Chemical characterization and *in vitro* toxicity on human bronchial epithelial cells BEAS-2B of $PM_{2.5}$ from an urban site under industrial emission influence

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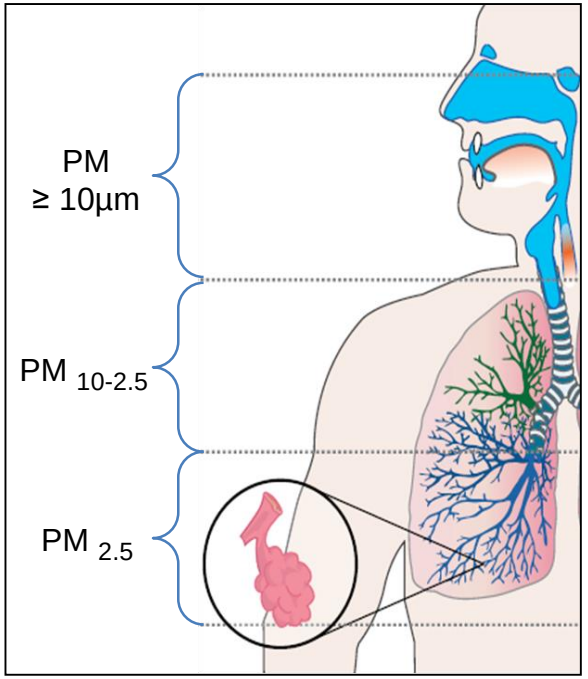
Health effects of Particulate Matter (PM)

Small size PM is attracting a particular attention:
the smaller the particle size,

- the higher time it remains suspended in air

  the higher probability of it being inhaled

- the higher ability to reach the deep lung



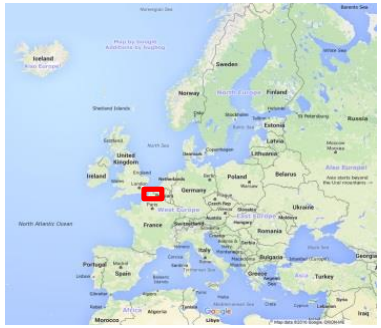
Epidemiological studies

↗ 10 µg/m^3 of $PM_{2.5}$ concentration:

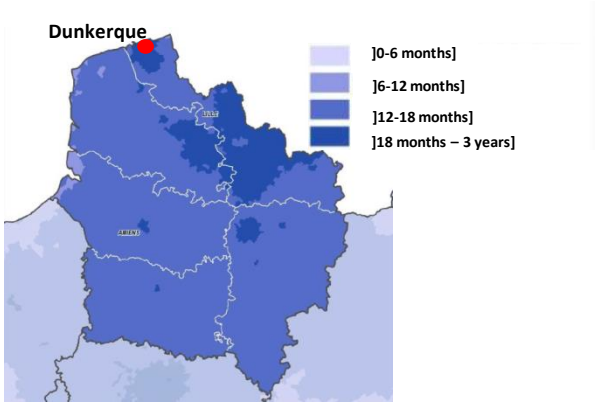
- ↗ 26 % mortality risk by cardio-vascular disease
  (Lepeule et al., 2012)
- ↗ 8 % incidence rate of lung cancer *(ERS, 2010)*
- ↗ Respiratory insufficiencies : asthma, chronic obstructive pulmonary diseases (COPD)
  (Silverman et Ito, 2009 ; Tsai et al., 2013)

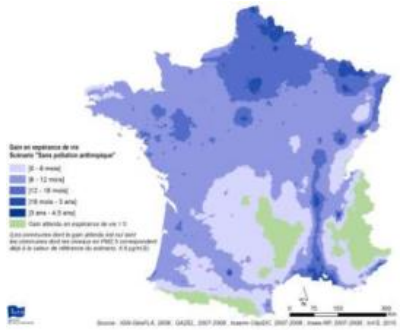
$PM_{2.5}$ and related health effects in the North of France

- Exposure to PM: 6 millions people in the Region
- Impact of PM on Life expectancy : average loss of 16 months *(InVS, 2015)*
- High road traffic density
- Local industrial emissions of particles in the atmosphere (>3000 tons/y) *(European-PRTR data)*



Gain in life expectancy in a scenario "without air pollution"

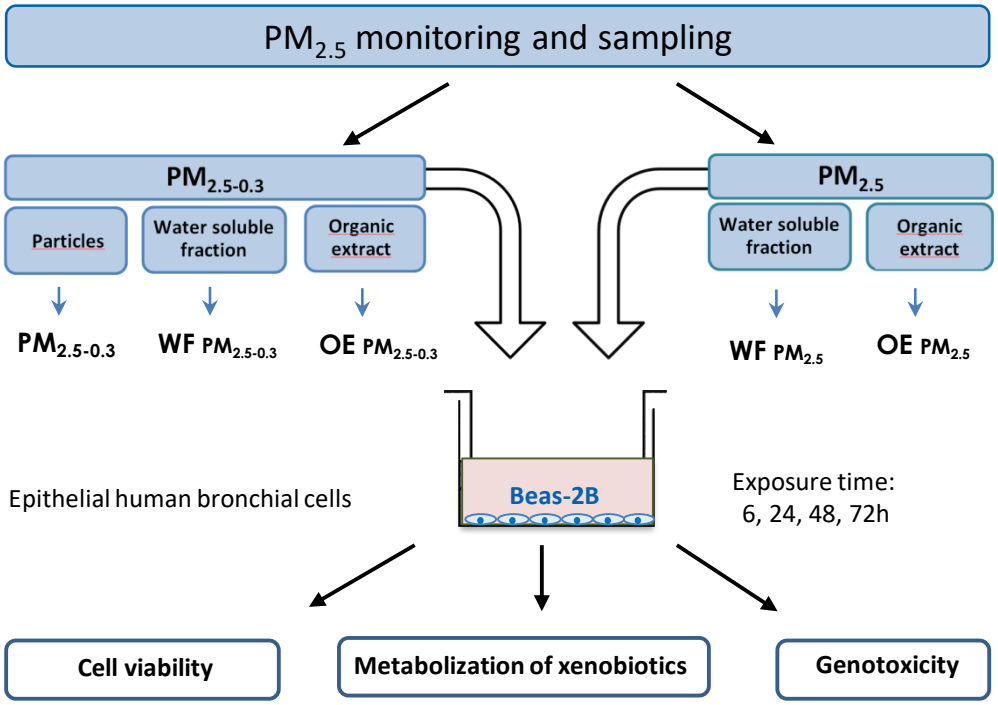




Objectives of the study

- Determine metals and PAHs concentrations in $PM_{2.5}$ and identify their sources in Dunkerque, a coastal urban site
- Compare toxic effects of $PM_{2.5}$ from *in vitro* experiments on epithelial lung cells, depending on their exposure to solid particles, water soluble or organic extracts

Experimental approach



Physico-chemical characterization :

- ⇨ PM composition
- ⇨ Source apportionment

***In-vitro* toxicity assessment :**

- ⇨ Effect of the different fractions
- ⇨ Effect of PM components in the solid state vs. in extracts

Methods

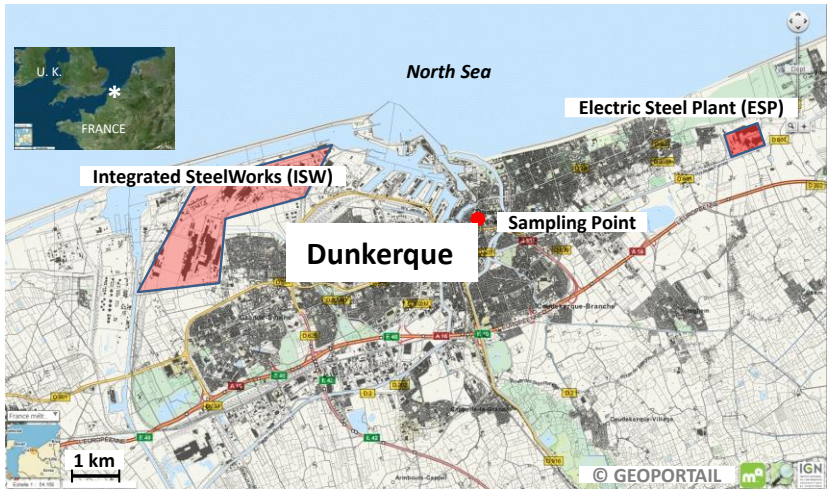
- $PM_{2.5}$ monitoring and sampling

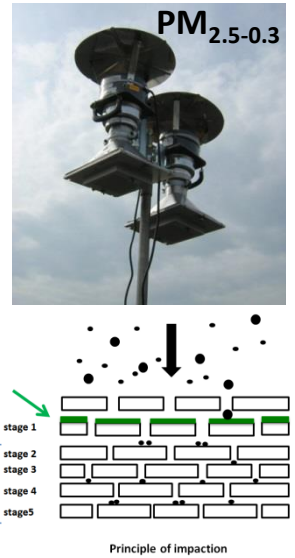
Location : Dunkerque, 200 000 inhabitants

Period : March to July, 2011

Devices : - MP101 RST, Environnement SA® for $PM_{2.5}$ concentration

- Sampler Digitel® DA 80 (30 m^3/h): 24h $PM_{2.5}$ samples (on filter)
- Cascade impactor STAPLEX ® (68 m^3/h): 5 days $PM_{2.5-0.3}$ samples (on plate)





- $PM_{2.5}$ chemical characterization

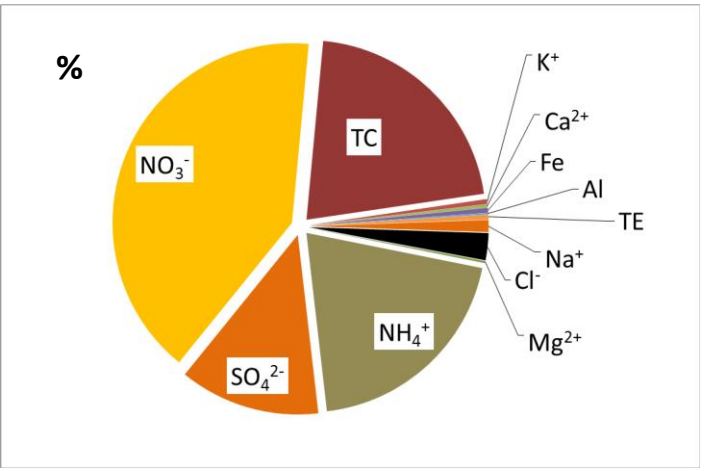
Major and trace elements (ICP-AES and ICP-MS), water soluble ions (IC)

Total carbon (CHON analyzer) and Polycyclic Aromatic Hydrocarbons PAHs (GC-MS)

$PM_{2.5}$ chemical composition

- Average $PM_{2.5}$ concentration (march- july 2011) : 14 µg/m^3

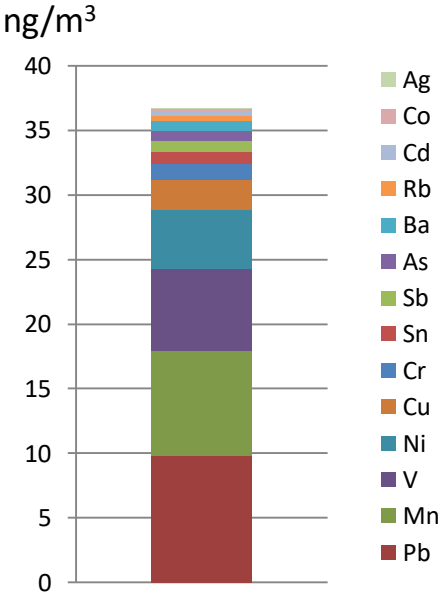
Major Components



- NO_3^-, SO_4^{2-}, NH_4^+ and carbon :

  > 90% of $PM_{2.5}$ mass

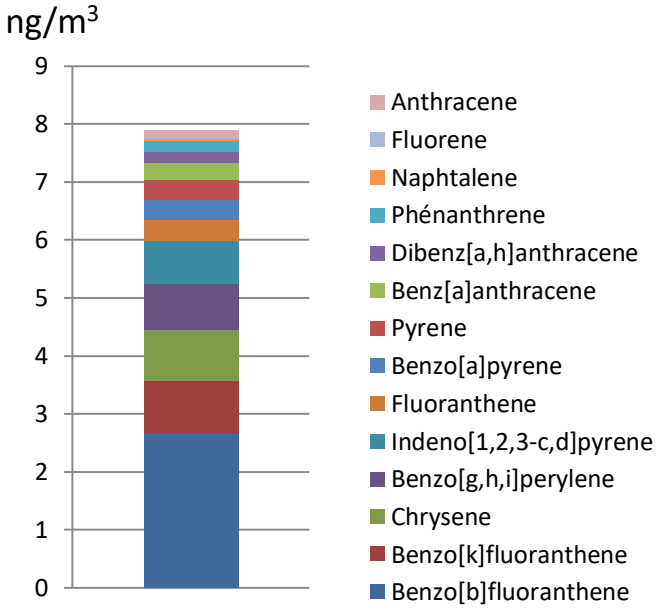
Metals



- Metals explained mainly by emissions from steelmaking industry, traffic non-exhaust and heavy fuel oil combustion

Ledoux et al., 2017

PAHs

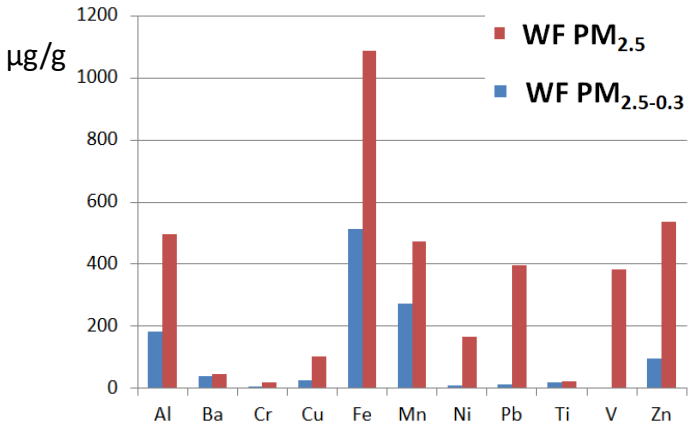


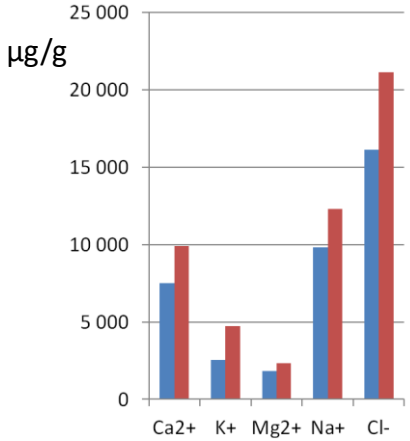
- PAHs ratios (Fla/Pyr, InPy/B_{ghi}P) convenient with diesel exhaust, heavy fuel oil combustion and cokemaking industry emissions

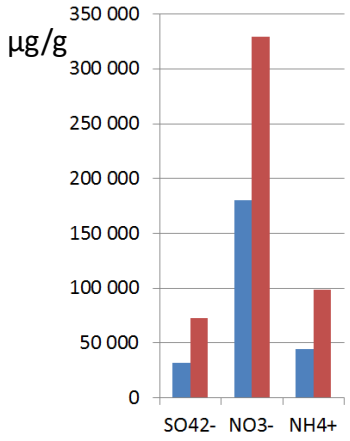
Cazier et al. 2016, Landkocz et al. 2017

Composition of PM extracts

- Water soluble fraction (WF) : particles placed in pure water





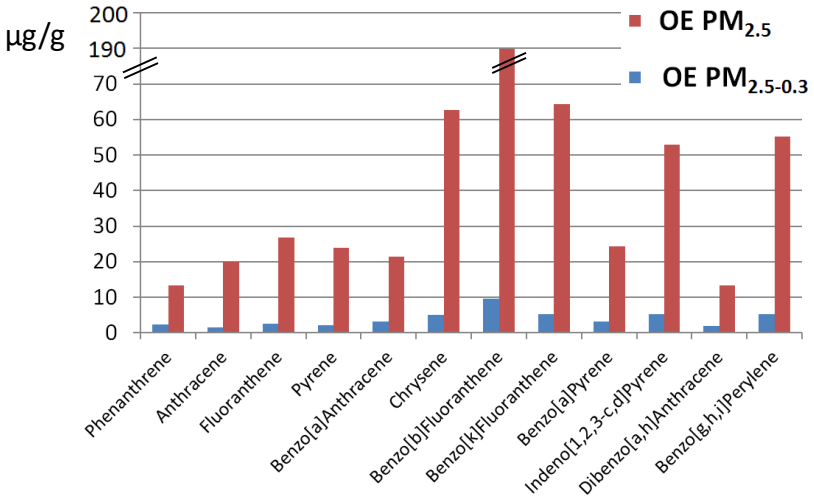


- Proportion of elements in $PM_{2.5-0.3}$ / $PM_{2.5}$:
  - Ba, Ti : > 75%
  - Al, Fe, Mn : between 75 and 25 %
  - Cr, Cu, Ni, Pb, V, Zn: < 25%

- Water soluble ions(Ca^{2+}, K^{+}) and sea salts (Na^{+}, Mg^{2+}, Cl^{-}) predominantly found in the $PM_{2.5-0.3}$, contrary to secondary inorganic ions

Composition of PM extracts

- Organic extracts (OE): Soxhlet extraction using DCM
  Organic compounds concentrated in DMSO



	$PM_{2.5-0.3}$		$PM_{2.5}$	
	(µg/g)	(pg/m³)	(µg/g)	(pg/m³)
Phenanthrene (Phe)	2.4	33	13.2	186
Anthracene (Ant)	1.5	21	–	–
Fluoranthene (Fla)	2.5	35	26.9	378
Pyrene (Pyr)	2.2	31	23.9	336
Benz[a]anthracene (BaA)	3.2	46	21.3	330
Chrysene (Chr)	5.0	71	62.7	882
Benzo[b]fluoranthene (BbF)	9.5	134	189	2,670
Benzo[k]fluoranthene (BkF)	5.3	74	64.2	904
Benzo[a]pyrene (BaP)	3.2	44	24.4	343
Indeno[1,2,3-c,d]pyrene (InPy)	5.3	74	52.8	743
Dibenz[a,h]anthracene (DahA)	2.0	28	13.4	189
Benzo[ghi]perylene (BghiP)	5.2	73	55.2	777
Total PAHs	48.5	669	548	7.708

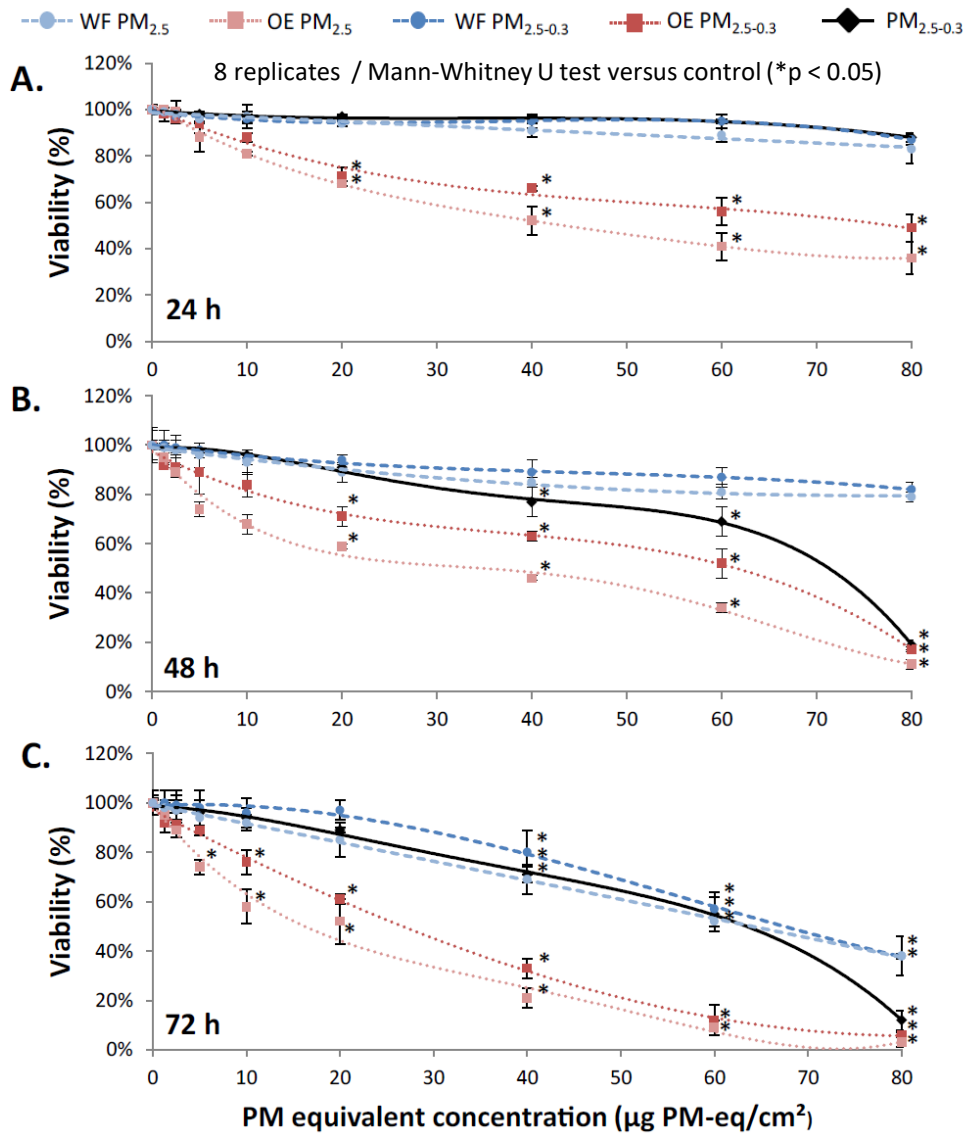
Landkocz et al. Env. Poll. 2017

- PAHs more concentrated in $PM_{2.5}$ including the ultrafine fraction than in $PM_{2.5-0.3}$
  - Phe, BaA, BaP, DahA : 5-10 fold higher
  - Other compounds > 10- fold higher

- Contribution of combustion processes, known to form hydrocarbon- rich particles predominantly in the ultrafine mode *(Kawanaka et al. 2009)*

Cell viability

from Extracellular LDH release measurements



- $PM_{2.5-0.3}$, OE and WF samples : decrease of cell viability in a time- and dose-dependent manner
- Organic extracts (OE): more cytotoxic than $PM_{2.5-0.3}$ and the two WF extracts
- Cytotoxicity :

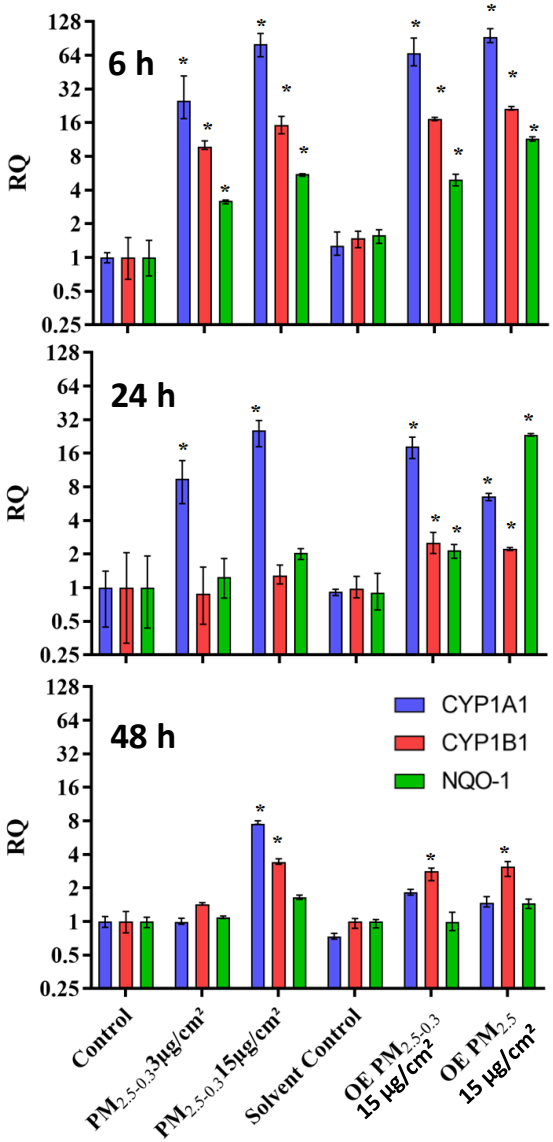
  OE $PM_{2.5}$ > OE $PM_{2.5-0.3}$

⇨ PAHs concentration : much higher in $PM_{2.5}$ than $PM_{2.5-0.3}$

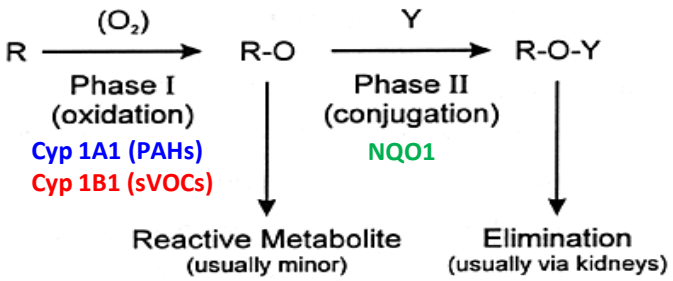
⇨ Cytotoxicity mainly governed by organic compounds, and particularly PAHs *(Oh et al. 2011, Topinka et al. 2013)*

- Tests using 2 doses : 3 and 15 µg/cm²

Gene expression of xenobiotic-metabolizing enzymes

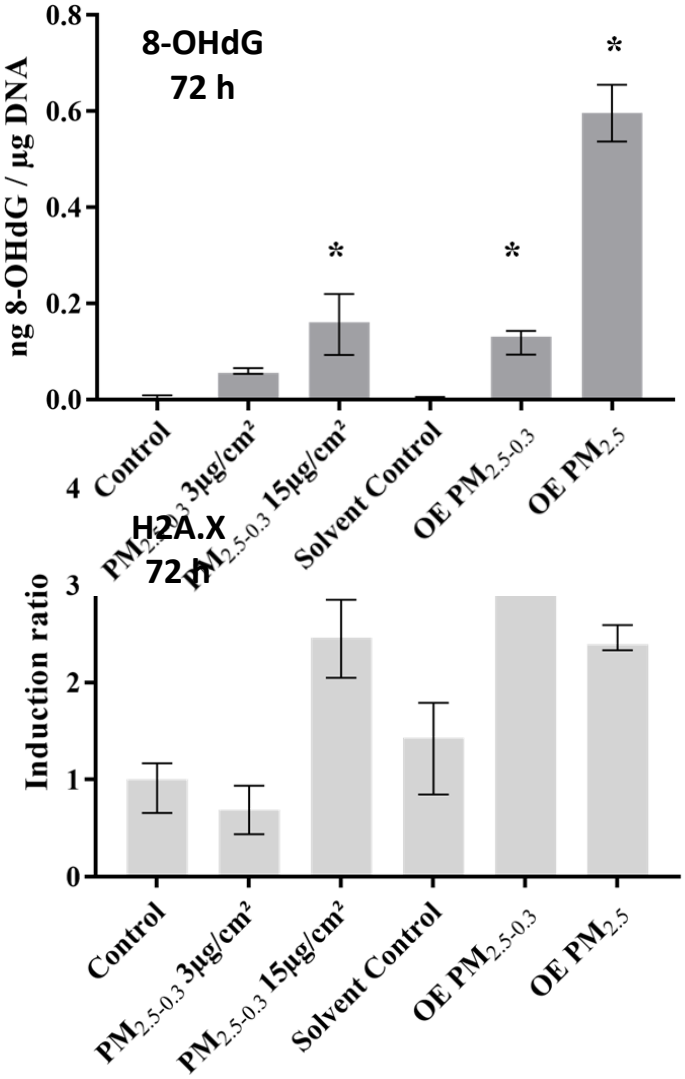


6 replicates / * Significant (Relative Quantity ,RQ<0.5 or RQ>2)



- CYP 1A1, CYP 1B1 and NQO-1 gene expression : induced in a dose-dependent manner (6h)
- CYP 1A1 gene expression : decrease over time, however, significant expression after 48h for $PM_{2.5-0.3}$ (15 µg/cm²) contrary to OEs
- Interpretation :
  - ⇨ 1/ higher bioavailability of PAHs in OEs
  - 2/ in $PM_{2.5-0.3}$, PAHs strongly bounded on particle surface and inside pores
  - 3) in $PM_{2.5-0.3,}$ a gene induction by metals can not be excluded *(Korashy et al. 2005)*

Genotoxicity



6 replicates / * p<0.05 between control and exposed (Mann Whitney U test)

- DNA oxidative alteration: Dose-dependent increase of 8-hydroxydesoxyguanosine 8-OHdG level *(Billet et al. 2018; Dergham et al. 2015)*
- Similar level : $PM_{2.5-0.3}$ ≈ OE $PM_{2.5-0.3}$, and OE $PM_{2.5}$ > OE $PM_{2.5-0.3}$

⇨ oxidative DNA alteration mainly linked to the organic fraction *(Høgsberg et al. 2013)*

- DNA Damage Response (repair mechanism of double strands breaks) *(Foster et al. 2005)*
- H2A.X : significant increase of phosphorylation for $PM_{2.5-0.3}$ (15 µg/cm²) and OEs :

⇨ 1/ organic compounds at high dose could limit the ability of cells to induce repair mechanism

2/ metals in $PM_{2.5-0.3}$ known to cause double strands breaks but also to inhibit proteins involved in the DNA repair pathway *(Morales et al. 2016)*

Conclusion

- Comparison of biological response of Beas 2B cells depending on the use of starting particles (as collected on plates) or water soluble and organic extracts
- Cytotoxicity and oxidative DNA alteration (8-OHdG) mainly governed by the organic fraction
- Considering Organic Extracts for *in vitro* toxicology tests does not reflect exactly the cell response (XME, DDR) in the presence of particles : role of the particle skeleton
- Further investigation on the signalisation pathways involving oxidative stress presented in the next talk….

Acknowledgements

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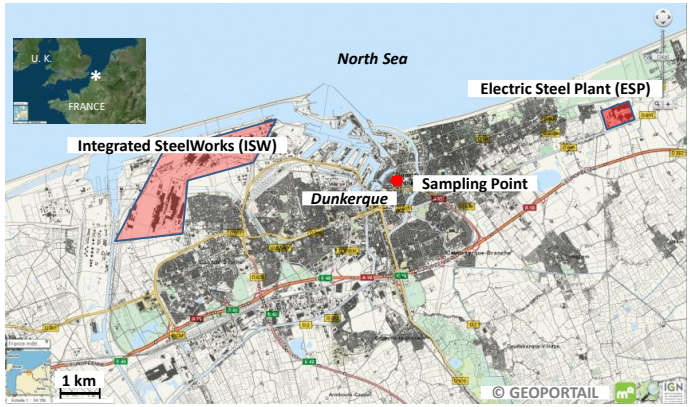
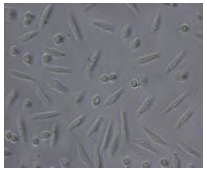
- the « Institut National du Cancer », France
- the European Union (EFRED)

Metal Concentration Roses



wind sector	elements	assignment
WNW	Fe, Mn, Cd, Rb, Ag, Pb, Zn	⇨ Integrated steelworks
ENE	Zn, Cr, Cu	⇨ Electric Steel plant
SE-SW	Cu, Sb, Ba, Pb	⇨ Trafic
SW- NE	Ni, V	⇨ Heavy fuel oil combustion

$PM_{2.5}$ toxic effects study



- Experiments using epithelial human bronchial cells (BEAS 2B) in culture : « *in vitro* » study

- Test considering $PM_{2.5-0.3}$ and extracts :
  Cytotoxicity, gene expression of XME, genotoxicity (8-OHdG, H2A.X)

	Preparation	$PM_{2.5}$	$PM_{2.5-0.3}$
Particles		DA80 filter	**$PM_{2.5-0.3}$ recovered on impaction plates**
Organic Extract	Soxhlet extraction using DCM. Organic compounds concentrated in DMSO	**OE $PM_{2.5}$**	**OE $PM_{2.5-0.3}$**
Water soluble fraction	Solubilization in pure water	**WF $PM_{2.5}$**	**WF $PM_{2.5-0.3}$**

- Exposure time: 6, 24, 48 and 72 h