TOXICITY DOES NOT VANISH INTO THIN AIR

molecular mechanisms of air pollutant mixtures

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Background

Although the air quality in the Czech Republic has improved greatly during past decades, air pollution remains to be hot environmental issue in some regions such as Ostrava agglomeration. Especially in winter when emissions from transportation and industry are boosted by emissions from local heating and trans border sources from Poland.

It has been shown that compounds occurring in the air possess the ability to disrupt hormone, immune or reproductive system. And since the exposure to polluted air can be lowered but never omitted it is very important to assess the bioactivity of air pollution.

Objective 1

Do the air pollutant mixtures elicit endocrine disruptive activities?

Objective 2

How are these activities distributed

- between gas and particulate phase?
- with respect to particle size?
- with respect to pollutant polarity?

Do they prevail in winter or in summer?

Objective 3

Are these activities bioaccessible?

Objective 4

Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants routinely measured as pollution indicators. They also represent a diverse group of structure derivatives, like oxygenated PAHs or nitro-PAHs.

How these can contribute to the air toxicity?

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Toxic potentials of particulate and gaseous air pollutant mixtures and the role of PAHs and their derivatives

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Lab story

Twelve days, two active air samplers:

1st sampler: PM_{10} phase + gas phase
→ extracts fractionated according to polarity

Polarity fractions			
NF	F1	F2	F3
non fractionated	non polar	semi-polar	polar

2nd sampler: 6 size fractions of PM_{10}

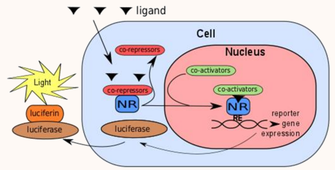
Size-based distribution of PM [µm]					
A	B	C	D	E	F
7.2 - 10	3 - 7.2	1.5 - 3	0.95 - 1.5	0.49 - 0.95	< 0.49

Organic extraction (DCM)

In vitro testing addressed:

- activity on 3 nuclear receptors: anti-/estrogenicity, anti-/androgenicity and thyroid receptor-mediated activity
- activity on cytosolic aryl-hydrocarbon receptor: AhR-mediated activity
- cytotoxicity for human bronchial cells (BEAS-2B)

And we used reporter-gene bioassays based on human cell lines. And did you know that bioassays are quite cool for testing the toxicity of environmental mixtures?
They can cover the interactions among compounds present in the mixture, like synergism or antagonism, while being relatively cheap, fast and relevant for human health.



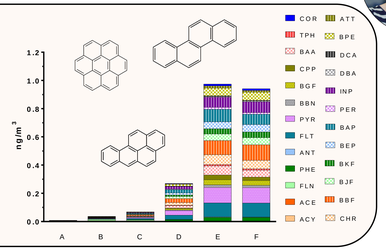
PM phase samples were also extracted to simulated lung fluid (SLF).

Chemical analyses on GS-MS covered:
parent PAHs (n=25), **OPAHs** (n=11) and **NPAHs** (n=18)

The contribution of PAHs to effects observed *in vitro* was calculated as (BEQ_{chem}) from their concentration in the extract and REP values (relative effect potency) found in the literature or in the UE EPA CompTox database.

$$BEQ_{chem} = \sum_{i=1}^{n} REP_i \cdot c_{a,i} \cdot \frac{MW_{RC}}{MW_i}$$

The contribution of studied compounds was also studied by mixture reconstitution. Based on the concentration measured in the original environmental sample, mixtures composed of all PAHs but also mixtures containing the studied sub-groups separetely (parent PAHs, OPAHs and NPAHs) were made and again tested in bioassays.



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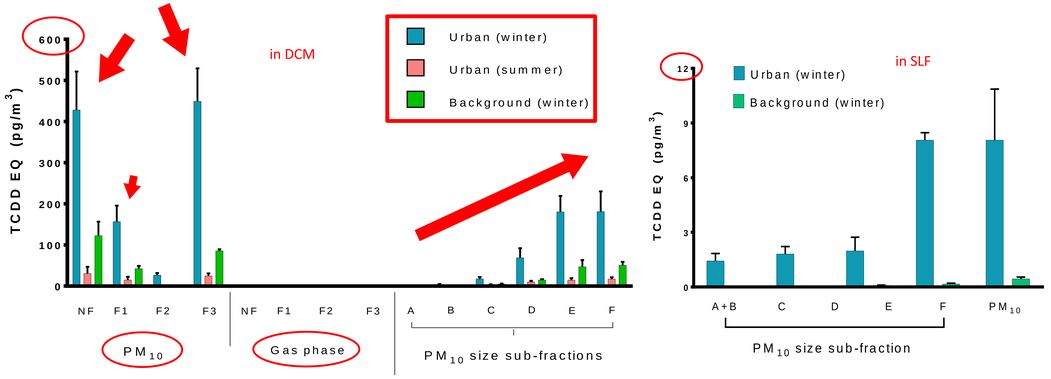
Results

Was studied activity detected?	Urban winter		Urban summer		Background winter	
	Gas	PM	Gas	PM	Gas	PM
AhR-mediated activity	X	✓	X	✓	X	✓
estrogenicity	X	✓	X	✓	X	✓
antiestrogenicity	X	X	X	X	X	X
androgenicity	X	✓	X	✓	X	✓
antiandrogenicity	✓	X	✓	X	✓	X
TR-mediated activity	✓	✓	✓	✓	✓	✓
cytotoxicity for lung cells	✓	✓	✓	✓	✓	✓



Results of reporter gene-based bioassay for the AhR-activation are expressed as bioanalytical equivalent of reference compound (TCDD). Left graph (results of organic extracts) shows that this activity was detected only in the PM phase, it increased inversely with the PM size, it was mostly elicited by the polar fraction (F3) and that it was observed in all 3 sampling campaigns. Right graph shows that similar pattern was observed in corresponding SLF extracts, however the levels are significantly lower. Both graphs show mean+SD.

The AhR-mediated activity is given here as an example. Except for antiestrogenicity, in organic extracts all targeted activities were detected (table above) but often with different distribution patterns. For all studied effects, the activity of corresponding lung fluid extract was quite low (in average 2 %), yet still quantifiable, especially in urban winter samples. The only exception was TR-mediated activity which reached quite higher levels in SLF extracts.

Conclusions

- air pollutants possess **endocrine disruptive potentials** and significant **cytotoxicity for lung cells**
- these effects are elicited mainly by chemicals in the **polar fraction** with size-dependent distribution: predominantly in fractions E and F → **easily inhaled PM** finer than 1 µm
- most of the effects showed **low bioaccessibility, except for thyroid receptor activation which should be further studied**
- effect modelling showed a huge data gaps regarding the toxicological information (REP availability) even for routinely measured air pollutants like PAHs
- bioassay testing of the reconstructed mixtures revealed that **PAHs play only minor role** in the studied effects
- *in vitro* bioassays showed that air pollutant mixtures can interact with important receptors involved in endocrine signalling network, thus can affect health of exposed population via different modes of action
- bioassays are suitable tool for air pollution toxicity evaluation when the effect drivers remain unknown