

The Role of Dynamic 3D Models in Evaluating MXenes Toxicity: A Focus on Pulmonary and Hepatic Cells



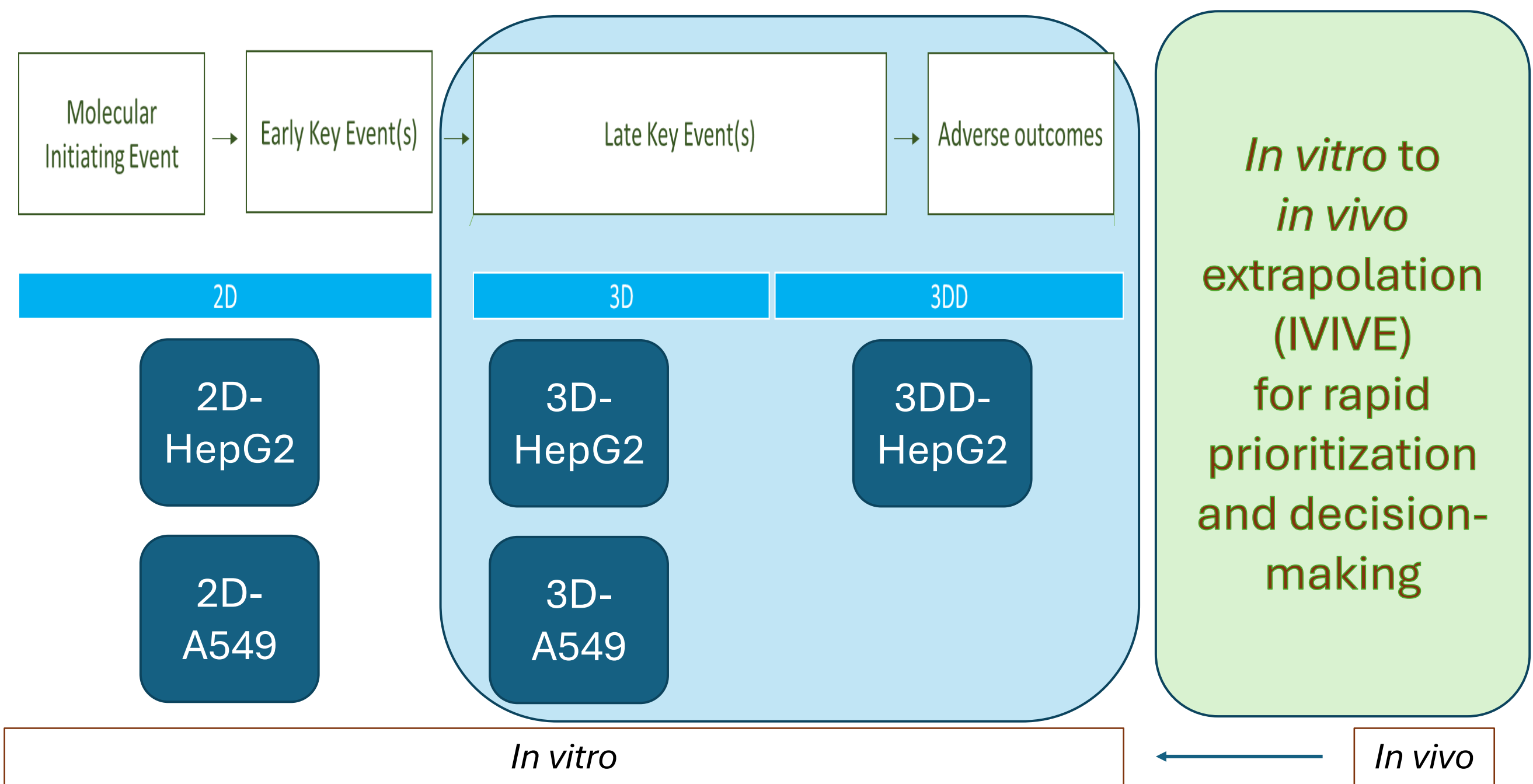
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Background	MXenes and Graphene possess outstanding physicochemical properties. They have strong application potential in electronics, energy storage, and biomedicine.
Challenge	Current nanoparticle regulations present critical gaps, particularly in human health risk assessment . There is a growing need for Safe and Sustainable by Design (SSbD) aligned evaluation strategies.
Aim	To bridge the <i>in vitro</i> – <i>in vivo</i> gap in nanosafety research.
Approach	Advanced <i>in vitro</i> models, including static and dynamic 3D cellular systems , were applied and compared to conventional 2D cultures.
Impact	These methodologies offer promising tools for improving the hazard assessment of 2D-nanomaterials , such as MXenes .

Framework linking *in vivo* lung and liver injury to *in vitro* models through cellular events and quantitative endpoints to predict organ damage.

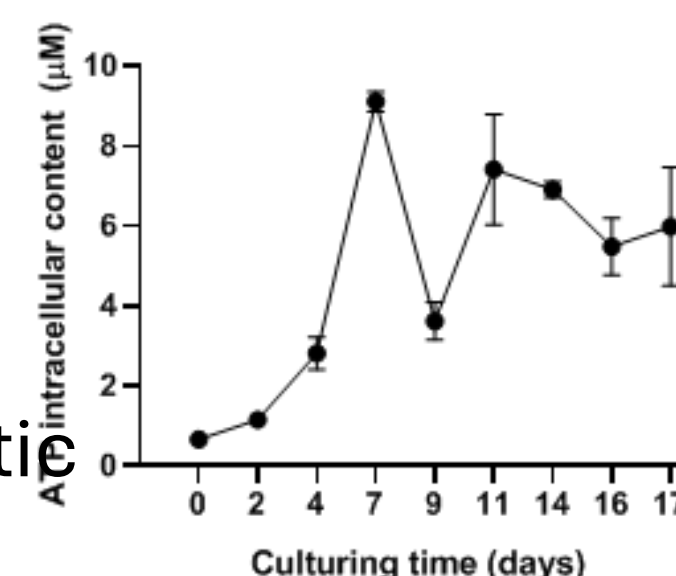
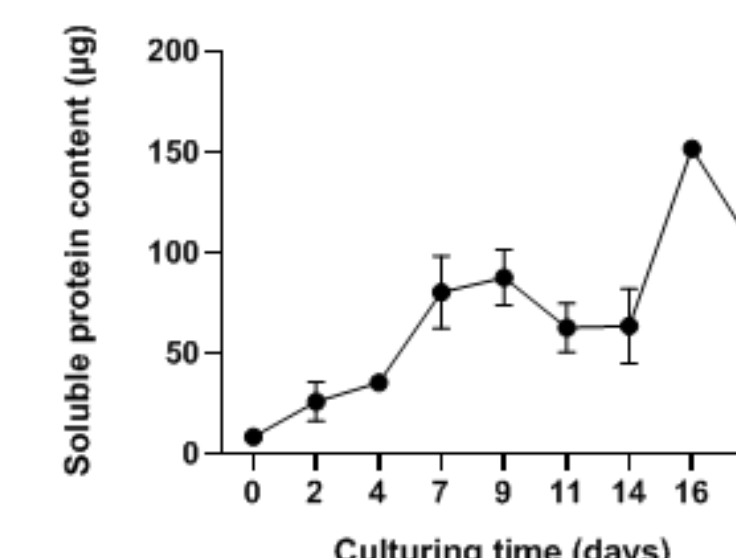
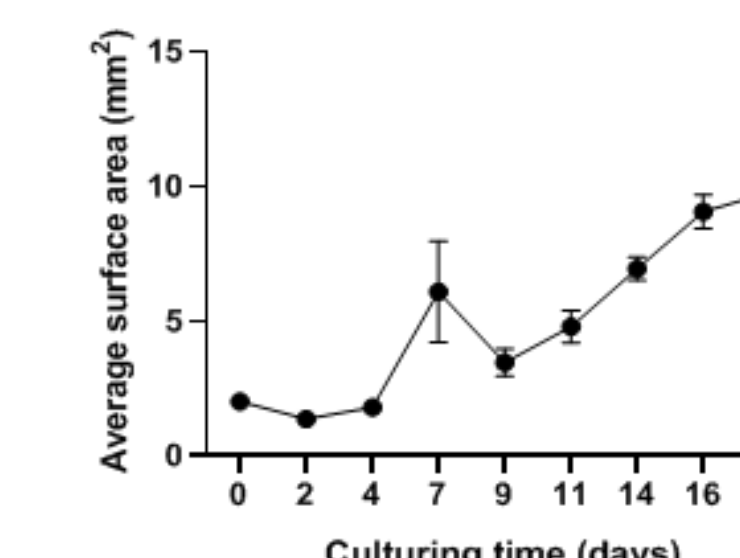
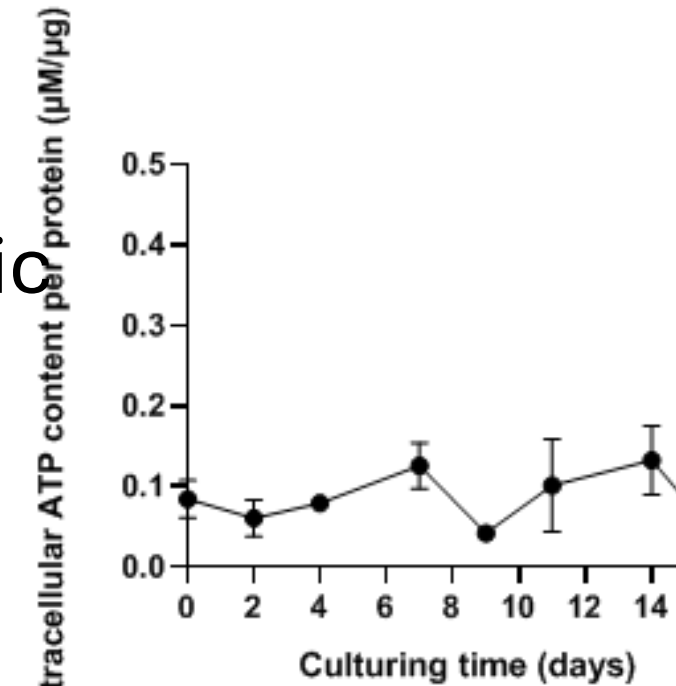
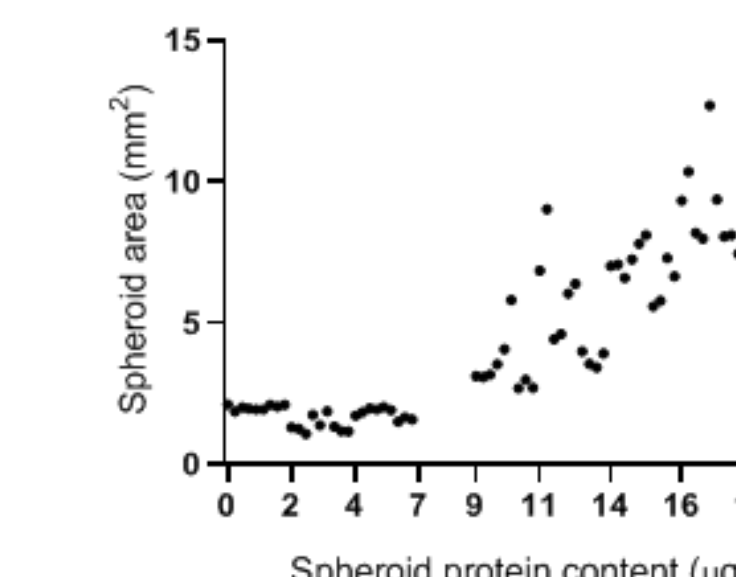
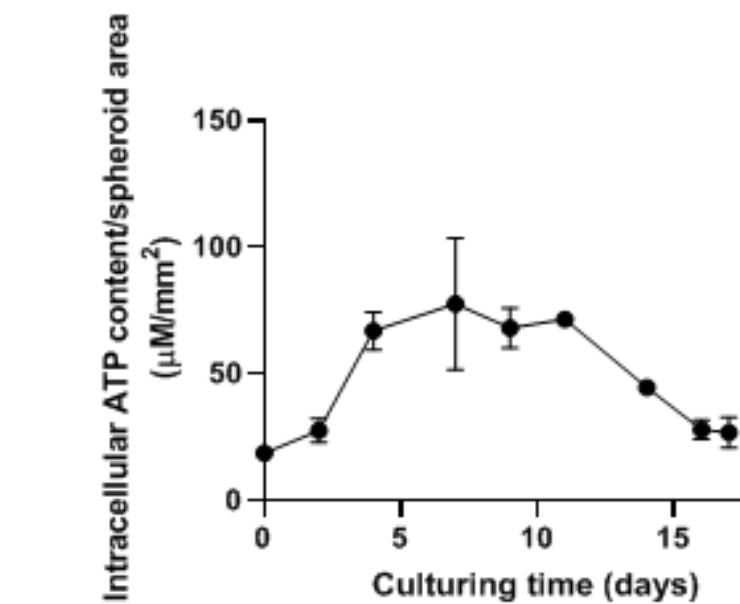


In vitro assays using 2D models showed no toxicity within the tested concentration range, as traditionally expected. However, in advanced models, an EC₅₀ was detected, suggesting that MXenes may interfere with cell behavior.

Advanced 3D cellular models provide a more physiologically relevant platform for evaluating the toxicity, metabolism, and pharmacokinetics of emerging nanomaterials such as MXenes, thereby improving our understanding of their potential health risks.

What did we find?

3D *In Vitro* Models: A More Reliable Approach for Advanced Toxicological Assessment

Methodological validation using HepG2 cells		Functional analysis of dynamic 3D HepG2 cells (3DD) using the Clinostar System		Dynamic 3D HepG2 (3DD) model validation using a hepatotoxic agent (valproic acid, VPA)									
	HepG2 monoculture maintained under standard conditions.		3D construct generated under static conditions and embedded in methylcellulose for 3 days.	<table border="1"><thead><tr><th>Model</th><th>IC50 VPA (mM)</th></tr></thead><tbody><tr><td>2D HepG2</td><td>10.38 (2.30 to 18.46)</td></tr><tr><td>3D HepG2</td><td>10.48 (6.158 to 24.18)</td></tr><tr><td>3DD HepG2</td><td>18.06 (13.04 to 25.51)</td></tr></tbody></table>		Model	IC50 VPA (mM)	2D HepG2	10.38 (2.30 to 18.46)	3D HepG2	10.48 (6.158 to 24.18)	3DD HepG2	18.06 (13.04 to 25.51)
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	Spheroids cultured under dynamic conditions (3DD) for 3+17 days	↓ Acute toxicity assessment											
													
													

The model was successfully validated using a well-established hepatotoxic control (VPA), as a replicate LC₅₀ of 18 mM was reported by Fey and Wrzesinski et al., 2012, in a publication that also demonstrated a correlation with in vivo data (R² = 0.85).

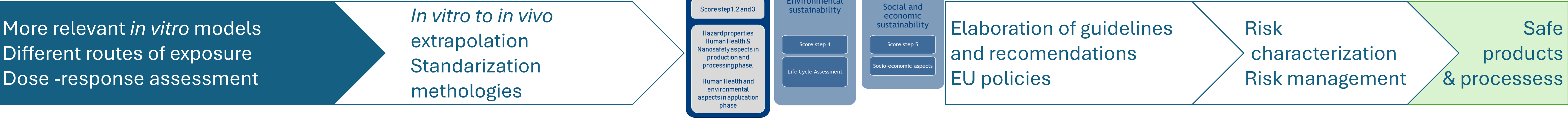
Advanced *In Vitro* Models for Realistic Nanosafety Assessment of MXenes: Bridging 2D-3D Responses Under SSbD Principles

Assess cell viability in 2D cell models exposed to Mxenes for 24 h	Lung and liver-spheroids grown under static conditions (3D) were exposed to MXenes in plates for 24h	Liver-spheroids grown under dynamic conditions (3DD) were exposed to MXenes in plates for 24h	IVIVE
 No dose-response relationship was observed within the concentration range recommended by NIOSH guideline ^{2,3} .	 IC₅₀ (µg/mL) 107.4 (7.5-180.5)	 IC₅₀ (µg/mL) 15.96 (10.49-26.25)	 IC₅₀ (µg/mL) 40.88 (23.29-94.30)

The data reveal IC₅₀ values undetectable by 2D systems, suggesting that MXenes may affect cellular behavior in ways not captured by these models. The enhanced sensitivity of 3D models is likely due to their more complex structure and function, making them more physiologically relevant.

Transferable Knowledge to Toxicology Assessment

These observations can help improve the understanding of more reliable *in vitro* models and gather information on inhalation and oral toxicity endpoints, contributing to the safety assessment step of the SSbD framework.



References

¹Fey SJ, Wrzesinski K. Determination of drug toxicity using 3D spheroids constructed from an immortal human hepatocyte cell line. Toxicol Sci. 2012 Jun;127(2):403-11. doi: 10.1093/toxsci/kfs122. Epub 2012 Mar 27. PMID: 22454432; PMCID: PMC3355318.
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Acknowledgments:
The **SAFARI** – Safe and Sustainable by Design Graphene/MXenes Hybrids project has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101135965.



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