Occupational exposure to LTA Nanozeolites: Strategies of Exposure Monitoring and Toxicity Evaluation

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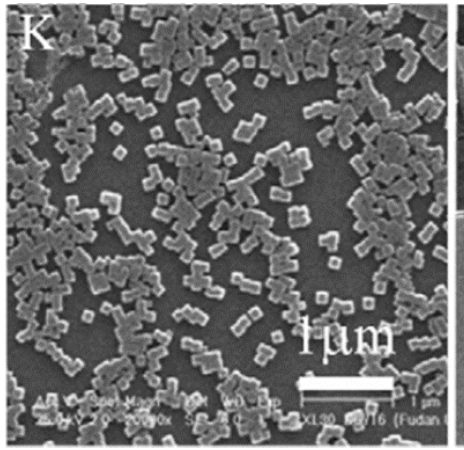
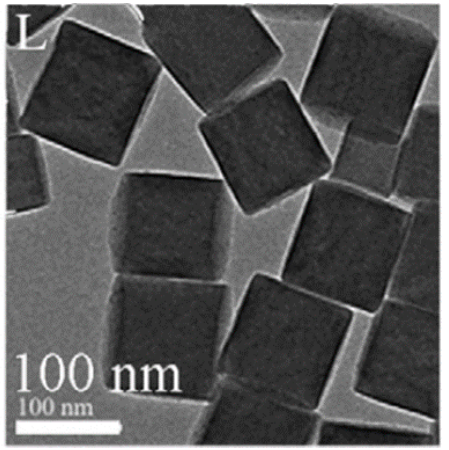
R&D Manufacturing Facility: case study

Main nanozeolites applications:

- Displays
- Energy Devices
- Micro-sensors (MEMS)
- Sensors
- Sealed-off Devices
- Indoor Lighting
- Vacuum Insulated Devices





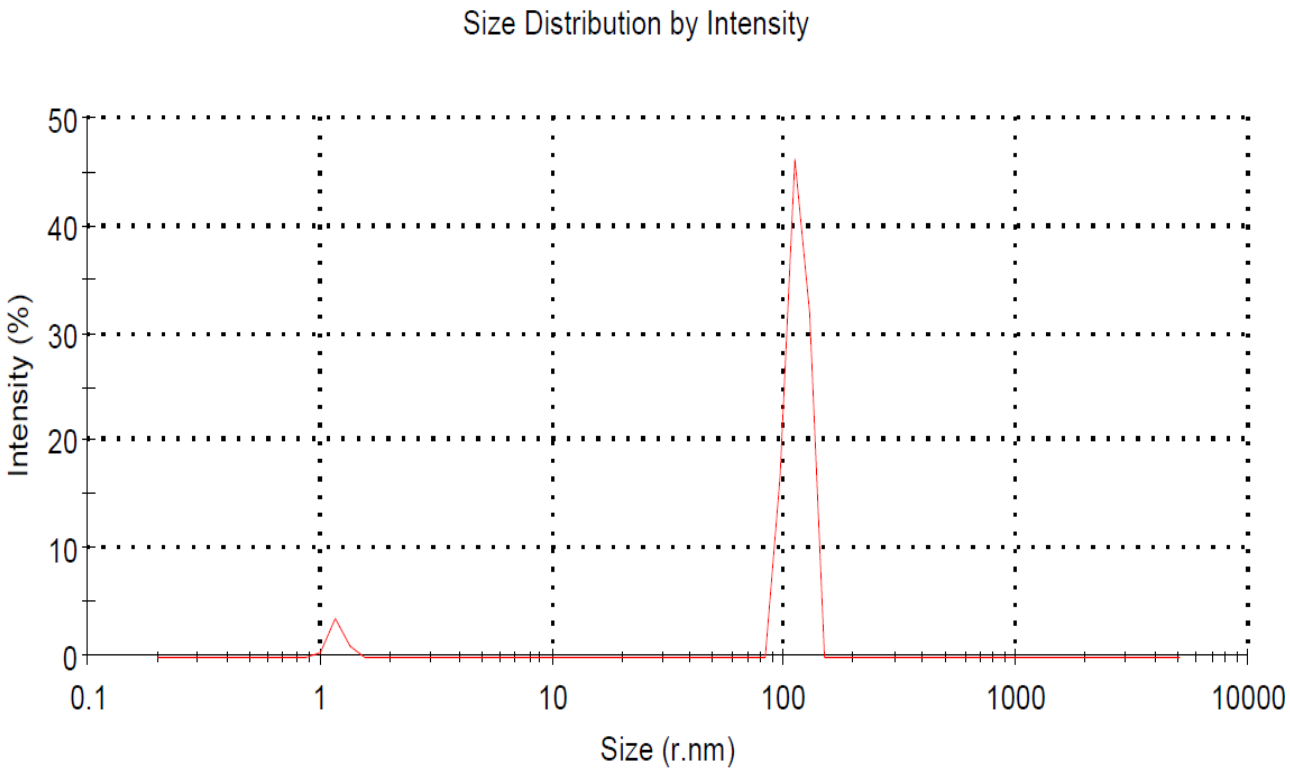
Manufacturing and processing operations were housed within about 5 m high and 1300 m^2 structure and organized on different laboratories and section

Annual Nanozeolites production totaled some 10kg/year .

"[...] the toxicities of zeolite nanoparticles depend on their size, composition and shape...aluminum-containing nanozeolites, such as LTA, show a dose-dependent toxic manner."

Kihara et al, 2011. *Effect of composition, morphology and size of nanozeolite on its in vitro cytotoxicity.* ***J. Biosci. Bioeng. 111(6): 725-730***

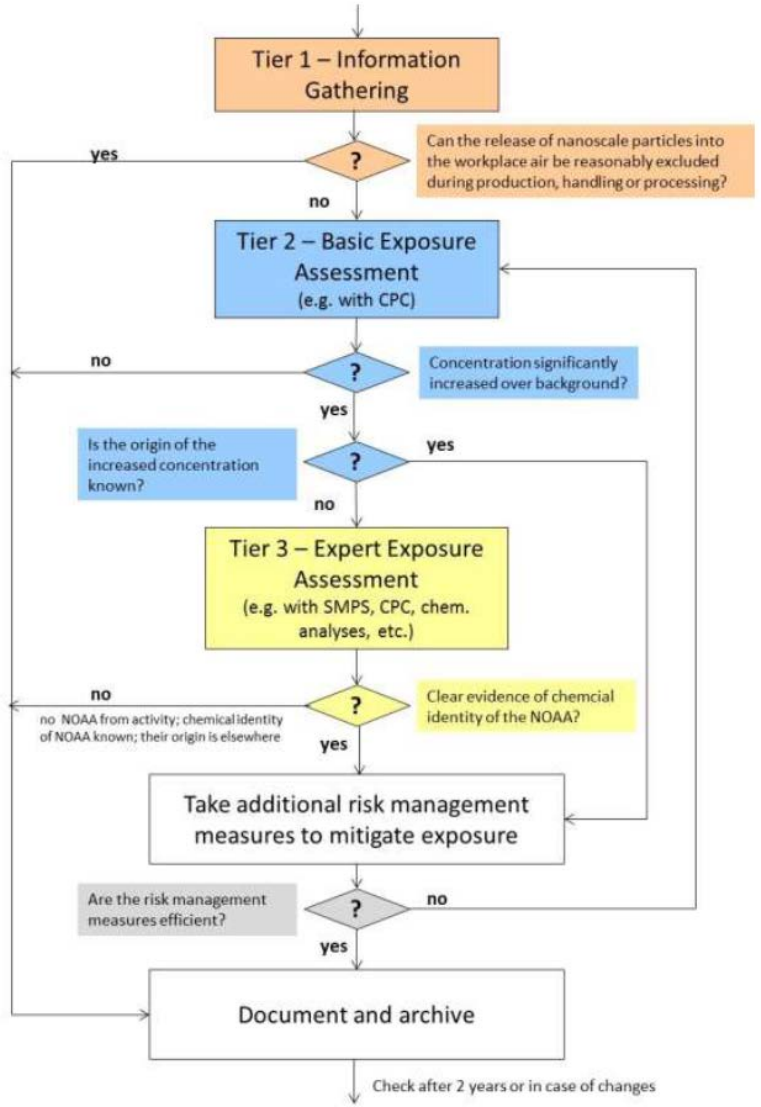
- Description of nanomaterial:
  - Technical name: LTA nanozeolites
  - Common form: powder
  - Chemical composition: $Na_wAl_xSi_yO_z$
  - Structure: crystal
  - Dimension: 70% < 100nm
- ...
- Produced/handled quantity: confidential
- Technology: confidential



The production process consists of the following points:

synthesis || chemical surface coating || drying || activation || mixing with organics matrix || packaging

Harmonized tiered approach for NOAA exposure assessment in the workplace



Multi-metric and tiered approach

- *Collection of information and initial analysis*
- *Study of NOAA production processes and workplaces*
- *Real Time preliminary measurements and sampling with portable instruments*
- *Laboratory Simulations with test materials (NOAA produced)*
- *Intensive campaign of measures and sampling with integrated device*
- *Detailed off-line analysis of multi-metric data*

Tier 1 | **Tier 2** | **Tier 3**

ENV/JM/MONO(2015)19

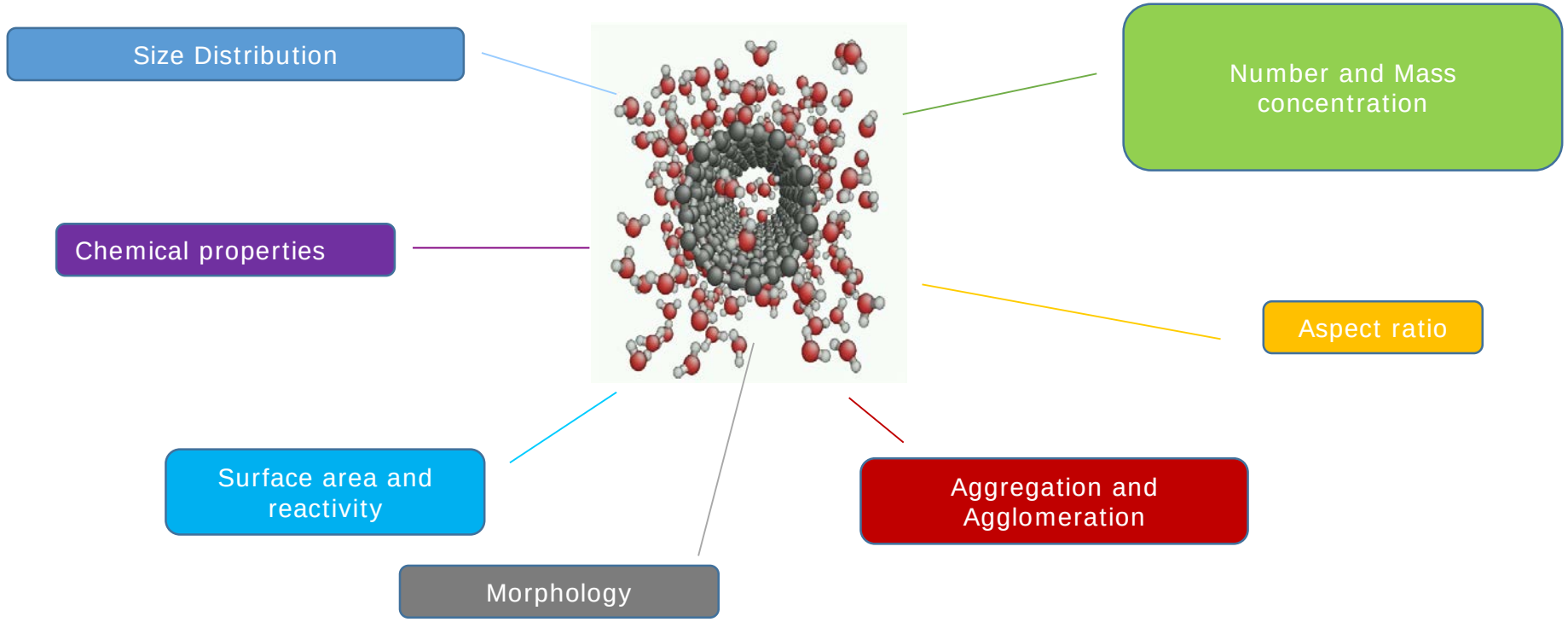
Harmonized tiered approach to measure and assess the potential exposure to airborne emissions of engineered nano-objects and their agglomerates and aggregates at workplaces.

Source: ISO, 2015

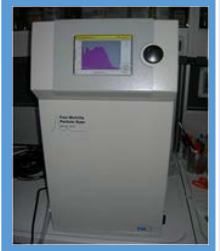
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2015)19&doclanguage=en

What parameter should we measure?

Multiple parameters may influence NMs health effects



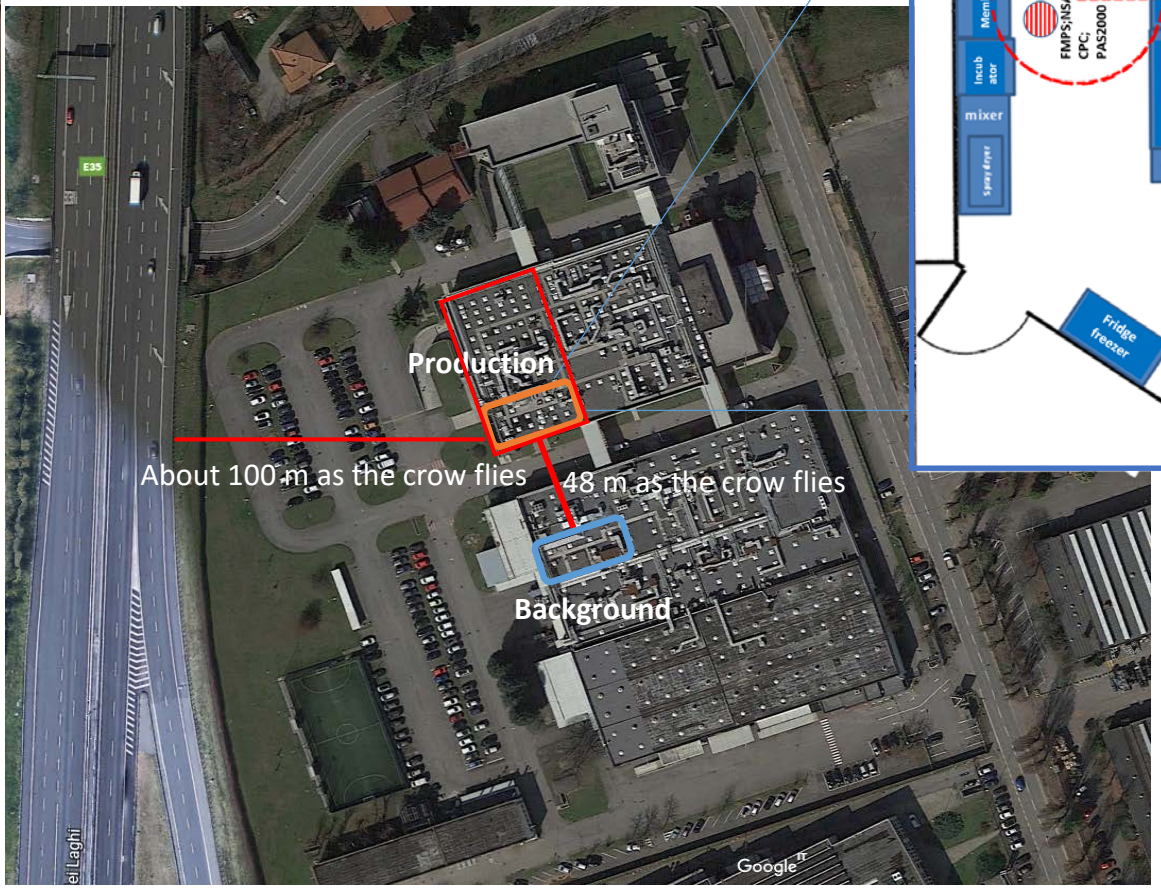
Instruments involved in preliminary and intensive measurements

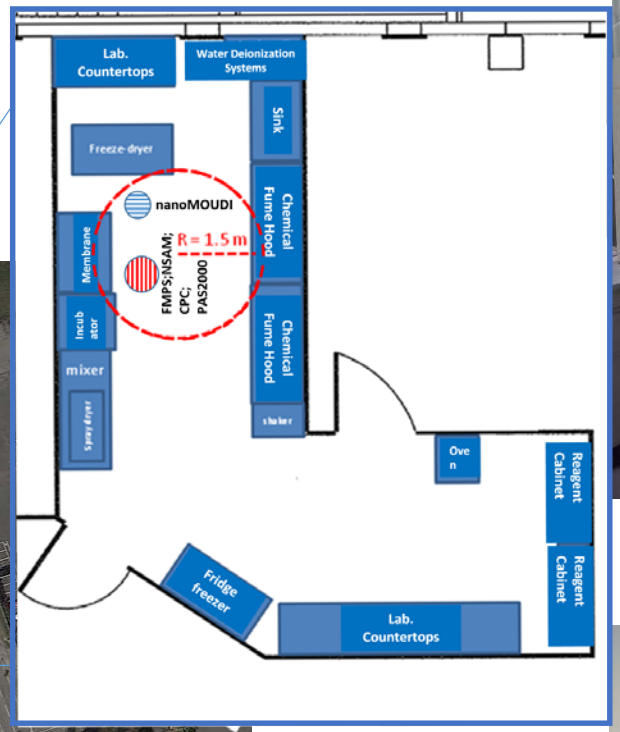
	Instrument	Class	Principle of operation	Output	Size range (nm)	Time resolution (s)	Flux (L/min)	Detection limits	Accuracy
	CPC TSI Inc. Mod. 3007	Real-time	Optical detection	PNC (part./cm^3)	10 – 1,000	1	0.7	1 – 100,000 part./cm^3	±20%
	FMPS TSI Inc. Mod. 3091	Real-time	Electrical mobility	PNC (part./cm^3) Size distribution	5,6 - 560	1	10	Small part.: 100-1x10^7 part./cm^3 Large part.: 1-1x10^5 part./cm^3	±15% MDC*
	NSAM TSI Inc. Mod. 3550	Real-time	Diffusion charging	Surface area running avg (µm^2/cm^3) and total (µm^2) TB or A fractions	10 – 1,000	1	2.5	TB: 0 - 2,500 µm^2/cm^3 A: 0 – 10,000 µm^2/cm^3	±20%
	O_3 Analyzer TEI Inc. Mod. 49 C	Real-time	UV photometric measurement	O_3 concentration (ppb)	-	20	1-3	>1 ppb	-
	PAS2000 EcoChem Inc.	Real-time	Photoelectric Ionization	PAH (ng/m^3)	10 – 1,000	10	2	>3 ng/m^3	±30%
	nanoMOUDI MSP Mod. 122 R	Time-integrated Area sampler	Aerodynamic diameter	Particle gravimetric mass Size distribution Samples for off-line analysis	10 – 18,000	-	30	-	-
	SIOUTAS	Time-integrated Personal sampler	Aerodynamic diameter	Particle gravimetric mass Size distribution Samples for off-line analysis	250 – 2,500	-	9	-	-

*Mean Diameter Counting (MCD) vs SMPS TSI/3936 for particles <100nm of polystyrene latex (PSL). Source: Asbach et al., J Nanopart Res (2009) 11:1593–1609

Experimental set-up

Background Far Field (FF)

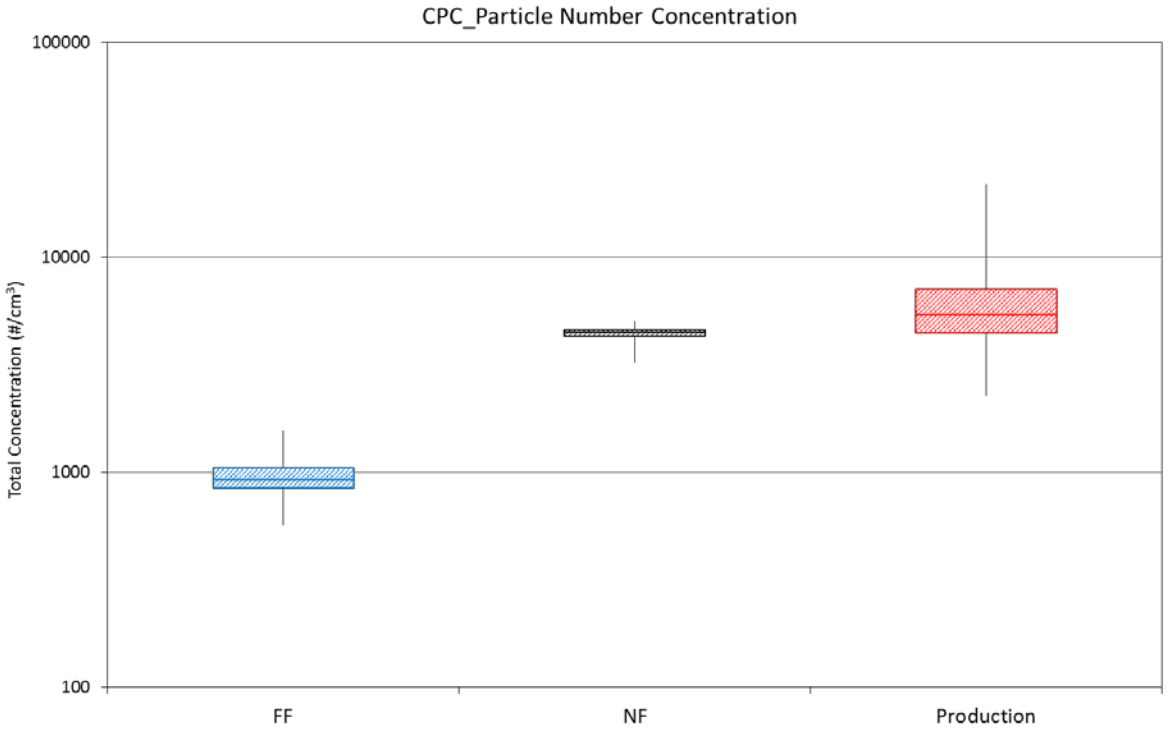


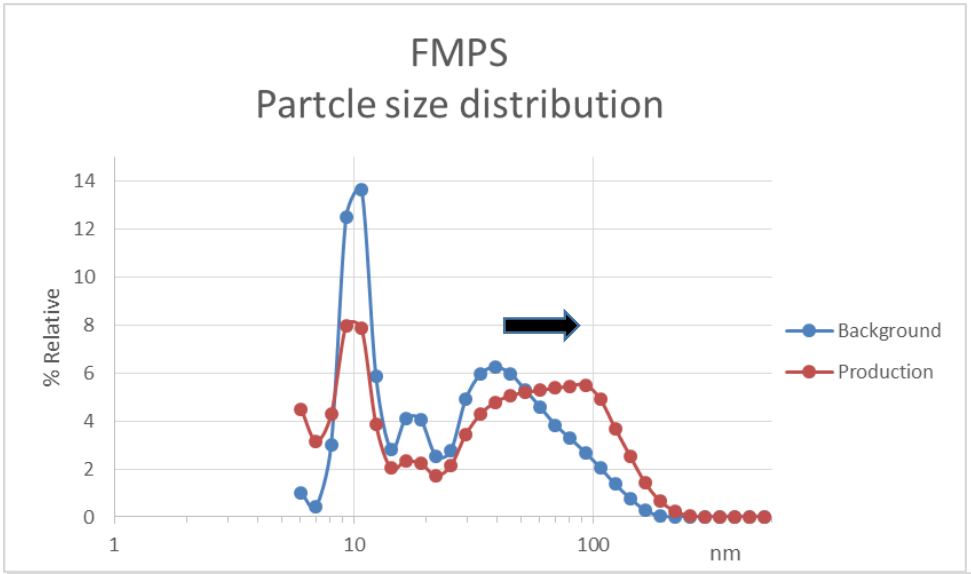


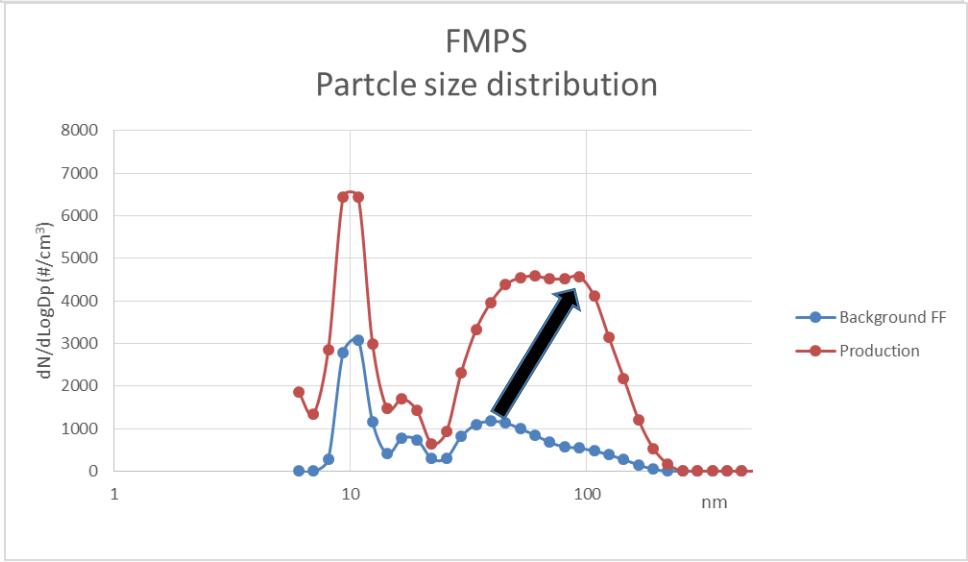
Near Field Background (NF) and Process Monitoring

Personal Sampling

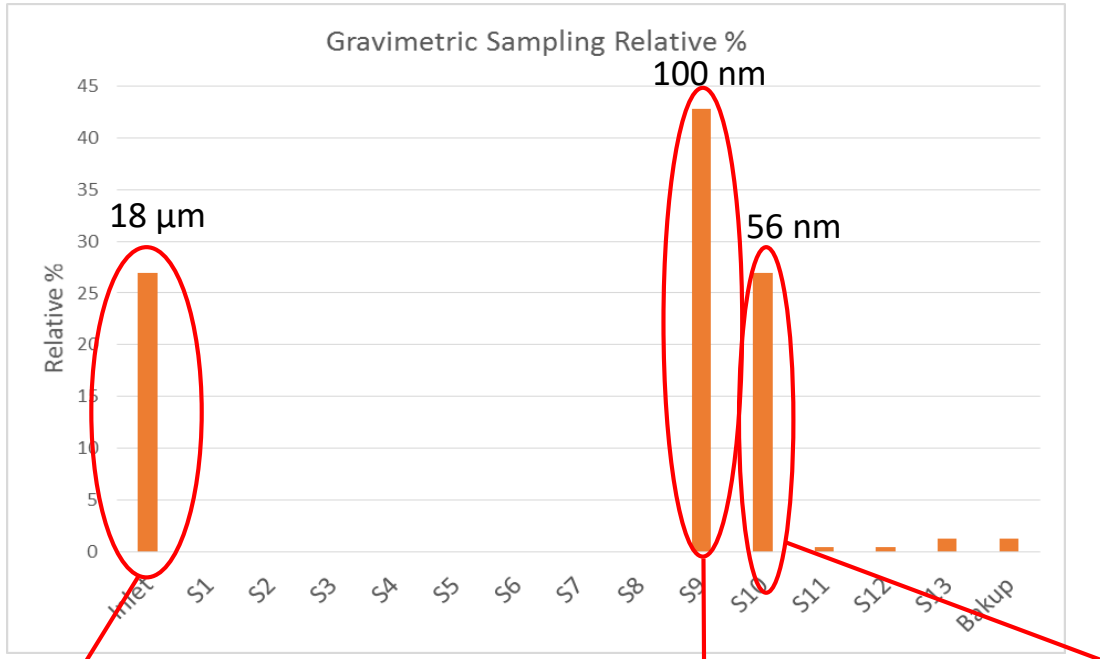
Real Time Measurement Results

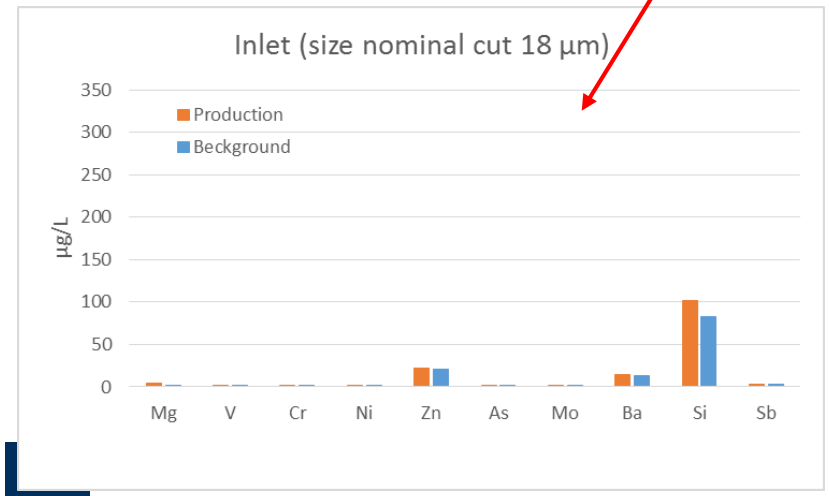


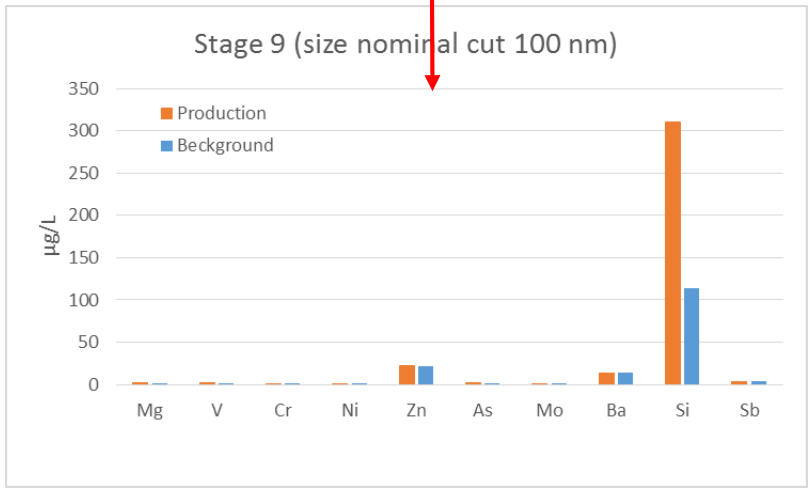


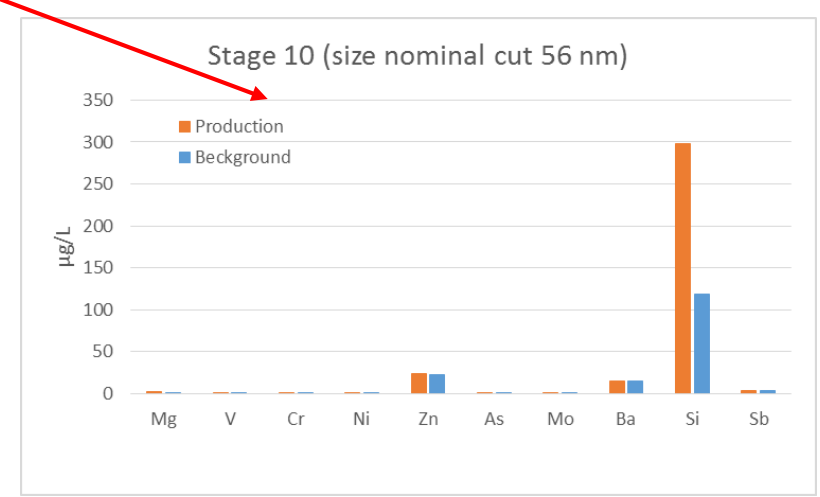


Off-Line ICP-MS analysis



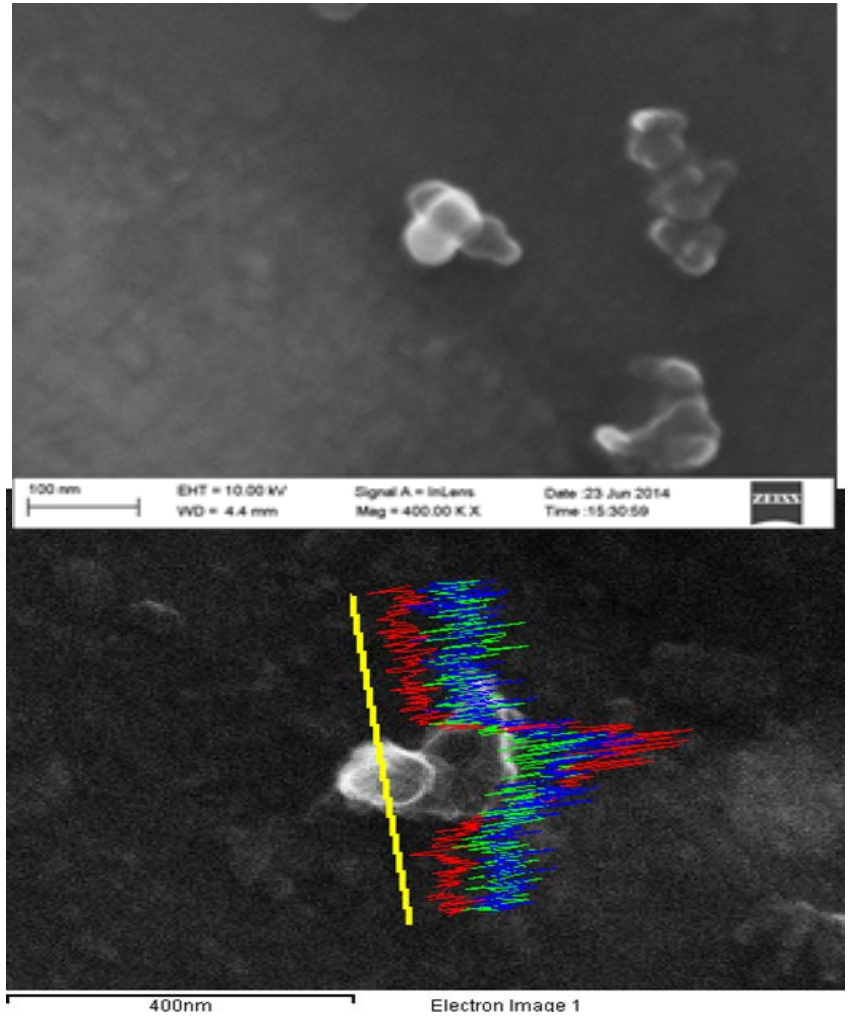






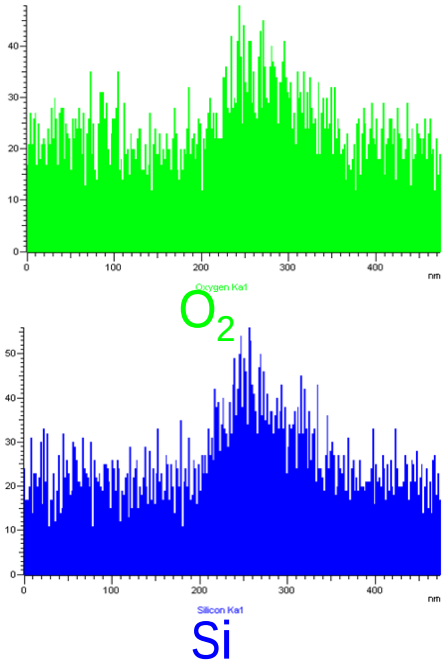
Off-Line FegSEM-EDX analysis results

nanoMoudi sampler S10 plate: nominal dimension 56-100nm

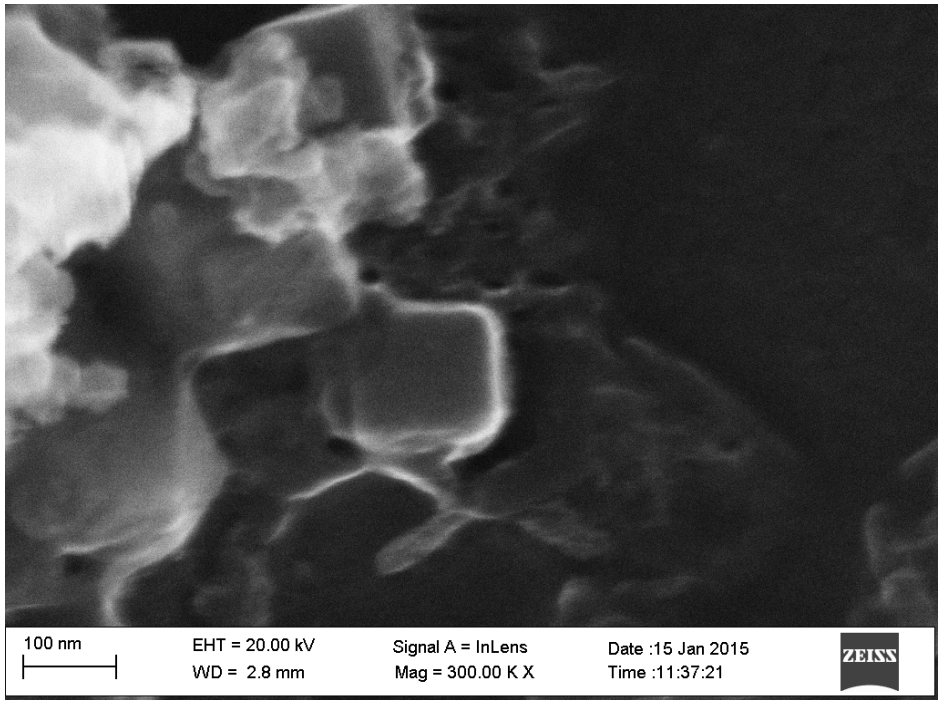


Courtesy of D. Lega and A. Antonini, INAIL

Close to particles and aggregates Si and O_2 EDX signals increase



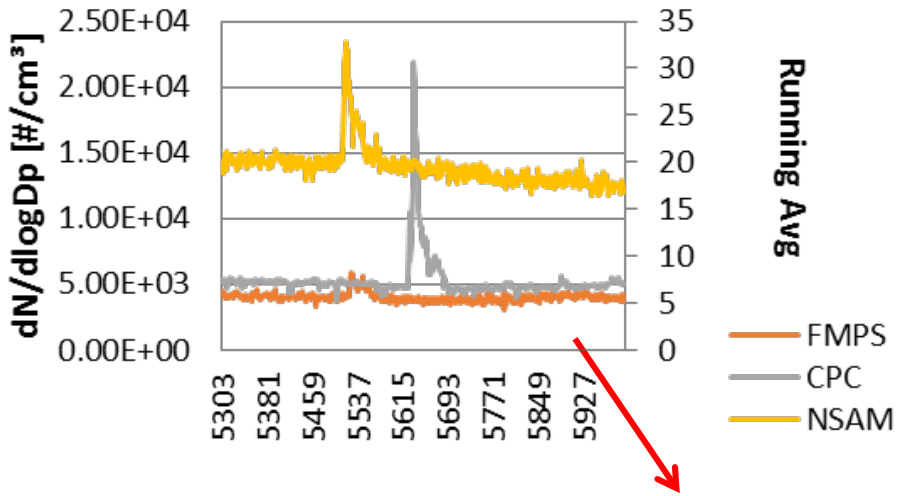
Sioutas personal sampler



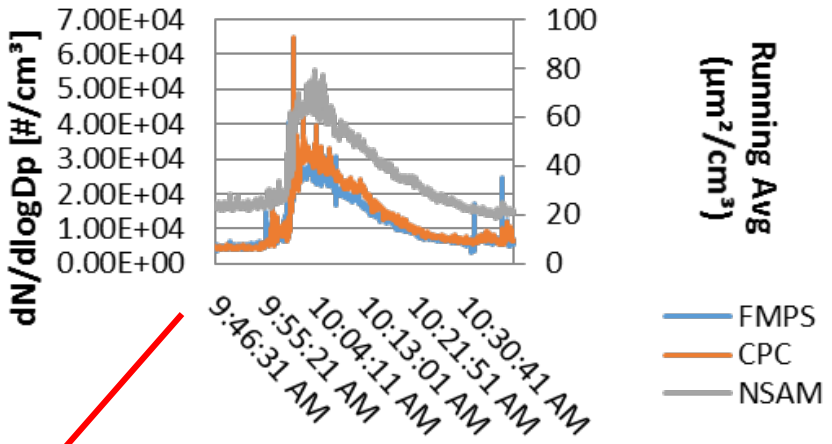
Nanozeolite aggregates on personal sampler filter

Far from the Background values: production and outdoor activities

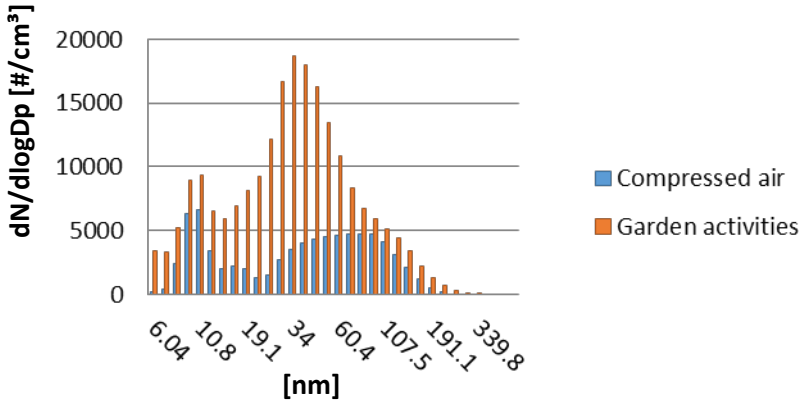
Compressed Air



Garden activities

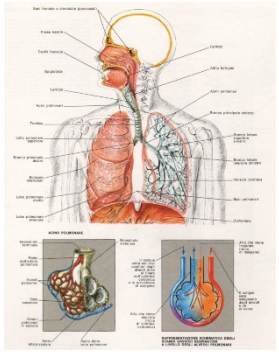


Compressed air-Garden activities

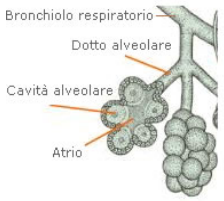


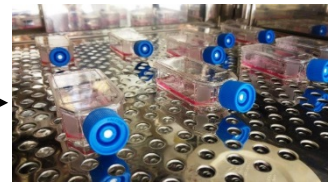
In vitro model

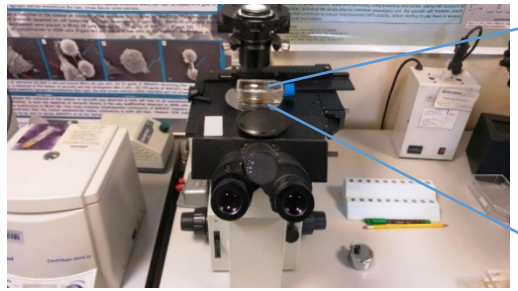
Evaluation of cytotoxic, genotoxic and oxidative effects of exposure to nanozeolites

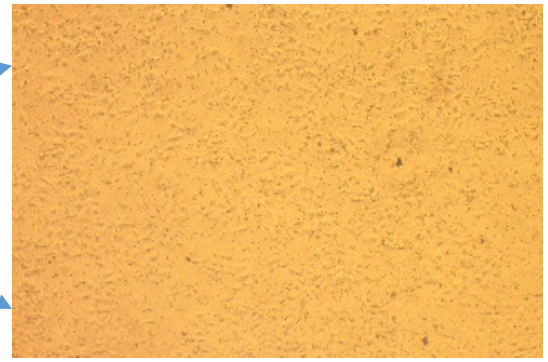


Human alveolar epithelial cells (A549)

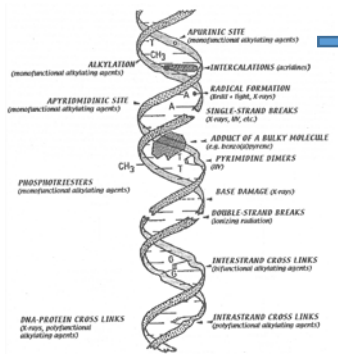


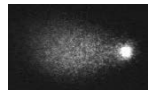






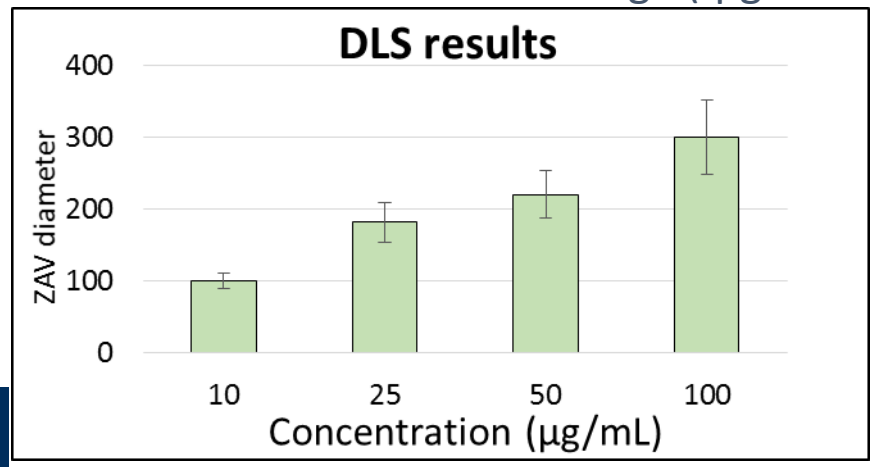
- Cell viability (GUAVA cytofluorimetric assay)
- Apoptosis (GUAVA cytofluorimetric assay)
- Membrane damage (Increase of LDH release)
- Direct and oxidative DNA damage (fpg-comet assay)





Exposure conditions:

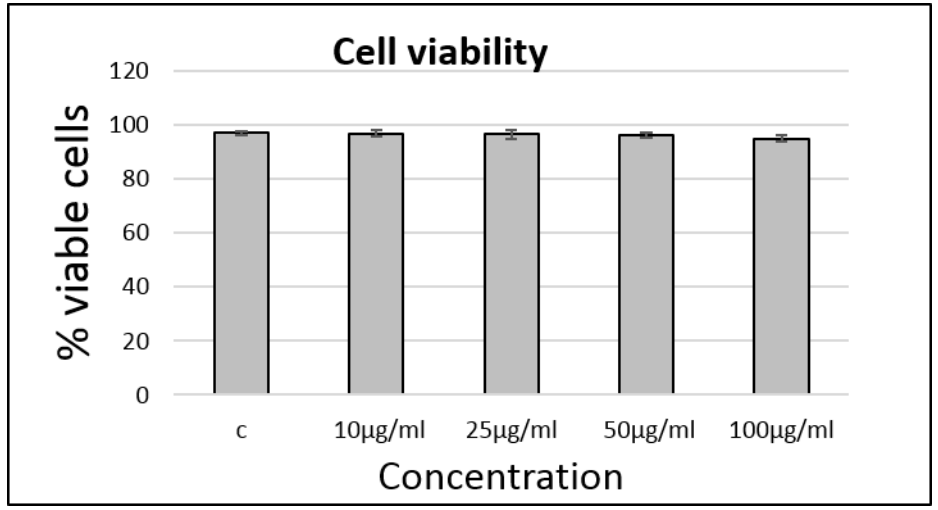
- stock solution 2mg/ml
- 10 min sonication
- BSA 15 mg/ml
- 4h and 24h duration
- 10, 25, 50 and 100 µg/ml in RPMI 1640 medium

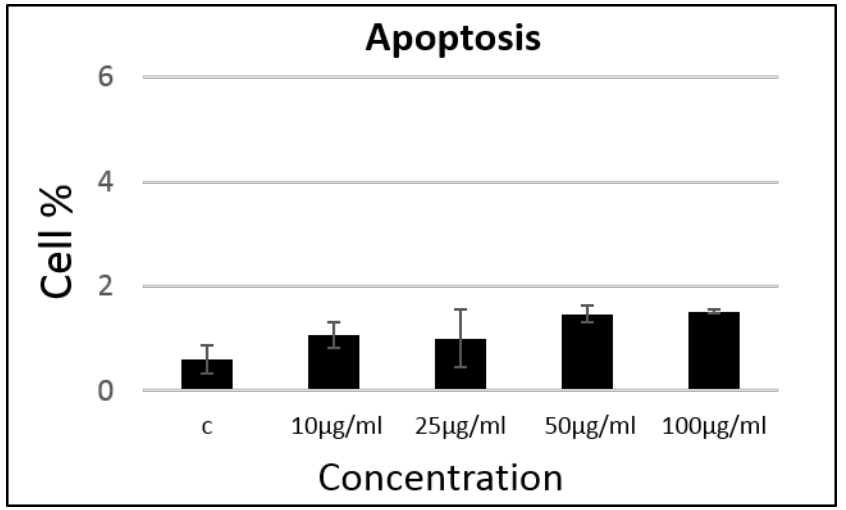


Nanozeolite dissolution in cell medium analysed by DLS: dose-dependent increase of ZAV diameter at t0

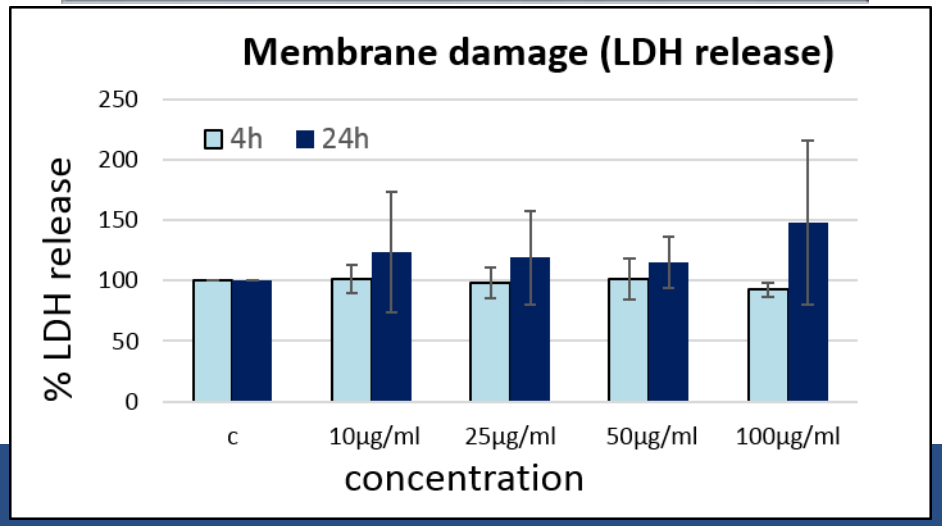
CYTOTOXICITY

Lack of significant cytotoxic effects



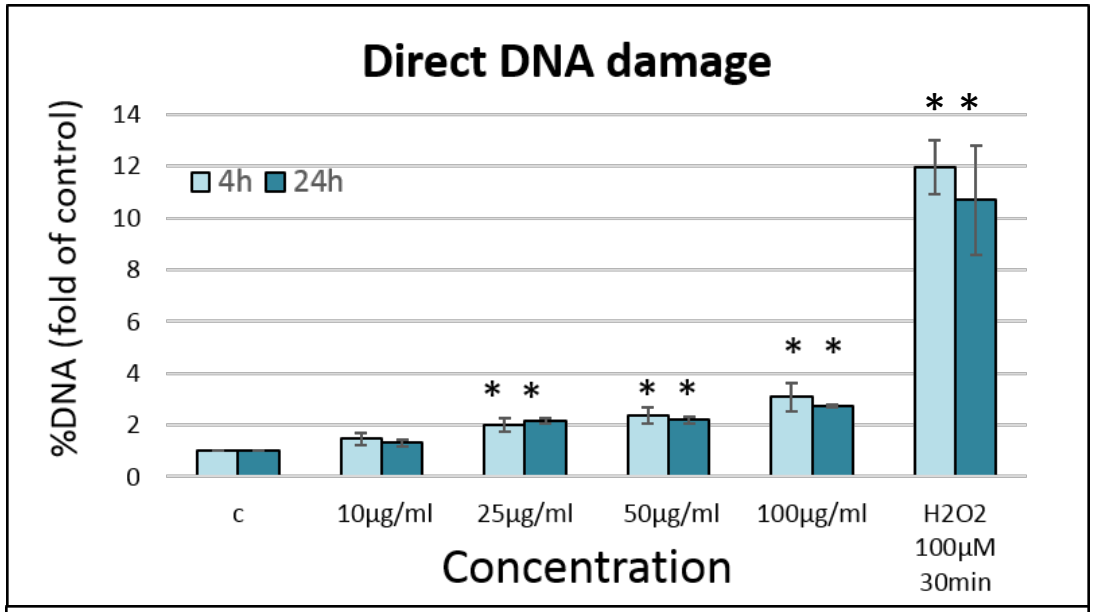


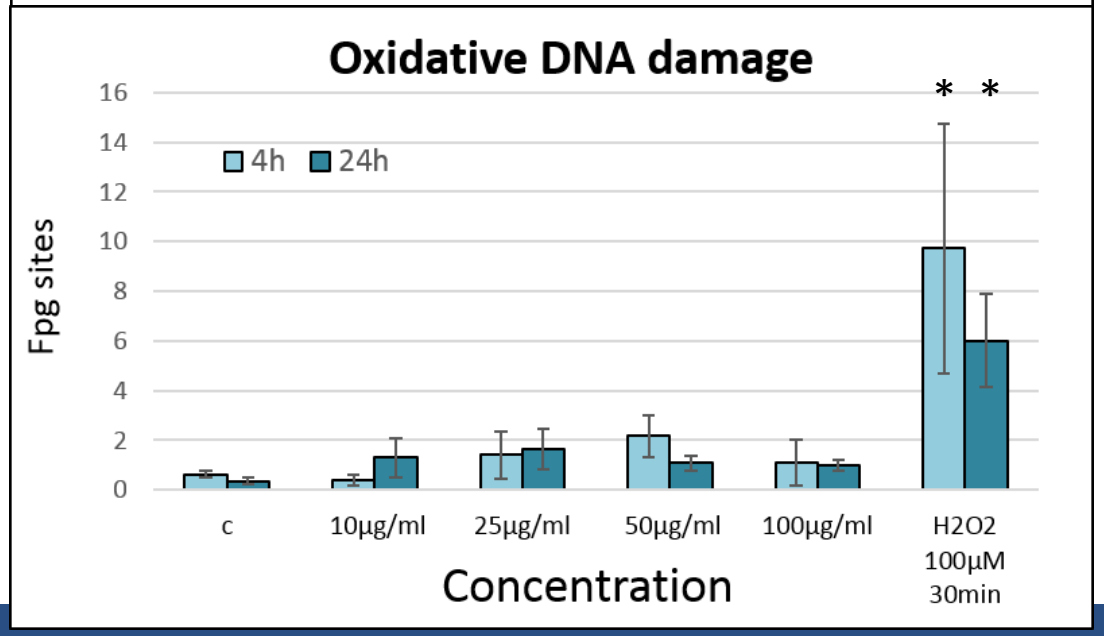
We did not find any reduction of viable cell percentage

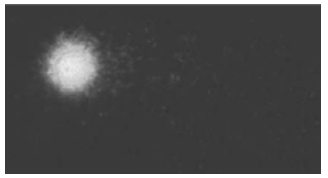


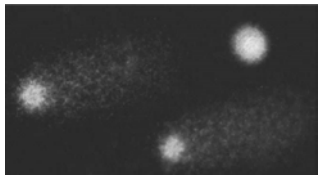
Slight increase of apototic cell percentage at 50 e 100 µg/mL associated to a slight increase of LDH releasae at 100 µg/mL

GENOTOXICITY (fpg-comet assay)









We found moderate induction of direct DNA damage beginning from 25 µg/mL and a slight induction of oxidative DNA damage

Conclusions

A multi-parametric approach allows us to collect data useful for:

- A comprehensive assessment of airborne nanoparticles in the workplace
- The distinction of background from airborne produced nanomaterials
- The identification of production phases in which exposure may occur
- The evaluation of outdoor influences on the measurements

The results represent the first ones data integrating the exposure scenario characterization and the cyto-genotoxic effects of LTA NZs

It is important to highlight the need to perform further studies to better quantify the real exposure condition to be correlated to possible induced toxicity.

Thank you for your attention!

Our NANO-working group…

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