Comparison of cytotoxic, genotoxic and epigenetic effects of heated tobacco product, electronic cigarette and conventional cigarette emissions in human bronchial epithelial cells

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INTRODUCTION

Tobacco use is a major public health problem, causing 8 million deaths each year worldwide. Cigarette smoke exposure is responsible for almost 30% of cancer deaths and the cause of almost 90% of lung cancer. Smoking cessation is, at present, the only effective way to slow down the progression of cancer. Recently, new alternatives to conventional cigarettes, such as electronic cigarettes (e-cigs) and heated tobacco products (HTP) have emerged on the market. They are generally perceived as low-risk substitutes for cigarette and have rapidly gained popularity in the absence of any real evidence of their safety.

In this context, we had undertaken an emerging *in vitro* study, in pulmonary BEAS-2B cells exposed using a smoking machine, in order to compare the cytotoxic, genotoxic and epigenetic effects of emissions from HTP (iQOS), e-cigs (18W and 30W) and conventional cigarette (3R4F).

MATERIALS AND METHODS

Cigarette
3R4F

E-cigs
Modbox 0,5 Ω
(18W and 30W)

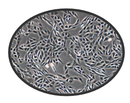
HTP
iQOS

Emission generation

Smoking machine
Vitrocell® VC1

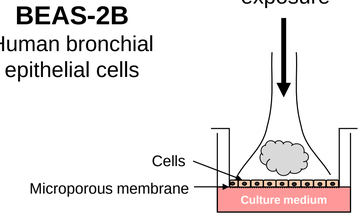
Health Canada Intense puff profile:
Volume: 55 mL; Duration: 2 s, Frequency : 2/min

Cellular model



BEAS-2B
Human bronchial epithelial cells

Air-liquid interface exposure

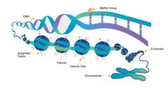


Endpoints

Cytotoxicity
- Intracellular ATP
- Nicotine amount

Genotoxicity
- Primary DNA damage
- Oxydative DNA damage
- Chromosomal aberrations

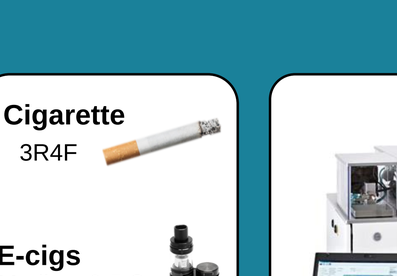
Epigenetic effects
- Global DNA methylation
- Histone modifications
- microRNA deregulations



RESULTS

Cytotoxicity

vs puff number

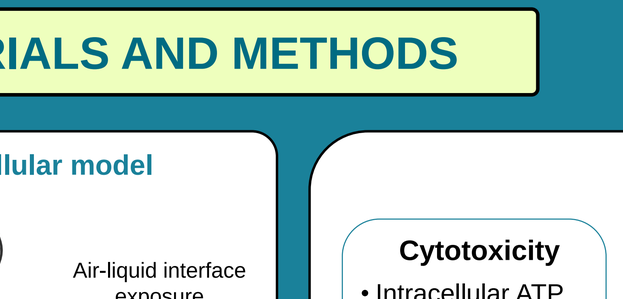


vs amount of nicotine

➔ 24h after exposure, **HTP** emissions induced reduced cytotoxicity compared to cigarette 3R4F smoke but higher than e-cig aerosols

Genotoxicity

Comet assay

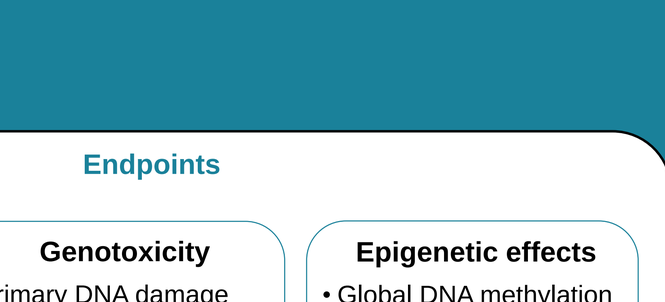


➔ **E-cigs**: No (oxidative) DNA damage

➔ **HTP**: Increase in oxidative DNA damage

➔ **3R4F**: Increase in oxidative and non oxidative DNA damage

Micronucleus test

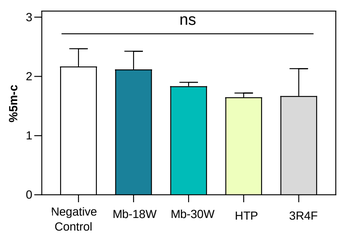


➔ **3R4F**: Slight increase in micronuclei after 10 puffs

➔ **E-cigs & HTP**: No chromosomal aberrations

Epigenetic effects

Global DNA methylation



➔ No DNA methylation

Mb-18W, Mb-30W (120 puffs)

HTP (120 puffs)

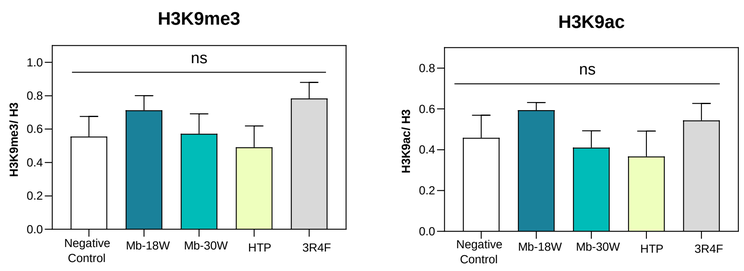
3R4F (4 puffs)

Negative control: air

ns: not significant

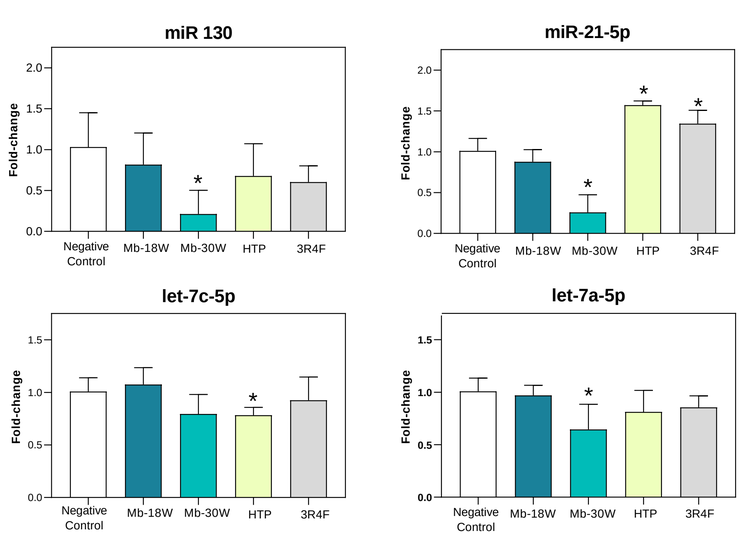
* p <0.01 (Mann-Whitney)

Histone modifications



➔ No histone modulations

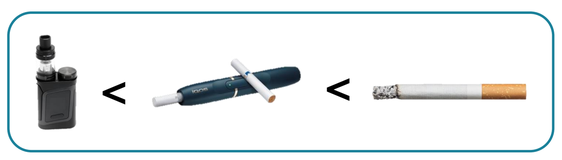
microRNA deregulations



➔ Deregulation of these 4 microRNA could be involved in lung carcinogenesis

CONCLUSION

- **This preliminary study demonstrates that HTP aerosol showed reduced cytotoxicity and genotoxicity compared to cigarette smoke but higher than e-cig aerosols in human bronchial epithelial cells.**
- **Surprisingly, cigarette smoke does not induce histone modulation or DNA methylation in acutely exposed BEAS-2B cells.**
- **The development of an *in vitro* repeated exposure protocol and animal studies should allow a better assessment of the the epigenetic impact of the emissions and their toxicity over the long term.**



This work benefited from the French National Cancer Institute (INCa) and the French Institute for Public Health Research (IReSP): Contracts N° INCa_13648 and N° INCa-IReSP_15748