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Section: Toxicology

Czech Academy of Sciences

Institute of Experimental Medicine, CAS

Genotoxicity of Organic Extracts of Particulate Emissions from Conventional Gasoline and Alternative Fuels

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Biofuels

Biofuels are liquid fuels produced from renewable biological sources (e.g. plants)

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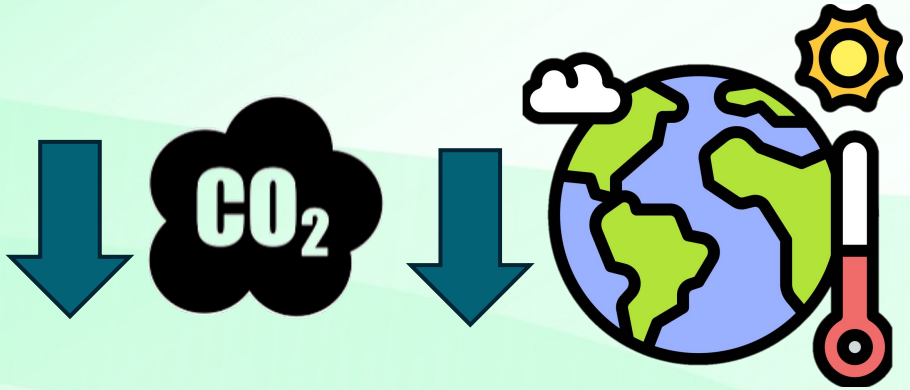
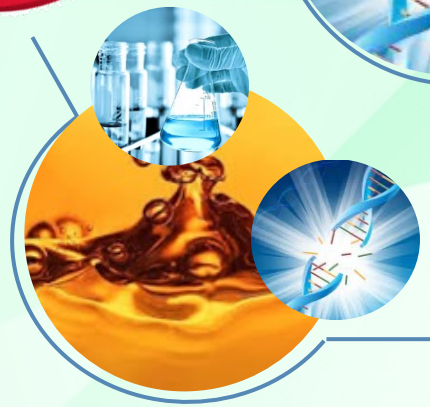


Conventional fossil fuels:
- Gasoline
- Diesel

Bio-additives:
- Ethanol
- Methanol
- Hydrogenated vegetable oil
- …

Aims



To **collect and characterize PM emissions** produced by **conventional gasoline fuel and alternative fuels** containing bio-additives

To **compare genotoxic potencies** of organic PM extracts in vitro

To find a **potential link between the fuel type, genotoxic compounds and genotoxic effects**

To contribute to the scientific knowledge on the health effects of biofuels emissions

To provide information for designing less toxic (bio)fuels

To decrease health burden of traffic-related emissions

Methods

Conventional fuel

Alternative fuels with bio-additives

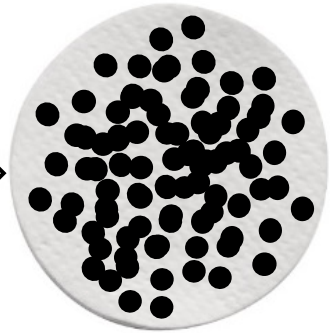
Gasoline (E0)

Gasoline + 25% i-buthanol (i-But)

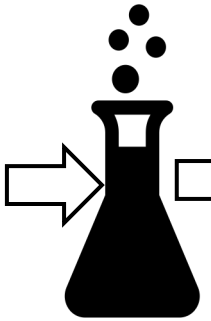
Gasoline + 25% N-buthanol (N-but)

Gasoline + 15% EtOH (E15)

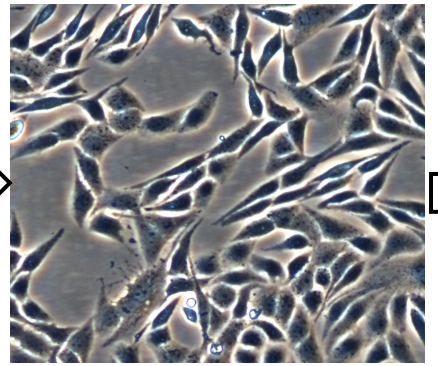
Chassis dynamometer facility



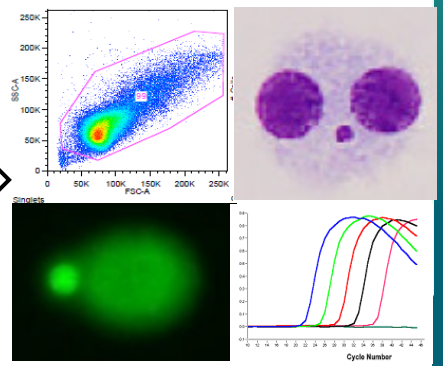
Filters



Organic extracts



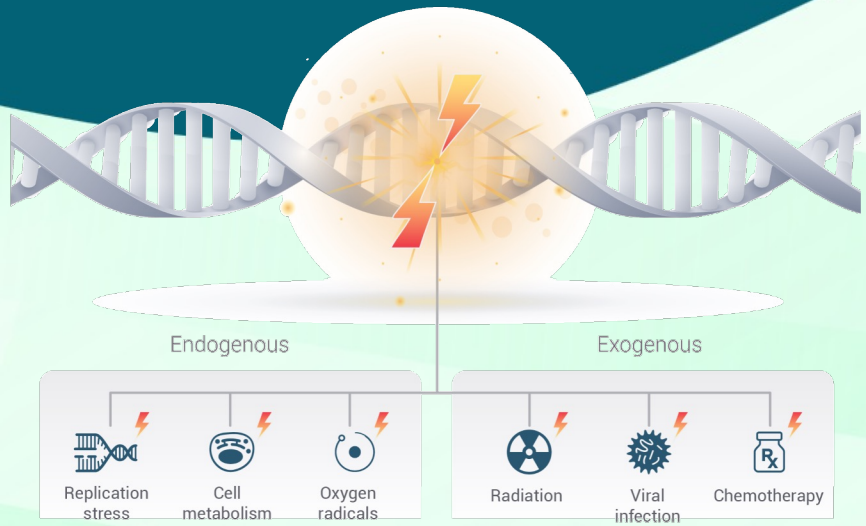
BEAS-2B cells



Genotoxicity testing

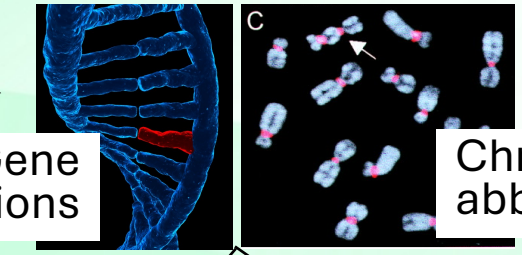
Genotoxicity

Property of an agent to cause damage to genetic information



https://www.sierraoncology.com/science/ddr/

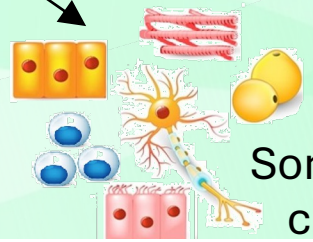
Not repaired



Gene mutations

Chromosomal abberations

Germ cells

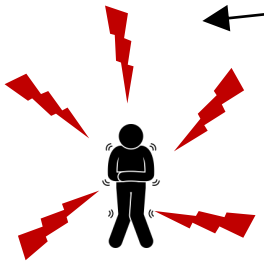


Somatic cells

One of the most important endpoints in **regulatory toxicology**

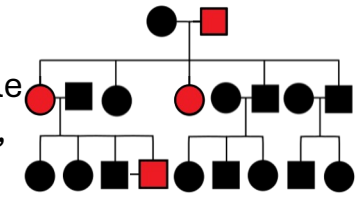
Link to various **diseases**

E.g. Diabetes, cardiovacular and neurodegenerative diseases



Multifactorial diseases

E.g. Cystic fibrosis, sickle cell anemia, hemophilia



Genetic diseases (single gene)

Inferility

E.g. Diabetes, cardiovacular and neuro-degenerative diseases

Other diseases

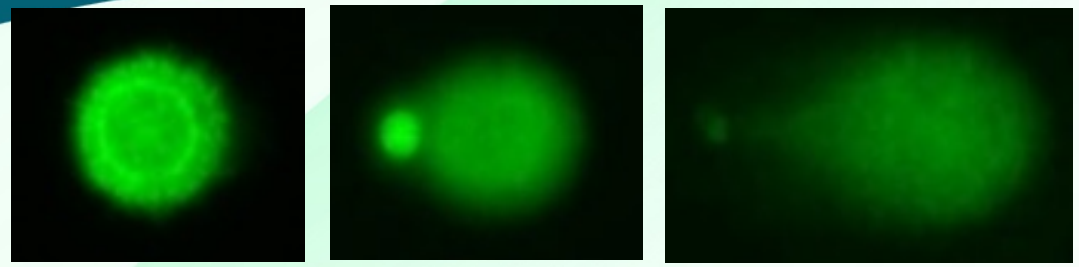
Aging

Cancer

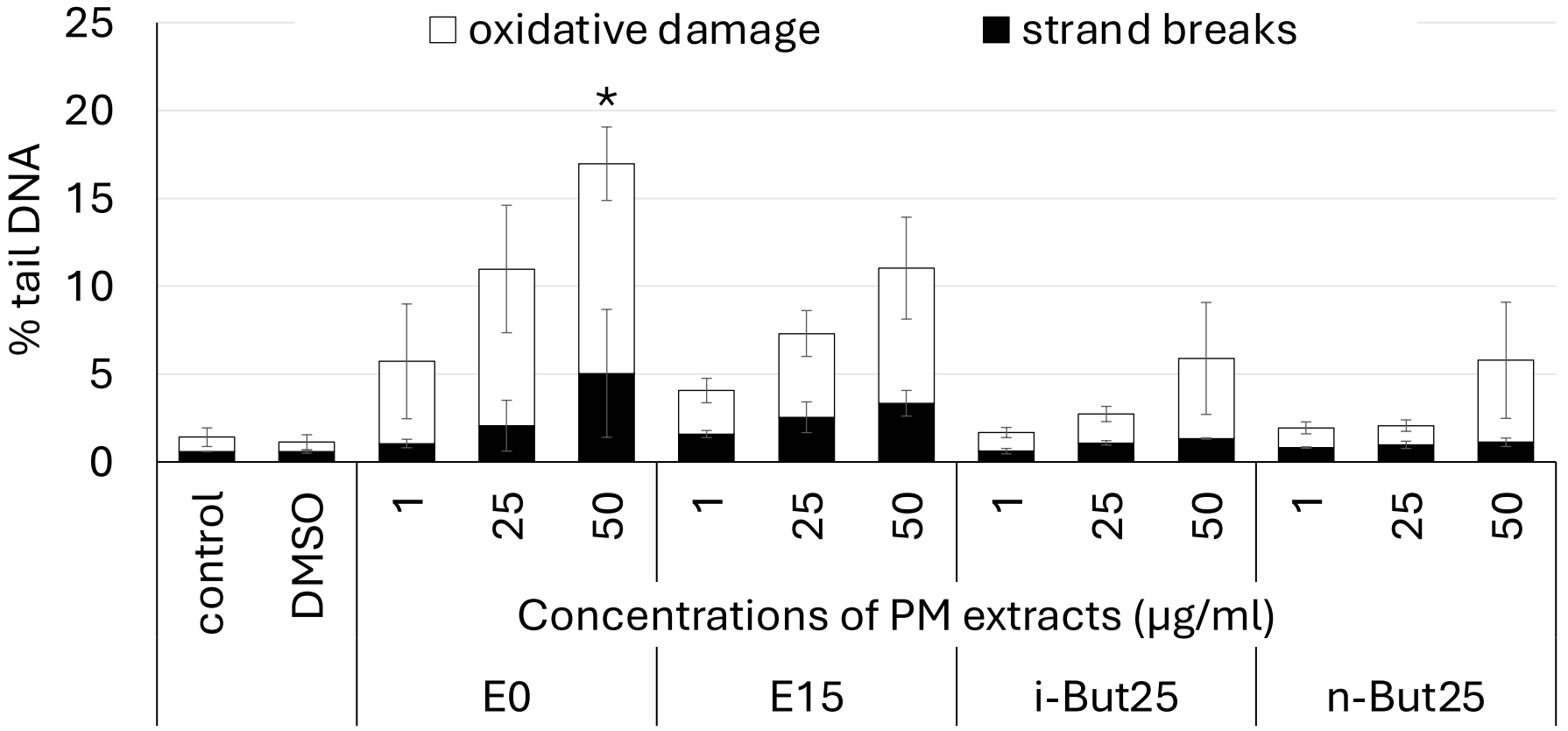
Chemical analysis

	Unit	E0	E15	n-But25	i-But25
PM mass	mg/km	1.7	1.8	0.9	1.3
Σ of PAHs	ng/mg PM	959	999	1294	816
Benzo[a]pyrene *	ng/mg PM	29	28	25	20
Benz[a]anthracene*	ng/mg PM	75	72	89	66
Chrysene*	ng/mg PM	66	63	74	59
Benzo[b]fluoranthene*	ng/mg PM	40	38	35	27
Benzo[k]fluoranthene*	ng/mg PM	20	17	16	15
Indeno[1,2,3-cd]pyrene*	ng/mg PM	27	26	24	19
Dibenz[a,h]anthracene*	ng/mg PM	0.5	0.5	n.d.	0.4
Sum of oxygenated PAHs	ng/mg PM	208	216	426	298
Sum of nitrated PAHs	pg/mg PM	231	142	276	187
Sum of dinitrated PAHs	pg/mg PM	0	0	9	4

Comet assay + FPG enzyme



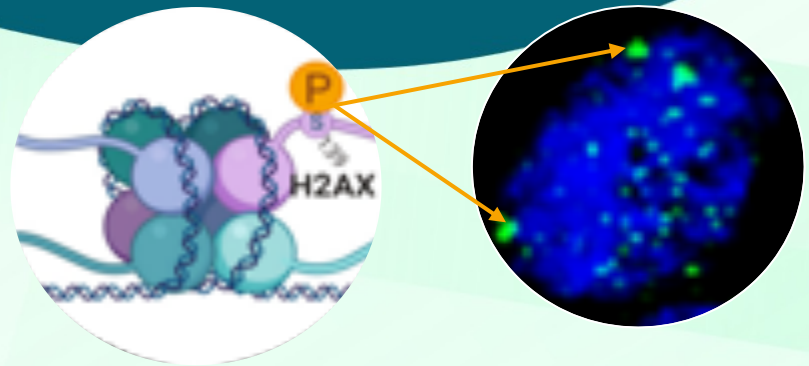
DNA damage = tail DNA



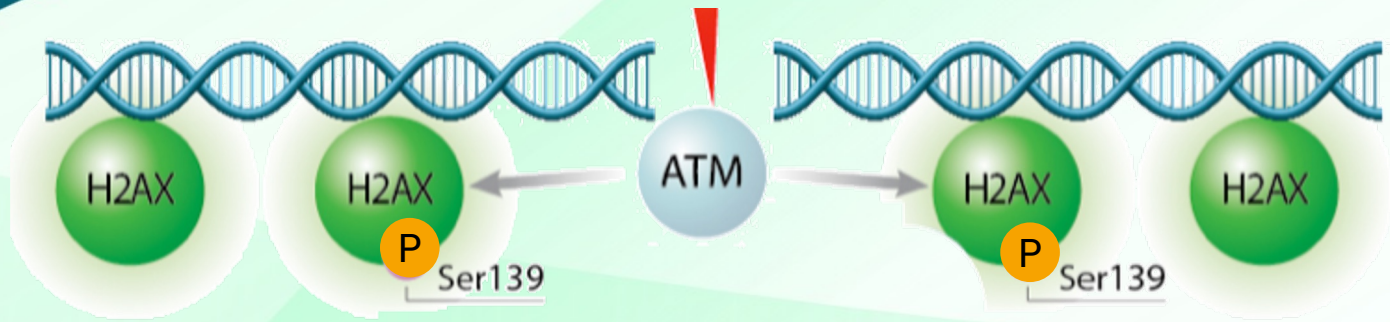
E0 and E15 extracts caused several fold increase of DNA damaged sites, particularly oxidized bases

* *significantly increased comparing to DMSO ($p<0.05$)*

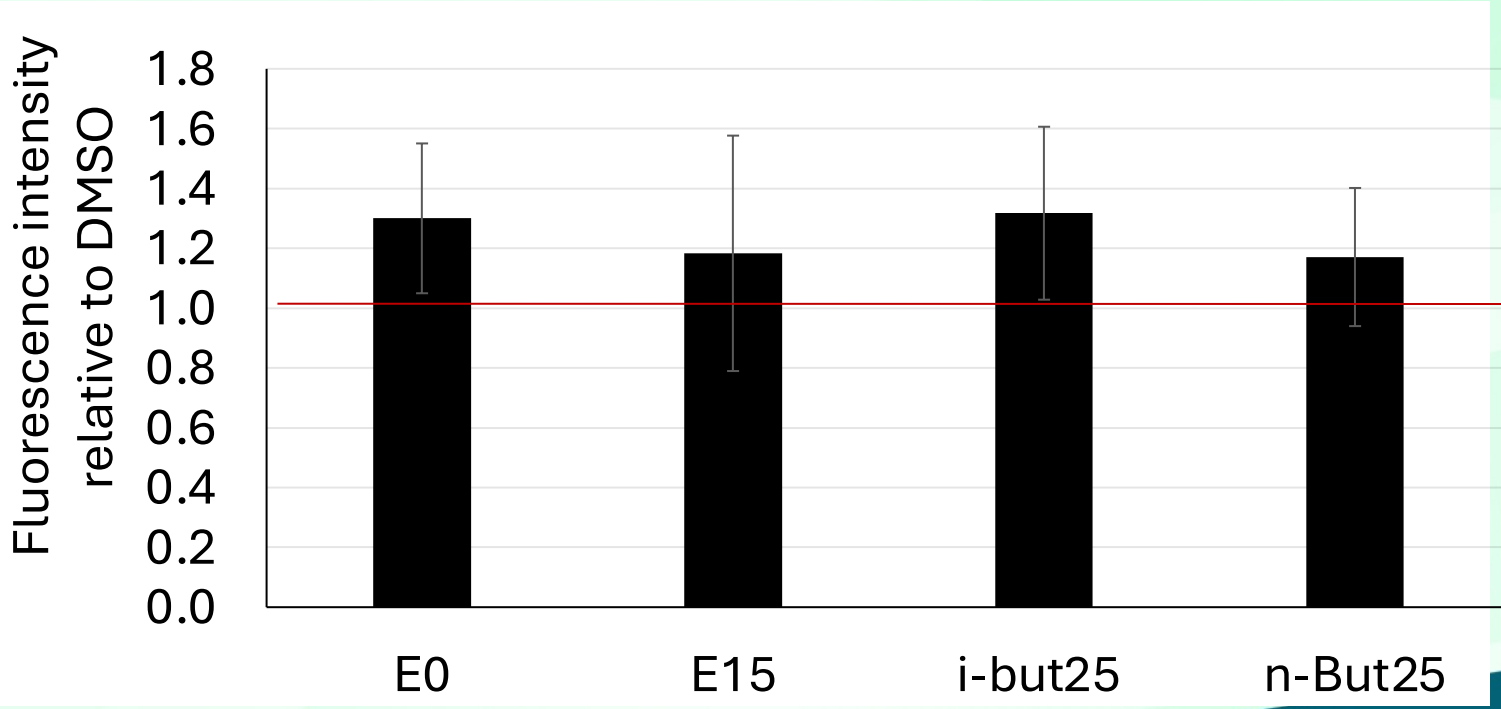
γH2AX (flow cytometry)



Detection of DNA double strand breaks

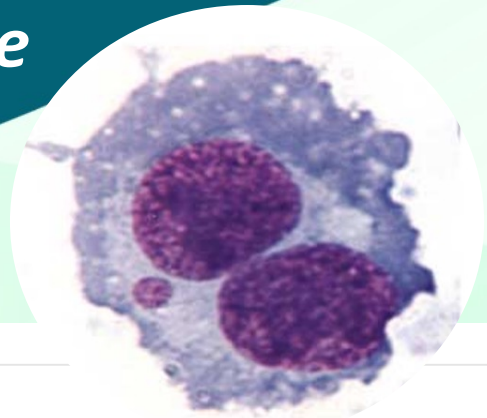


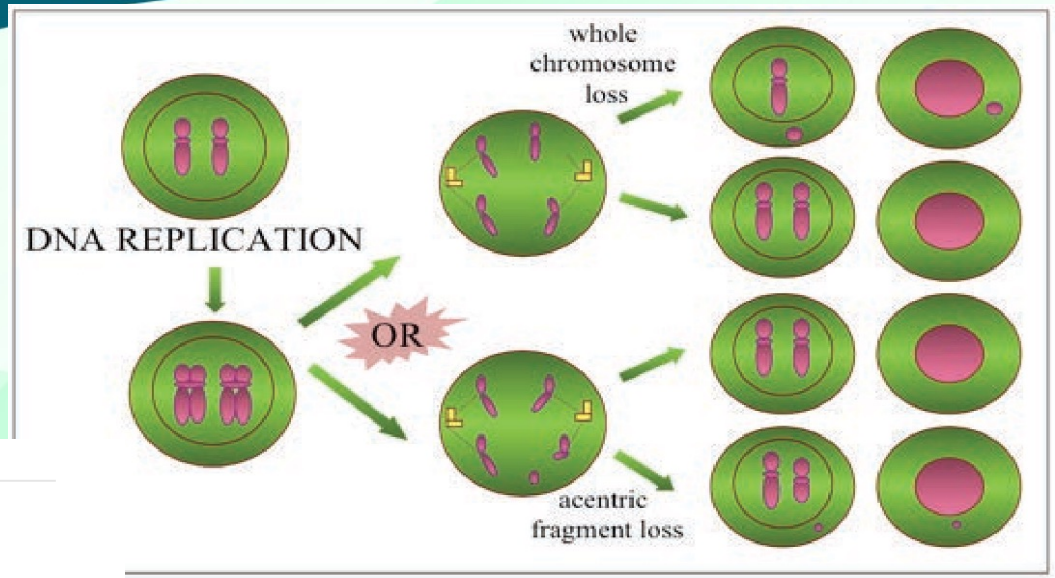
https://measurebiology.org/w/images/9/91/Fa16_M1D5_H2AX-P.png



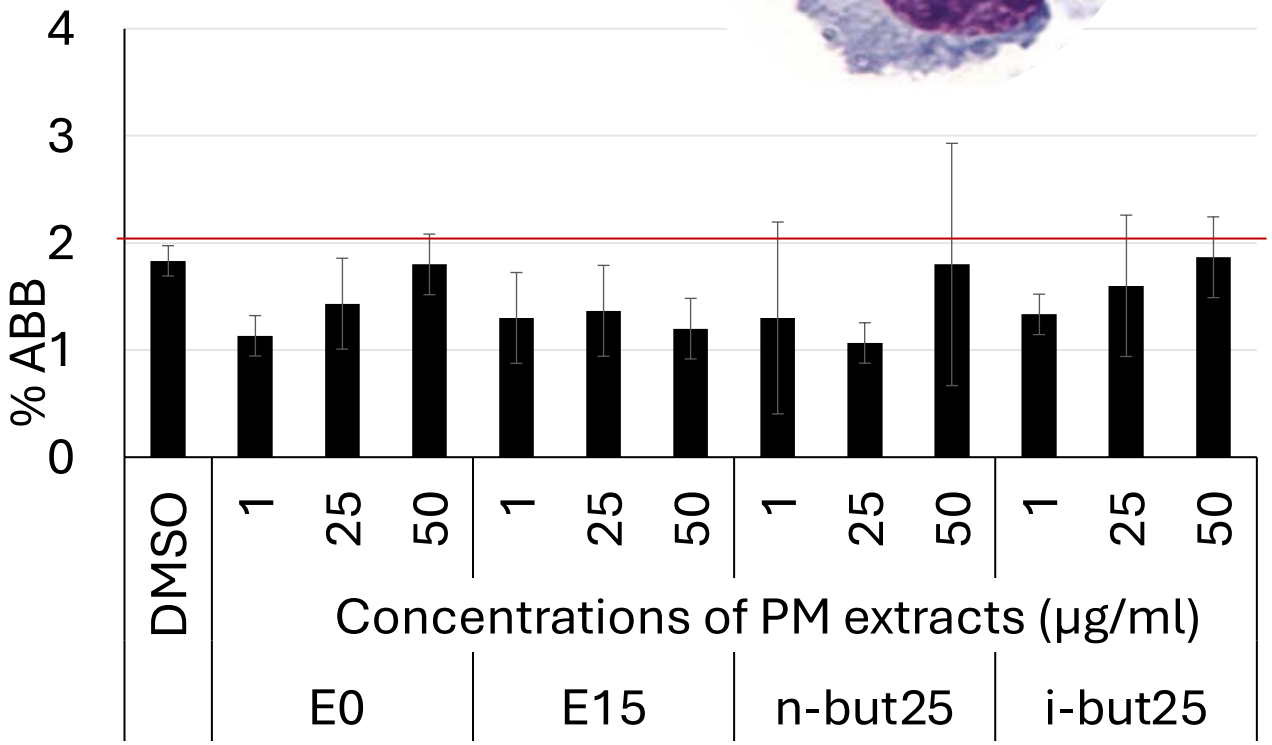
Slight (not significant) increase of γH2AX observed after the treatment with E0 and i-But25 extracts (~1.3 fold induction)

Micronucleus assay
Chromosomal damage





Cerqueira ED, Meireles JR. The Research and Biology of Cancer. 2012:1-26.

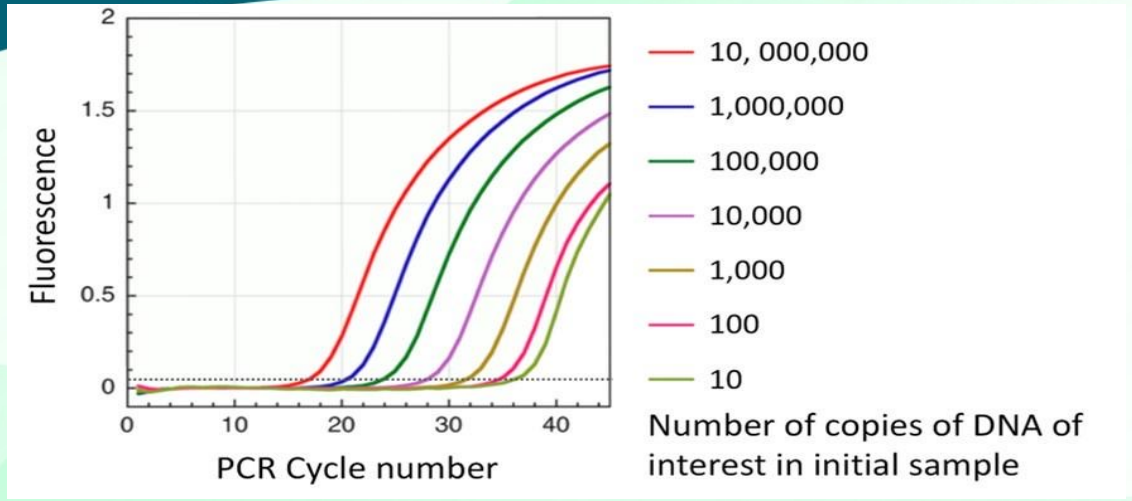


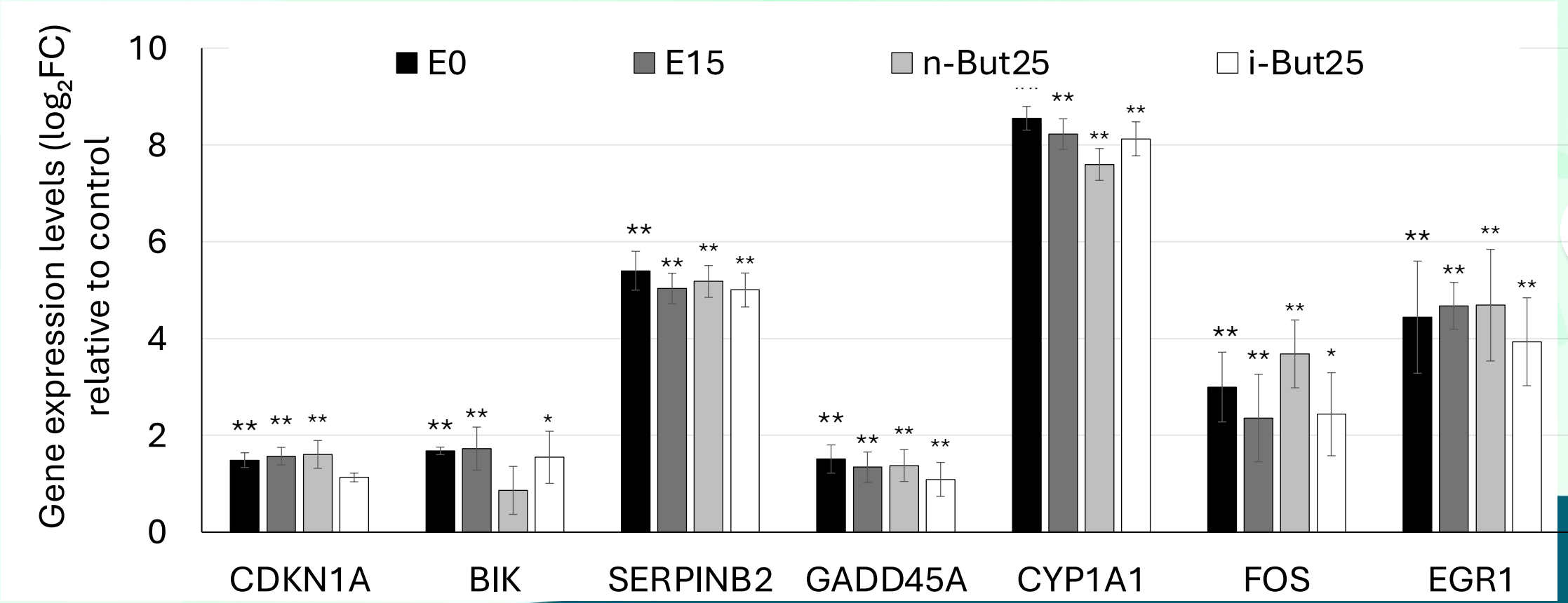
Lower doses of all PM extracts exhibited rather inhibitory effect (not significant) on micronuclei formation comparing to DMSO

% ABB = % aberrant binucleated cells w/ micronuclei

qPCR analysis of changes in expression of selected genes

https://equine.ca.uky.edu/content/science-sleuths-science-shapes-diagnostic-tests-pcr-qpcr-%E2%80%93-what%E2%80%99s-difference



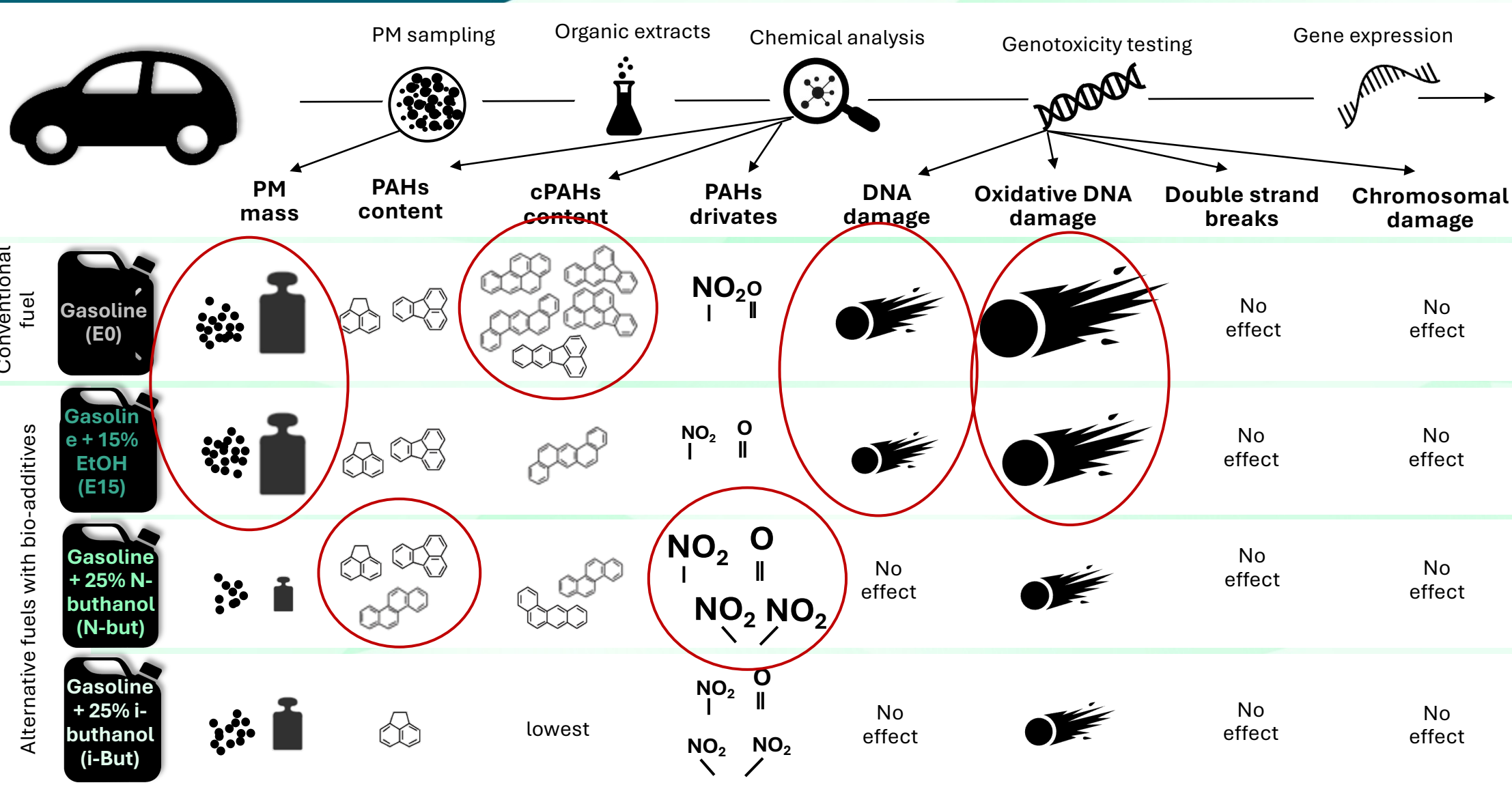


qPCR analysis of changes in expression of selected genes

Genes with increased expression

Selected genes	Pathway	Biological function
FOS	implicated in the protection against **genotoxic stress**	immediate-early stress response
EGR1	regulation of genes involved in **DNA repair**, cell **survival**, and **apoptosis**	
CDKN1A	key mediator of the **p53 response**, **inhibitor of cell cycle**	DNA damage response
BIK	**pro-apoptotic** function	
GADD45A	implicated in regulation of DNA repair, cell cycle control, senescence and genotoxic stress	
SERPINB2	target of **p53 and AhR**, activated by **DNA damage response** pathway	Metabolism of PAHs
CYP1A1	target of **AhR**, crucial role in **metabolic activation of PAHs**	

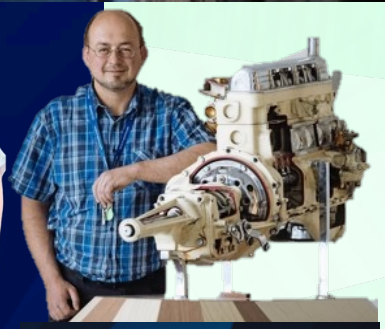
Summary of the results



Conclusions

- Despite the **highest** total mass of **PAHs** and their **derivatives** found in **n-But25** extract, **E0 and E15** extracts exhibited **higher genotoxic potency**, possibly due to the higher content of **carcinogenic PAHs**
- **i-But25** extract had the **lowest concentration of PAHs** and induced **lower toxic response**
- **no** PM extract induced **double-strand breaks** and **chromosomal damage** evaluated as a frequency of micronuclei
- gene expression analysis revealed activation of **DNA damage response** suggesting a possible impact on cell fate including cell **cycle arrest** and **apoptosis**, and toxic response mediated by activated **AhR**

Acknowledgement



Thank you for your attention

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