Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Advanced Materials Earliest Assessment (AMEA)

Wendel Wohlleben[1] Michael Persson[2], Blanca Suarez-Merino[3], Anders Baun[4], Veronica Di Battista[1,4], Susan Dekkers[5], Eugene van Someren[5], Dirk Broßell[6], Burkhard Stahlmecke[7], Martin Wiemann[8], Otmar Schmid[9], Andrea Haase[10]

[1]BASF SE, Carl-Bosch-Str. 38, 67056 Ludwigshafen, Germany
[2]Chalmers CIT, Chalmers Industriteknik, Sven Hultins Plats 1, 412 58 Göteborg, Sweden
[3]TEMAS Solutions GmbH, Lätteweg 5, 5212 Hausen, Switzerland
[4]DTU Sustain, Technical University of Denmark, Building 115, 2800 Kgs. Lyngby, Denmark
[5]TNO, Risk Analysis for Products in Development, The Netherlands
[6]Federal Institute for Occupational Safety and Health, Nöldnerstr. 40-42, 10317 Berlin, Germany
[7]Institut für Umwelt & Energie, Technik & Analytik e. V., Bliersheimer Str. 58-60, 47229 Duisburg, Germany
[8]IBE R&D Institute for Lung Health gGmbH, Münster, Germany
[9]Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[10]German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety
E-mail contact: wendel.wohlleben@basf.com

SUPPORTING INFORMATION

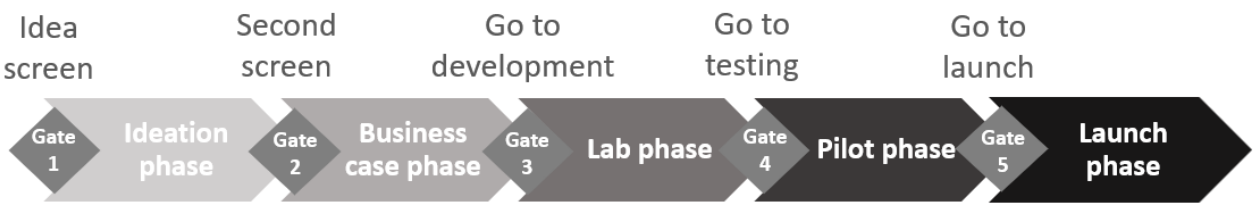

Figure SI_1: Portfolio management of innovation projects by the StageGate[1] process. Each gate serves as filter: The available innovation budget is thus assigned the most promising projects, whereas the majority of projects is stopped.

Additional background on section 2.2 “Data requirements for early phases of StageGate”:

The best practice in industry integrates criteria of safety and sustainability into the decisions to be made at each gate (Figure SI_1).[2] Technology readiness level (TRL) scale was originally defined by NASA as “a type of measurement system used to assess the maturity level of a particular technology. TRL scale uses the parameter that evaluates the maturity of a technology according to a series of indicators that go from 1 (the basic principles are documented) to 9 (the technology is released, and industrial production is started”.[3] The TRL scale was introduced in EU funded projects in 2012 and is currently the point of reference for determining the development or maturity of a research and its readiness for the market uptake and potential investments.[4] The initial TRL at ideation phase is typically 1 to 3, but may also be higher for incremental innovation. At the end of the lab phase phase, the TRL should have reached 5 to 6. By definition, a market launch (Figure SI_1) constitutes TRL 7 to 8.[4] At each gate, also the dimensions of the technical probability of success, the probability of commercial success, the raw materials sourcing, and the overall dimension of socio-economic assessment are typically supported by company-specific tiered tools, and in order to avoid overlap with existing tools, these dimensions are beyond scope of AMEA.

Considering that sustainability assessment is comparative in nature (and not absolute), the scarcity of data at low TRL plays a major role in the feasibility of any assessment framework. Data scarcity generally relates to one of the three following situations:

A. No data can be provided for a certain data requirement, because it is not technically possible at this innovation stage (e.g. due to an inadequately defined production process), or because the resources to gather the data would consume an inappropriate share of the R&D budget that can be sustained by the expected commercial value (ECV)[5] of the targeted product, application and region (P-A-R).[6].

B. Generic data was derived from sector-specific knowledge, or from grouping and read-across. This assigns - for a certain endpoint - *one* value to *all* versions of the AdMa, and allows an evaluation of the importance of certain SSbD dimensions, e.g. mapping against planetary boundaries,[7] and possibly a prioritization of testing in the next phase. However, this assessment cannot discriminate between AdMa versions, and thus provides no design target.

C. Specific data was measured or gathered for each specific version of the AdMa, and also for a corresponding CoMa. This allows to differentiate versions of the AdMa against each other and against the CoMa benchmark. This assessment will directly provide valuable design targets for the next iterations of the AdMa during R&D.

By selecting only the most relevant assessments at early innovation stages (Table 1, Figure 2), AMEA strives to build the assessment on C) specific data for each version of the AdMa.

Table SI_1: Case studies to exemplify the categorization by AMEA dimensions

Material	**Material consists of particles, or contains particles, or none thereof?**	**Material is a nanomaterial or nano-enabled, or none thereof?**	**Materials is conventional or advanced?**
Perovskite-oxides for exhaust catalysts	Consists of particles. Final application (in specific case: catalyst) contains particles.	Nano-enabled, but no nanomaterial: Median size is above the applicable regional (EU) regulatory definition, but performance depends on large surface	Advanced: rational design for qualitatively new functional performance of oxygen storage capacity *and* catalytic activity
TiO_2	Consists of particles. Final application (e.g. paint or cosmetics) contains particles.	Some grades are nanomaterials (Fig. SI_2). Some grades are no nanomaterials.	TiO_2 pigments (non-nano) and UV filters (nano) fulfill all CoMa indicators (Fig. 1). TiO_2 nanofibers and nanocubes are AdMa, due to targeted inherent structural features (Fig. SI_2) and rational design for other functional uses than pigments.
Quantum Dots	Consists of particles. Final application (in specific case: TV screen) contains particles.	Nanomaterial	Borderline: known since more than a decade, but the structural design of the specific case[8] is complex and large-scale commercial use is still recent and limited to few suppliers
Aerogel-fiber mats for façade insulation	Does not consist of particles, does not contain particles.	Nano-enabled, but no nanomaterial: Pore size is nanoscale, but internally porous materials are out of scope of the applicable regional (EU) regulatory definition. The enhanced thermal insulation (Knudsen effect) depends on nanopores.[9, 10]	Borderline. Inherent structural features are very different from CoMa (mineral wool or polymer foams), and the >10% enhanced performance is significant, but there are several established producers. However, market penetration is still limited to higher-cost niche applications.[11-13]

Details on the exemplary IATA-based hazard screening of CoMa and AdMa forms of TiO_2

The scope of the case study as explained in section 2.4 includes TiO_2 as white pigment and also nanoforms of TiO_2 serving as UV filter in transparent sunscreens, and finally also TiO_2 nanofibers and nanocubes with targeted structural features (Figure SI_2). Only the comparative hazard screening by new approach methods (NAM) as recommended by Table 1, specifically in chemico NAMs and in vitro NAMs, shall be exemplified in the following.

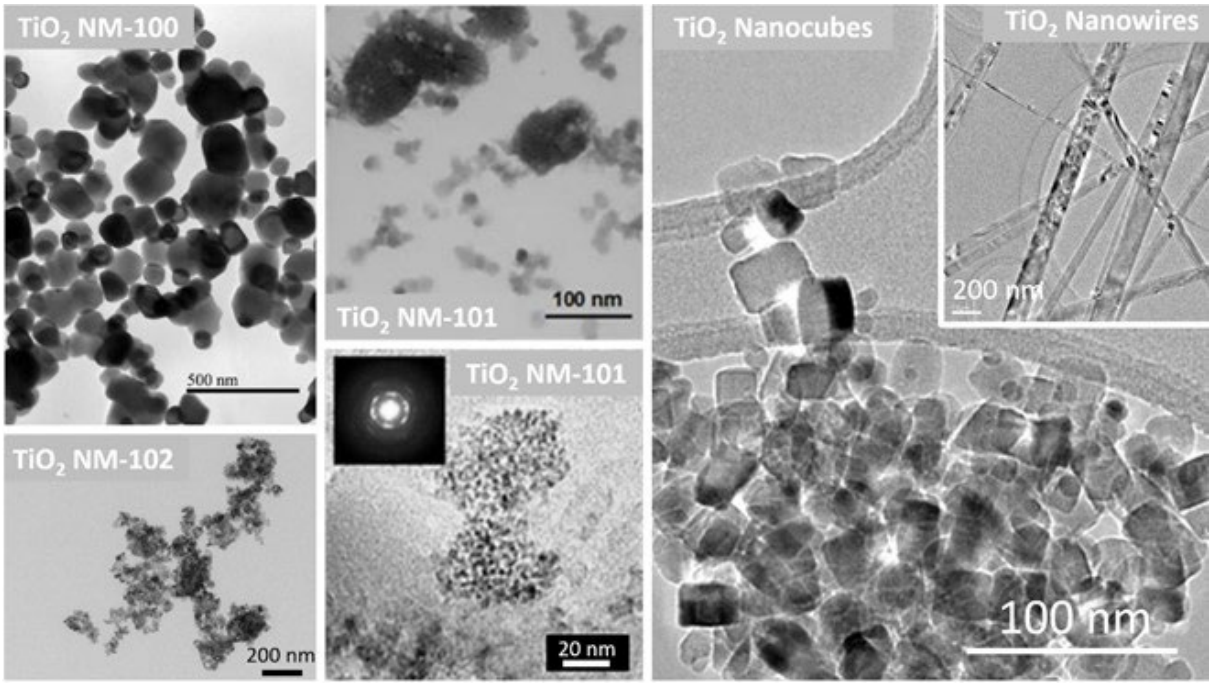

Figure SI_2 CoMa and AdMa forms of TiO_2 and results of their hazard screening a) Electron microscopic imaging shows qualitatively similar CoMa forms (NM-100, NM-101, NM-102) and targeted structural features of AdMa (nanocubes, nanowires). Partially reproduced from the JRC Report on TiO_2 nanomaterials;[14] image of the TiO_2 nanocubes was provided by Polona.umek@ijs.sl, image of TiO_2 nanowires was taken from the manufacturers website (http://www.novarials.com/ProductsTiONWsA.html, accessed 17.1.2022).

A suitable IATA exists and recommends testing of biodissolution, surface reactivity and in vitro macrophage interaction [15, 16]. Methods were applied as recommended by the IATA, and published earlier.[15, 17] By their composition and structure, all materials were within the applicability domain of the assays recommended by the IATA.

Nanowires were compared in two grades of purity: The industrial grade (IG) fibers differed from the research-grade (RG) fibers by a significantly higher dissolution rate (Table SI_2). This may also be related to Ti-containing impurities. The AdMa RG fiber, the AdMa cubes and two CoMa particle benchmarks (both from JRC) shared a very similar reactivity in surface dose metrics (Table SI_2). Measurements were performed in the dark, without UV activation. Differences in the mass-dose reactivity were within a factor 3, which is a cutoff for sufficient similarity in the ECETOC NanoApp, supporting the joint registration of different nanoforms [18, 19]. Accordingly, the differences between AdMa and CoMa in that range may also not be relevant for SSbD decisions.

The TiO_2_fibre RG, the cubes and two particle benchmarks (both from JRC) also shared a very slow dissolution rate (Table SI_2). Differences in the dissolution behavior were metrologically significant, but not biologically significant by the GRACIOUS criteria of similarity [17, 20, 21]. By the standardised detection of radical generation via electron paramagnetic resonance,[22] no significant reactivity was found on the TiO_2 fibers (data not shown), but reactivity of the JRC particles was significant and in similar ranges up to 3-fold above water blank [23]. The different shapes also differed in the specific surface area, necessitating a comparison both in mass metrics and in surface metrics. The surface metrics was proposed as more relevant to identify similar reaction mechanisms, i.e. qualitative similarity. The scaling with BET may then be used to justify a category with systematic trends between the better-known CoMa and the AdMa.[15]

Table SI_2 Results on both CoMa and AdMa forms of TiO_2 by IATA-based comparison of the extrinsic properties of reactivity and dissolution rate (in chemico NAM):

RG: research grade. IG: Industry grade; n.d.: not determined

Material	Shape	BET m^2/g	Reactivity sBOD nmol TEU/m^2	Dissolution rate ng/cm^2/h	Reactivity mBOD nmol TEU/mg	Dissolution halftime Days
			Surface dose metrics		Mass dose metrics	
TiO2_fibre RG	Fibre	9.46	4 ± 1	0.15 ± 0.023	0.04 ± 0.01	>2000
TiO2_fibre IG	Fibre	9.46	0 ± 3	1.08 ± 0.046	0 ± 0.03	282
TiO2_cube	Cube	88.18	8.5 ± 1.9	0.004 ± 0.001	0.4 ± 0.09	>2000
TiO2 NM105	Particle	51	13 ± 0	0.046 ± 0.005	0.6 ± 0.02	>1000
TiO2 NM102	Particle	80	8 ± 0.1	n.d.	0.6 ± 0.02	n.d.

Due to the rationale that a close contact is necessary to reveal possible form-dependent effects of nanomaterials, the bioactivity of these TiO_2 varieties was studied with phagocytic cells, namely alveolar macrophages (NR8383 cells) under serum-free conditions. All TiO_2 formed aggregates/agglomerates under cell culture conditions and/or settled to the bottom of the cell culture dish during a standardized exposure period of 16 h. This enabled their quantitative ingestion by a defined number of cell and led to a largely defined cellular burden within the cell collective, such that biological effects of the different TiO_2 qualities became directly comparable. Therefore, we used "mass per volume" as an appropriate dose metric for biologic effects which were reflected by the activity/concentration of lactate dehydrogenase (LDH), glucuronidase (GLU), tumor necrosis factor α (TNFα), and hydrogen peroxide (H_2O_2) in the cell culture medium. To simplify the evaluation, Figure SI_2 provides color-coded "in vitro LOAECs" calculated as statistically significant against vehicle-treated controls.

Name	Size/Shape from TEM Data	BET (m^2/g)	LOAECs LDH	LOAECs GLU	LOAECs H_2O_2	LOAECs TNFα	Crystal-linity	Shape
TiO2 nanowire 100/20 (RG)	Fiberes diameter 100nm. Length 20 µm	n.m.					Anatase	Fiber
TiO2 nanowire 100/20 (RG) *crushed*	Fiberes diameter 100nm. Length < 10 µm	n.m.						roughly spherical
TiO2 nanocubes	Aggregates , primary particle size: 10-40 nm (from TEM picture)	n.m.						
TiO2 NM-100	Aggregates size from 30-700 nm; primary particles: 50-90 nm (JRC Report)	9.2						
TiO2 NM-101	Aggregates Size from 10-170nm; primary particles: 6 nm (JRC Report)	316.1						
TiO2 NM-102	Crystallite < 50 nm. Aggregates 100-500 nm; prim. particles 21 ±10nm (JRC Report)	78						
TiO2 NM-103	Prim. particle size: 20-100nm (small elongated, prismatic), aggreates from 40-400nm (JRC Report)	50.8					Rutile	
TiO2 NM-104	Primary particle size: 8-200nm, aggreates from 20-500nm (JRC Report)	56.3						
TiO2 NM-105	Primary particle size: 10-34nm (small elongated, prismatic; agglomerate/aggreates with fractal structure (JRC Report)	46.2						

Figure SI_3 Bioactivity in a macrophage assay (in vitro NAM), which is recommended by an applicable IATA.[15, 24] Color coding of the in vitro Low Observed Adverse Effect Concentrations (LOAECs) for lactate dehydrogenase (LDH, glucuronidase (GLU), hydrogen peroxide (H_2O_2) and tumor necrosis factor α (TNFα)

in [µg/mL]: purple ≤22.5, red ≤ 45, orange ≤ 90, yellow ≤ 180, green> 180. Additional benchmarks of low reactivity (e.g. well-known $BaSO_4$ NM220 from JRC repository or corundum dust) and high reactivity (e.g. crystalline quartz) are applicable.[24] n.m.: see Table SI_2.

With respect to roughly spherical particles (Figure SI_2), main differences were found between anatase and rutile materials with respect to cytotoxicity (LDH and GLU), with anatase materials being less cytotoxic. Differences between small, medium and large BET surfaces of anatase materials (NM-100, NM-101, NM-102) were hardly found which may be due to the overall low activity and to a more or less uniform formation of agglomerates, visible at the bottom of the cell culture vessels (data not shown). TiO_2 nanocubes, despite their sharper edges (see Figure SI_2), exhibited no apparent bioactivity. Again, this material exhibited some degree of aggregation under cell culture conditions.

In contrast, the TiO_2 nanowires elicited pronounced effects in NR8383 cells and increased all four parameters at low concentrations (Figure SI_2). Because the length of this rigid fibers outscored the diameter of NR8383 macrophages (12-15 µm) frustrated phagocytosis was frequently seen to occur. This finding may provide the main SSbD design recommendation. Unfortunately the only available fibers by Novarials differed from the other AdMa and from the CoMa also by crystallinity, specified by the manufacturer as “The wetcake form of TiO_2 nanowires are in the form of H_2TiO_3, monoclinic crystal phase, it can be changed to anatase upon heating to around 550C.” The attribution of differences to either shape of crystallinity thus requires a further control experiment. We prepared crushed TiO_2 nanowires by extensive mortaring. This led to shortened TiO_2 nanowire fragments which were taken up completely by the macrophages without eliciting any frustrated phagocytosis. Of note, cytotoxicity (LDH) and TNFα formation were reduced by 50-75 %, while the release of glucuronidase and H_2O_2 were abolished (Figure SI_2).

Overall, the comparison of fully ingestible anatase TiO_2 NMs (spheres vs. cubes vs. crushed fiber fragments) showed that effects on the NR8383 alveolar macrophage model with its 4 parameters LDH, GLU, H_2O_2 and TNFα were comparatively low.

In contrast, the pronounced effects of the rigid TiO_2 nanofibers were attributable to their elongated form and not to crystallinity or other differences, as shown by experiments with crushed TiO_2 nanofibers. It may be suspected that there is a length-dependent increase in bioactivity and/or even a length-dependent threshold value, based on the discrepancy of fiber length and cell size.

In summary, the results confirmed pronounced effects of the rigid TiO_2 nanofiber AdMa and attributed them to the fiber shape. This finding alone does not necessarily exclude this part of the SSbD design space from further development, but the SSbD weighing of the dimension of occupational safety against the dimension of benefits during use would require important benefits for this material to be more sustainable than alternatives, and would still necessitate risk management measures. In contrast, the effects were not attributed to the crystallinity, thus leaving the SSbD design space open for crystallinity.

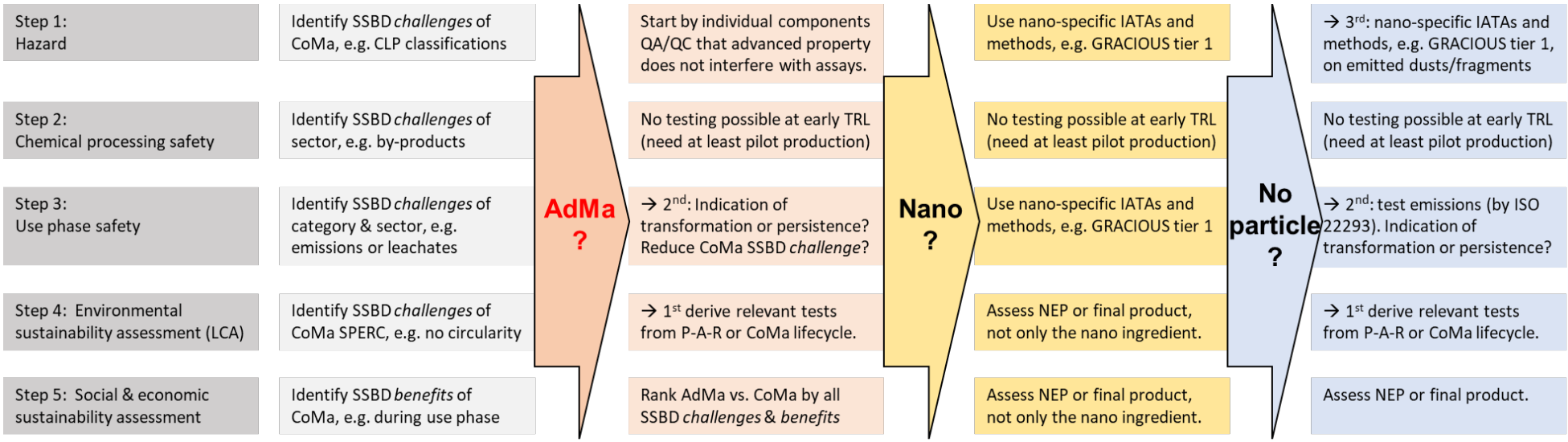

Figure SI_4: Mapping of elements of the AMEA guidance in Table 1 to the five "steps" of JRC's draft SSbD framework, focusing exclusively on low-TRL screenings in the ideation phase, business case phase, and lab phase. The implications of identification as advanced, multicomponent, nano or nano-enabled by AMEA are cumulative: a material that is AdMa and nano-enabled triggers both of the requirements or recommendations. As a result of the mapping, the numberings 1st, 2nd, 3rd indicate a logical order of steps that is flexibly adjusted to the case by the AMEA, and is thus often different from the fixed order of steps in the JRC draft SSbD framework.

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