The use of fish cell lines in geno-ecotoxicology assessment

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BACKGROUND: a crucial awareness…

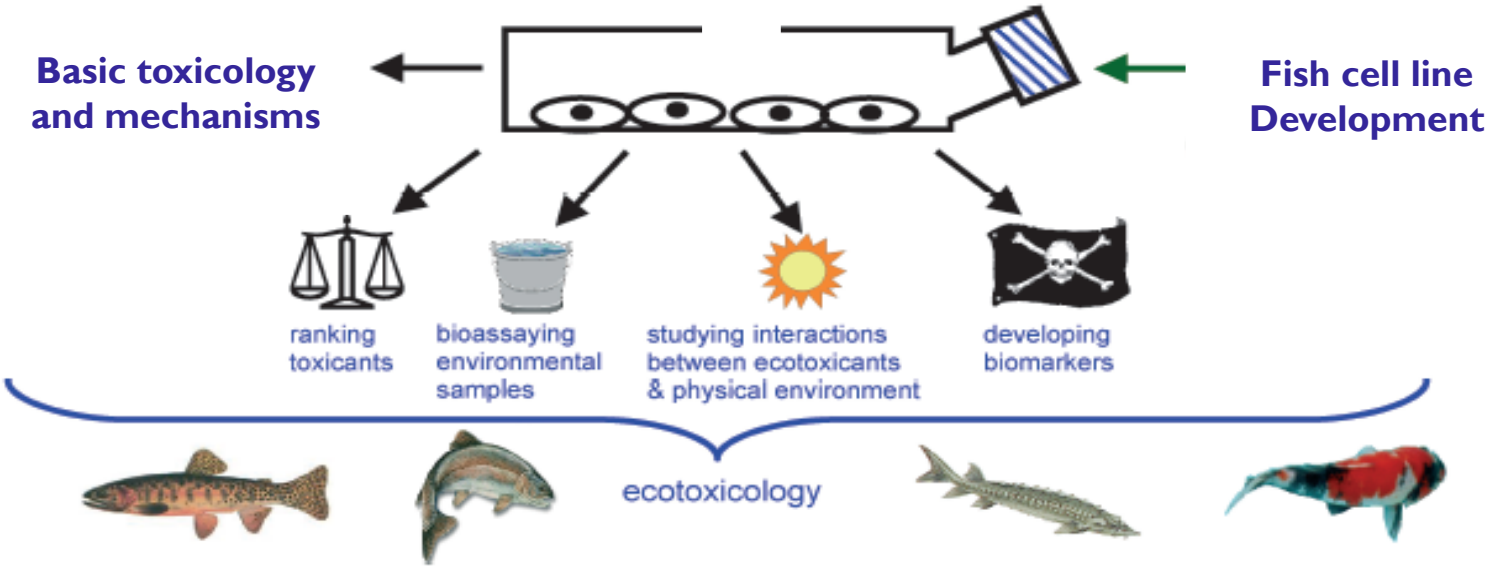
Man-made contamination of aquatic environment raises the necessity to assess hazards and risks for aquatic organisms including **fish**

Many levels should be covered:

- Basic (eco)toxicological research
- Environmental surveys & monitoring (WFD 2015)
- Regulatory toxicity tests

Thus, ethical, technical, scientific and economic reasons support the development of *in vitro* methods for ecotoxicology studies

Fish cell lines : ALTERNATIVE SYSTEMS IN ECOTOXICOLOGY

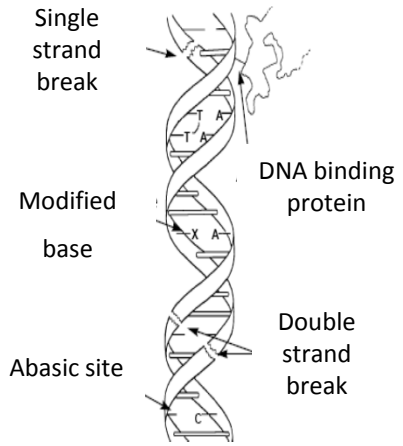


Adapted from Bols *et al.* 2005

Main advantages:

- More than 150 fish cell lines stemming from over 30 different species
- Retain specific characteristics of fish: ectothermia, resistance to osmotic variations, metabolic, biotransformation and DNA repair capacities, tissues such as gill.
- Simple to work with compared to mammal cell lines
- Fits the 3Rs concept: reduce, replace & refine the use of vertebrates

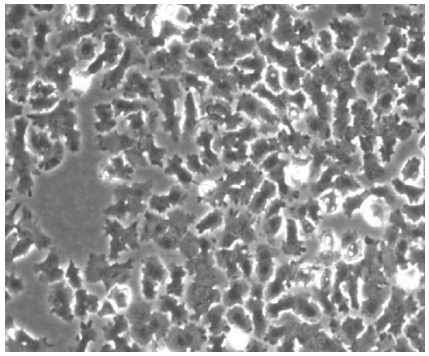
DNA: Potential target for xenobiotics



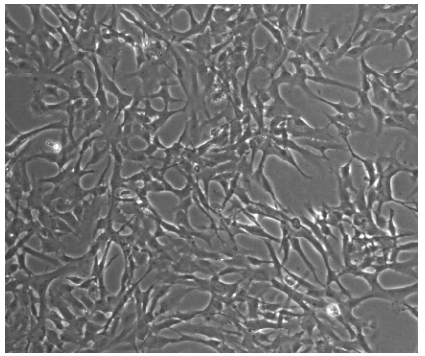
Why does genotoxicity testing warrant inclusion in hazard and risk assessment processes ?

- About a third of contaminants in the aquatic environment are suspected to be directly or indirectly genotoxic
- A genotoxin alters the genetic material at non-lethal and non-cytotoxic concentrations
- Genotoxins often have delayed effects (month, year…) which are crucially important at population and community levels

GENOTOXINS have high ecotoxicological relevance in situation of chronic exposure to low doses and to multiple contaminants



How can fish cell lines help?



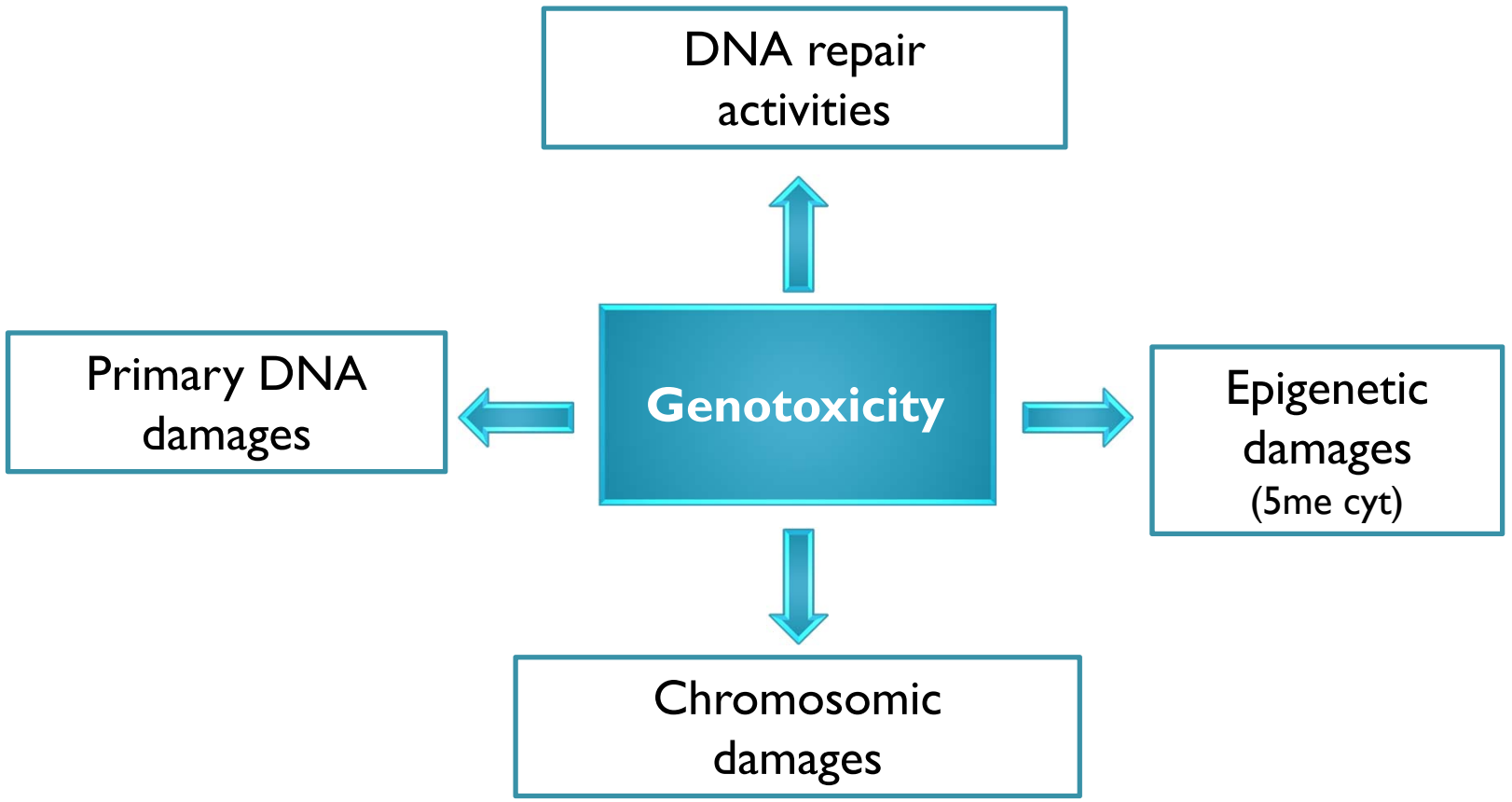
- For pure chemicals and complex matrix genotoxic hazard evaluation:

Alternative or in addition to bacterial genotoxicity testing systems such as Ames, Umu C, SOS chromotest, Rec-assay

- To study mechanisms of genotoxicity

- **To identify/set up new genotoxicity biomarkers**

Genotoxicity Biomarkers

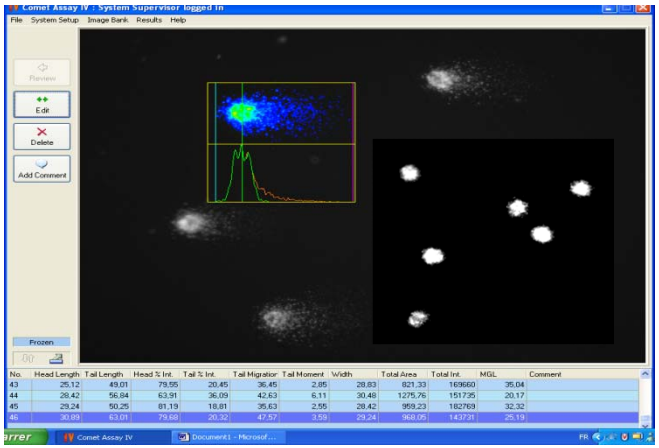


Interest of a **multi-biomarkers approach** to optimize the hazard and risk assessment in multi-contamination scenario

Primary DNA damages: the need for a sensitive tool to detect low contamination levels

The Alkaline Comet assay: a sensitive and versatile tool to quantify single and double DNA strand breaks

Measure the DNA breakdown at individual cellular level
Based on electrophoretic properties of DNA in agarose at pH>13



Quantification of the level of DNA breaks by Image analysis

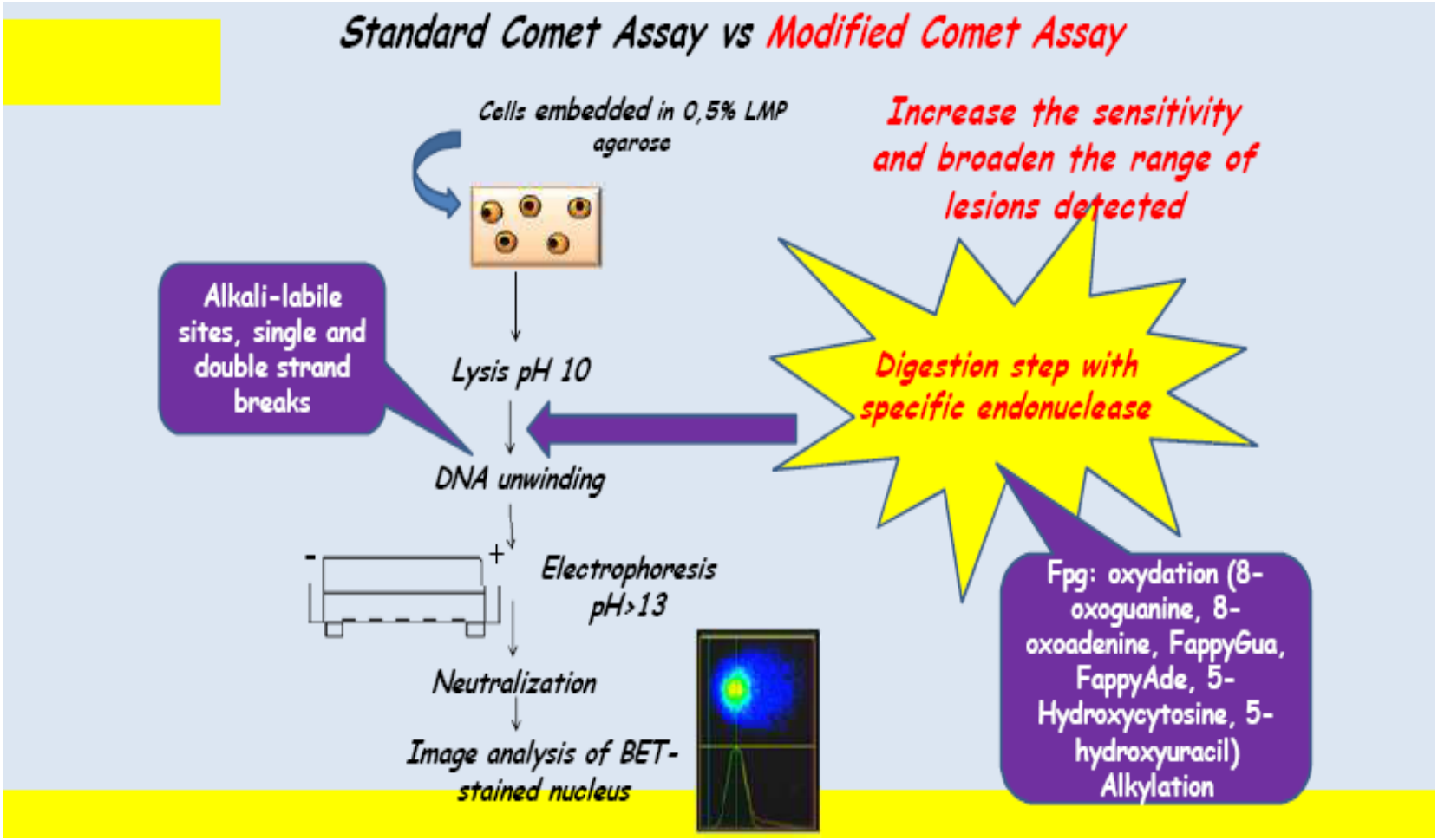
Sensitivity and specificity of the assay can be improve by the use of an additionnal step using restriction endonuclease

*Mechanistic purposes: specific enzymes (OGG1, Endo III, Alk A, T4endoV)

***Improved sensitivity: Formamido pyrimidine glycosylase (Fpg)**

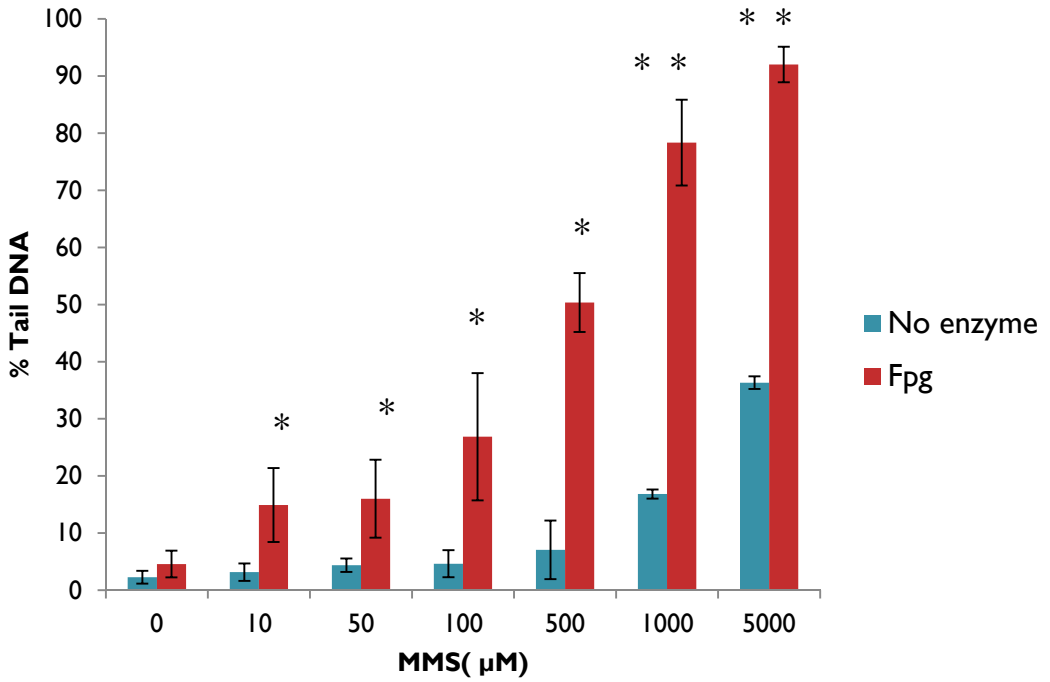
*DNA methylation level: methyltransferases (HpaII, HhaI, McrBC)

Need for a sensitive tool to detect low levels of primary DNA damage in fish cell lines

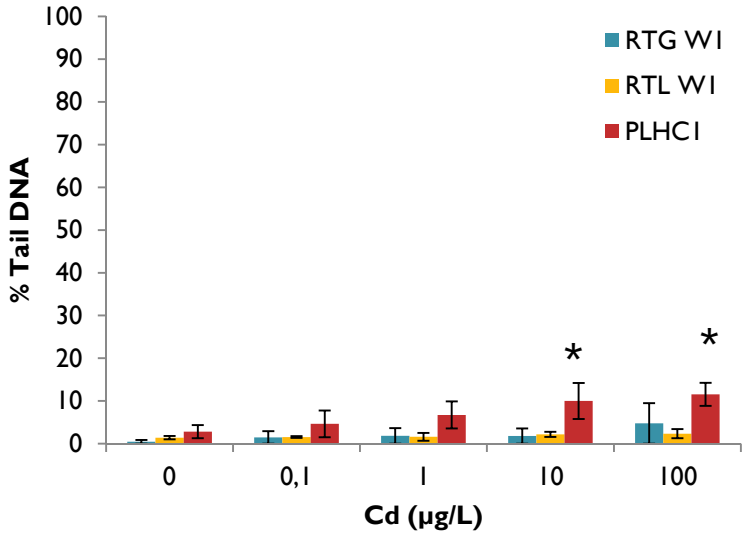


Sensitive genotoxicity testing in fish cell lines using the Fpg-modified comet assay

RTG W1 exposed to the model genotoxicant MMS (alkylating agent)



ROS mediated genotoxicity: cadmium

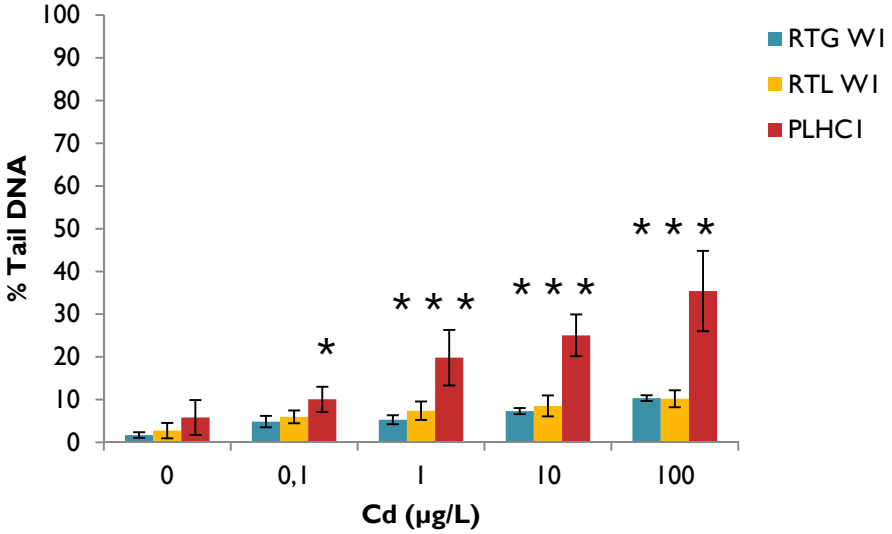


With Simple Comet Assay

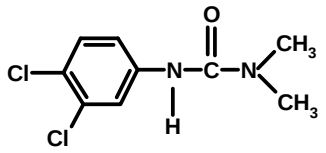
Low or no genotoxicity

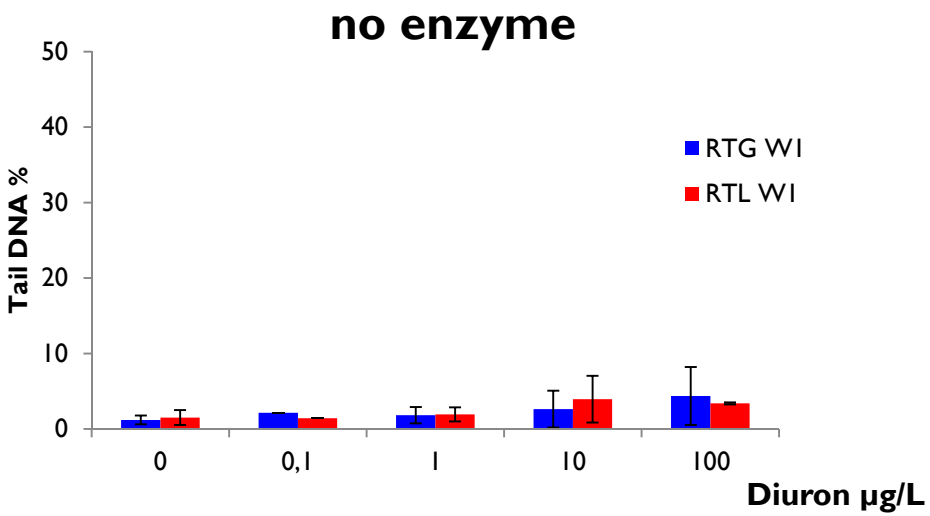
With Fpg modified Comet Assay

2 orders of magnitude improvement



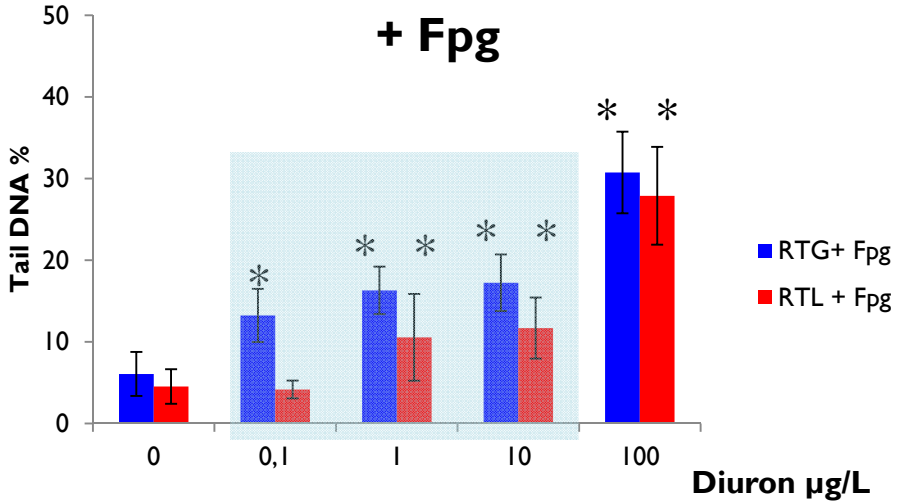
Vineyard pesticides: the case of Diuron





Simple comet assay :

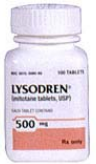
No DNA damage



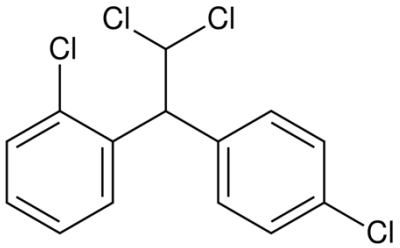
Fpg-modified comet assay :

Significant DNA damage
at 0.1µg/L for **RTG W1**
at 1 µg/L for **RTL W1**

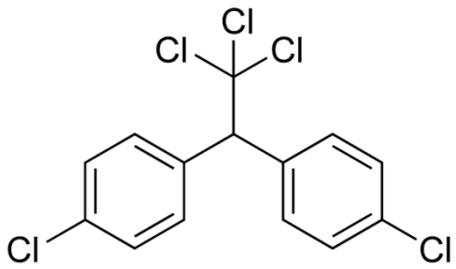
Environmental Risk assessment for the MITOTANE, an anti-cancer drug



- Used against metastatic adrenal corsical carcinoma
- Daily dose: 6 -12 g / day / patient
- Low bioavailability: 65% excreted without metabolization
- Mechanism of action and toxicity almost unknown
- Structure very close to that of DDT
- **High bioconcentration factor: 7330**
- Long half-life: 190 days



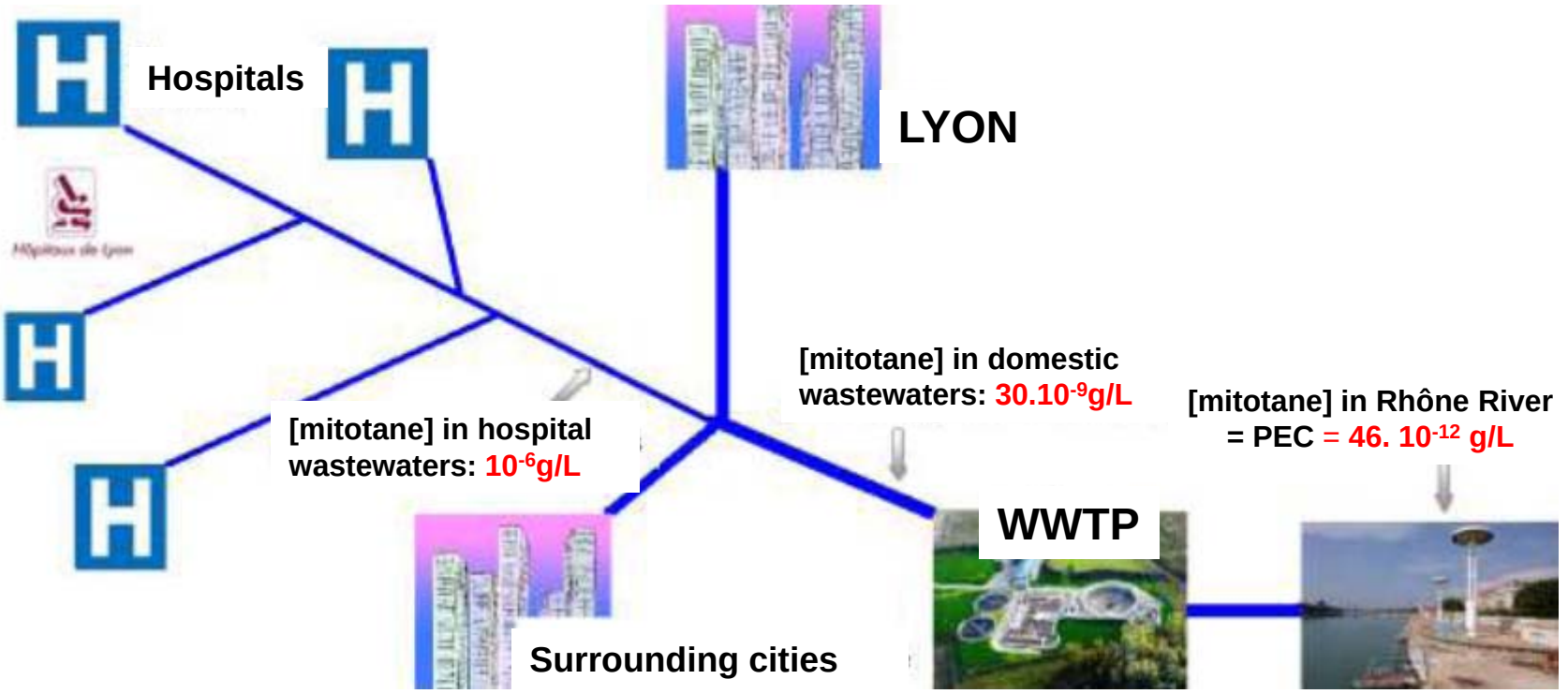
Mitotane= o-p' DDD



p-p' DDT

The risk assessment scenario

Mitotane: 1200 g / year



Predicted **E**nvironmental **C**oncentration = 46. 10^{-12} g/L

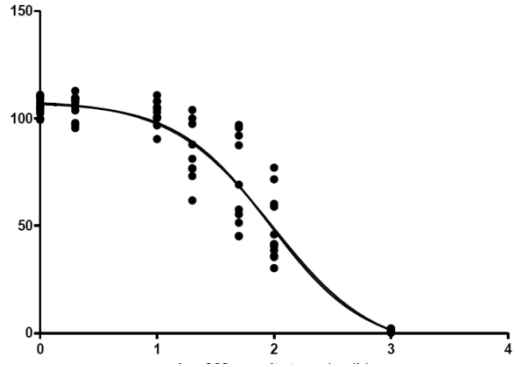
Predicted **N**o **E**ffect **C**oncentration = 72.10^{-9} g/L

PEC/PNEC <<< 1

No RISK

Predicted intra-fish concentration (Body residue): **340. 10^{-9} g/L**
(PEC x BCF)

Mitotane PLHC1 cell line cytotoxicity

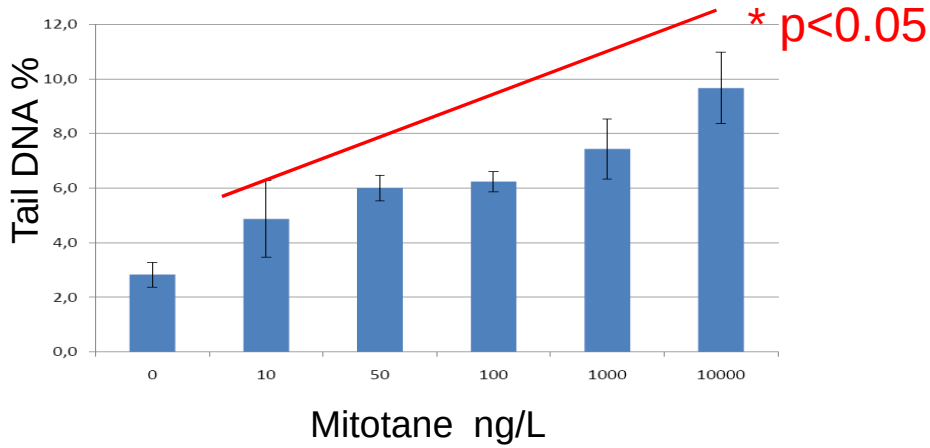


CE_{10} = 6µg/L
CE_{50} =18µg/L

>> **340. 10^{-9} g/L**

No RISK

Mitotane PLHC1 cell line genotoxicity (Fpg-modified comet assay)

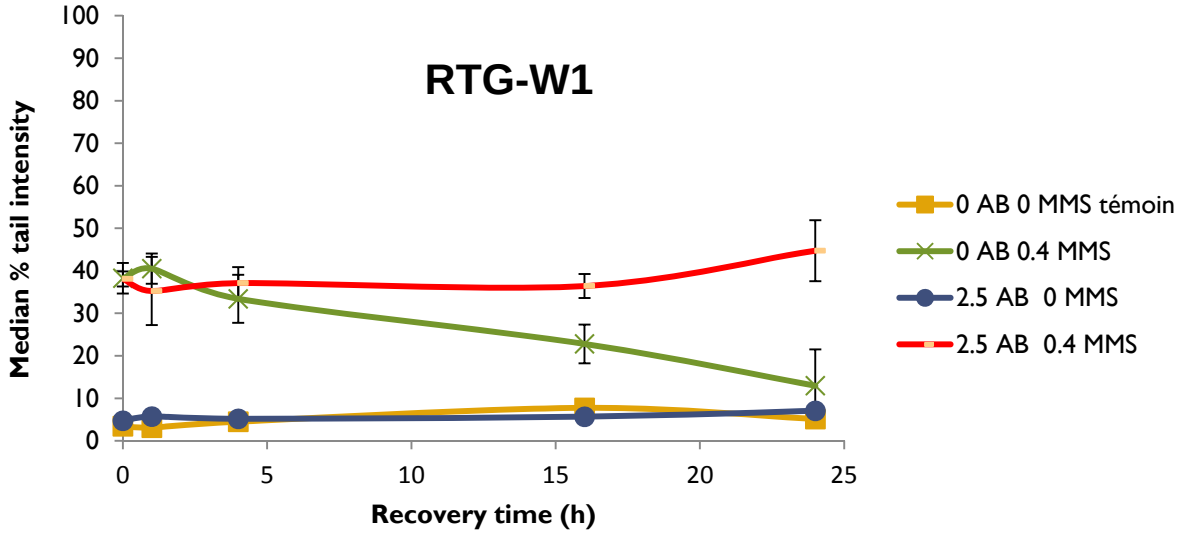


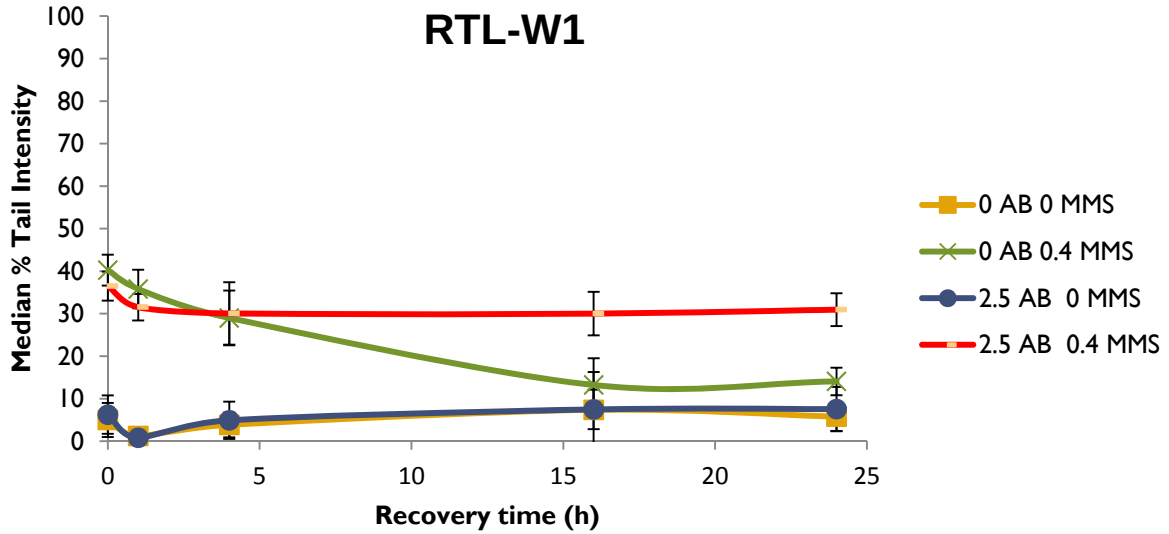
First effect concentration: 50ng/L << **340. 10^{-9} g/L**

RISK

DNA repair activities : the need for a simple and sensitive assay

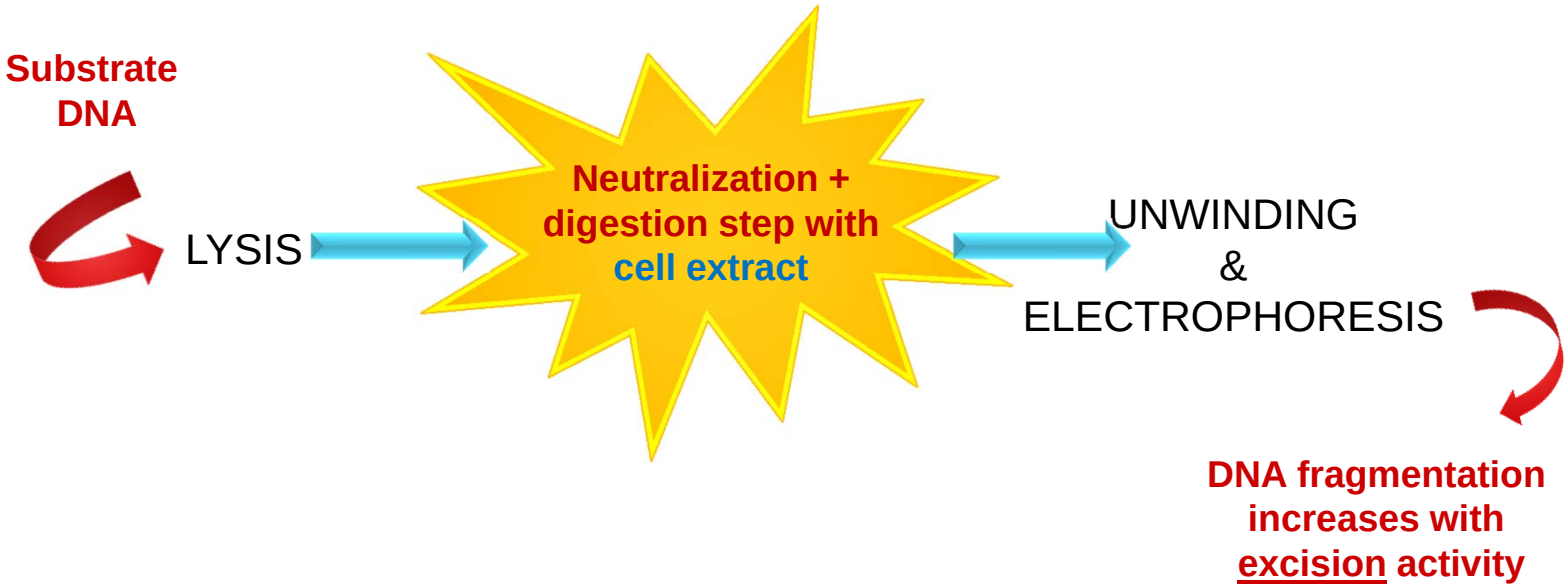
Do fish cell lines have DNA repair activities?





Evaluation of the DNA repair capacities

Modified version of the Alkaline Comet assay



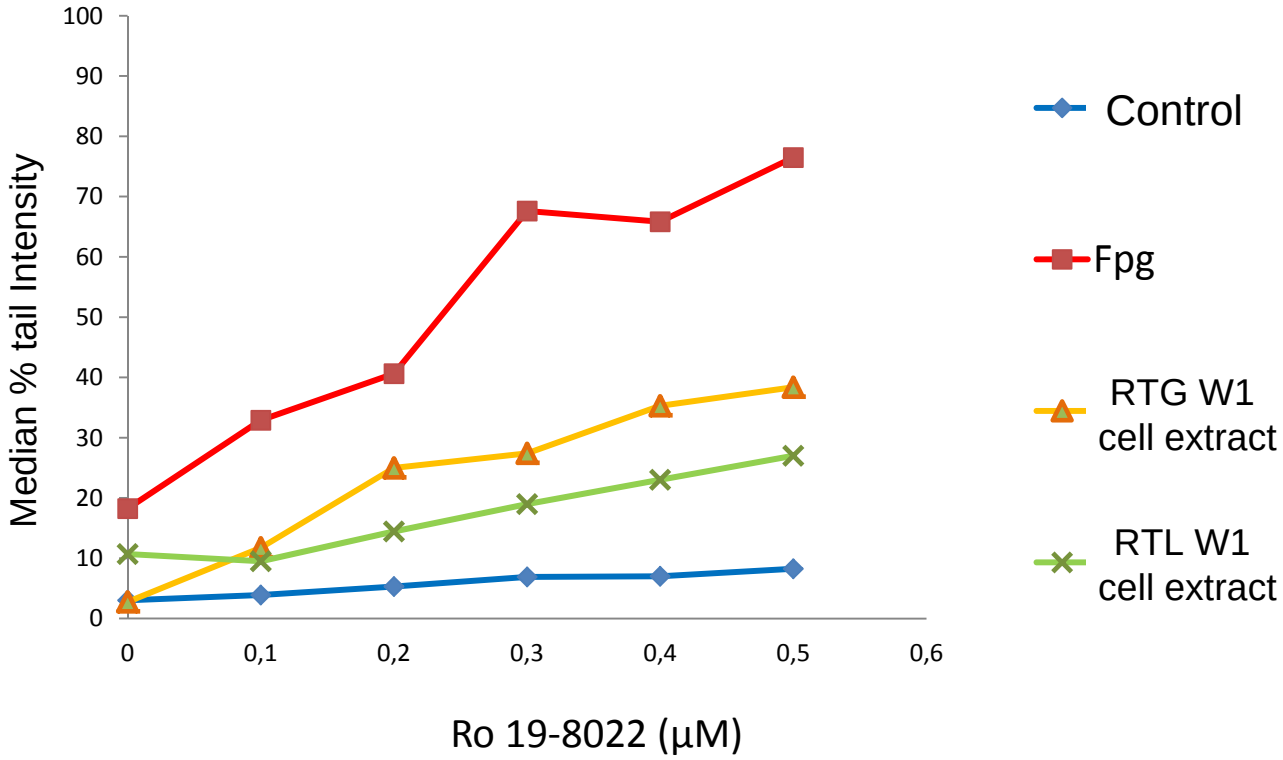
Applied to the two main repair mechanisms resulting in SSB:

Base Excision Repair (BERc) that recognizes base oxydation, alkylation, hydrolysis, deamination…(substrate DNA = 8OH Gua)

Nucleotide Excision Repair (NERc) that recognizes bulky adducts, helix distorting lesions such as pyrimidine dimers, 6,4 photoproducts…(substrate DNA = pyrimidine dimers)

BER activity in RTG and RTL W1 cell lines

Ro19-8022 + light ⟹ 8OH DGua, a model lesion for BER



Conclusions

- There is a clear need for alternative *in vitro* methods in ecotoxicology

- Permanent fish cell lines can represent a very useful tool particularly in the field of genotoxicity hazard evaluation

- They are easy to handle and are sensitive when using modified versions of comet assay to assess primary DNA damage and DNA repair capacities

and perspectives …

- Application to genotoxicity assessment of environmental samples

- Transfert of the new biomarkers *in vivo*

- Carry on to explore new genotoxicity biomarkers (epigenetic changes, GADD reporter cell line)

THANK YOU VERY MUCH FOR

YOUR ATTENTION !