UV detector → fractionation → Toxicity assay

→ multi-well format of toxicity assay necessary

Ames II

- Same strains, except TA100 → TAMix
- Agar → liquid culture
- Colony counting → colour change counting





Adaptations Ames II for environmental samples: sample preparation

- **Extraction and concentration (10.000x) of water**
- **Compared different methods at ambient pH:**
  - **XAD SPE** — **classic, practical limitations**
  - **SPE with OASIS HLB** — **new, easy**
    - Elution with ACN — general
    - Elution with EtAC — hormones
  - **LLE with EtAC (for hormones)** — **classic, practical limitations**
- **Water spiked with broad mixture**
- **Recoveries analyzed by LC-MS**

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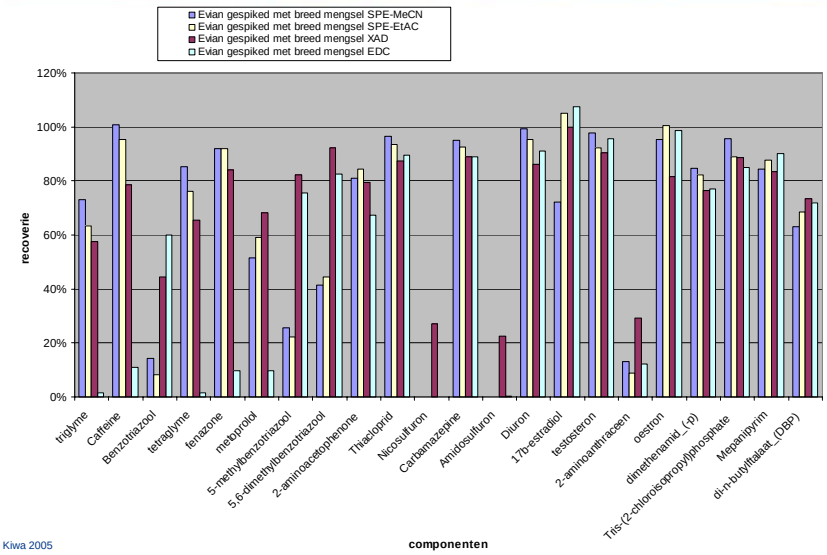
Results comparison sample preparations



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Adaptations Ames II for environmental samples: statistics

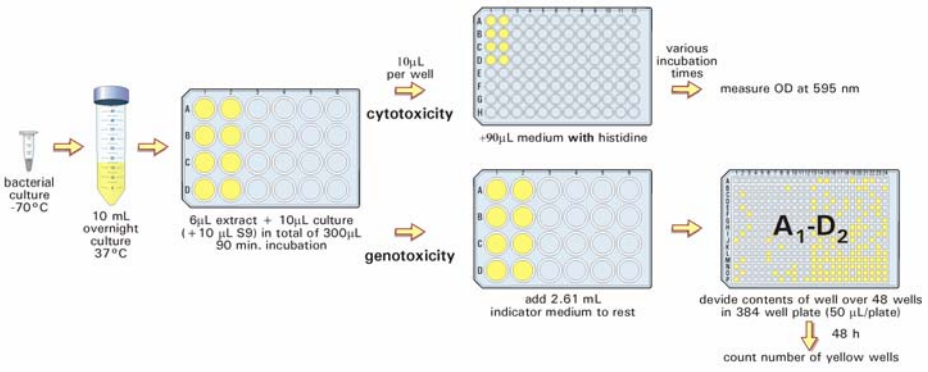
- Low levels → when significant positive result?
- Statistical method

$$DL_{response} = \overline{NC} + t_r \cdot \left(\frac{s_r}{\sqrt{m}} \right)$$

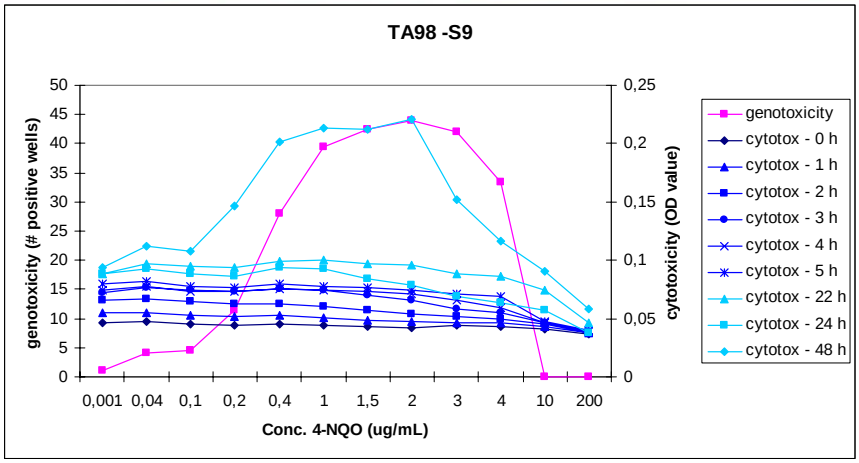
$DL_{response}$	= response detection limit
$\overline{NC}$	= average response negative control in test
t_r	=student t-value, from reproducibilty test
s_r	= st. dev. of neg. control in reproducibilty test
m	= number of replicates

Check for cytotoxicity

- No dose-response curve → check cytotoxicity
- Designed new method



Optimal incubation time cytotoxicity method



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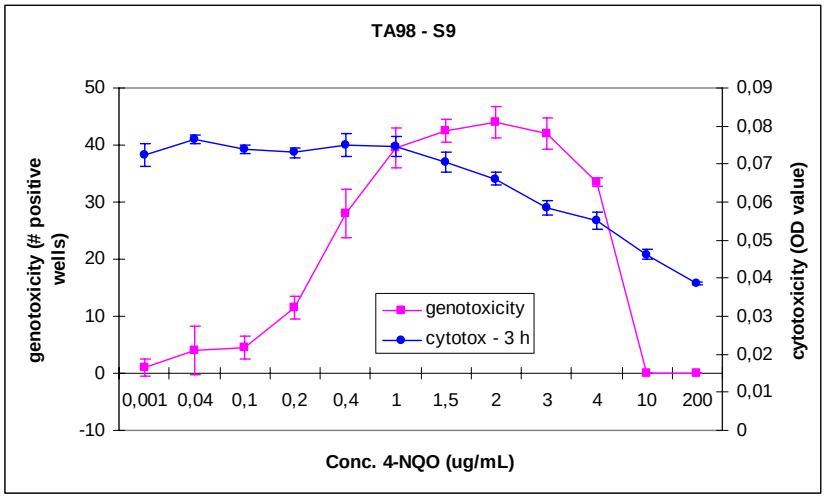
Results cytotoxicity method



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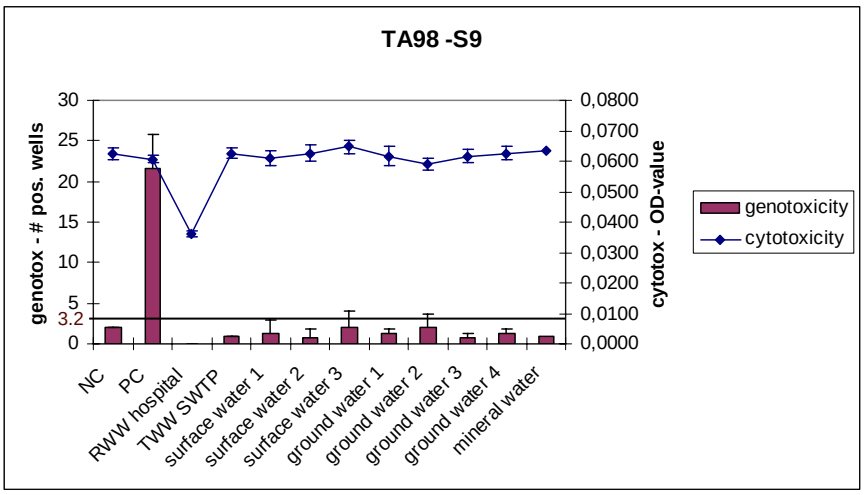
First results application Ames II



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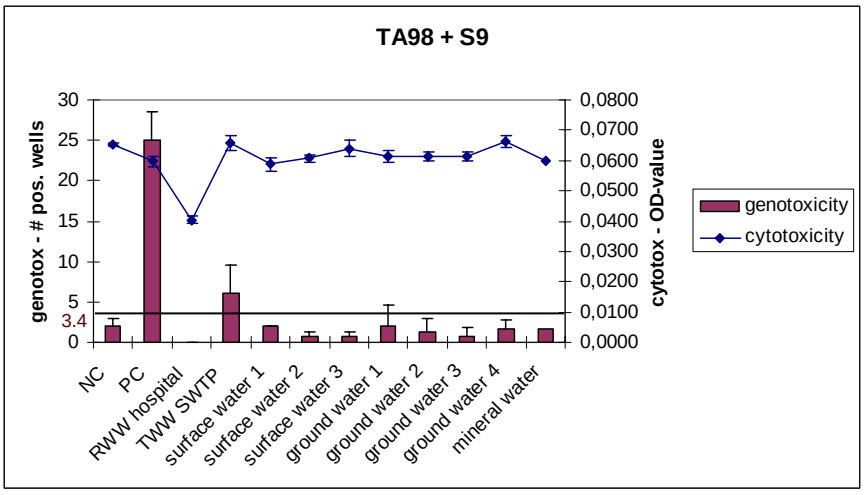
First results of application Ames II



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Present and future research

- Choice between Comet and micronucleus
- What level of test response is unacceptable?
- Toxicity sensors with GMOs

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Toxicity sensor with GMOs

- Bacteria luminesce after exposure to toxicants
  - Plasmid with *promotor - luxCDABE*
- Panel of strains to cover all necessary toxic effects
  - Different promotors → different strains → respond to different toxicities
  - Also human toxicity
- Response in 1 hour → continuous monitoring
- Immobilization on optical fibres simplifies and eases regulatory acceptance
  - Developed by Ben-Gurion Univeristy from Israel
  - Kiwa WR has license to perform field test with these GMO-sensors

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