Safety Testing of Emerging Products: The iCare Project and Its Contribution to Product Life Cycle Information

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Challenges and Gaps in Transitioning from Substance-Based to Product-Based Safety Assessment

Ensuring safety of emerging and innovative products is an essential step for a transition towards a sustainable future. To support EU Green Deal ambitions and the implementation of the broader United Nation's 2030 Agenda, EU strategies (e.g. EU chemical strategy for sustainability) are being translated into new directives and regulations (Figure 1). These complement existing EU regulations aimed at protecting human health and the environment (e.g. the Classification, Labelling and Packaging (CLP) regulation) by addressing product specific considerations. **However, important challenges remain, notably due to limited understanding of the toxicity mechanisms of harmful chemicals and materials such as neurotoxicants** [1].

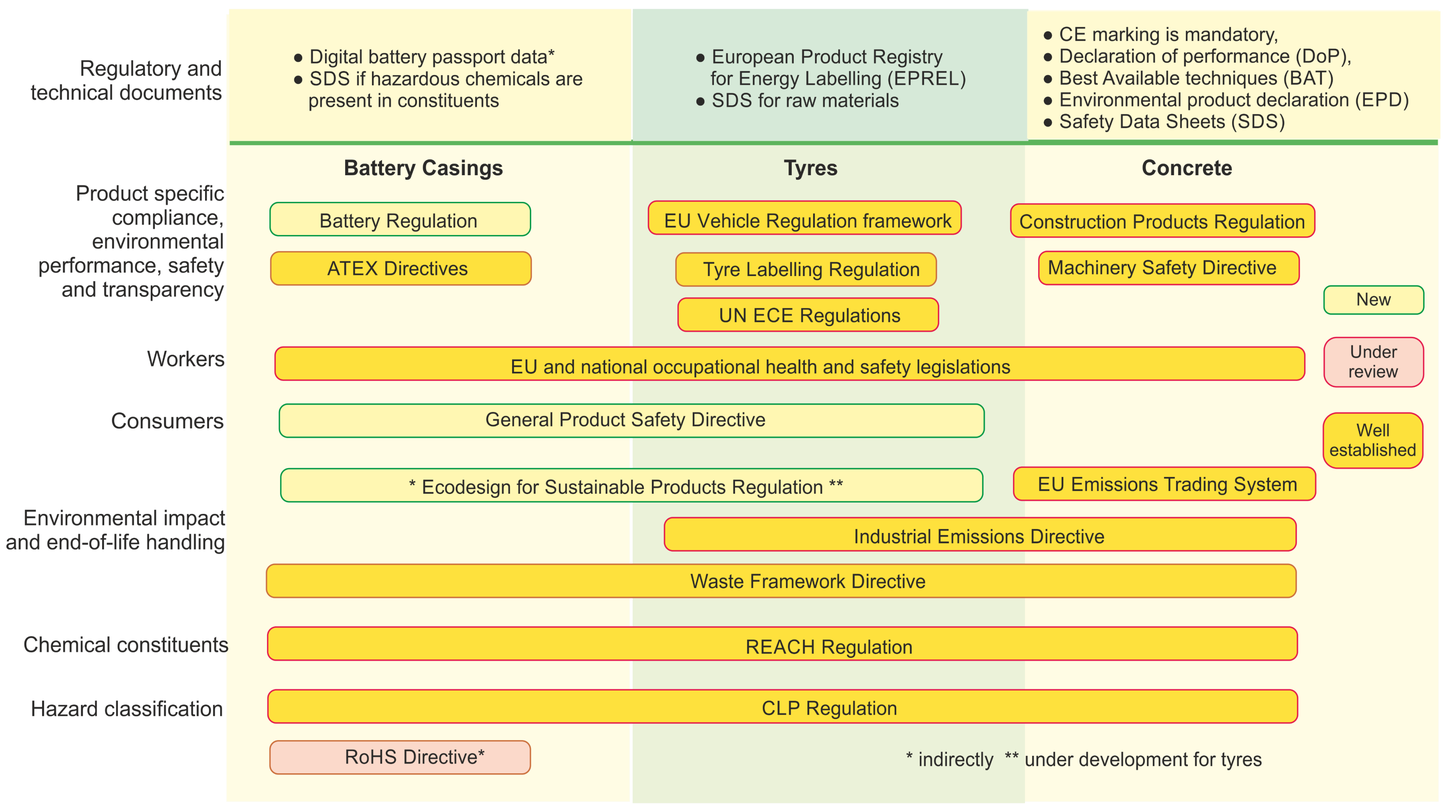

Figure 1 – Regulatory landscape for 3 of the iCare Use Cases

The iCare project is developing an integrated model system to characterize and predict the neurotoxic potential of pristine advanced nanomaterials and particles released during their life cycles.

Advanced Materials Life Cycle Sampling

Performance standards were adapted to generate representative samples of the use and the end of life of the selected nano-enable product prototypes (e.g. ISO 9352:2012 for abrasive wear resistance of plastics)**.**

However, concentration and size selection protocols were deployed on the released particles collected to accommodate the adapted neuro-(eco)toxicity assays.

Out of 35 samples, 14 passed the assays' requirements for neurotoxic potential analysis (i.e. size of released particles below 10 µm, solid content 1 g/L and dispersible), including 6 pristine graphene 2D materials (Figure 2).

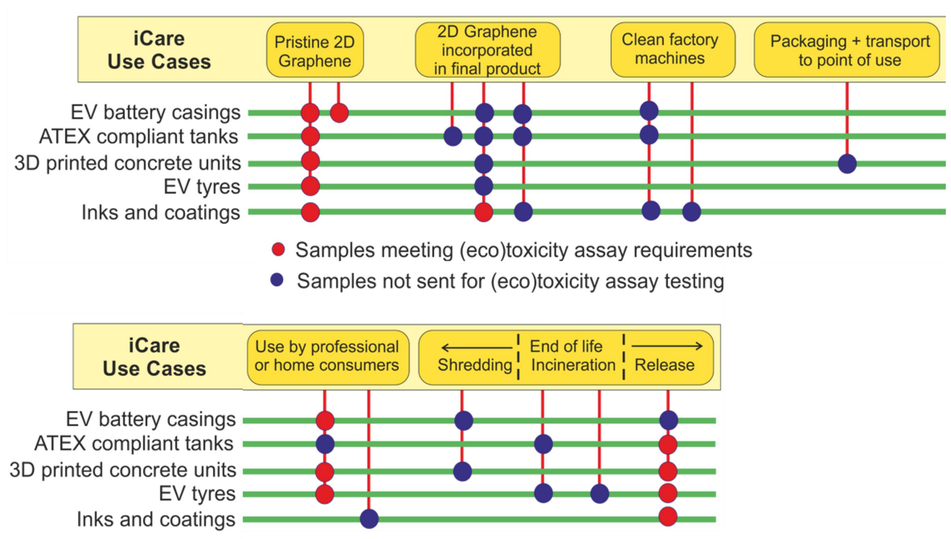

Figure 2 – Sampling points along iCare use cases life cycle stages

Integrated safety assessment and Neuro-Nanotoxicity

In iCare, (neuro)toxicity profiling of pristine and transformed nanomaterials is conducted using abiotic, *in vitro* and *in vivo* assays developed, adapted and integrated into a testing approach using human and fish cell lines and invertebrates (Table 1).

Table 1 – Example of regulatory relevant endpoints tackle by iCare assays for battery casings

Regulatory Requirement	Regulation(s)	Required Endpoint	iCare Assay(s)	Status
Acute Toxicity (Aquatic/Environmental)	REACH, Battery Reg.	Fish toxicity	RTgill-W1, OmB cell lines	Fish cell alternatives to OECD TG 203
Human Acute Toxicity	REACH, CLP, Battery Regulation	Cytotoxicity	Alamar Blue (SH-SY5Y, HMC-3, BBB), Planaria assays	Not validated for regulatory use
Genotoxicity / Mutagenicity	REACH, CLP	DNA damage, mutation potential	Planaria, Comet and diffusion assays	Not validated for regulatory use
Carcinogenicity	REACH	Long-term mutagenicity or cancer risk	ROS, Planaria genotoxicity	Needs long-term or cell transformation assay (e.g., Bhas 42)
Reproductive / Developmental Toxicity	REACH Annex IX/X	Reproductive/developmental endpoints	C. elegans reproduction, development growth	Not accepted alone; useful as weight-of-evidence
Neurotoxicity	REACH Annex IX/X, Battery Regulation	Neuroactivity / CNS endpoints, Neuro Barrier Permeabilit (BBB Integrity)	SH-SY5Y assays, TEER, Dextran permeability on BBB model, C. elegans neuro endpoints	Strong evidence of neurotoxic hazard screening
Inflammatory Response / Immunotoxicity	REACH Annex IX, Battery Regulation	Cytokine/inflammation signaling	ELISA (SH-SY5Y, HMC-3, BBB)	Supports mechanistic understanding of toxicity
Oxidative Stress / ROS Generation	REACH, CLP, Battery Regulation	ROS/reactive species, oxidative damage	Resazurin, HRP-Phenol-Red, ROS assay, Flow Cytometry (Planaria)	Strong screening capability
Environmental Toxicity (Soil/Invertebrates)	REACH Annex IX/X	Soil/worm toxicity	*C. elegans* survival, Planaria mobility, histology	Not validated for all endpoints, but useful for environmental hazard assessment
Abiotic Reactivity	Battery Regulation, REACH (exposure route)	Redox reactivity, H_2O_2 generation	Abiotic assays: Resazurin-DTT/TCEP, HRP-Phenol red	Key for assessing post-use exposure during recycling
Internalization / Histological Damage	Battery Regulation, REACH Annex X	Tissue alteration	Planaria histology	Not standardized for regulatory use, but informative

(BBB = Blood-Brain-Barrier, ROS = Reactive Oxygen Species, TEER = Transepithelial-Transendothelial Electrical Resistance)

Next steps

The iCare project supports the prioritization of samples to be tested along the life cycle of a product by developing a screening (neuro)toxicity approach applicable for (nano)materials.

The most predictive assays will be validated to generate regulatory-relevant data, supporting hazard classification and labelling of advanced (nano)materials while providing mechanistic insight into (neuro)toxic effects across their life cycle.

In vitro–in vivo extrapolation will enable the use of relevant (eco)toxicity assays in life cycle assessments, and support the implementation of product specific regulations, and recommended Safe and Sustainable-By-Design framework [3].

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References

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[2] Tal et al. *Frontiers in toxicology* 6 (2024): 1359507
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