Finnish Institute of Occupational Health

Hazard characterization of graphene-based nanomaterials in energy production and storage

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Joint action

THE PROJECT IN A NUTSHELL

Duration of the project: 2.5 years

Starting date: 01.01.2021

Partners:

- University of Trieste (UniTs)
- The Finnish Institute of Occupational Health (FIOH; Coordinator)

Budget: 348 k€

Funded by:

- The Finnish Work Environment Fund (FWEF)
- The Italian Workers Compensation Authority (INAIL)
- UniTs
- FIOH

THE ADVISORY COMMITTEE

Companies

- Amaia Zurutuza (Graphenea)
- Martin Lohe (Sixonia-Tech)
- Julio Gómez (Avanzare)
- Santiago Blanco de la Torre (Grupo Antolín)

Funding organizations

- Paolo Bragatto (INAIL)
- Mikael Saarinen (FWEF)
- Carita Aschan (FIOH)

AIM OF THE PROJECT

The overall aim of this project is **to identify and characterize the toxicological hazard that graphene-based nanomaterials (GBMs) used in energy production and storage may posed at the occupational level**

OBJECTIVES OF THE PROJECT

1. ***Adapt toxicity test guidelines (TGs) for advanced materials and apply them for GBMs testing***
   - Employing the latest principles and procedures developed within the OECD Manufactured Nanomaterials Working Party (MNMWP) programme
   - Comparing *in vitro* results with the human biomonitoring data on the same toxicological endpoints that will be obtained by FIOH within the EU Graphene Flagship programme

2. ***Contribute to elucidating the mechanisms of action at the basis of human toxic responses after inhalation exposure to GBMs***
   - Using *in vitro* approaches able to differentiate between primary (interaction with target cellular components) and secondary (mediated by an inflammatory response) mechanisms of actions

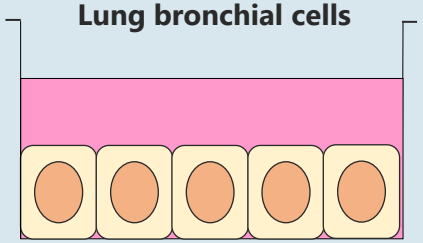
3. ***Assess how the physico-chemical properties of GBMs can affect their toxicity***
   - Evaluating GBMs with different physico-chemical properties for their *in vitro* effects on targeted cells → Safe(r)-by-Design

METHODOLOGY

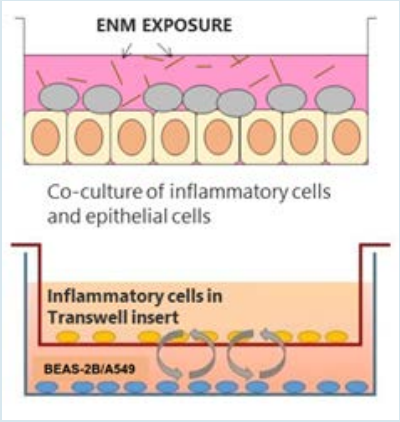
GBMs
(FLG, GO, rGO, composites)

Physico–chemical and biological properties:
- Shape
- No. of layers
- Oxidation state, etc
- Endotoxin content

MONOCULTURES



CO-CULTURES



Cellular uptake
ROS formation
Immunotoxicity
Genotoxicity

INHALATION EXPOSURE

ROS formation
Immunotoxicity
Genotoxicity

***GMBs' properties and toxicity* correlations**

In vitro- human biomarkers correlation

PLANNED SCHEDULE

Year	1												2												3					
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
T1																														
T2																														
T3																														
T4																														
T5																														
T6																														
T7																														

T1: Characterization of GBMs
T2: Adjustment of test protocols for assessing GBMs
T3: *In vitro* toxicity assessment of primary effects of GBMs
T4: *In vitro* toxicity assessment of secondary effects of GBMs
T5: Establishing correlations between physico-chemical properties of GBMs and their *in vitro* toxicity
T6: Correlations between *in vitro* toxicity data and human data from workers exposed to GBMs
T7: Dissemination of the results

T1. CHARACTERIZATION OF GBMs

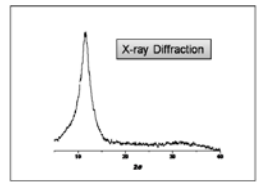
GRAPHENEA

AVANZARE

SIXONIA-TECH

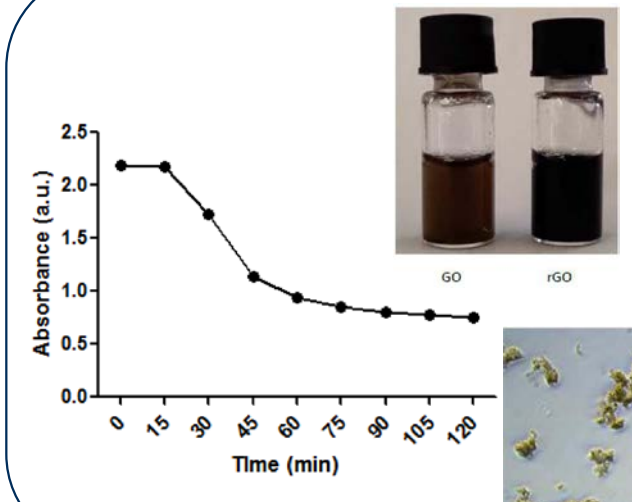
GRUPO ANTOLÍN

Appearance	Black Powder
Raman, I_G/I_D	~ 1
Carbon Content (EA*)	~ 48 %
Oxygen Content	~ 45 %
Sulphur Content	< 2 %
Nitrogen Content	< 0.5 %



GBMs MATERIALS

- Graphene oxide (GO) *vs.* rGO
- Powdered GO *vs.* stable GO dispersion
- rGO with different oxidation status
- rGO with different flakes dimension and number of layers
- Different functionalized rGO



UNIVERSITÀ DEGLI STUDI DI TRIESTE

Endotoxin analyses (LAL test)

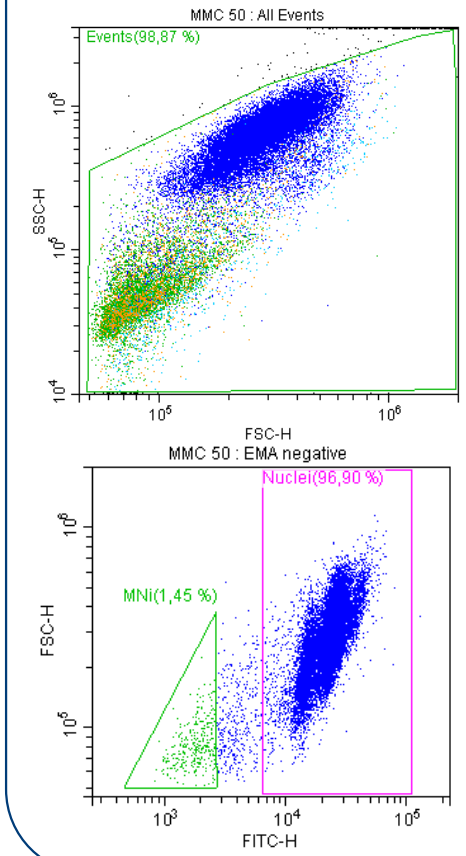
Finnish Institute of Occupational Health

INTERFERENCE WITH THE MATERIALS!!

Genotoxicity assays

Assay	Detected event	Pros	Cons	Decision
Micronucleus	Chromosome damage	Ongoing adaptation to NMs (OECD WPNM) Used in the co-culture system	GBMs may interfere with MNi scoring	✓
Mammalian gene mutation	Gene mutation	Ongoing adaptation to NMs (RiskGone)	Unclear whether applicable to 16HBE cells Time-consuming	X
Comet	DNA damage (mainly single DNA strand breaks)	Suitable for MNs Oxidative damage can be detected	No validated TG Possible interference with GBMs	✓
γ-H2AX	DNA damage (double DNA strand breaks)	More relevant type of DNA damage	No validated TG Already covered by the MNi assay	X

Flow cytometry-based micronucleus assay

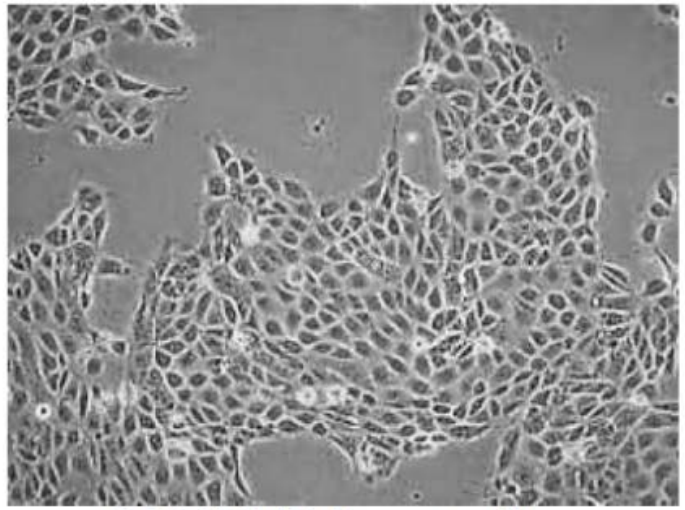


UniTs – FIOH collaboration

Lab	Cell line	Endpoint	Aim	Hazard characterization
FIOH/UniTs	16HBE14o- (Human bronchial epithelial cell line)	Cytotoxicity	Choosing sub-toxic or non-toxic doses	Cytotoxicity Oxidative stress Inflammation Genotoxicity
UniTs	16HBE14o-	ROS generation	Evaluation of the oxidative stress potential	
UniTs	16HBE14o-	Release of cytokines	Evaluation of the inflammatory potential	
FIOH	16HBE14o-	Genotoxicity (2 assays)	Evaluation of the genotoxic potential	
UniTs	THP-1 (human monocytic cell line)	Cytotoxicity	Choosing sub-toxic or non-toxic doses	
UniTs	THP-1	Release of cytokines	Evaluation of the inflammatory potential	
UniTs	THP-1	ROS generation	Evaluation of the oxidative stress potential	

Both institutions are using same cell lines, dispersion method, dose range and exposure times

Bronchial epithelial cells + macrophage-like cells → Co-culture system (secondary toxic effects)



16HBE14o- cells

CELL VIABILITY

ROS PRODUCTION

GENOTOXICITY

Conclusions

- rGO is more potent than GO in causing cytotoxic effects
- Powdered GO is more potent than GO stabilized in dispersion
- Unclear association with O_2 content of rGO
- Evaluated rGO did not induce DNA damage
- Some rGO are able to induce chromosome damage, although this capacity is not related to the O_2 content

NEXT STEPS

T1: Characterization of GBMs

- Transmission electron microscopy analysis (size and shape) (UniTs)
- Close contact with companies to obtain characterization data (UniTs & FIOH)

***T3: In vitro* toxicity assessment of primary effects of GBMs**

- The release of pro-inflammatory cytokines and chemokines by THP-1 cells exposed to GBMs (UniTs) will at first be evaluated to select the most inflammogenic conditions to be studied later on in the co-culture system.
- Completing genotoxicity testing (FIOH)

T4: *In vitro* toxicity assessment of secondary effects of GBMs

- Secondary toxic effects mediated by THP-1-released cytokines and pro-inflammatory factors will be evaluated in the human bronchial epithelial cells co-cultured with GBM-exposed THP-1 cells by means of genotoxicity (FIOH) and inflammatory response (UniTs)

NEXT STEPS (II)

T5: Establishing correlations between physico-chemical properties of GBMs and their in vitro toxicity

- Data on physico-chemical characterization of GBMs obtained in Task 1 will be correlated to the toxicity data obtained in Tasks 3 and 4, to identify which physico-chemical properties could mostly affect the toxic response to inhaled GBMs.
- This information will be relevant in choosing safer materials (Safe-by-Design approaches)and it can help GBMs grouping when registering them under European regulations

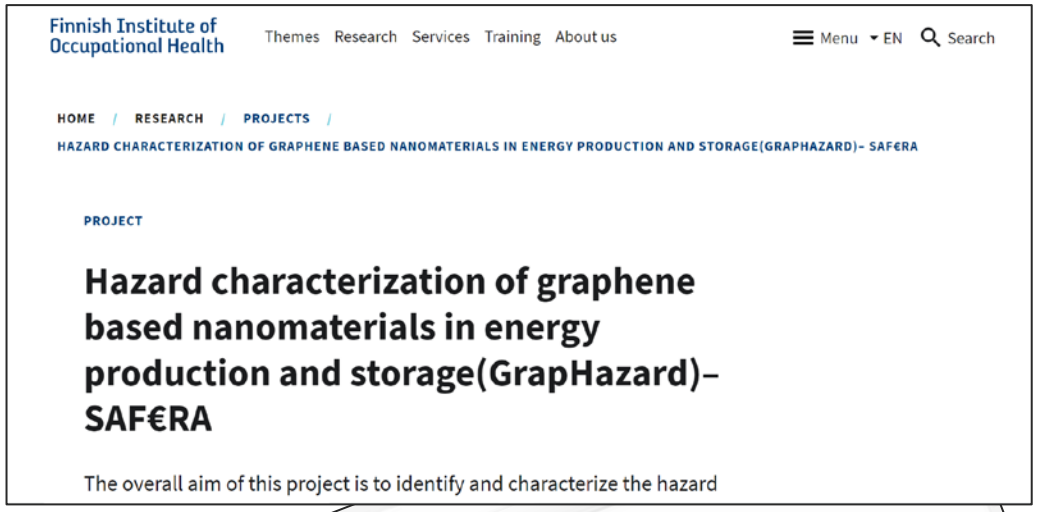
T6: Correlations between in vitro toxicity data and human data from workers exposed to GBMs [FIOH (leader), UniTs] (M18-M30)

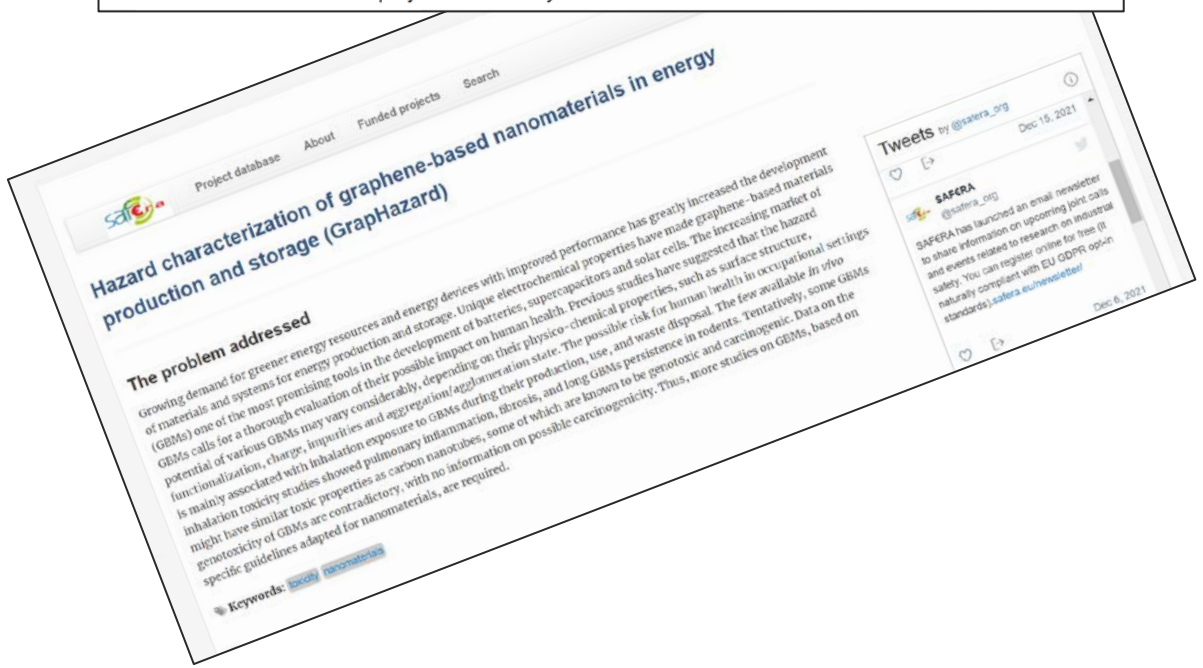
Data from human biomonitoring studies performed within the Graphene Flagship will be correlated with the toxicity data obtained in Tasks 3 and 4, to assess

i) whether the *in vitro* settings are predictive of human response, and
ii) the mechanisms of action operating in possible toxic responses observed in humans exposed to GBMs.

T7. DISSEMINATION

- Advertisement of GrapHazard in several websites and social media
- SAF€RA Symposium (Rome, 19-20 May 2022)
- Scientific congresses
  - "Nano-week" 2022 (Cyprus, June 2022)
  - International Congress of Toxicology (ICT2022, Maastricht, September 2022)
  - Finnish Nanosafety seminar (Helsinki, October 2022)
  - Congress of the Italian Society of Pharmacology (Rome, November 2022)
  - Congress of the Italian Society of Toxicology (Bologna, February 2023)
- Scientific publications
  - Domenech *et al.* Genotoxicity of Graphene-based materials (under revision)





PERSONNEL INVOLVED

UniTs

- Marco Pelin
- Aurelia Tubaro
- Silvio Sosa
- Cristina Ponti
- Michela Carlin
- Clara Passerino

FIOH

- Gerard Vales
- Adriana Rodríguez Garraus
- Kukka Aimonen
- Mira Hartikainen
- Satu Suhonen
- Outi Kulo (project assistant)
- Julia Catalán
- Tomi Kanerva (FIOH, Graphene Flagship)

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THANK YOU!

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