Comparison of chemical composition and toxicity effects of emissions from electronic cigarettes, heated tobacco products and tobacco cigarettes in human bronchial epithelial cells

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Conflicts of interest

The authors have no conflicts of interest to disclose concerning the presentation

Electronic cigarettes (e-cig)

Heated Tobacco Products (HTP)

IQOS® (Philip Morris)

New, less harmful alternatives to cigarettes?

E-liquide : Propylene glycol
Glycerol
+/- nicotine
+/- aroma

- ✓ Device with heating blade ≤ 350°C
- ✓ Heets (Tobacco + glycerol)

Studies mainly conducted by the tobacco industry → comparison of toxicity / cig

www.iqos.com :

IQOS heats tobacco instead of burning it, producing on average 95% less harmful chemicals compared to cigarettes*.

Switching completely to IQOS is less harmful than continuing to smoke**.

3rd generation models are the most used today **However** few toxicological studies

Objectives of the study

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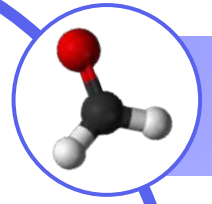
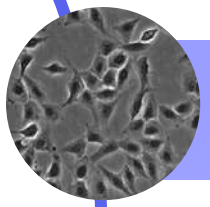
Chemical characterization and *in vitro* toxicity study of electronic cigarette emissions and heated tobacco products

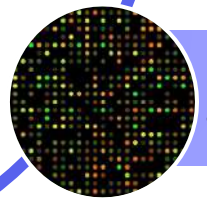


1. Comparison of the chemical composition of the different emissions



2. Comparison of emission cytotoxicity in human bronchial epithelial cells

3. Analysis of oxidative stress and possible genetic alterations



4. Identification, by transcriptomic analysis, of the signaling pathways associated with the toxicity of HTP and e-cig emissions

Experimental protocol

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IQOS

Heated tobacco

Control –
Air

E-cigarette ⟷ **Cigarette**

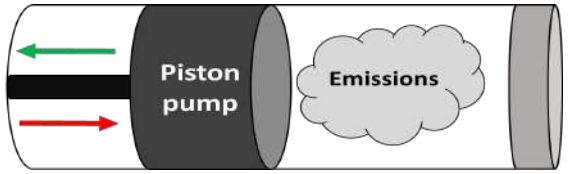
Modbox (Mb)
0.5 Ω
18 or 30 W

Blond tobacco
16 mg/mL nicotine

3R4F

Generation of aerosols

Smoking machine (Vitrocell)

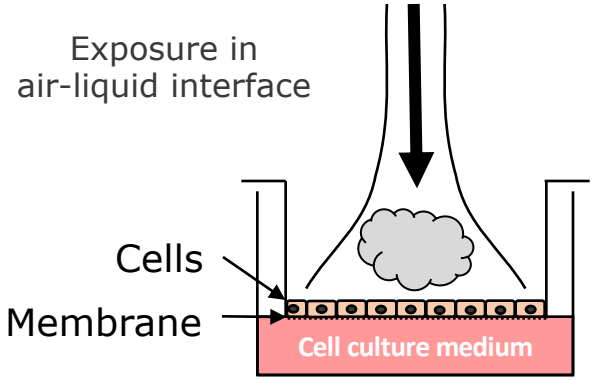


Puff Profile (HCI)
Volume : 55 mL
Duration : 2 s
Frequency : 2 puffs/min

Cell model:

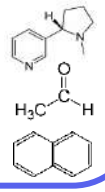
Human bronchial epithelial cells (BEAS-2B cell line)

Exposure in air-liquid interface



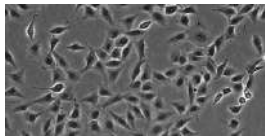
Chemical characterization

- Nicotine
- Carbonyl compounds
- Polycyclic aromatic hydrocarbons



In vitro toxicity

- Cell viability
- Oxidative stress
- Inflammation
- Genotoxicity
- Transcriptomic analyzes



Comparison of the chemical composition of the different emissions

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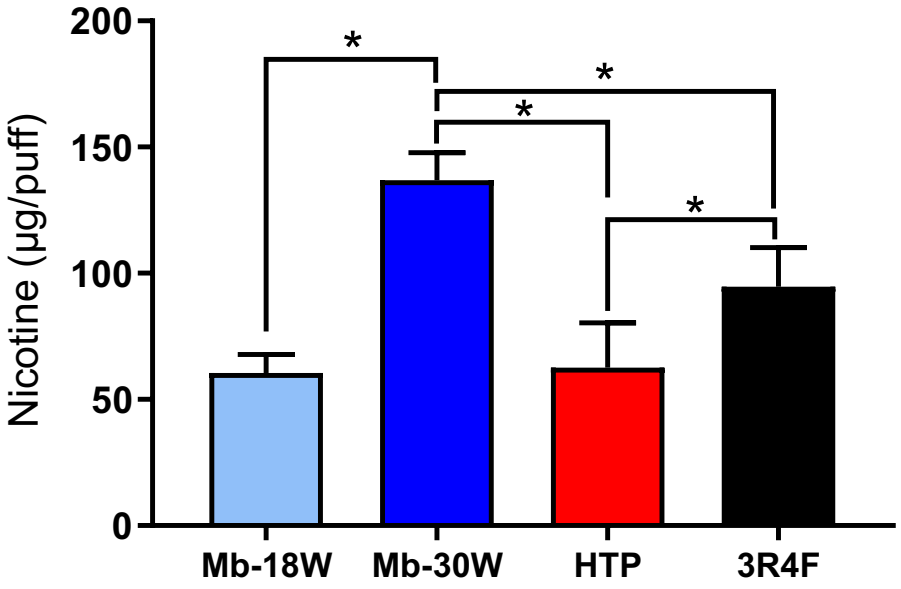
Nicotine



→ HTP: 30% less nicotine in a puff compared to cigarette

→ E-cig: the level of nicotine delivered is highly dependent of the power

Mb-30W > Mb-18W

Mann-Whitney : * $p < 0.05$ (n=4)

Dusautoir *et al.*, 2021

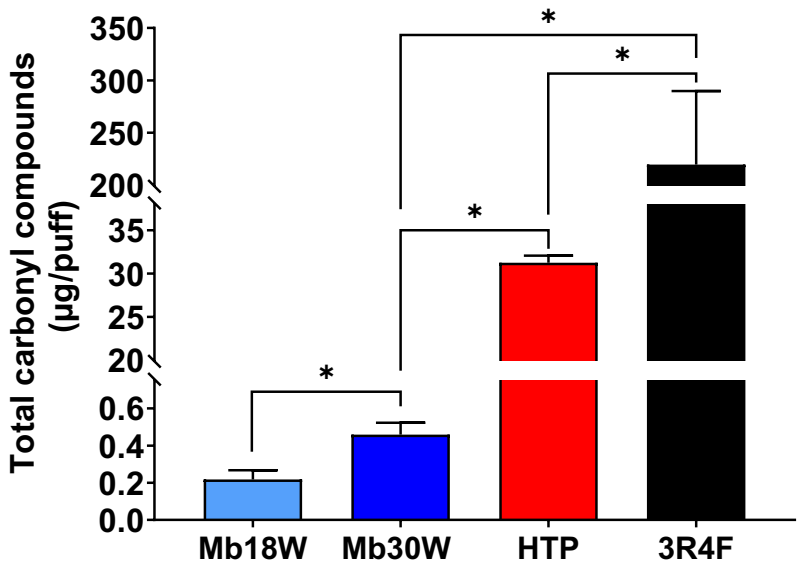
Comparison of the chemical composition of the different emissions

Carbonyl compounds

UHPLC System with UV/VIS

Detector

Formaldehyde, Acetaldehyde, Propanone, Propanal, Methyl vinyl ketone, Crotonaldehyde, Methyl ethyl ketone, Methylpropenal, Butanal Benzaldehyde, Isopentanal, Pentanal, Glyoxal, o-Tolualdehyde, m-Tolualdehyde, p-Tolualdehyde, Methylglyoxal, Hexanal, 2,5-Dimethylbenzaldehyde



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Comparison of the chemical composition of the different emissions

Carbonyl compounds

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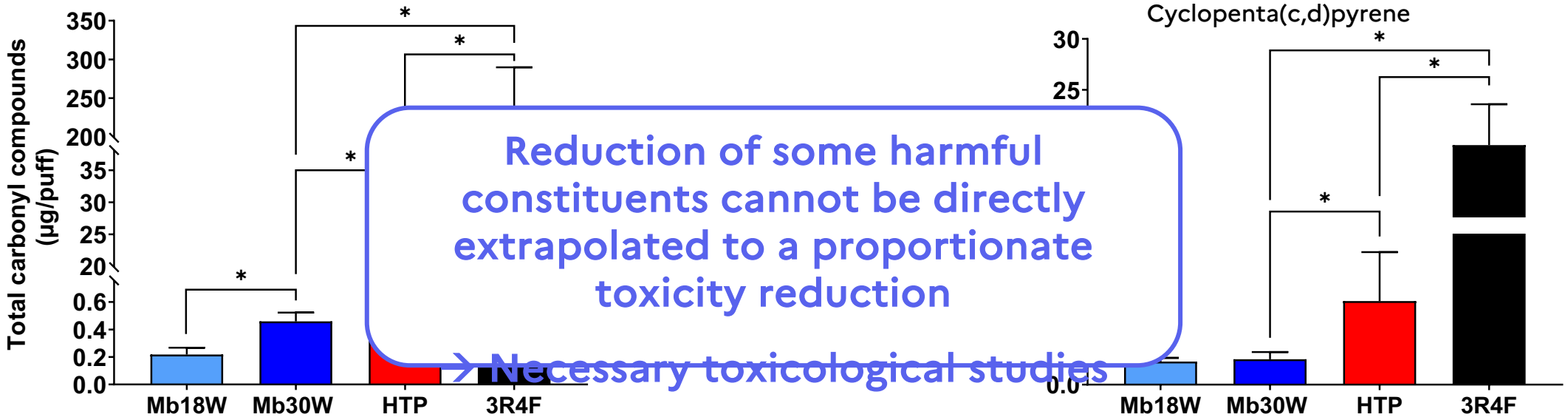
Polycyclic Aromatic Hydrocarbons (PAH)

HPLC coupled with fluorescence

detector

Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(c)phenanthrene, Benzo(a)anthracene, Chrysene, 5-Methylchrysene, Benzo(e)pyrene, Benzo(b)fluoranthene , Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,l)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, Indeno(1,2,3-c,d)pyrene, Dibenzo(a,e)pyrene, Anthanthrene, Coronene, Cyclopenta(c,d)pyrene



Reduction of some harmful constituents cannot be directly extrapolated to a proportionate toxicity reduction

→ Necessary toxicological studies

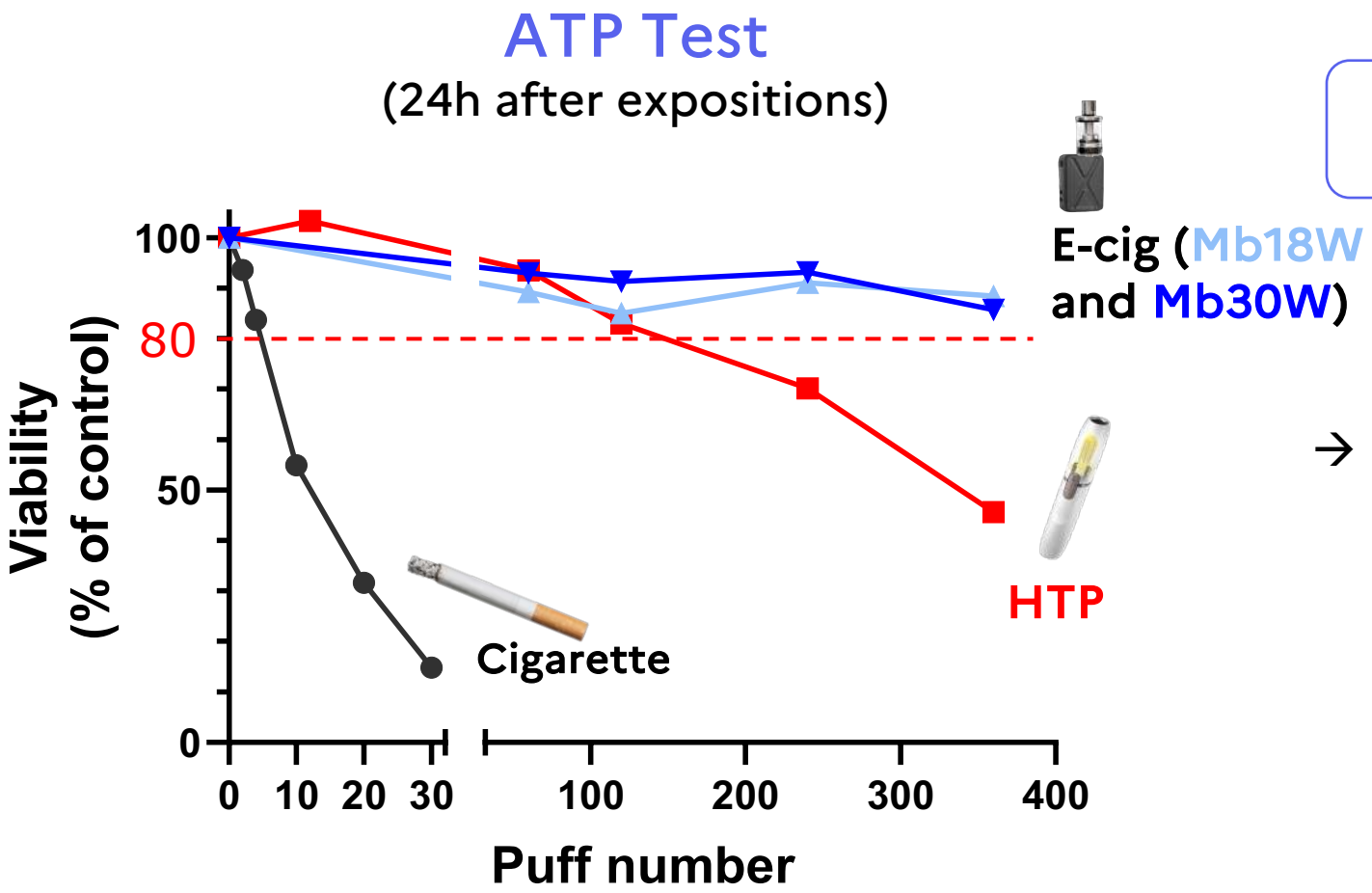
Cigarette > HTP > E-cig

Mb-30W > Mb-18W

Cigarette > HTP > E-cig

Mann-Whitney : * $p < 0.05$ (n=4)

Cytotoxicity



Cigarette > HTP > E-cig

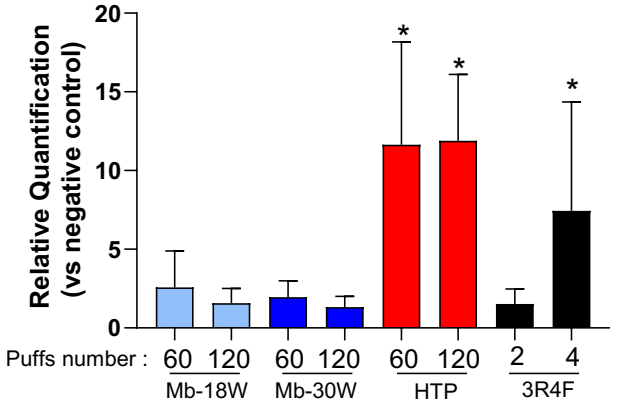
→ Choice of sub-toxic doses (max 20% cytotoxicity) for measurements of:

- oxidative stress
- genetic alterations
- transcriptomic analyzes

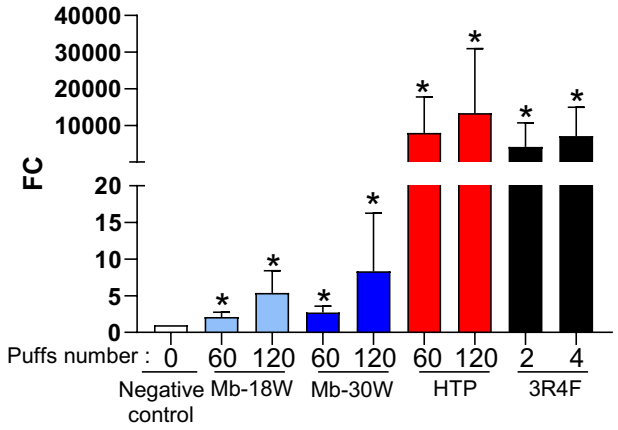
Oxidative stress

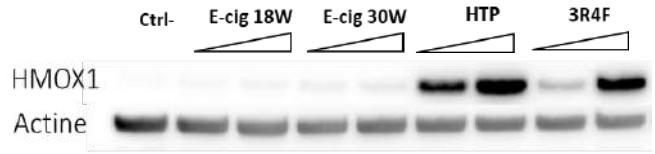
HMOX1

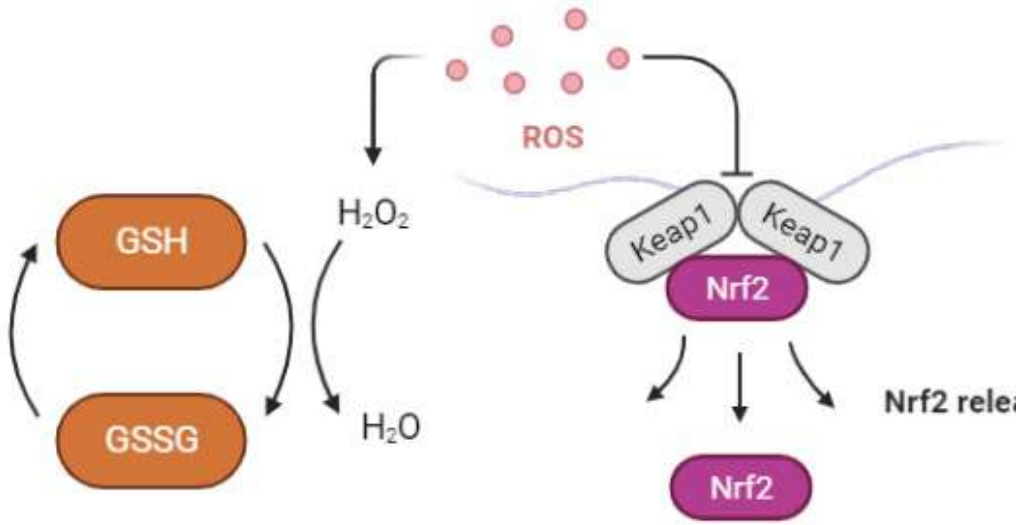
mRNA



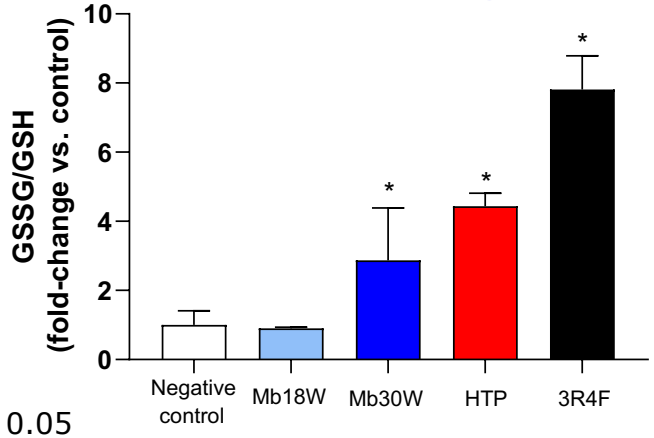
Protein





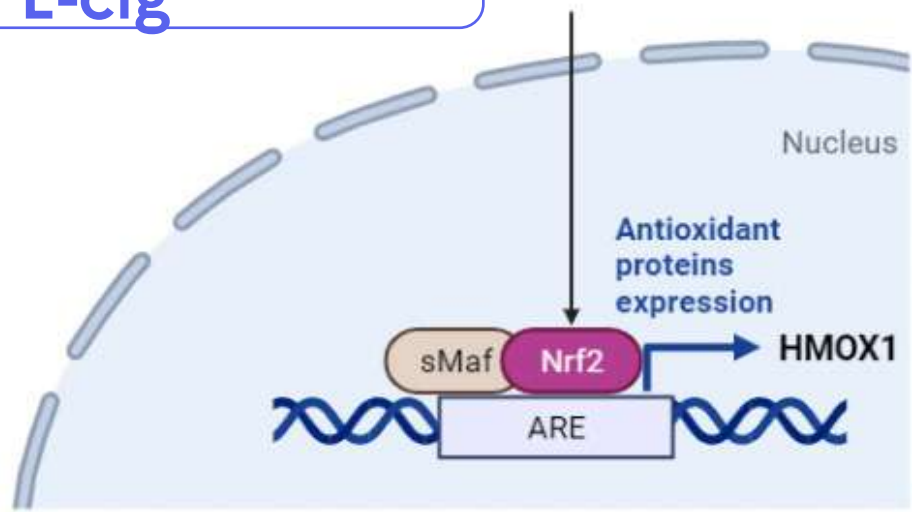


Oxidized/reduced glutathione ratio



Mann-Whitney : * $p < 0.05$

Cigarette and HTP > E-cig



Analyse of primary DNA lesions

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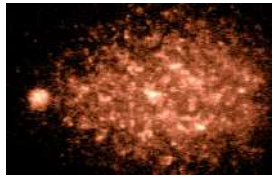
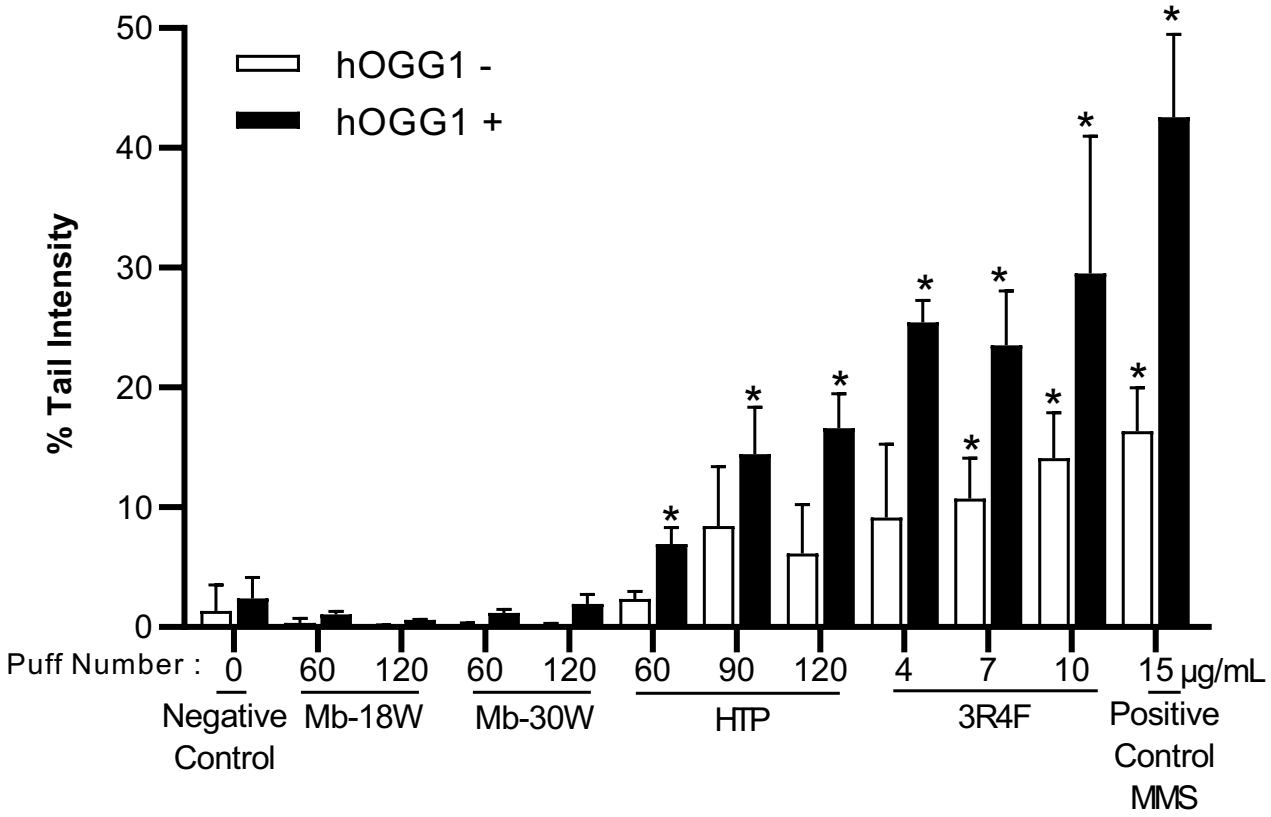
Comet assay

→ Detection of DNA strand breaks

Undamaged nucleus



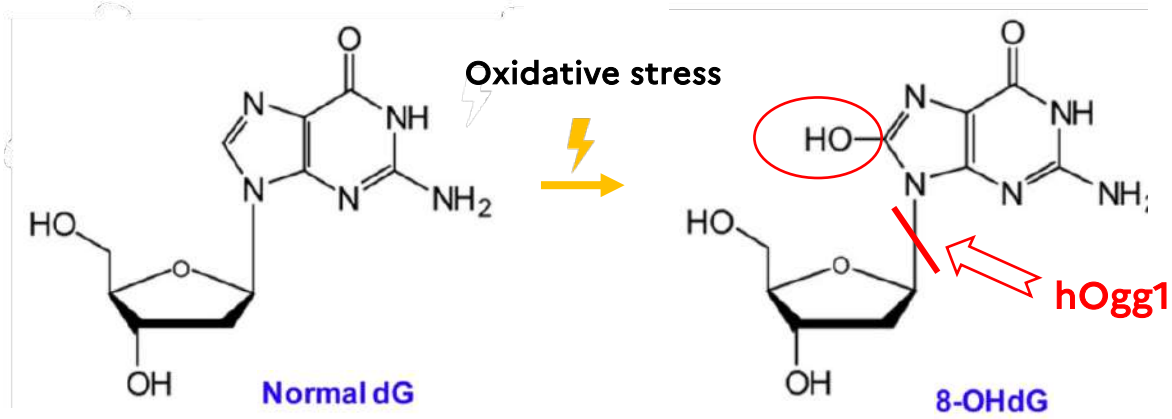
Damaged nucleus



Mann-Whitney : * p < 0.01 (n=4)

8-oxoguanine DNA glycosylase 1 (hOgg1) :

→ detection of oxidative DNA damage

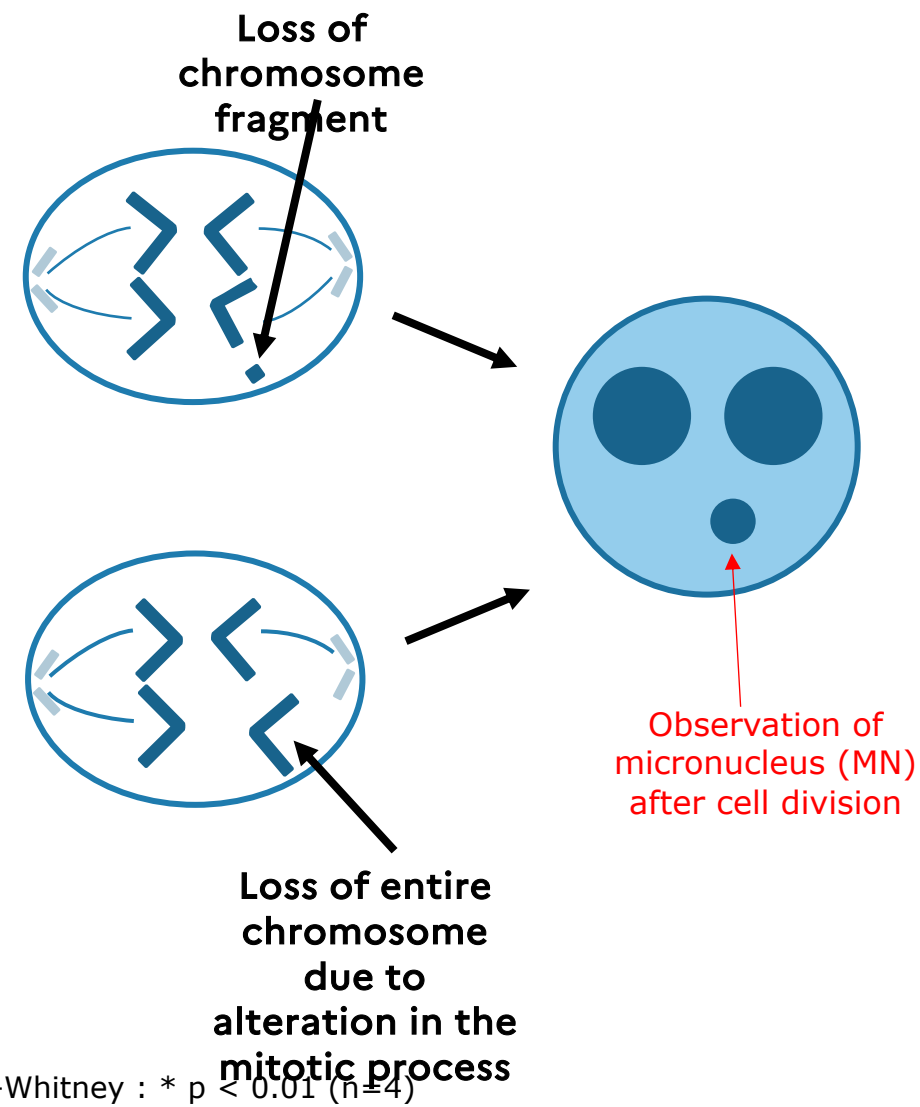


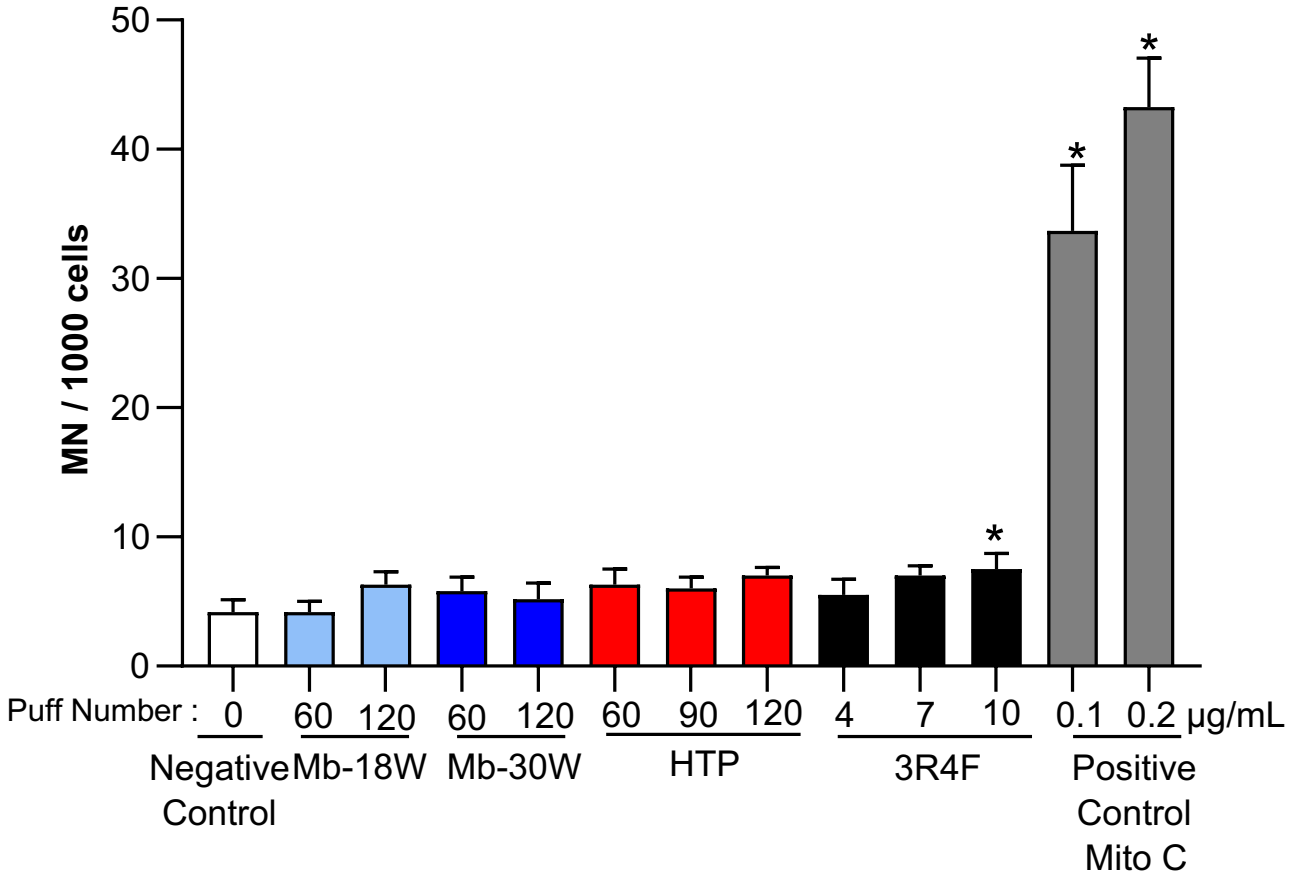
3R4F Cigarette > HTP > E-cig

DNA strand breaks	+	−	−
Oxidative damage	+	+	−

Measurement of chromosomal aberrations

Micronucleus test

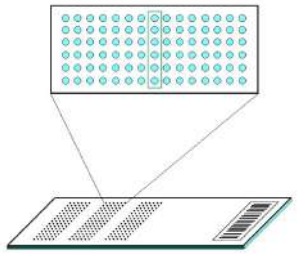




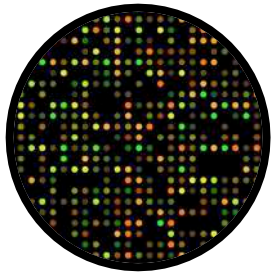
No chromosomal aberration with HTP and e-cigs

Mann-Whitney : * $p < 0.01$ (n=4)

Transcriptomic analysis



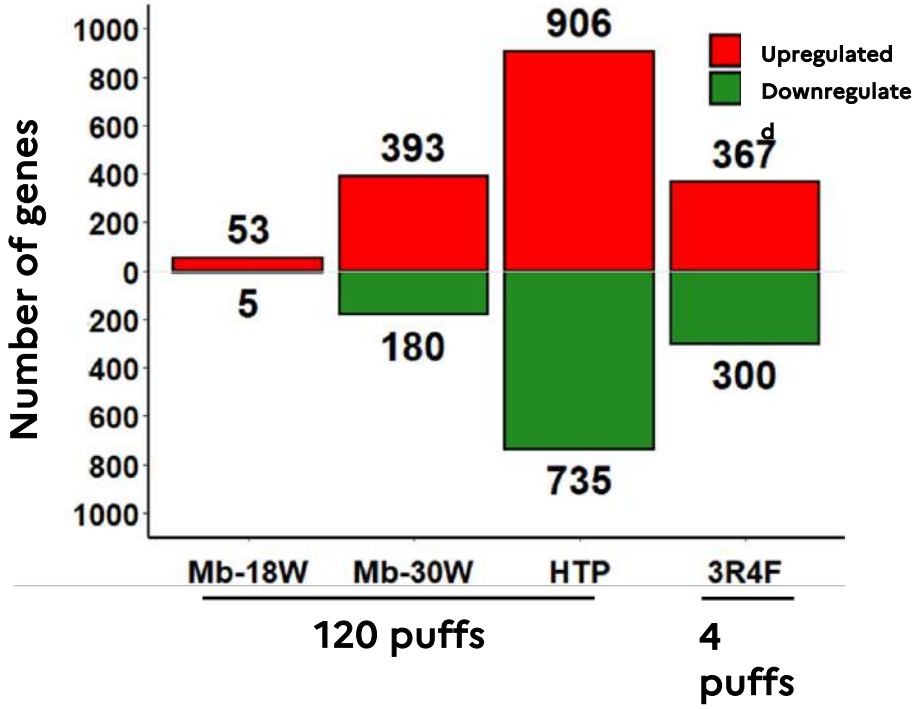
Pangenomic cDNA chip



Analysis of relative intensities

|Fold-change|>1.5
p<0.05

Filter applied to genes



n=4

HTP → most deregulated transcripts

E-cig → less deregulation compared to HTP
more deregulated transcripts for 30 W than 18 W

3R4F cigarette → less deregulated transcripts than HTP but exposure of 4 puffs vs 120 puffs for HTP (similar cytotoxicity at these 2 doses)

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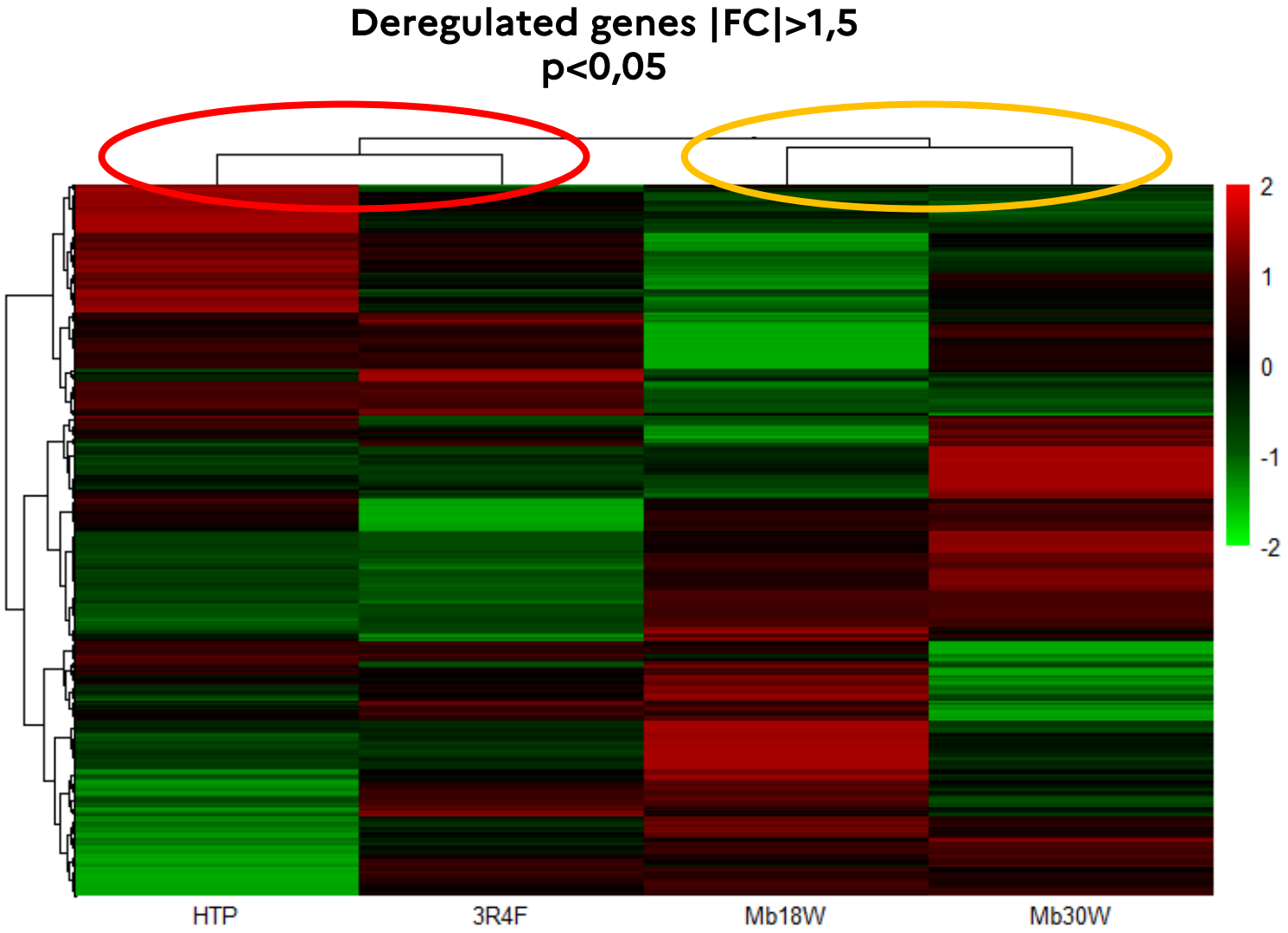
Clustering according to the transcriptomic profile of each de

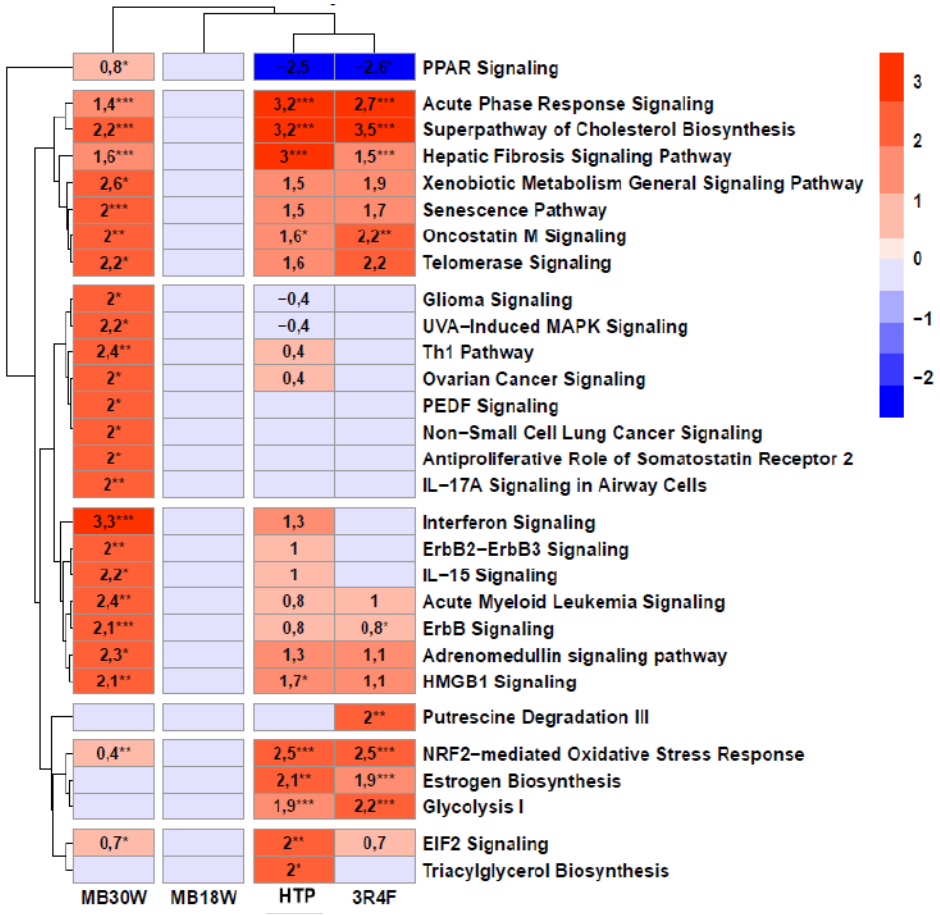


→ A first subgroup includes tobacco products (HTP and cigarette)

→ A second subgroup includes E-cigs (Mb-18W and Mb-30W)

Transcriptomic analysis

Dysregulated biological functions and signaling pathways



Transcriptomic deregulations with e-cig but only with the highest power

- ↗ interferon signalling
- ↗ xenobiotic metabolism
- ↗ cholesterol biosynthesis
- ↗ senescence pathway

Similar deregulations between HTP and 3R4F

- ↗ NrF2 pathway → oxidative stress
- ↗ glycolysis
- ↗ cholesterol biosynthesis
- ↗ cell proliferation and survival

→ Carcinogenesis processes

Conclusions and perspectives

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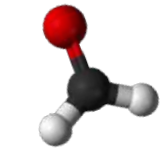
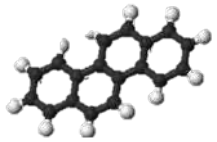
Quantities of carbonyl compounds and PAHs





Acute *in vitro* toxi

+	+	−	Cytotoxicity
+	+	+	Oxidative stress
+	+	−	DNA damage
+	−	−	Chromosomal aberrations
+	+	+	Inflammation*

*Dusautoir *et al.*, 2021

Common pathways of carcinogenesis

Inflammatory Xenobiotic metabolism

Transcriptomic analyzes

only with the highest power

Conclusions and perspectives

Fewer toxic compounds

Depend on the power of use

Low toxicity after acute exposure

Oxidative stress
Inflammation

Impact after chronic exposure?

Perspectives :

- Validation in primary culture cells

- Long-term toxicity of e-cig and heated tobacco emissions ?
  → *in vivo* studies with repeated exposures

Acknowledgments

Dr Sébastien Anthérieu
Dr Romain Dusautoir
Dr Marie Lenski
Thomas Martinez
Dr Jean-Marc Lo Guidice
Pr Guillaume Garçon
Dr Nicolas Beauval
Pr Delphine Allorge

Dr Anne Platel
Dr Fabrice Nesslany

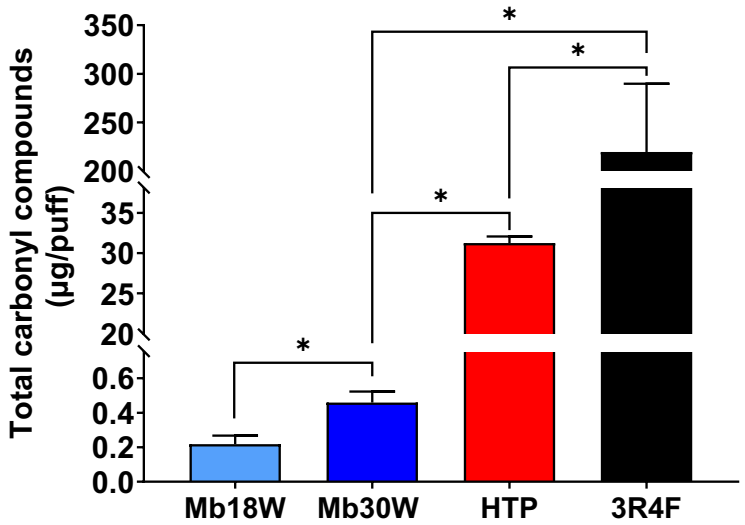
Dr Marie Verrièle
Dr Isabelle Fronval
Dr Véronique Riffault
Pr Nadine Locoge

Thank you for your attention

Project funded by INCa and IReSP :
ntrats n°INCa_13648 and n°INCa-IReSP_15748

Comparison of the chemical composition of the different emissions

Carbonyl compounds



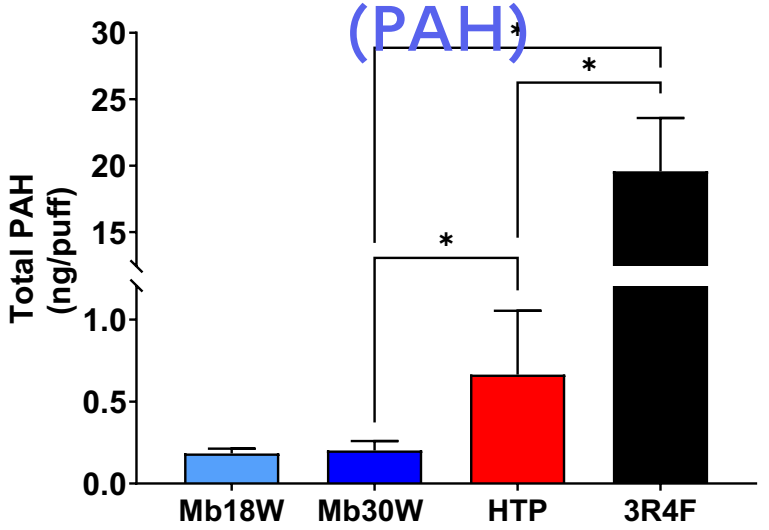
Cigarette > HTP > E-cig

Mb-30W > Mb-18W

➔ HTP and 3R4F: Acetaldehyde, acetone (tobacco burning)

➔ E-cig: Acetaldehyde and formaldehyde (thermal degradation of PG/G)

Polycyclic Aromatic Hydrocarbons (PAH)



Cigarette > HTP > E-cig

➔ 3R4F: tobacco combustion → PAH +++

➔ HTP : presence of harmful compounds (BaP)

➔ E-cig: Limited pyrolysis process

Mann-Whitney : * $p < 0.05$ (n=4)

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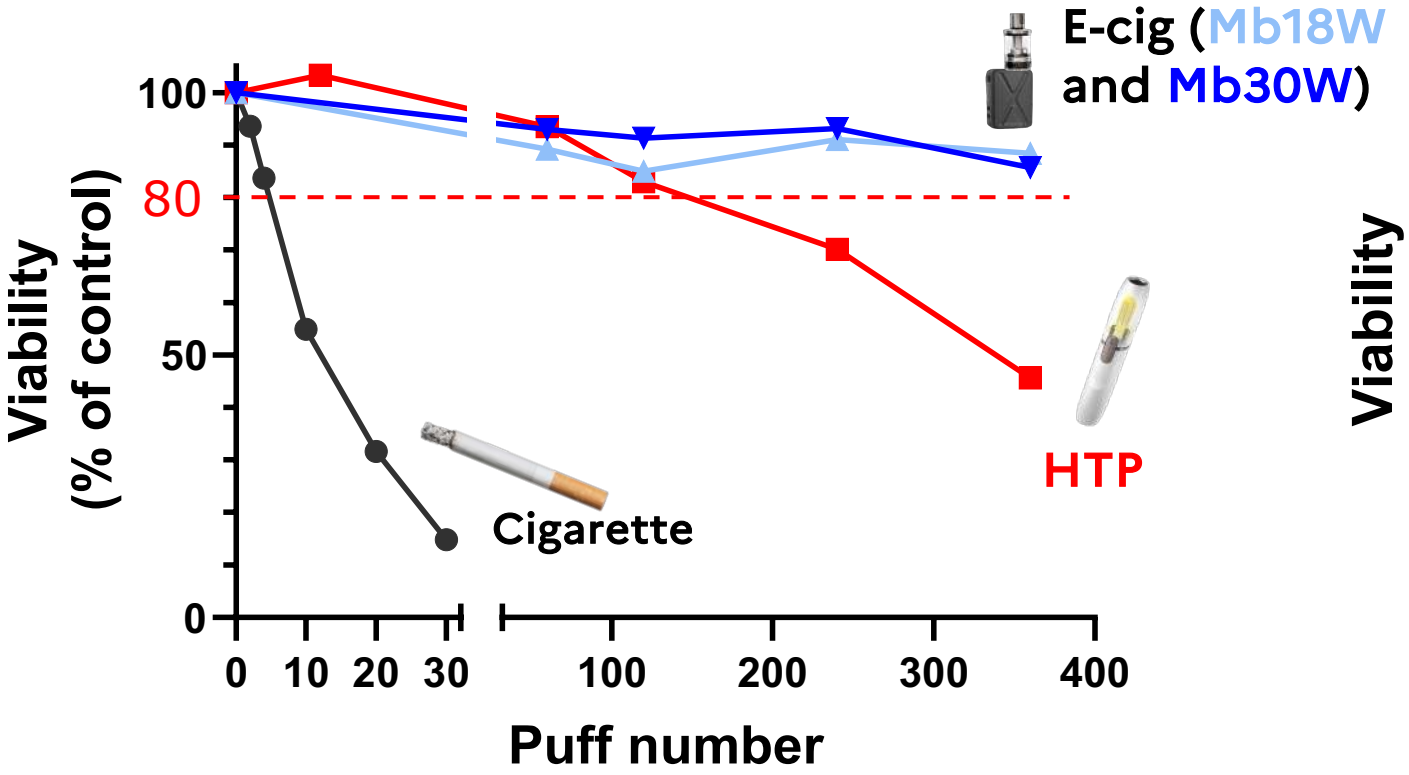
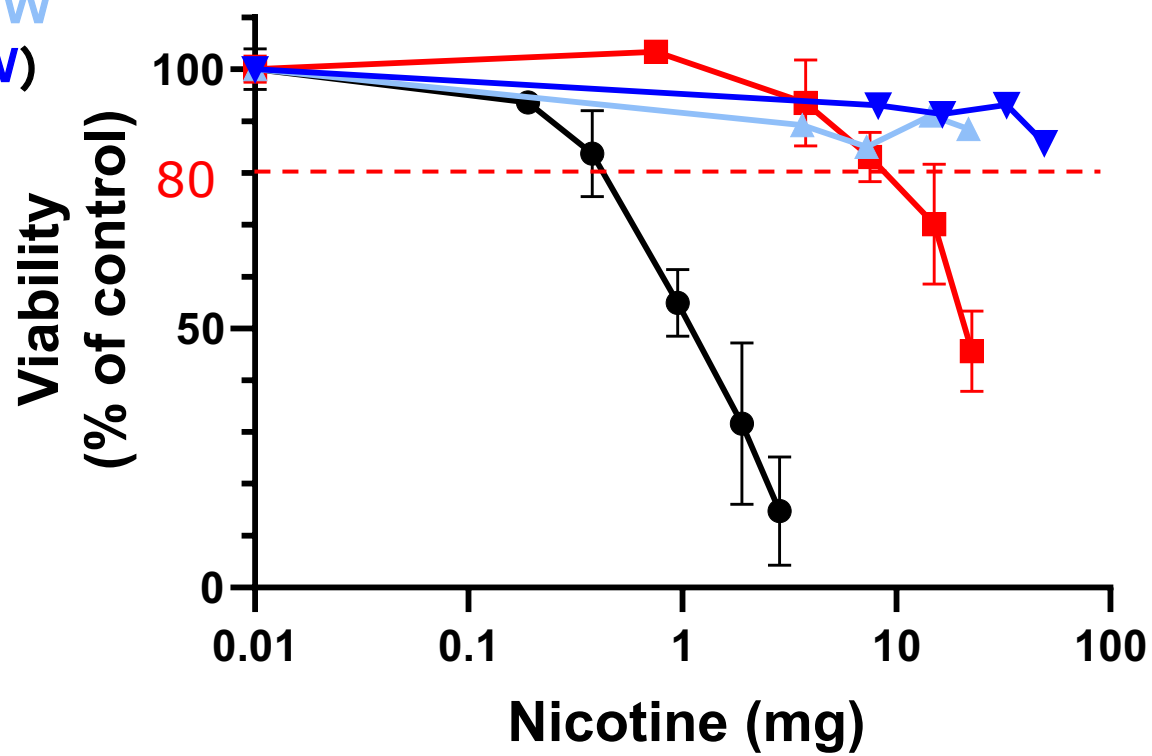
ATP Test





Cigarette > HTP > E-cig