



## **Cytotoxic and genotoxic effects of two heterocyclic amines in functional human hepatoma HepaRG cells**

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## Heterocyclic amines (HCAs) in few words...

- **PhiP and MeIQx : the most abundant HCAs found in human environment**

- ✓ major exposure through cooking of meat and fish
- ✓ also detected in cigarette smoke

- **Studies in experimental animals**

- ✓ PhiP and MeIQx are carcinogenic at multiple tissue sites and in multiple species
- ✓ Only MeIQx consistently induces liver tumors in mice and rats
- ✓ Carcinogenic effects modulated by interactions between HCAs and variable production of reactive metabolites

- **Studies in humans :**

- ✓ Epidemiological data : increased risk of cancer

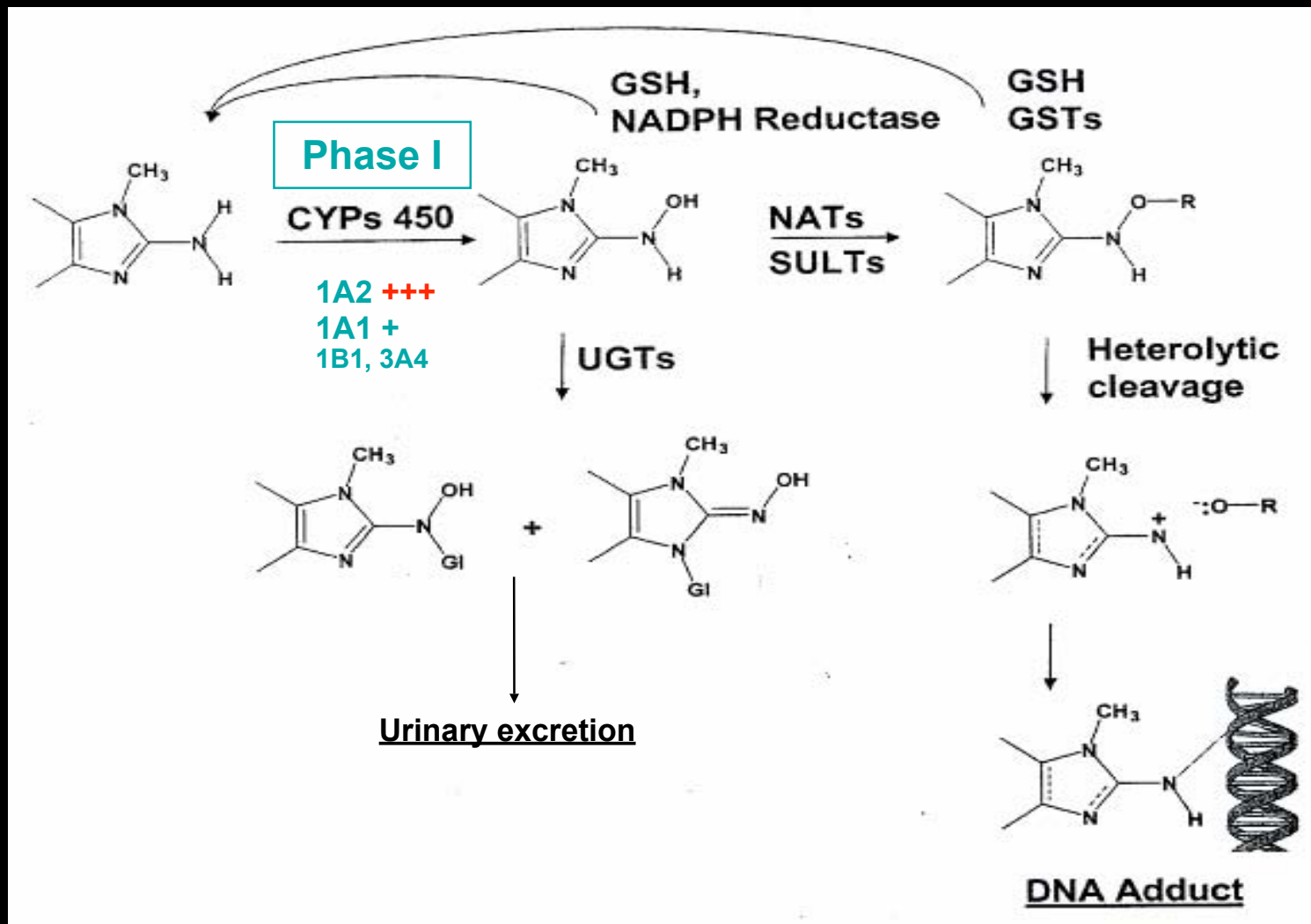
PhiP : breast and gastric cancers

MeIQx : colon adenoma, lung and possibly

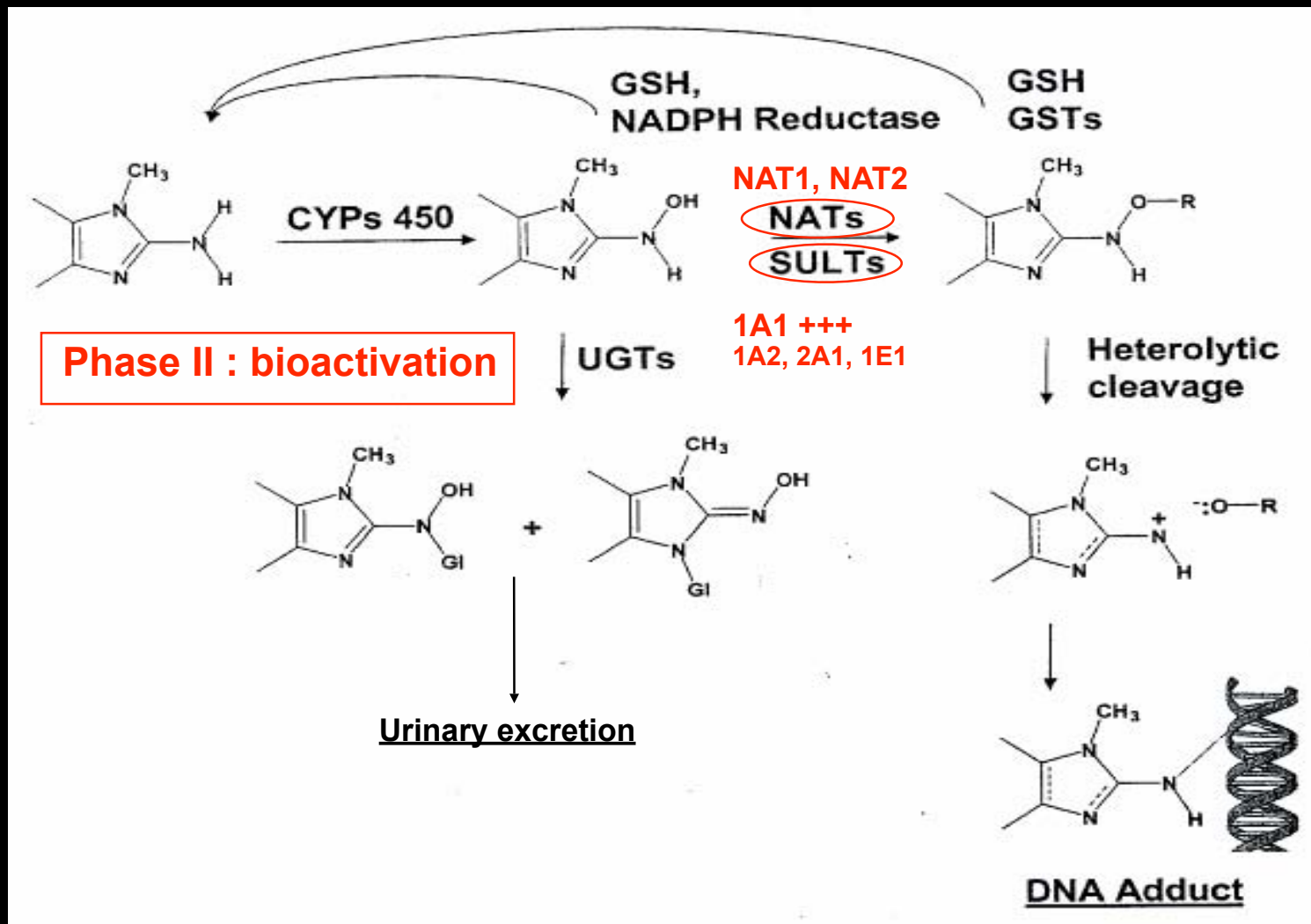
breast cancers

- ✓ Little is known regarding metabolism, chronic toxicity and genotoxicity of HCAs

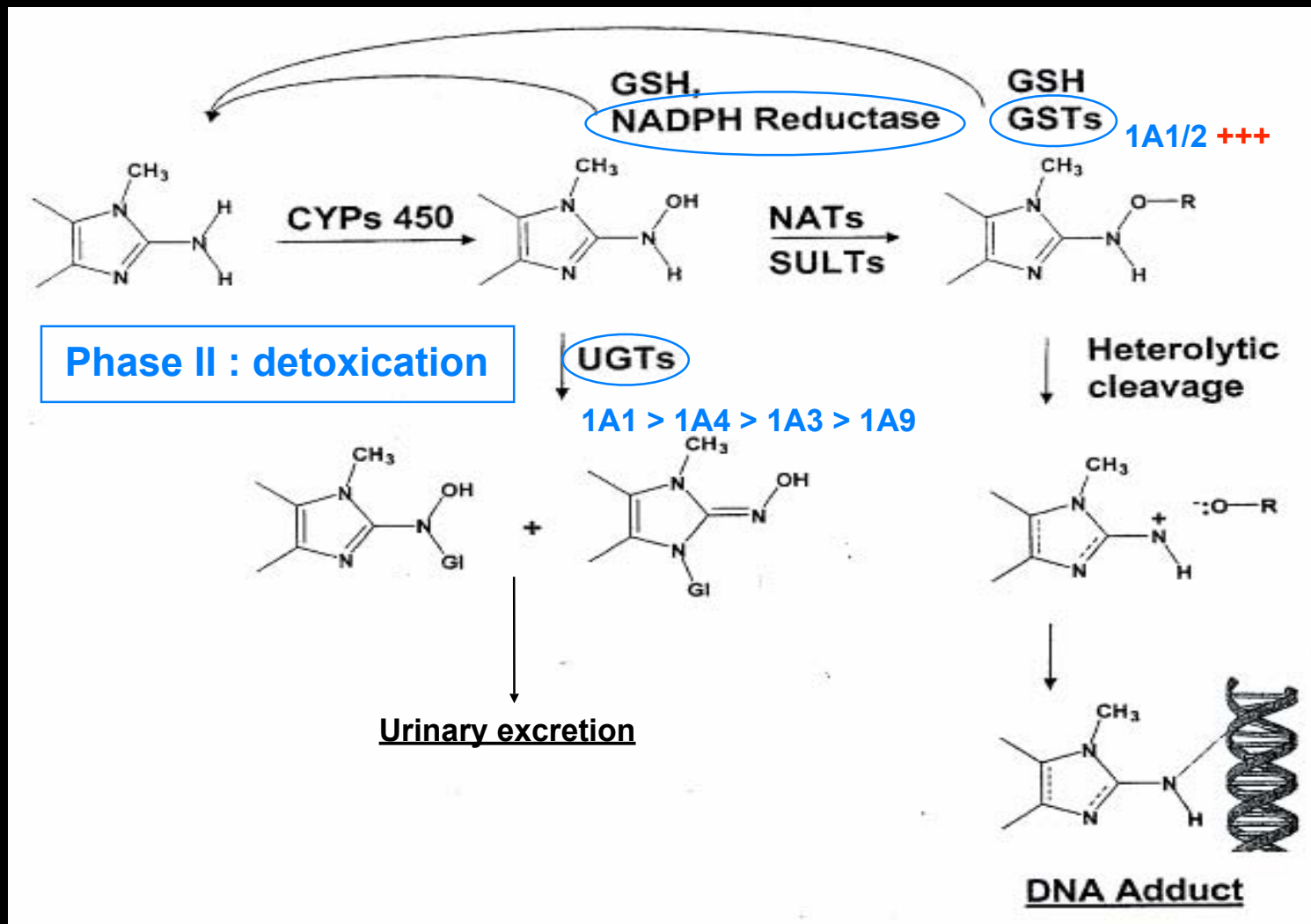
## Enzymes involved in phase I HCAs biotransformation



Enzymes involved in phase II : bioactivation to reactive metabolites

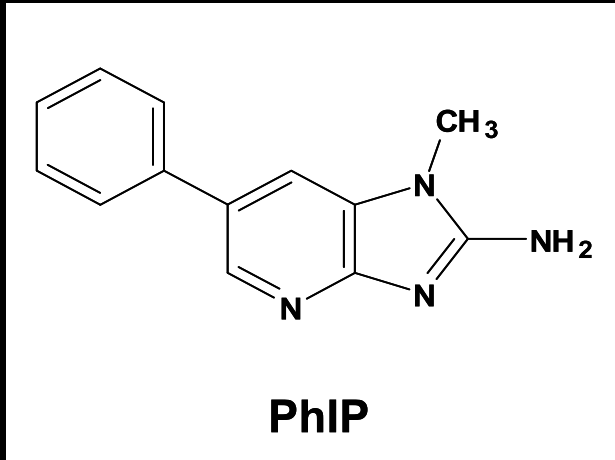


## Enzymes involved in phase II : detoxication pathways



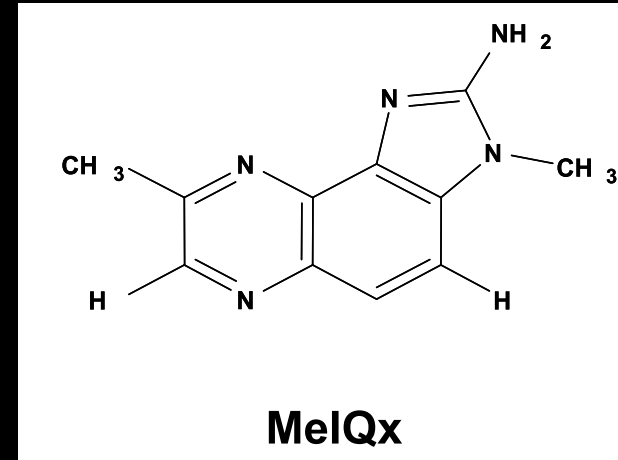
## Objective of the study

The two most abundant HCAs concomitantly found in human environment :



**PhIP**

2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine



**MeIQx**

2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline

### Objective

Evaluate the chronic hepatic toxicity and genotoxicity of PhIP and MeIQx, individually and in combination, using the metabolically competent human hepatoma HepaRG cells

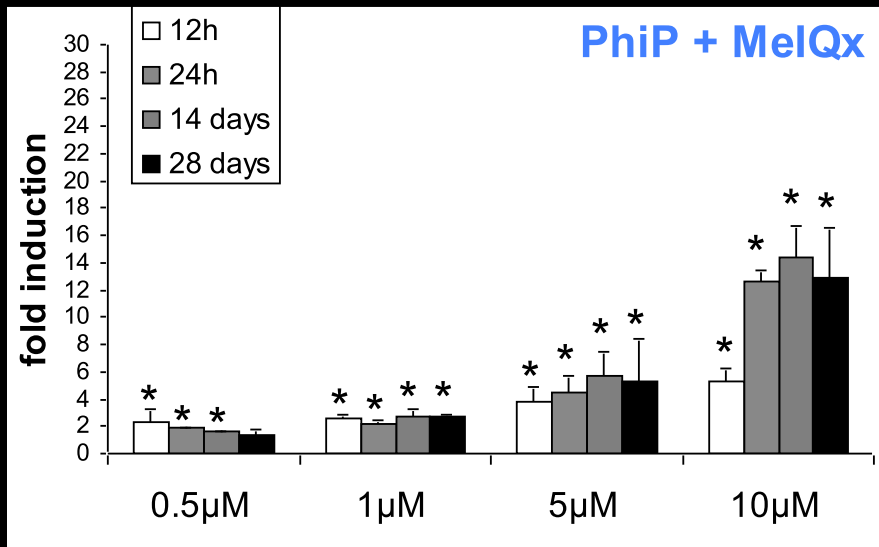
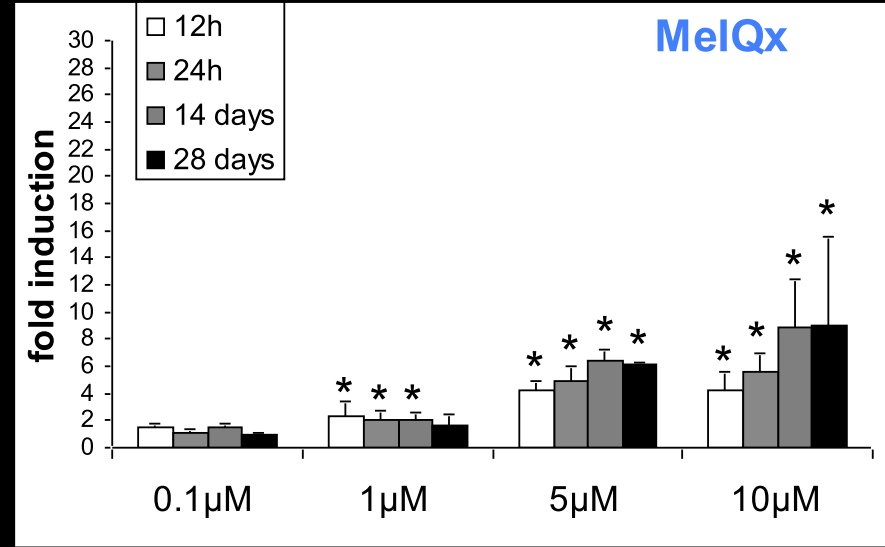
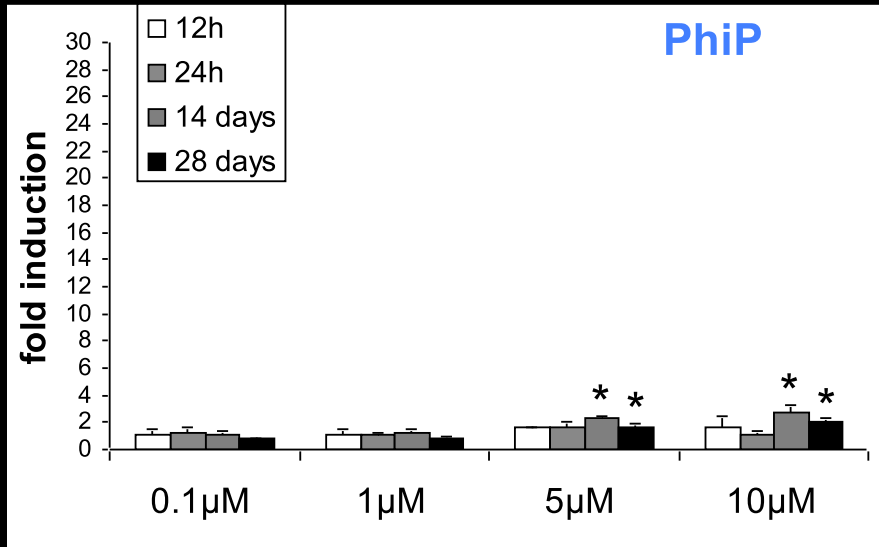
# Study design



## Cytotoxicity of HCAs in differentiated hepaRG cells

- PhiP and/or MeIQx (from 0.1 to 10 $\mu$ M) triggered no cytotoxicity in HepaRG cells
  - by intracellular ATP content measurements
  - by neutral red incorporation measurements
- No morphological changes were observed whatever the time of exposure
- PhiP and/or MeIQx exposure was not associated with increased caspase-3 activity suggesting no significant effect on apoptosis

# Expression of metabolizing enzymes in response to HCAs : **CYP1A2**



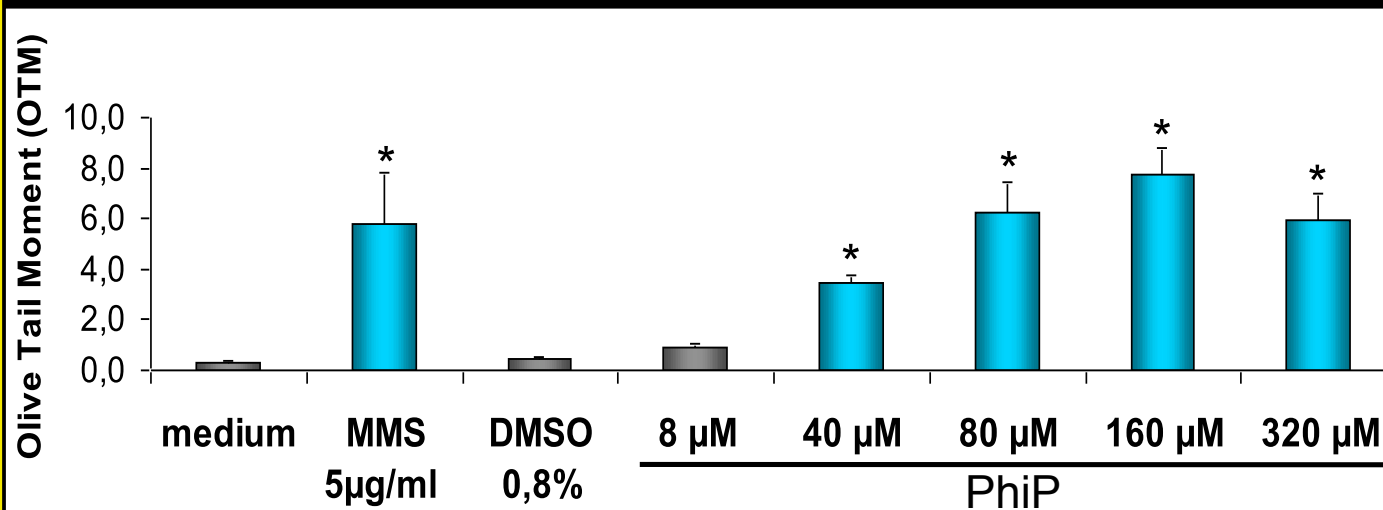
n=3, \* p<0.05 vs control

- **PhiP** : Maximal induction after 14 days (x2.8 at 10 $\mu$ M)
- **MelQx** : Maximal induction after a long term exposure (x9 at 10 $\mu$ M)
- **Mixture** : Maximal induction after 14 days (x14.4 at 10  $\mu$ M)

## Expression of metabolizing enzymes in response to HCAs : **summary**

	PhiP	MeIQx	PhiP + MeIQx
<b>Phase I enzymes</b>			
<b>CYP1A2</b>	↗	↗↗	↗↗↗ (24 Hrs)
<b>CYP1A1</b>	↗	↗↗	↗↗↗ (24 Hrs)
<b>CYP1B1</b>	↗	↗	↗
<b>CYP3A4</b>	=	=	=
<b>Phase II enzymes</b>			
<b>bioactivation</b>			
<b>NAT1</b>	=	=	=
<b>NAT2</b>	=	=	=
<b>SULT1A1</b>	=	=	=
<b>SULT1A2</b>	=	=	=
<b>SULT2A1</b>	=	=	=
<b>detoxication</b>			
<b>UGT1A1</b>	=	↗	↗
<b>UGT1A3</b>	=	=	=
<b>UGT1A4</b>	=	=	=
<b>UGT1A9</b>	=	=	=
<b>GSTA1/2</b>	=	=	=
<b>NQO1</b>	=	=	=

## Genotoxicity of PhiP : the comet assay



after 24 hours

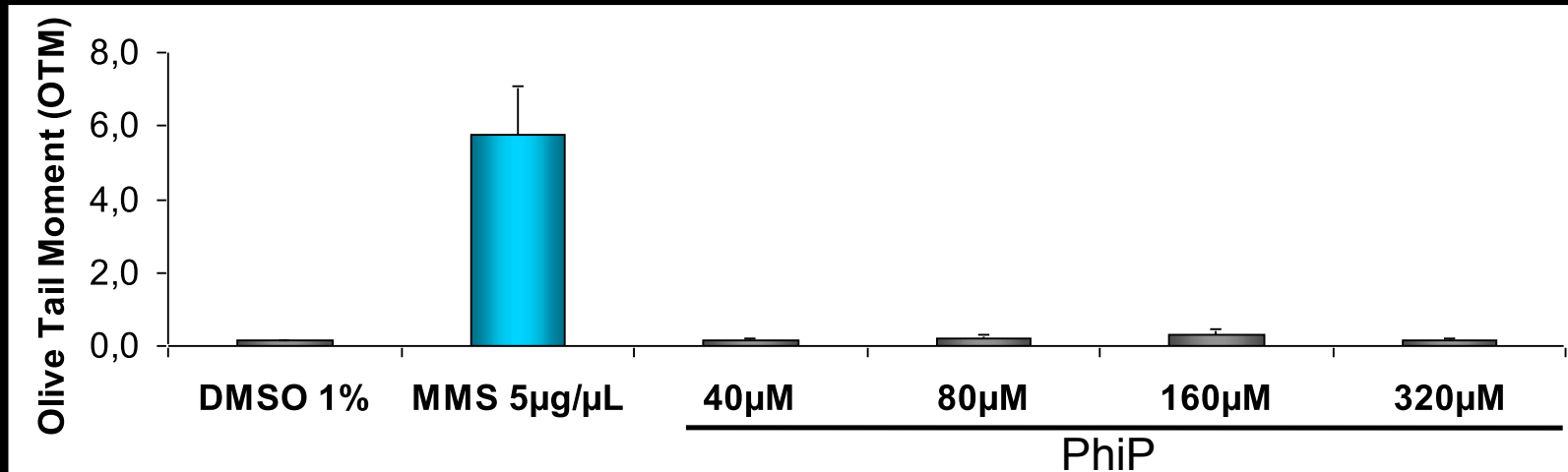
dose-dependent increase in DNA damages  
➤ Low Effect Concentration = 40 μM

n=3, \* p<0.05

- Cell viability remains above 80% whatever the concentration tested
- At 40 and 80 μM, there is no increase in caspase-3 activity suggesting no apoptosis
- ⇒ The genotoxicity detected by the comet assay is not explained by an increase in cytotoxicity or in apoptosis

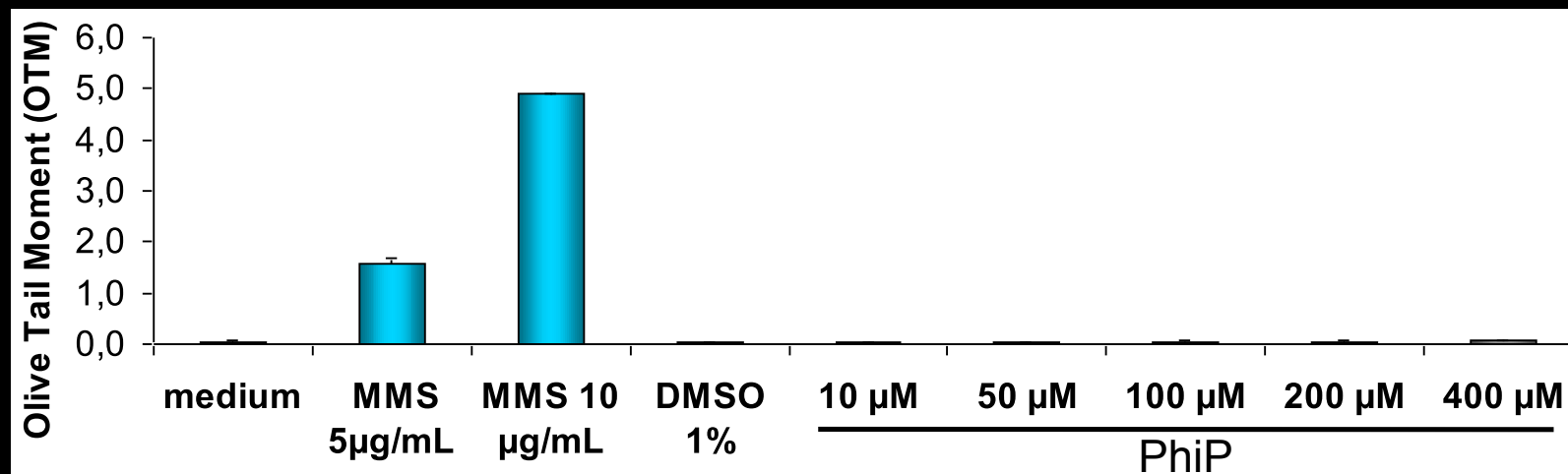
## Is metabolic functionality of HepaRG required for PhiP genotoxicity ?

- Comet assay in HepG2 cells (ATCC)



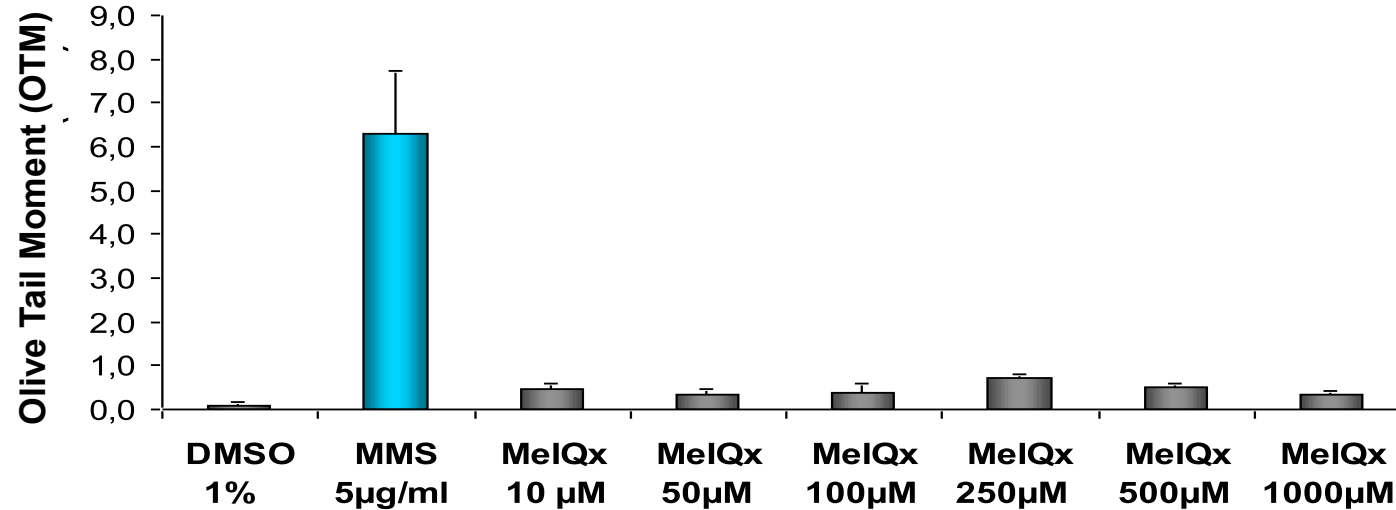
n=3

- Comet assay in undifferentiated HepaRG cells (day 5 after seeding)



n=2

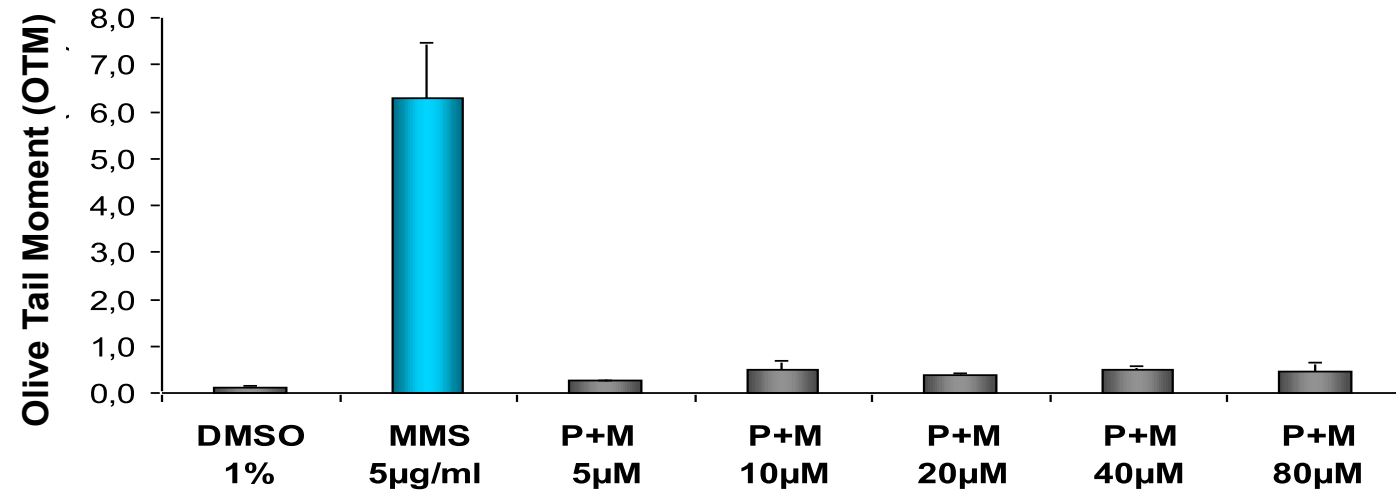
## Genotoxicity of MeIQx and the mixture : the comet assay



### MeIQx

No genotoxic effect after a 24h exposure

n=3



### PhiP + MeIQx mixture

No genotoxic effect after a 24h exposure

n=3

- ✓ Whatever the concentrations (from 0.1 to 10 $\mu$ M) and time of exposure (from 12 hrs to 28 days), there is no cytotoxicity of PhiP and/or MeIQx in differentiated HepaRG cells
  
- ✓ PhiP and/or MeIQx increase CYPs 1A2 et 1A1 mRNA expression
  - mixture > MeIQx > PhiP
  - induction by PhiP is only detected after a long-term exposure
  - induction by MeIQx and the mixture is maintained during long-term exposure
  
- ✓ CYP1B1 and UGT1A1 mRNA expression is slightly increased by MeIQx and the mixture
  
- ✓ Only PhiP induces DNA damage in differentiated HepaRG cells and metabolic activation is required for its genotoxicity

- Measurement of reactive oxygen species (ROS) production and cell cycle analysis may help to detect potential phenotype changes induced by HCAs
- Concerning the induction of metabolizing enzymes : measurement of protein expression and enzymatic activity are needed
  - ⇒ Mechanisms of induction remain to be elucidated
- There is a discrepancy between phase I enzyme induction by MeIQx and the absence of genotoxic effect
  - ⇒ Is differential genotoxicity related to UGT activity induction by MeIQx ?
  - ⇒ Confirmation by another tests is necessary (cytokinesis-block micronucleus assay, DNA adducts detection...)