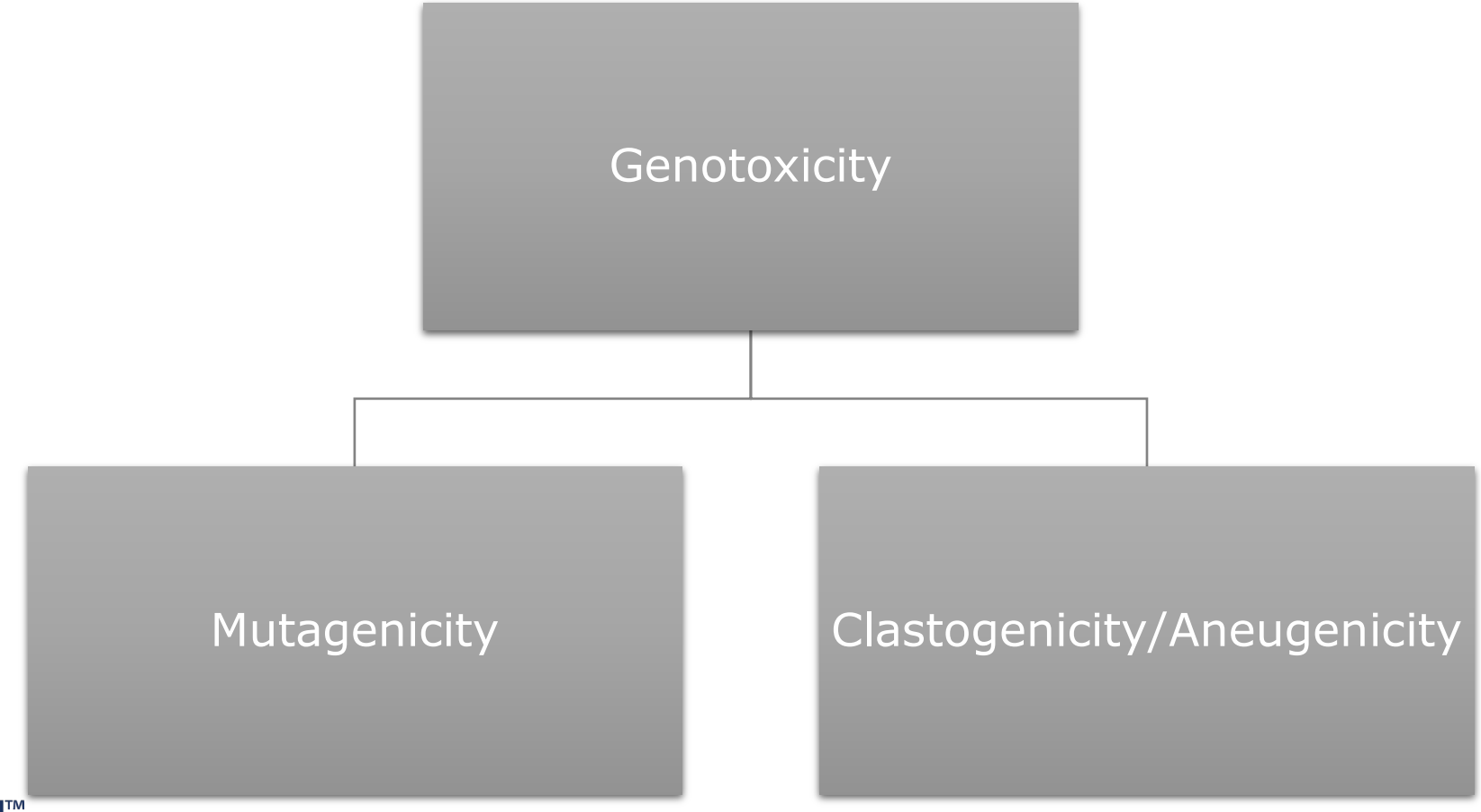
GTA 2023

Use of Chicken egg assay as an animal alternative testing model to evaluate the genotoxic potential of Drugs and chemicals

Yax Thakkar

INTRODUCTION

A mutagen causes genetic mutations versus a clastogen which induces chromosomal/DNA strand breakages



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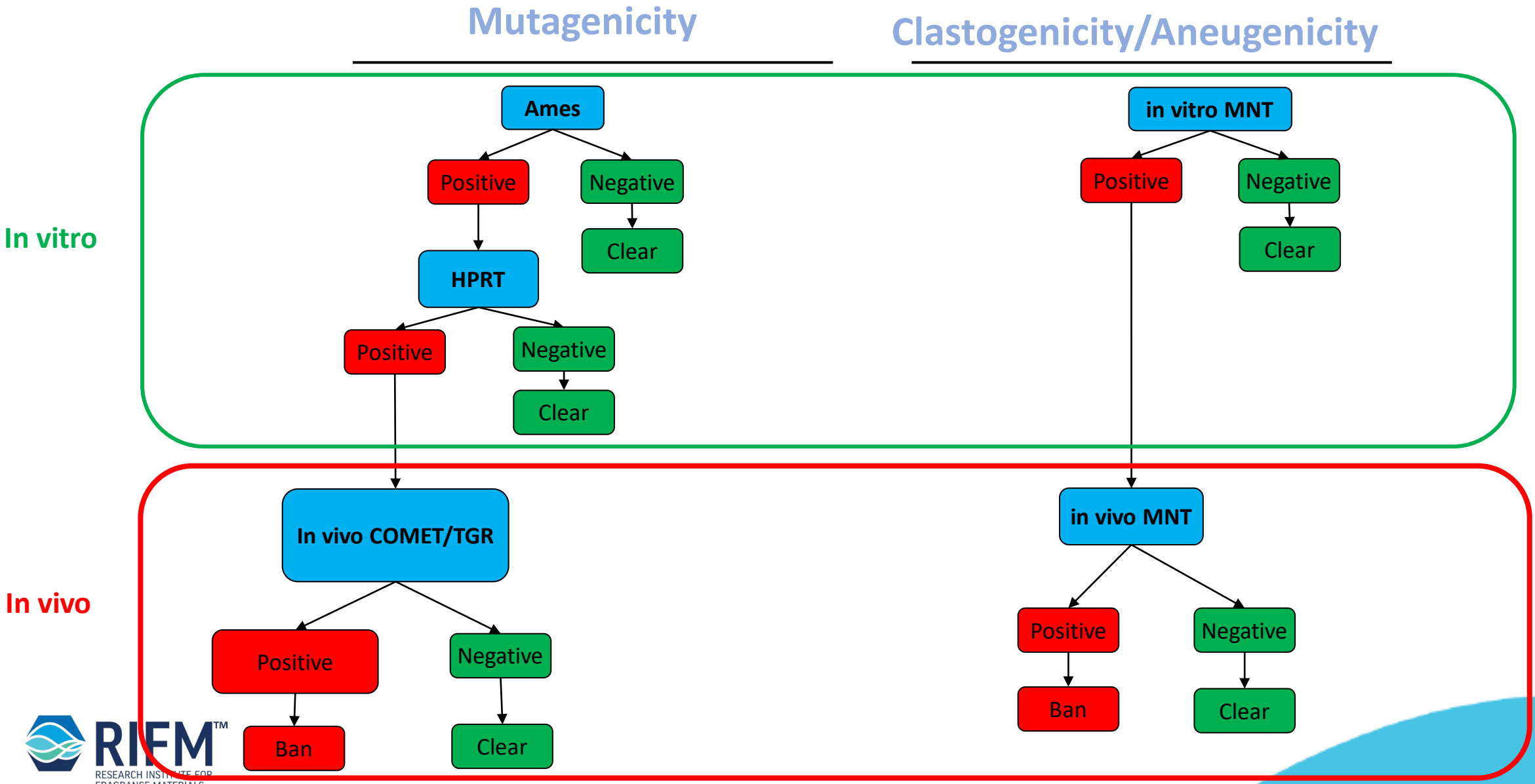
Step wise approach to genotoxicity testing



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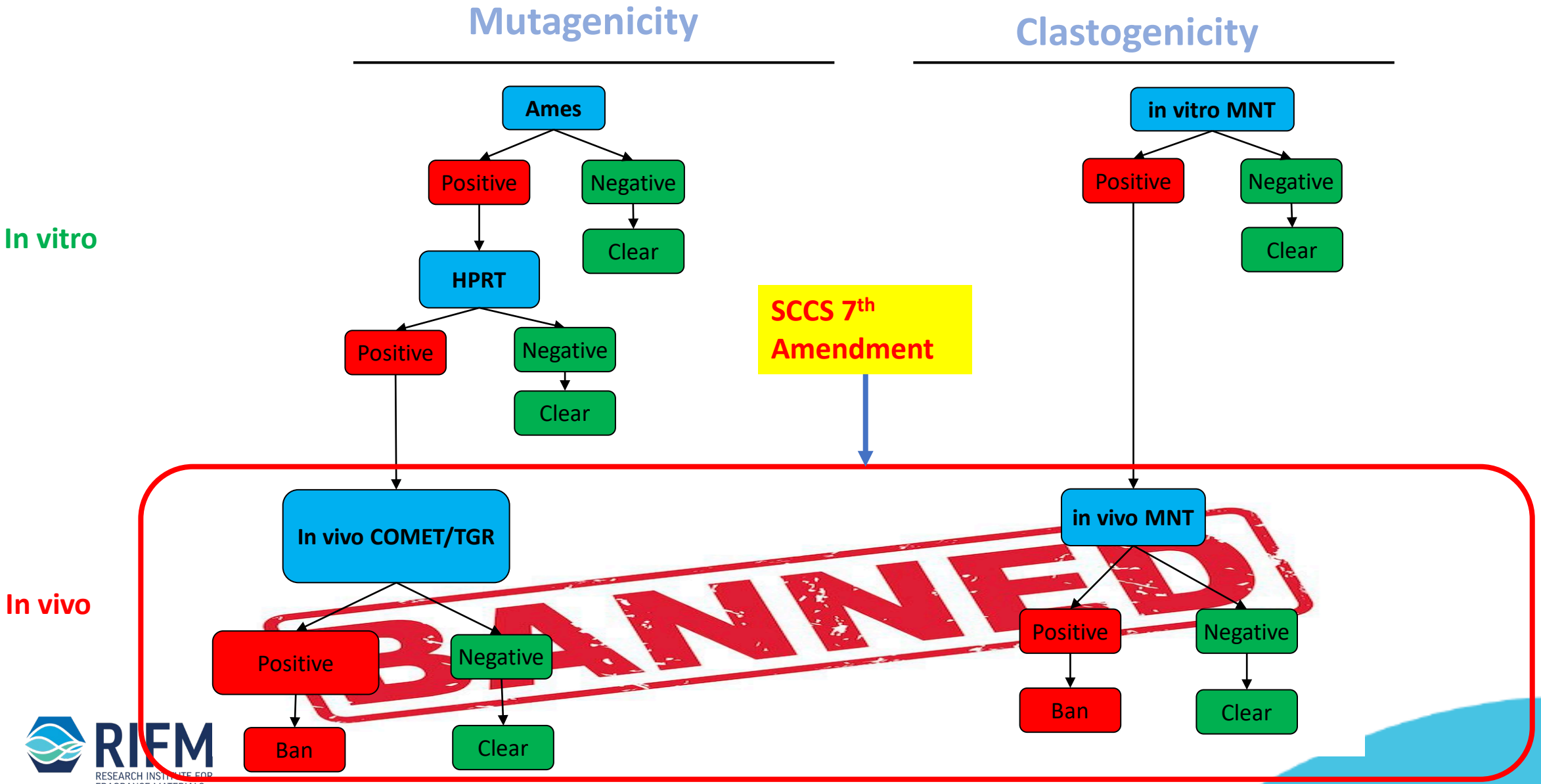
The SCCS (Scientific Committee on Consumer safety) 7th amendment ban on animal testing has significant impact on the follow-up *in vivo* testing



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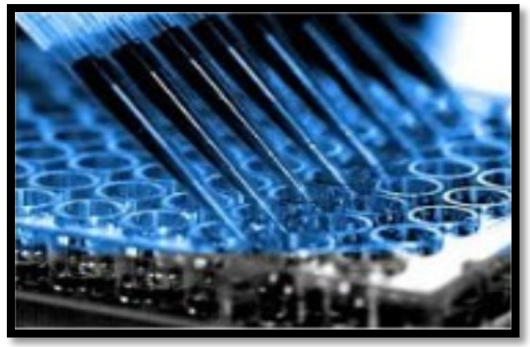
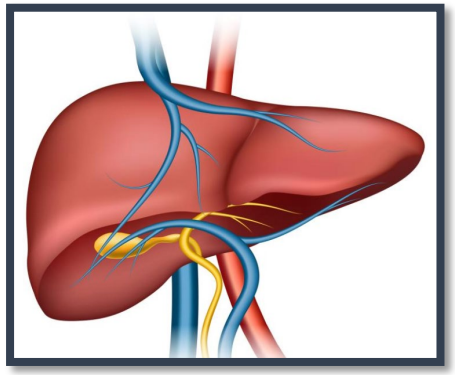
In vitro models have some serious drawbacks



False Positives (~25-30%)



Lack of detoxification

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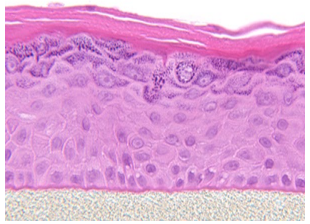
Need for more applicable animal alternative assays addressing misleading outcomes in the existing in vitro assays

Dermally Applied products



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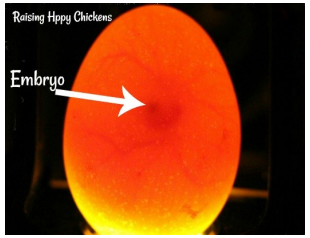
Oral intake (Flavoring agent)

Lip products



HET-MN

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Evaluation of the Chicken egg model to detect the genotoxic potential for Chemicals

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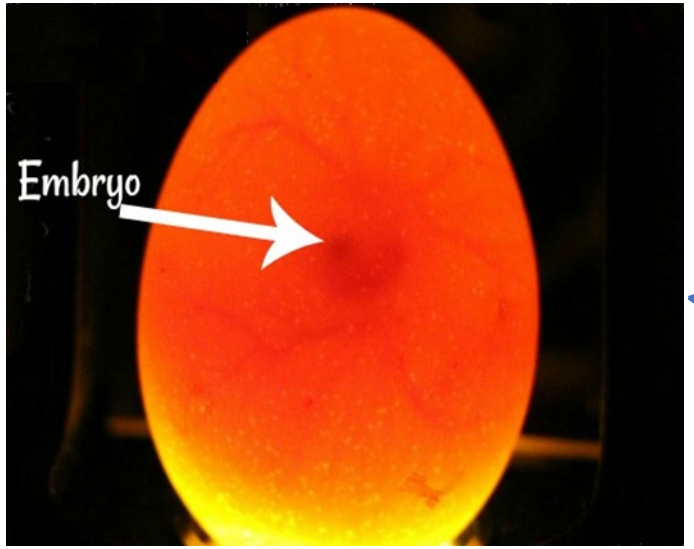
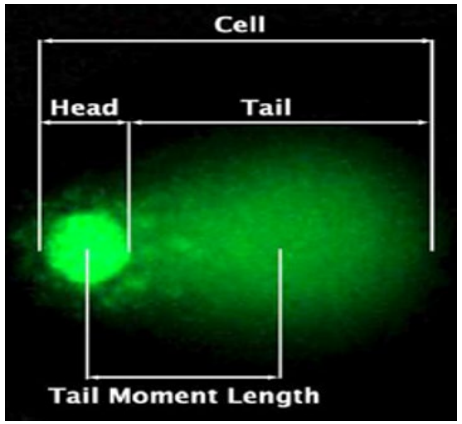
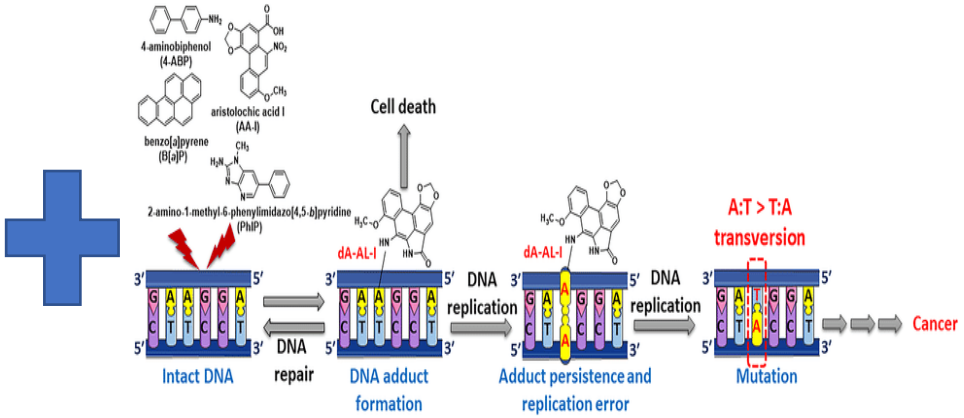
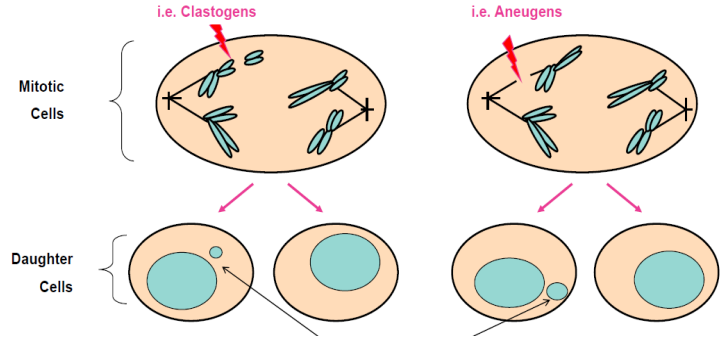
Genotoxicity end-points measured COMET/NPL and Micronucleus







DNA strand break (Comet assay)/DNA adduct formation (NPL assay)



Clastogenicity/Aneugenicity (Micronucleus assay)

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Selection of Materials for Pilot Study

- Criteria:
  - Materials α, ß unsaturated aldehyde
  - Positive data on at least one of the traditional in vitro Ames and/or in vitro micronucleus assay
  - Has been followed up in vivo COMET and/or micronucleus study

- 4 Materials were identified which satisfy the above criteria

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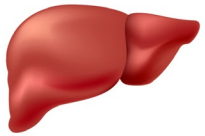
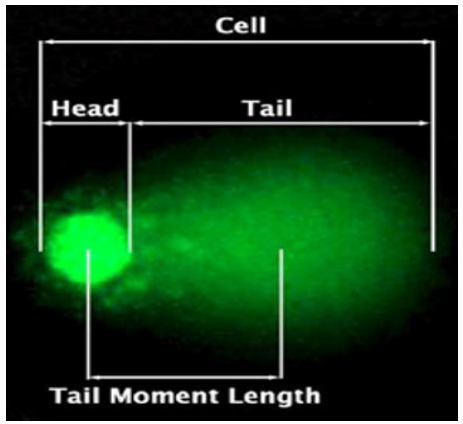
Study Design

CEGA COMET/NPL

Incubation period, days



Liver sample collection



DNA Adduct formation

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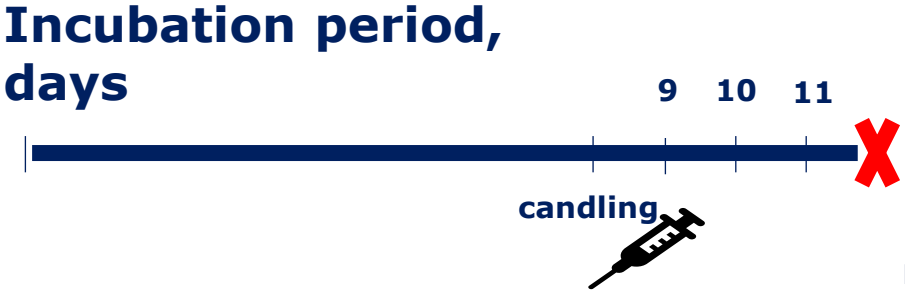
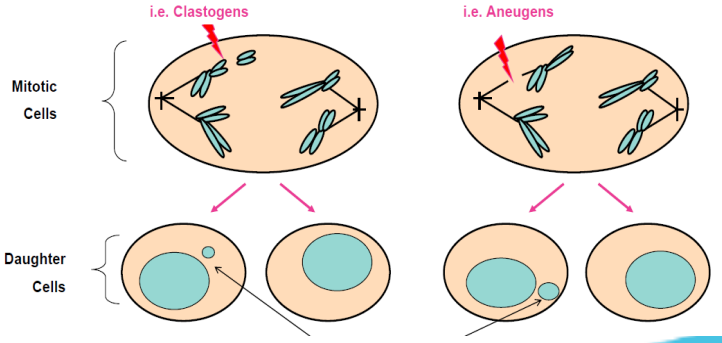
Incubation period, days



Fixation of umbilical artery and blood collection



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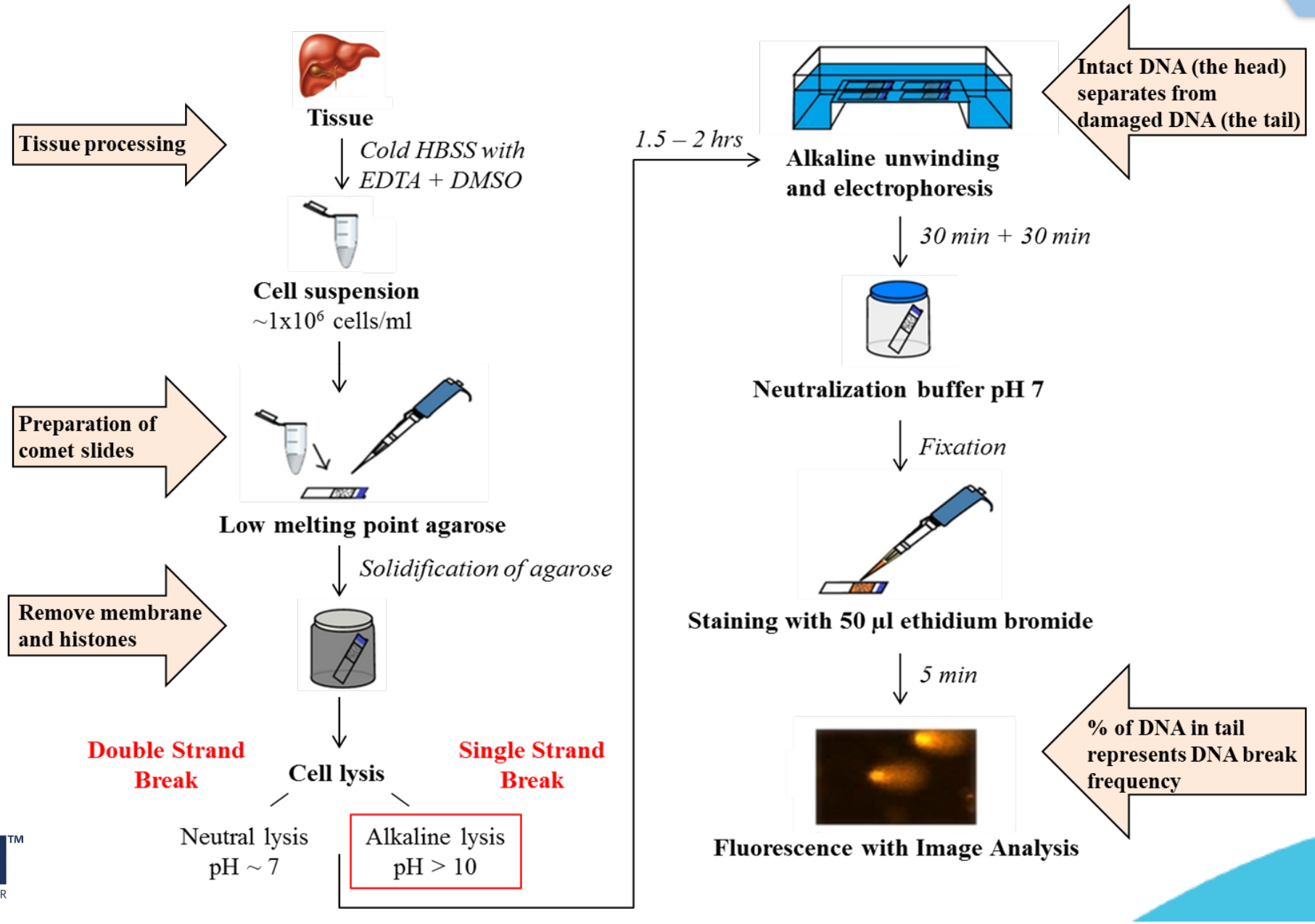
COMET ASSAY



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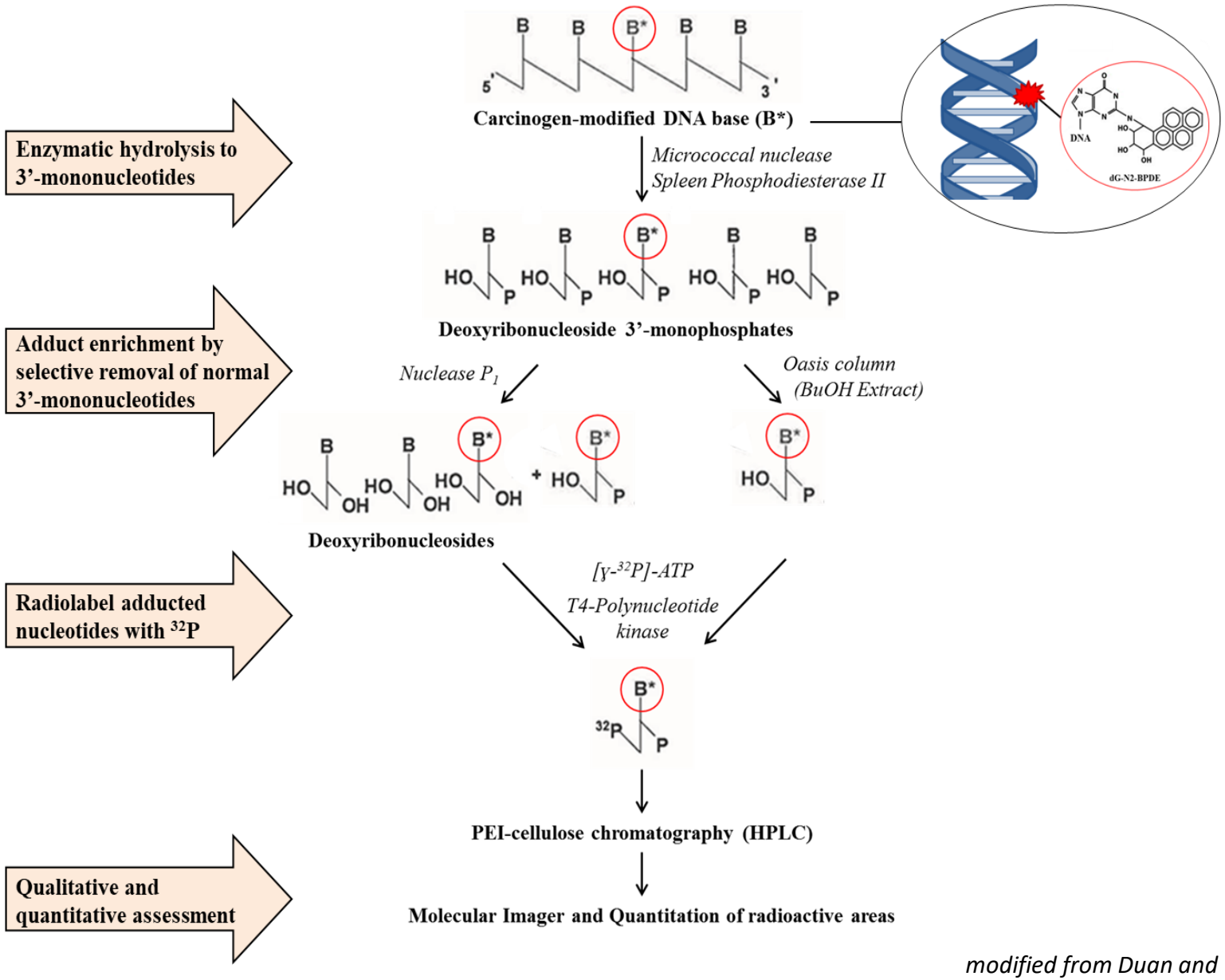
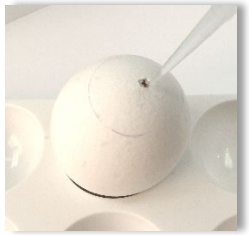
^{32}P-NUCLEOTIDE POSTLABELING ASSAY



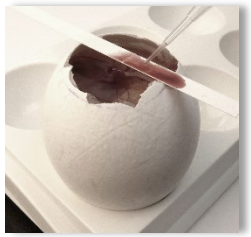
modified from Duan and Williams, Toxicol Open Access. 2016. 2

HET-MN methodology

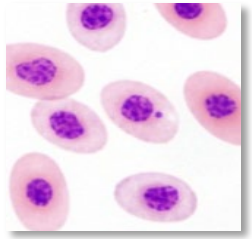
Application day 8

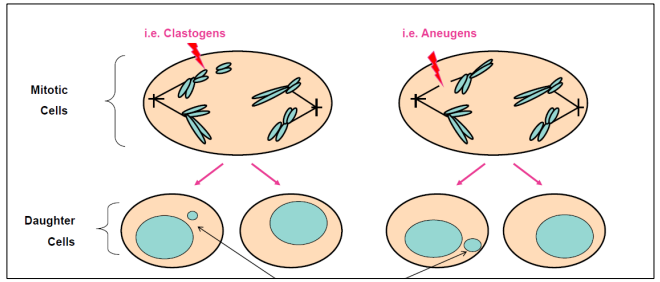



Sampling day

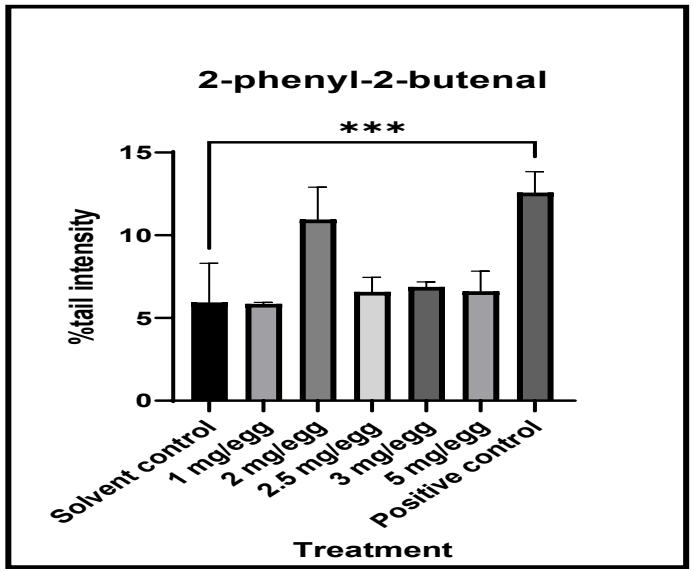
Analysis

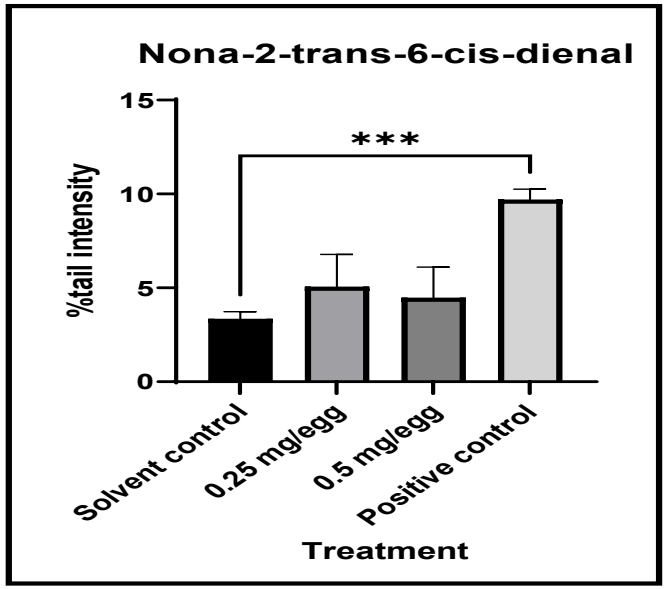





COMET assay results

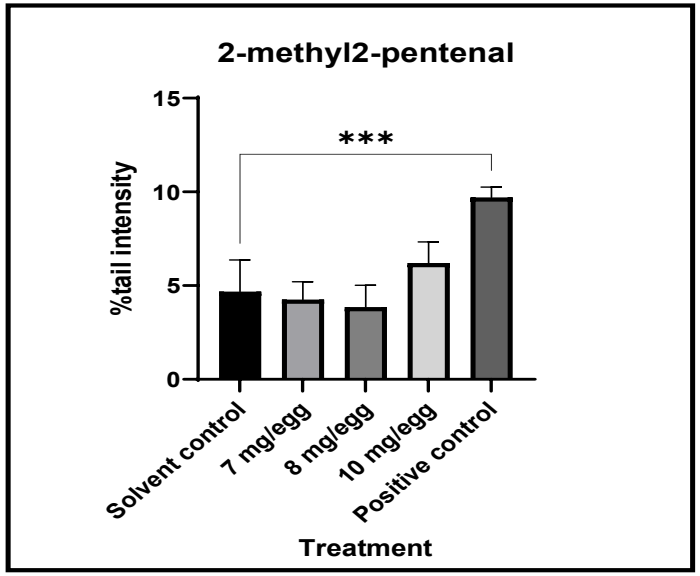
Negative

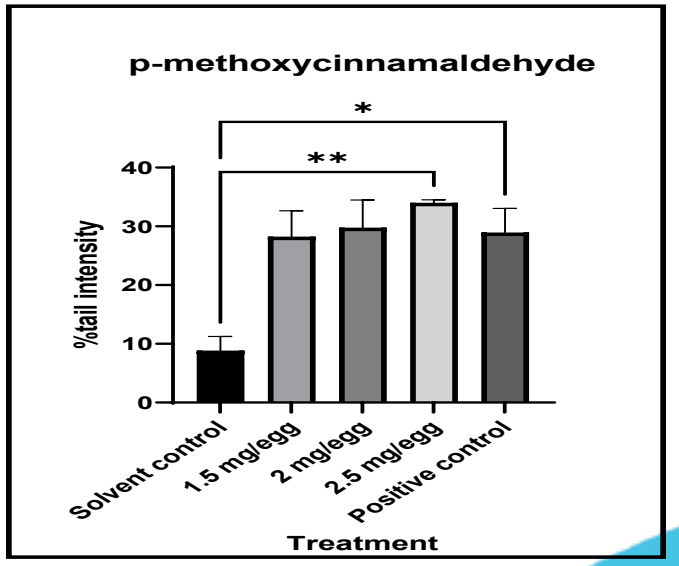




Negative

Negative





Positive

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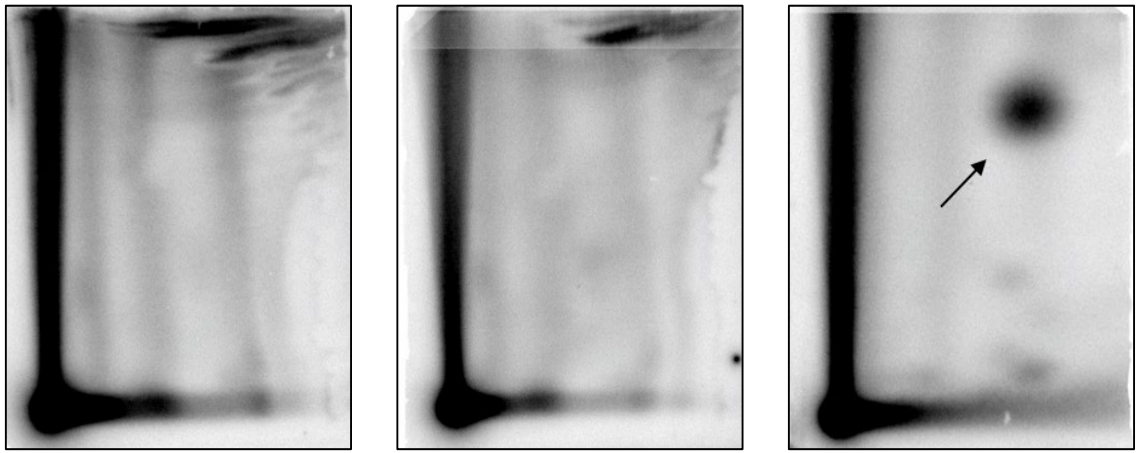
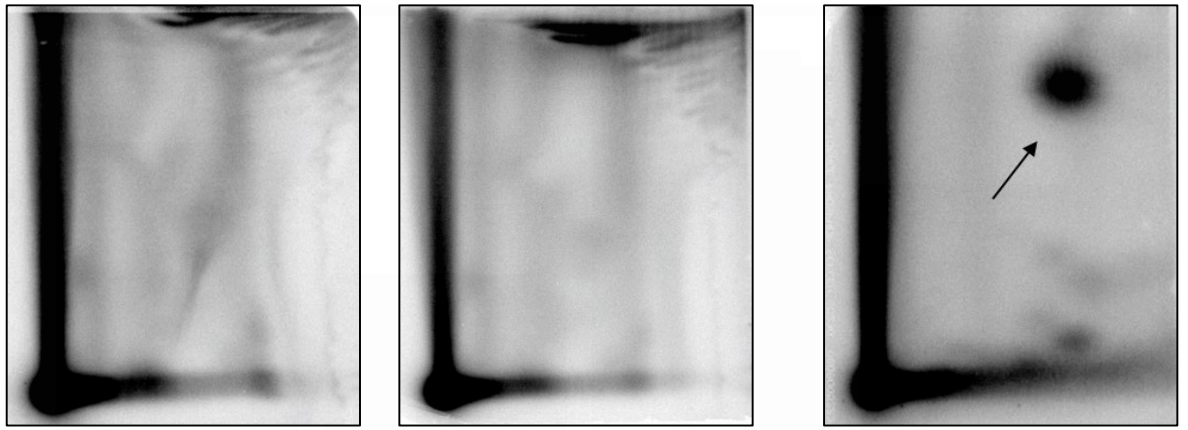
NPL assay results

2-Phenyl-2-butenal



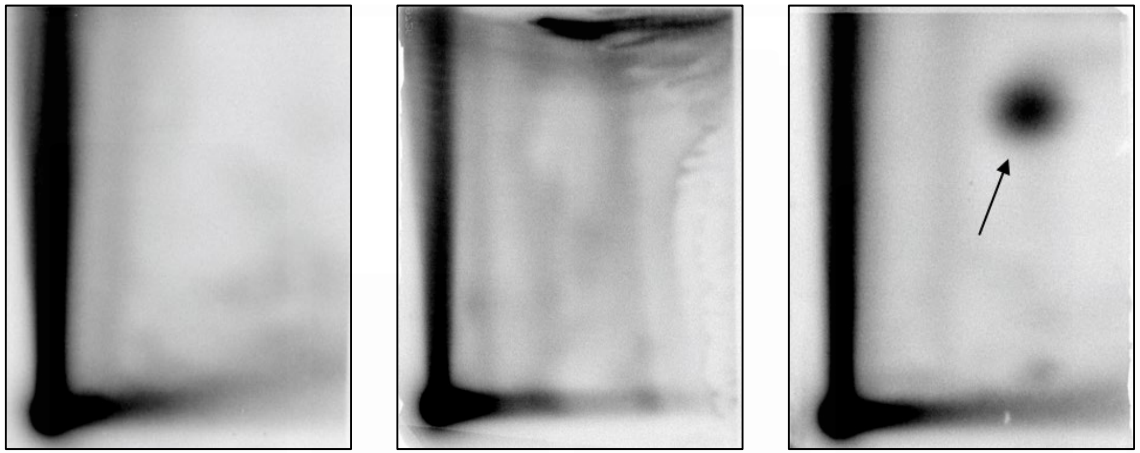
Vehicle, 20% HS 15 | 2-Phenyl-2-butenal, 5 mg/egg | Quinoline, 5 mg/egg

Nona-2-trans-6-cis-dienal



Vehicle, 20% HS 15 | Nona-2 trans-6-cis-dienal, 0.5 mg/egg | Quinoline, 5 mg/egg

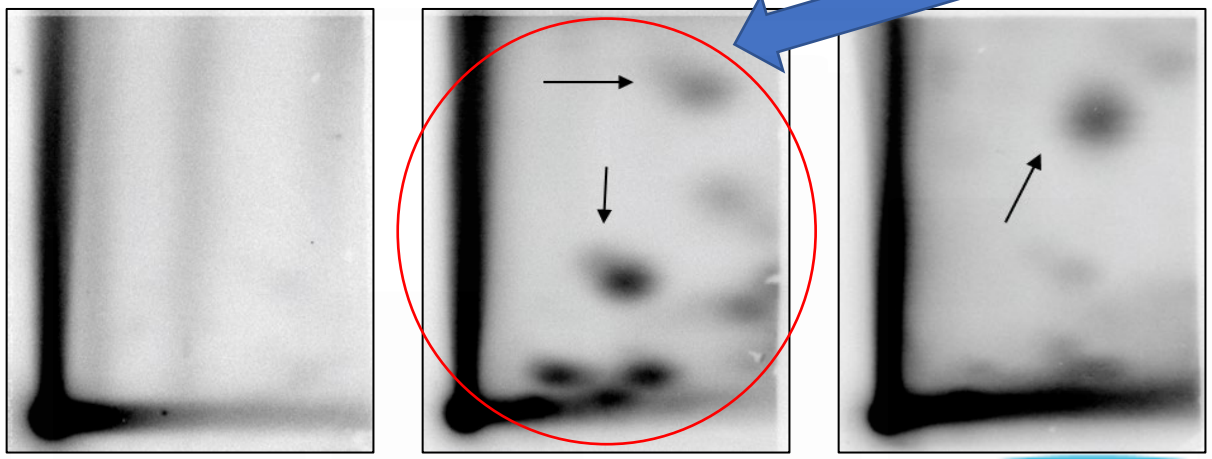
2-Methyl-2-pentenal



Vehicle, 20% HS 15 | 2-Methyl-2-pentenal, 8 mg/egg | Quinoline, 5 mg/egg

p-Methoxy cinnamaldehyde

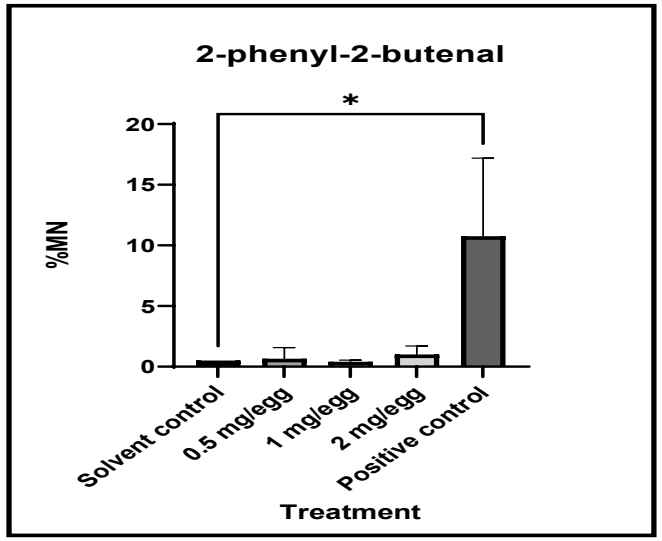
Positive

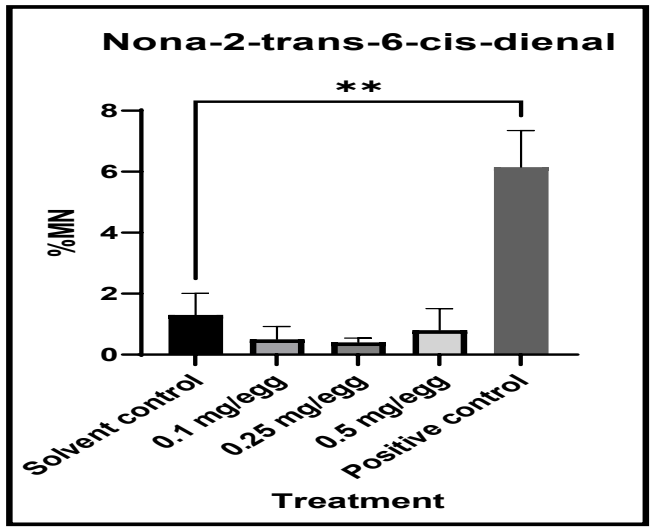


Vehicle, 20% HS 15 | p-Methoxy cinnamaldehyde, 2.5 mg/egg | Quinoline, 5 mg/egg

MNT assay results

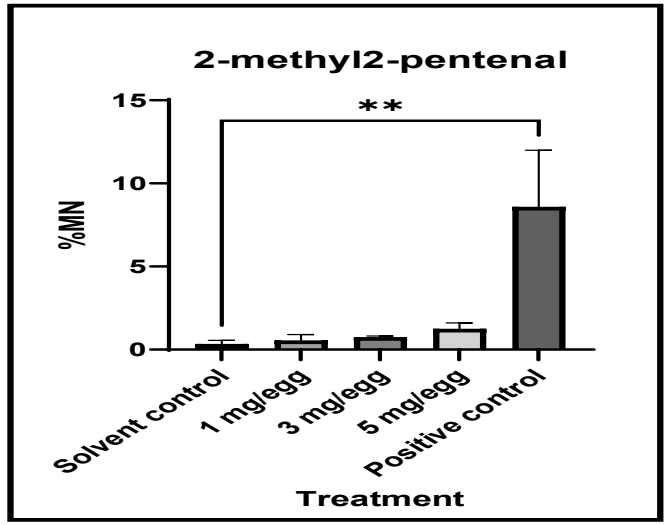
Negative

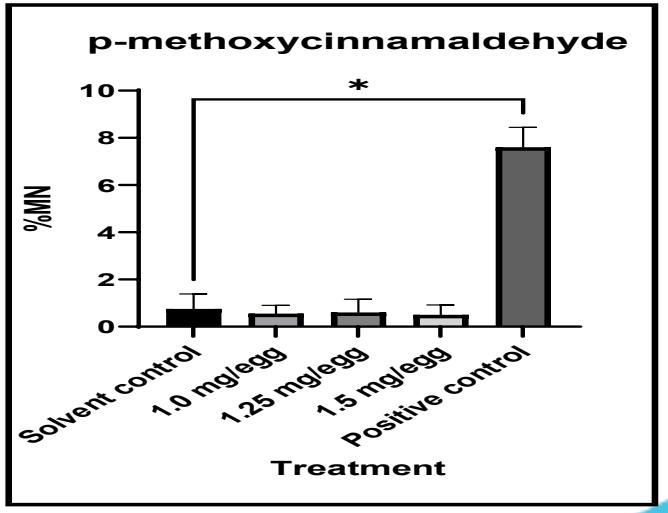




Negative

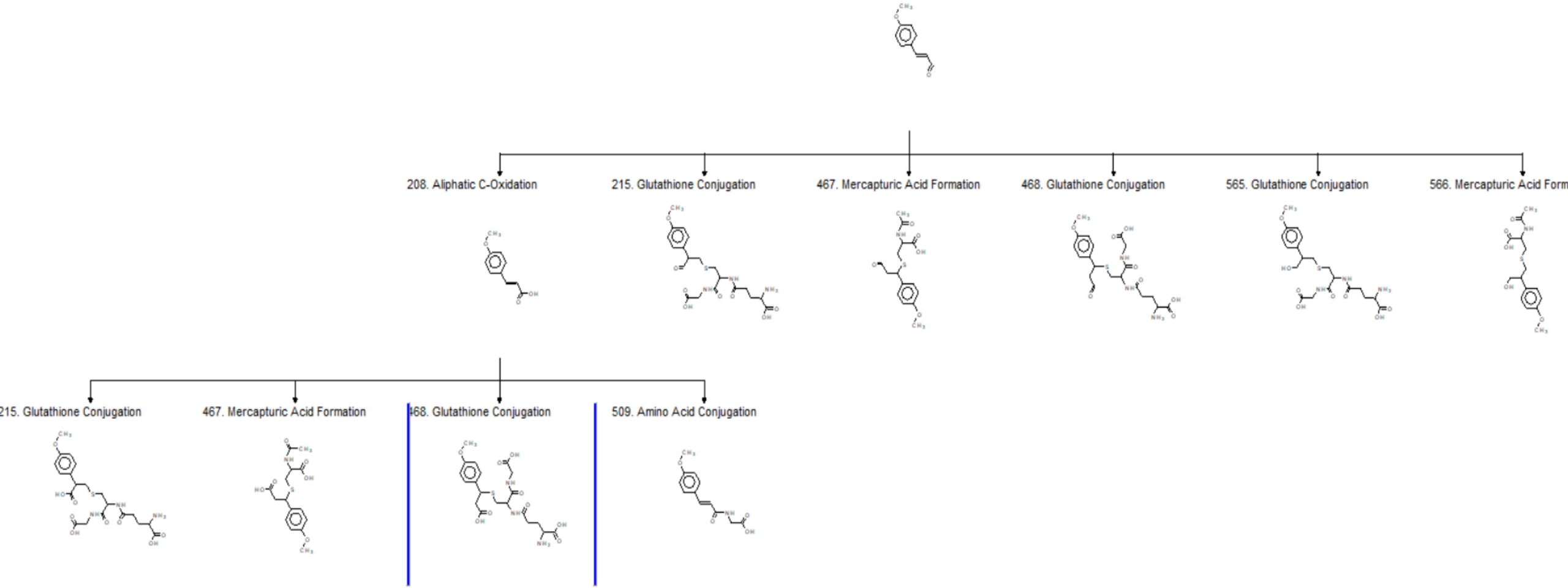
Negative





Negative

In-silco prediction



The *In ovo* model results from a pilot study conducted using fragrance materials showed promising concordance when compared to results from *in vivo* (animal) studies

Chemical Name	CAS #	Ames	In vitro MNT	In vivo COMET	In vivo MNT	CEGA COMET/NPL	HET-MN
2-phenyl-2-butenal	4411-89-6	+		-	-	-	-
2-methyl-2-pentenal	623-36-9	-	+	-	-	-	-
Nona-2-trans-6-cis dienal	557-48-2		+	-	-	-	-
p-methoxycinnamaldehyde	1963-36-6	+	+	-	-	+	-

Glutathione depletion → Repeat using N-acetyl cysteine/NADPH

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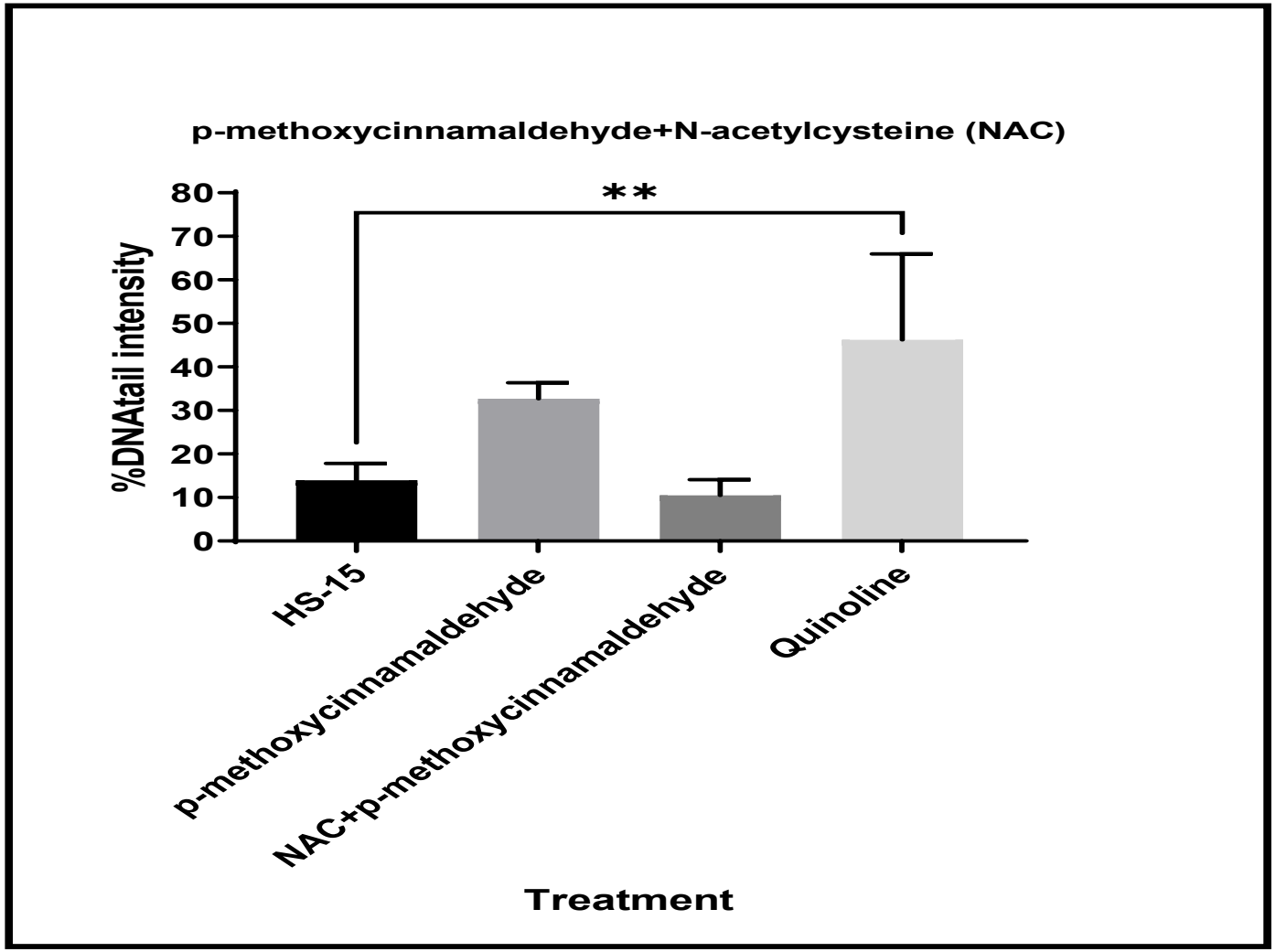

p-methoxycinnamaldehyde+N-acetylcysteine (NAC)
**
%DNAtail intensity
80
70
60
50
40
30
20
10
0
HS-15
p-methoxycinnamaldehyde
NAC+p-methoxycinnamaldehyde
Quinoline
Treatment

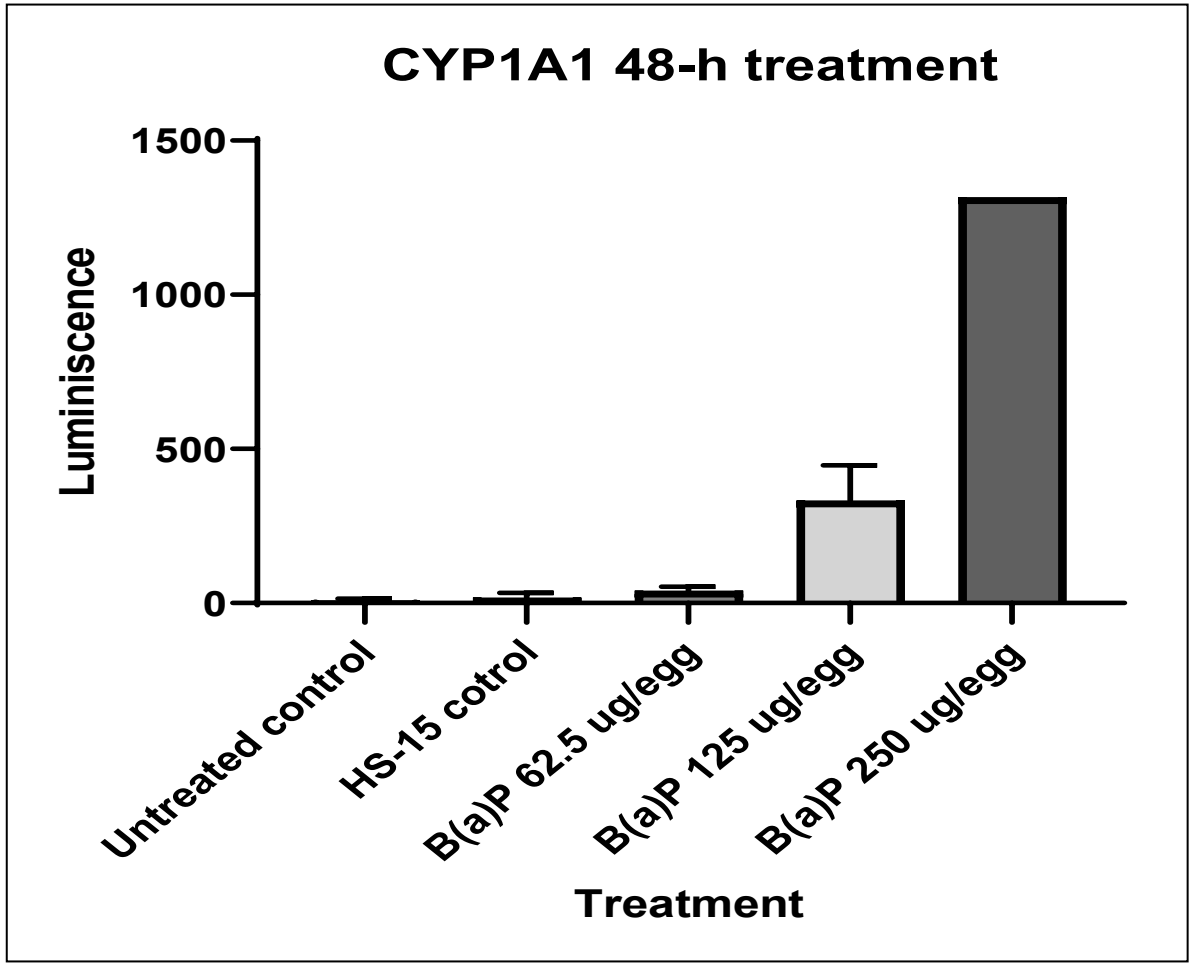
Negative

Enzyme activity studies

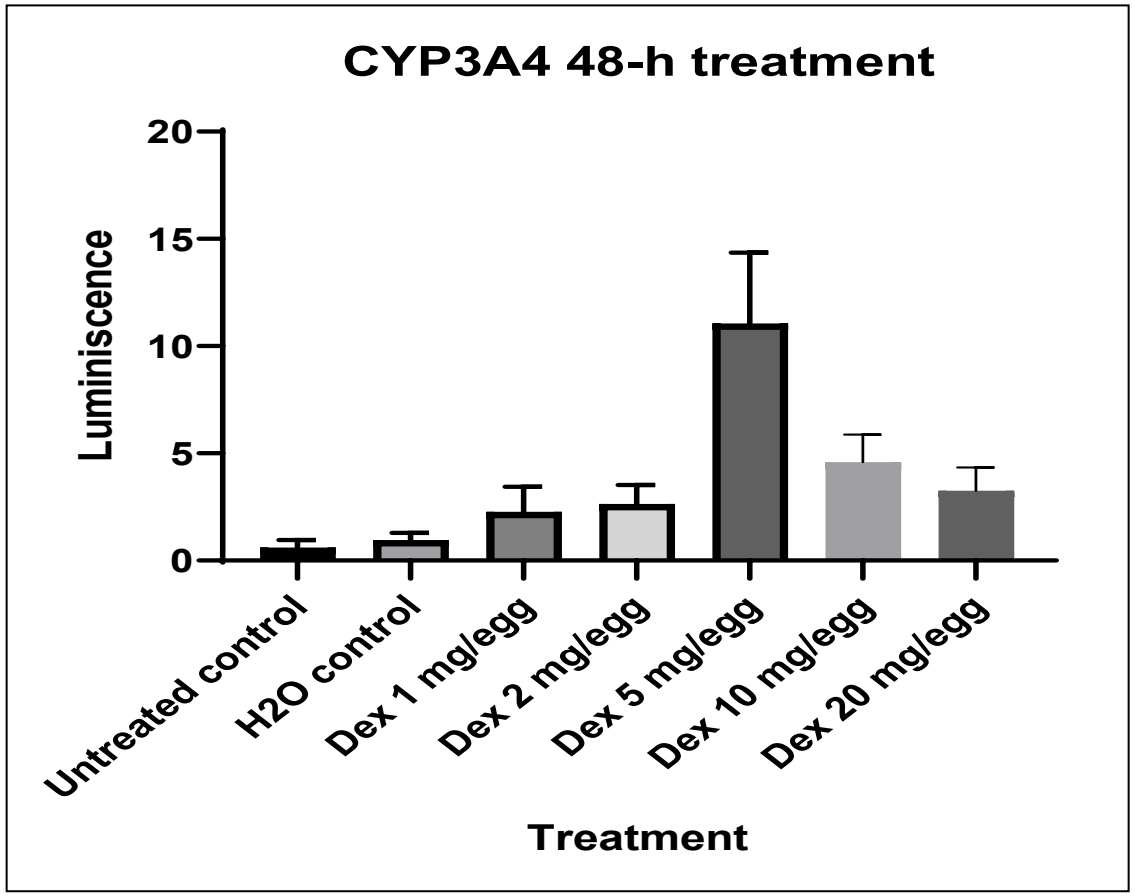
Phase-I enzymes

- CYP1A1
- CYP3A4
- CYP2C9

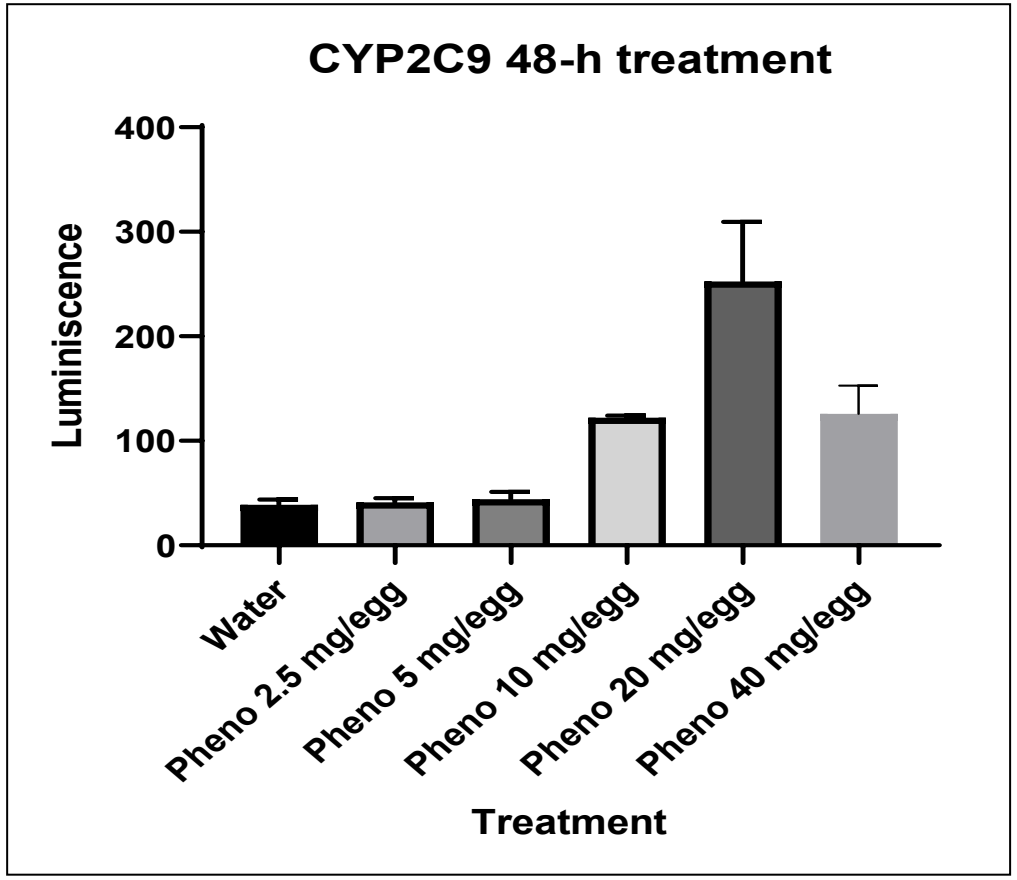
Increase in CYP1A1 activity in a dose dependent manner when treated with Benzo-a-pyrene



Increase in CYP3A4 activity in a dose dependent manner when treated with Dexamethasone



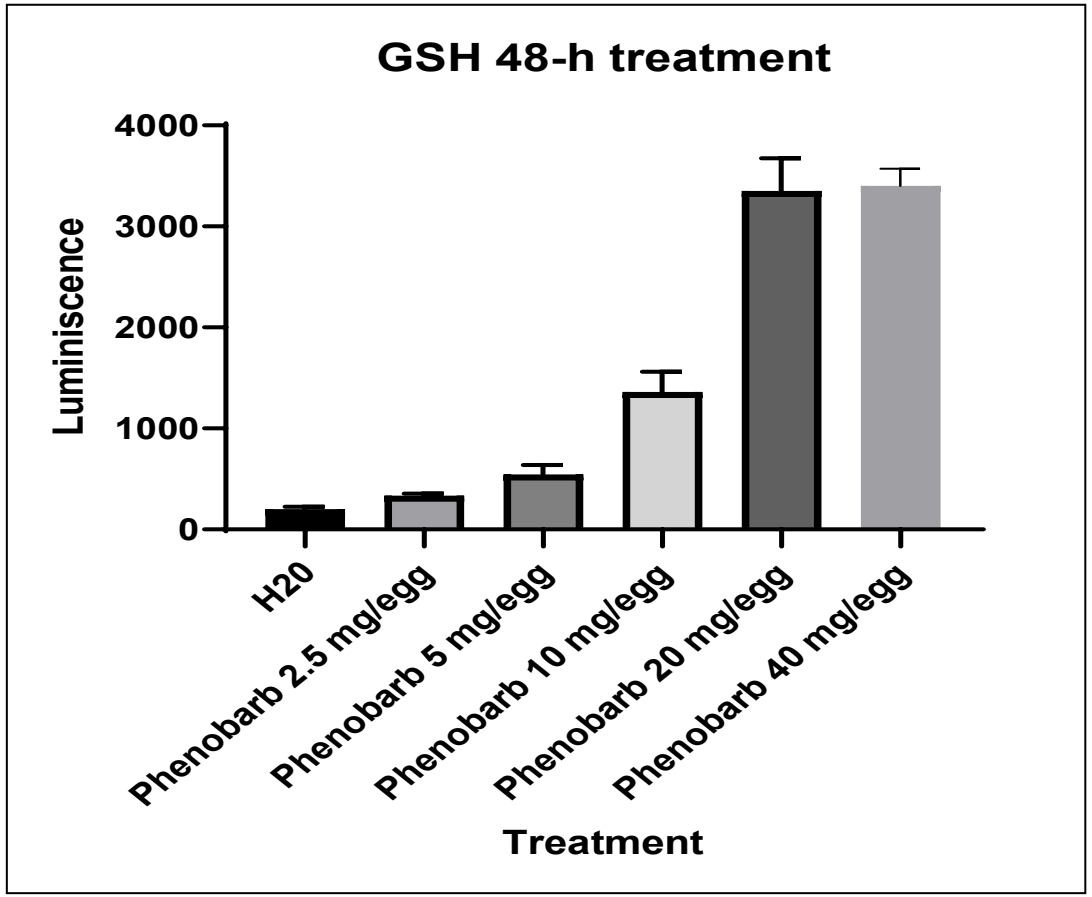
Increase in CYP2C9 activity in a dose dependent manner when treated with Phenobarbital



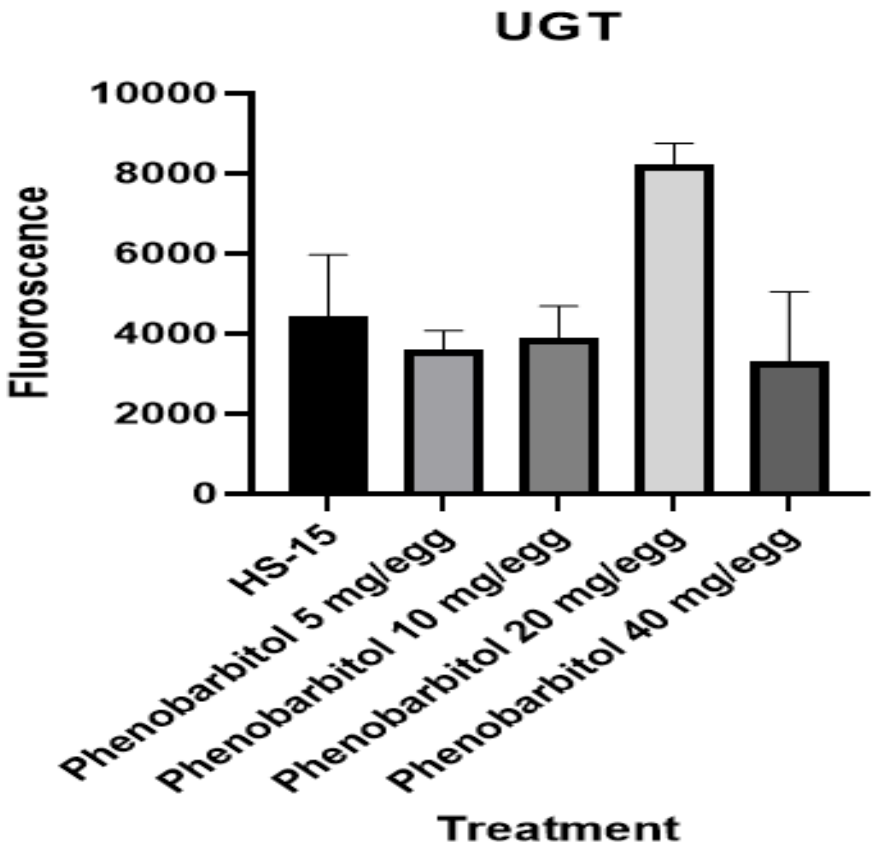
Phase-II enzymes

- GSH
- UGT

Increase in GSH activity in a dose dependent manner when treated with Phenobarbital

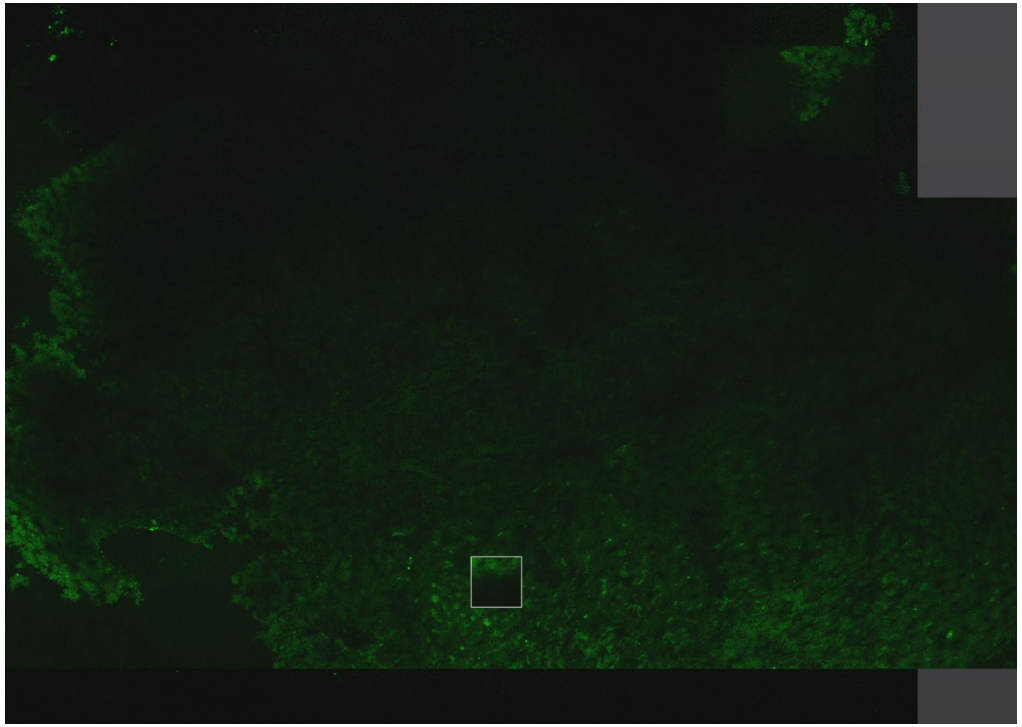


Increase in UGT activity in a dose dependent manner when treated with Phenobarbital

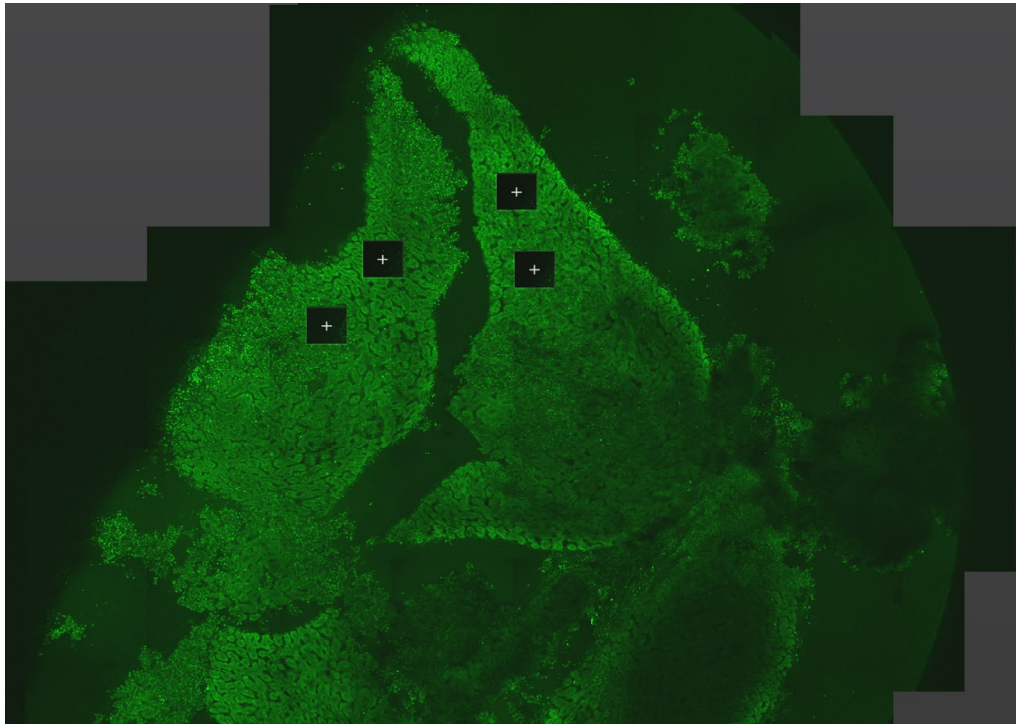


Target Tissue Exposure study

Acridine Orange treatment to demonstrate target tissue exposure

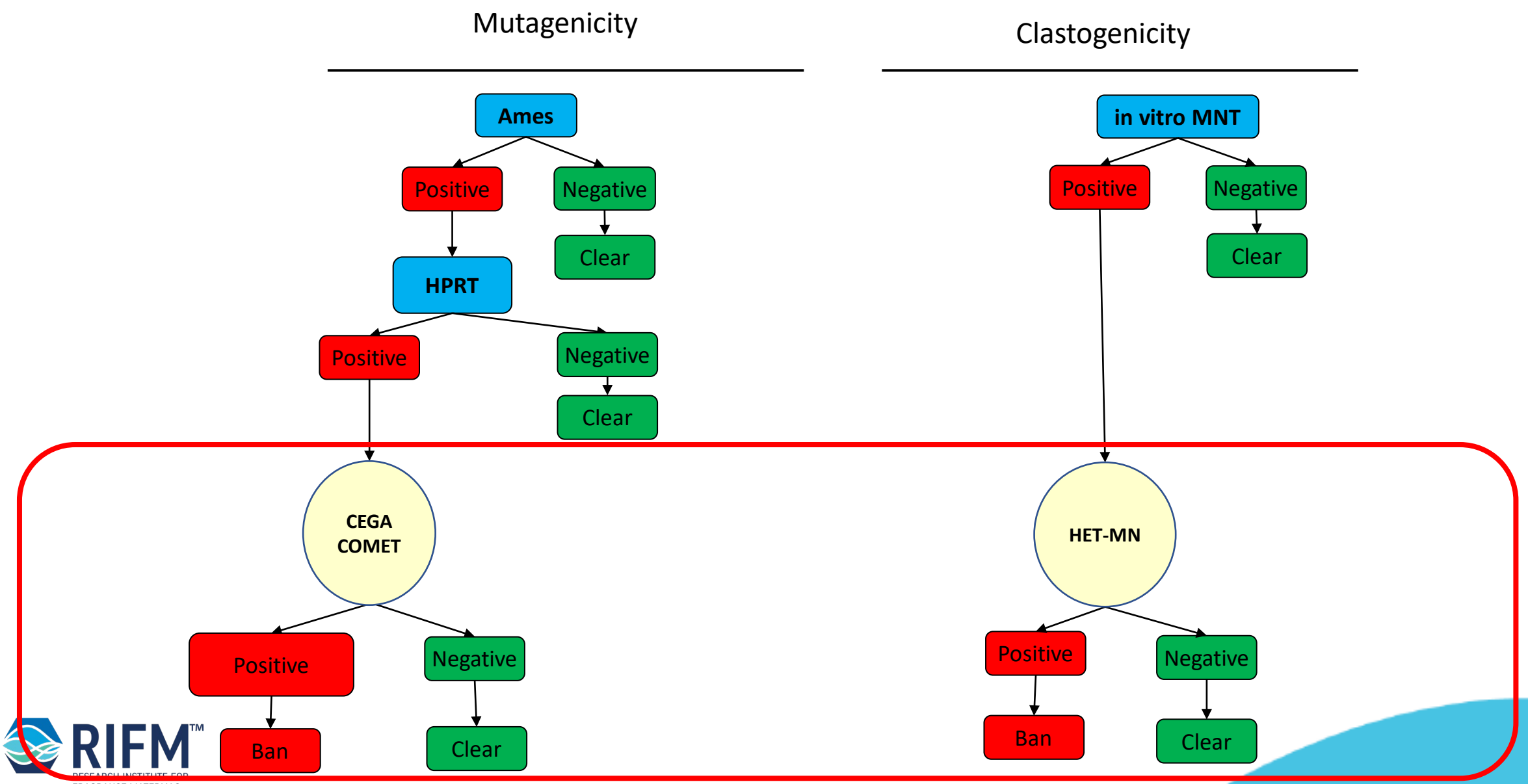


Water treatment Liver tissue



Acridine orange treatment
Liver tissue

Animal alternative Testing strategy



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Acknowledgements

Tetyana Cheairs, New York Medical College
Jiandong Duan, New York Medical College
Anne Marie Api, RIFM INC.

Thoughts/Suggestions/Questions?

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