The SAbyNA Platform: A Guidance Tool to support industry in the implementation of safe and sustainable by design concept for nanomaterials, processes and nano-enabled products

Cazzagon V.[1], Vanhauten R.[2], Hanlon J.[3,4], Sánchez Jiménez A.[3,5], Harrison S.[6], Auffan M.[7], Braakhuis H.[8], Boyles M.[3,9], Candalija A.[1], Katsumiti A.[10], Rodriguez-Llopis I.[10], Catalan J.[11,12], Cross R. K.[6], Lahive E.[6], Morel E.[6], Simeone F. [13], Delpivo C.[1], Clavaguera S.[14], Seddon R.[15], Salmatonidis A.[1], Barruetabeña L.[10], Traas L.[2], Lotti D.[16], Mays C.[17], Vazquez-Campos S.[1]*

Supporting Information

SI 1. Examples of Usability Cards included in the guidance module of the Platform.

RNF/NEPSbD 12	**Cytotoxicity of nanomaterials applicable in restoration and conservation**		
Scope / abstract: In this pilot study, we compared the toxic potential of representatives of three of the most common oxide materials applicable in restoration: TiO_2 (standard and purified P25, a mixture of prevailing anatase with rutile crystalline modifications), SiO_2 (bare A200, and R805, R9200 as coated forms of A200), and ZnO. Using two in vitro cytotoxicity assays, WST-1 and LDH, evaluating metabolic activity and cell membrane integrity, respectively, we preliminary ranked the tested substances according to their cytotoxic potential, which may be used for their prioritization for further testing and applications. After 24h exposure, a dose-dependent decrease in cell viability was only detected in ZnO NPs and uncoated silica (A200). Hydrophobic coated silicas (R805 and R9200) and TiO_2 NPs (purified and unpurified P25) did not exhibit cytotoxic effects up to the highest tested concentration of 250 µg/mL. Toxicological data related to the physico-chemical characteristics will be applicable in developing both more efficient and safer nano-based products for restoration and conservation.			
NFs chemistry: **SiO_2**	*Mechanism of concern:* **Surface reactivity**	*Key physico-chemical property for risk:* **Surface chemistry**	*Safe by design strategy apply:* **Coating**
Main content: In this document the authors work on silica NPs, trying to reduce cytotoxicity of silica NMs. In fact, silanol groups on the surface of bare silica are involved in ROS generation and can cause cytoxicity. The results showed that hydrophobic coating, as $-CH_3(CH_2)_7$ and $-CH_3$, prevents cytoxicity of silica NPs, which may be related to decreased abundance of surface silanol group and reactivity. The main limitation can be associated to the final functional property and application.			
Recommendations for use: (e.g. link to other resources)		*Conference Paper (free)*	
Source : ISBN 978-80-87294-89-5		**Nanocon 2018 - Proceedings 10th International Conference on Nanomaterials - Research & Application**	

Figure 1 SI1a. Example of Usability Card for SbD interventions for safer nanoproducts

SAbyNA RPSbD003	EN 1093-11:2001+A1:2008 Safety of machinery - Evaluation of the emission of airborne hazardous substances - Part 11: Decontamination index		
This standard describes a method for the measurement of the decontamination index of pollution control systems e. g. capture devices including local exhaust ventilation, water spray systems and, when appropriate, separation equipment installed on a machine. This method uses the real pollutant (EN 1093-1: 1998) and can be operated in room or field environments.			
Design topic	***Design strategy***	***Type of action***	***Level***
Emissions of hazardous materials and substances	*Emissions verification*	*Risk management measures (i.e. implementation of control measures)*	*Advanced*
Main content (extracted from EN 1093-11): The **decontamination index** is defined as the average of the ratios, obtained at a number of specified locations in the surroundings, of the ambient air quality improvement to the real pollutant mean concentration with the pollution control system not in operation. The principle of this measurement method consists in determining the **decontamination index**, the concentrations being measured at predetermined points around the machinery under inspection and in interpreting the value of this index, taking into account its range of variation and the influencing factors. Corrections can be necessary to take into account of air pollution caused by other operations ("the background level"). When particle size distribution is determined at the same time as pollutant concentration, a decontamination index for each size fraction can be determined (see for example EN 481 "Workplace atmospheres - Size fraction definitions for measurement of airborne particles"). The document provides guidance on the principle of the method, the determination of the concentration measurement points, the test method, the application to a specific group of machines, the influencing factors, the expression of results and the test report.			
Harmonized standard. Compliance with the normative clauses of this standard - within the limits of the scope - confers a presumption of conformity with the corresponding essential requirement 1.5.13.- Emissions of hazardous materials and substances of the EU Directive 2006/42/EC on Machinery, and associated EFTA regulations. Presumption of conformity stays valid only as long as a reference to this European standard is maintained in the list published in the Official Journal of the European Union.			*Standard* *Not nano-specific*
Source: https://standards.cen.eu/dyn/www/f?p=204:110:0::::FSP_PROJECT,FSP_ORG_ID:31115,6096&cs=1E6A640EA68CB94627A18B800863AEBDC			**CEN**

Figure 1 SI1b. Example of Usability Card for SbD interventions for safer processes.

SI2. GUIDEnano tool and Gracious blueprint

The SAbyNA Platform has been built up by making use of existing resources that could either be reimplemented into the tool or adapted to the purpose of SSbD. The key resources reused are the GUIDEnano risk assessment tool (https://tool.guidenano.eu/) developed in the H2020 GUIDEnano project (G.A. 604387) and the GRACIOUS blueprint from the GRACIOUS project (G.A. 760840).

The GUIDEnano tool is a nano specific risk assessment tool assessing human health and environmental risks along the entire life cycle of a NEP. It is primarily intended to support (regulatory) risk assessment of existing NFs and NEPs at the end of the design stage of the product development or when the NEP is already on the market. For this reason, its hazard assessment module is setting with the aim to use in-vivo hazard data and therefore not suited for early design stage hazard screening required for SSbD purposes. Nevertheless, GUIDEnano has been used to provide the core software architecture of the SAbyNA tool and a number of knowledge modules which have been reimplemented and further improved in SAbyNA such as the material and activity modules as well as the kinetic fate model to predict mass and particle concentrations over time in different connected indoor and outdoor compartments as input for both human and environmental exposure assessment of NFs. The GRACIOUS blueprint is a PDF document automatically generated from an operational test environment of the GRACIOUS grouping and read-across framework developed by the GRACIOUS project. Actually, the core-architecture of the GRACIOUS blueprint was also derived and adapted from GUIDEnano and new functionalities to support nanoform grouping, similarity assessment and alignment with ECHA use description were introduced. Also, a descriptor framework was developed to improve unique identification of data endpoints, assays, etc. to improve correct data mapping and exchange. This descriptor framework was used to map existing project data provided by eNanomapper ambit instances. Most functionalities of the GRACIOUS blueprint have been re-implemented and improved in the SAbyNA platform such as the forementioned data analysis section but also the IATA support framework which supported the integration of the SAbyNA developed hazard testing strategy for SbD.

SI3. Basis for the Sustainability and costs analysis model

The Sustainability and cost model provides background information in order to fill data gaps in the assessment of NEPs, even at early stage of development, covering multiple aspects.

At the inventory phase, default values are provided to evaluate the environmental implications of the processes that take place during the life cycle. For example, inventory data to evaluate different additive manufacturing processes have been gathered through an extensive literature review, including energy consumption and material loss.

Table 1 SI3 shows an extract of the information in the Sustainability and Cost model for the Additive Manufacturing (AM) processes.

Table 1 SI3. Example of energy consumption data gathered for a additive manufacturing processes

AM processes	Min (MJ/kg)	Max (MJ/kg)	Average (MJ/kg)	Reference
Stereolithography (SL), Polymer	13.9	41.4	27.1	(Kellens et al., 2017; Malshe et al., 2015)
Selective Laser Melting (SLM), Steel	15.5	163. 3	54.4	(Baumers et al., 2011; Kellens et al., 2017, 2011; Kokare et al., 2023; Peng et al., 2020; Priarone and Ingarao, 2017)
Selective Laser Melting (SLM), Al	85.9	169.2	128.2	(Faludi et al., 2017; Jiang et al., 2022; Kellens et al., 2017, 2011; Kokare et al., 2023; Ma et al., 2021; Peng et al., 2021; Priarone et al., 2018)
Selective Laser Sintering (SLS), Polymer	94,68	144.3	122.6	(Baumers et al., 2011; Kellens et al., 2017, 2011; Kokare et al., 2023; Kwon et al., 2020)
Electron Beam Melting (EBM), Ti alloys	59.9	399.5	164.9	(Baumers et al., 2011; Ingarao and Priarone, 2020; Kellens et al., 2017; Kokare et al., 2023; Le and Paris, 2018; Lyons et al., 2021)
Fused deposition modelling (FDM/FFF), PLA	9.5	83.2	39.7	(Enemuoh et al., 2021; Hopkins et al., 2021; Kokare et al., 2023; Ma et al., 2021; Napolitano et al., 2022; Ulkir, 2023; Zakaria et al., 2022)
Fused deposition modelling (FDM/FFF), ABS, PC	11.2	174.2	55.3	(Bezzina and Refalo, 2023; Garcia et al., 2021; Hernandez Korner et al., 2024; Kellens et al., 2011;

				Kokare et al., 2023; Ulkir, 2023; Yosofi et al., 2018)

The material loss ratio (waste and emissions to air) in different process steps is used within the model to calculate the mass flow over the production steps. By incorporating calculations based on these parameters, it is possible to perform a preliminary evaluation based on limited information (materials entering the manufacturing phase and definition of the process steps involved).

A similar strategy has been implemented in the use and end of life phases, adding Transfer Coefficients to calculate the flow of materials along these phases, leading to the estimation of materials released during the use phase (e.g., weathering), arriving to the end-of-life treatment installations, or emitted during the waste treatment (e.g., nanomaterials released to air).

Figure SI2 SI3 represents the concept applied for the calculation of the material flow over the production steps in the module developed for the Additive Manufacturing sector. The material inputs and outputs in pre-processing and post-processing operations are calculated from the material input in the manufacturing phase, considering the default values in the module. However, it is also possible to customize these values when specific data is available.

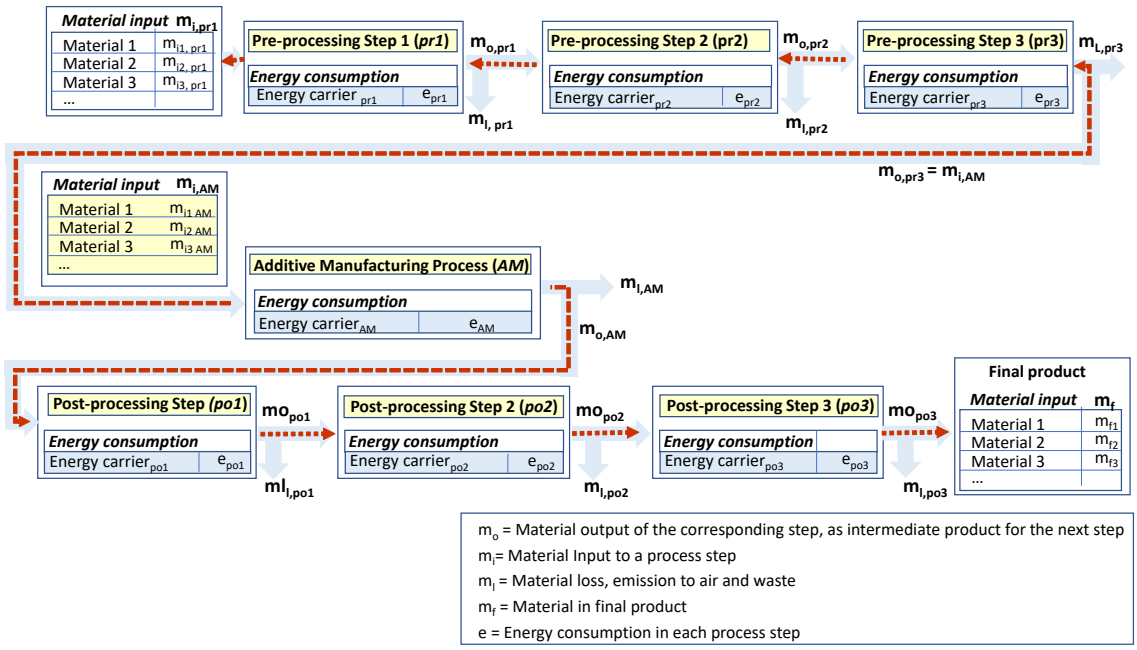


Figure 2 SI3. Material and energy inputs and outputs in the production phase within SAbyNA Additive Manufacturing Cost and Sustainability Model (pre= pre-processing step, AM= Additive Manufacturing process, post= post-processing step). Elements in yellow show the minimum data to be provided in the model, and elements in blue show the data that can be derived from the background data in the module or can be customized. Discontinuous red arrows represent the direction of the calculations of material inputs and outputs based on the data provided by the user and the default values of the tool.

SI4. Hazard categories used in the screening assessment of the Platform

The hazard information related to the CLP classification is contained in an internal database and comprises intrinsic hazard properties relevant to human and environmental health, as well as physical hazards. The Platform prioritises information retrieved from the Harmonised classification in Annex VI of CLP from the Classification and Labelling inventory, followed by information from Non-harmonised CLP Self-classification from REACH Registration Dossiers. It also contains information from the WHO report Lee et al., 2017 containing nano specific hazard labels. Additional hazards from the Candidate List of Substances of Very High Concern (SVHC) are also considered (https://echa.europa.eu/candidate-list-table). Classification for endocrine disruption (ED), PBT (Persistent Bio accumulative and Toxic), and PMT (Persistent Mobile and Toxic) are also considered although very limited information is available as they were only recently added as EU hazard labels (https://echa.europa.eu/new-hazard-classes-2023). Whenever available, the hazard data contained in the database refers to the NF, although this is not possible in many cases as the REACH registration of NMs or sets of similar NFs has only been mandatory for a few years. When NF-specific information for all the CLP categories is available and indicates no hazard classification, the NF raises a green flag indicating low concern. If data is missing for any of the hazard categories or it refers to the bulk form instead of to the NF, a precautionary orange flag is raised suggesting assessing the NF and its application more thoroughly. Whenever the NF classifies for any of the hazard categories, the platform retrieves that information allowing its classification into the criterion H1 (substances of very high concern), criterion H2 (substances of concern), and H3 (substances of low concern) categories defined in the JRC SSbD framework and reported in the SI3. According to the JRC SSbD framework (Caldeira et al., 2022), materials fulfilling the H1 criterion should be prioritised for substitution and/or re-design, the ones falling into the H2 criterion would be advised to substitute or re-design and to control the emissions/exposure, and the NF into the H3 criterion should reduce the toxic effects and ensure the safety along life cycle.

Table 2 SI4. Output given by the SAbyNA tool based on CLP classifications.

Red	Classified, add category
MISS	Missing information
Green	Data conclusive, no classification required

According to the JRC SSbD framework, hazard information for all endpoints should be complete and classify as green. In practice, this is never the case for NMs (yet). Many NMs are not produced in large amounts, and therefore REACH registration does not require hazard information on all endpoints. According to the JRC SSbD framework, data unavailability due to a low tonnage band is not a valid point for data waiving. Furthermore, there may only be information available for the bulk form of the core composition of the NM under investigation. This may falsely raise green flags for hazard of the NM. REACH registration of NMs or sets of similar NFs has only been mandatory for a few years. Therefore, it is to be expected that nano specific hazard classification will become more readily available in the future. In the meantime, the following assumptions are made by the SAbyNA tool to interpret CLP classifications of the bulk form:

- Green flag for bulk -> NM might still be harmful -> consider as orange, missing information

- Red flag for bulk -> red flag can be transferred to NM -> red flag
- Unknown whether data is from nano or bulk -> assume data represents bulk.

In some cases, missing information can be justified and therefore the data need can be waived. According to the JRC SSbD framework, data gaps can be justified with the right explanation. They list some chemical-specific examples. Here are some NM-specific examples:
-It is very unlikely that a NM is hazardous to the ozone layer. If they are not listed on the list of ozone-depleting substances (Annex I to Regulation 1005/2009), this justifies the data gap and gives a green flag for this endpoint.
-Any hazard endpoints related to gases can be waived.

Based on the three CLP hazard tables (human, environmental, physical hazards), the tool produces the following decisions:

- All GREEN for nanoform -> sufficient evidence, no concerns for hazard
- One or more ORANGE (nano-specific data missing) -> Potential concern, go to detailed hazard assessment to gather/generate data for exposure routes of concern.
- One or more RED -> SbD intervention required (reduce hazard or exposure or both), define hazard category to classify into criterion H1, H2 or H3 (Table 3 SI4).

Table 3 SI4. Hazard criterion definition according to JRC SSbD framework, hazard categories included in each criterion for human, environmental and physical hazard, and advise to user in the SAbyNA platform

Hazard criterion	Criterion definition	Hazard categories			Advice to user
		Human health hazards	Environmental hazards	Physical hazards	
H1	Substances of very high concern	Carcinogenicity Cat. 1A and 1B Germ cell mutagenicity Cat. 1A and 1B Reproductive / developmental toxicity Cat. 1A and 1B Endocrine disruption Cat. 1 (human health) Respiratory sensitisation Cat 1 Specific target organ toxicity - repeated exposure (STOT-RE) Cat. 1, including immunotoxicity and neurotoxicity	Persistent, bioaccumulative and toxic / very persistent and very bioaccumulative (PBT/vPvB) Persistent, mobile and toxic / very persistent and mobile (PMT/vPvM) Endocrine disruption Cat. 1 (environment)	-	Prioritise substitution/ Re-design
H2	Substances of concern	Skin sensitisation Cat 1 Carcinogenicity Cat. 2 Germ cell mutagenicity Cat. 2 Reproductive / developmental toxicity Cat. 2 Specific target organ toxicity - repeated exposure (STOT-RE) Cat. 2 Specific target organ toxicity - single exposure (STOT-SE) Cat. 1 and 2 Endocrine disruption Cat. 2 (human health)	Hazardous for the ozone layer Chronic environmental toxicity (chronic aquatic toxicity) Endocrine disruption Cat. 2 (environment)	-	Substitution/ Re-design/ Control emission-exposure

H3	Substances of low concern	Acute toxicity Skin corrosion Skin irritation Serious eye damage/eye irritation Aspiration hazard (Cat. 1) Specific target organ toxicity - single exposure (STOT-SE) Cat. 3	Acute environmental toxicity (acute aquatic toxicity)	Explosives Flammable gases, liquids and solids Aerosols Oxidising gases, liquids, solids Gases under pressure Self-reactive Pyrophoric liquids, solid Self-heating In contact with water emits flammable gas Organic peroxides Corrosivity Desensitised explosives	Reduce toxic effects/ Ensure safety along life cycle

SI5. Physico-chemical and technical function data used in the screening assessment of the PC-SWCNT

Screening data used in the Platform:

- Nanoform introduction: SWCNT (TUBALL™ MATRIX 822, OCSiAl)
- Technical functionality: antistatic agent

Intrinsic physicochemical properties of the considered nanoform

- Type of nanoform: NM_SWCNT (applied mask)
- Nanoform composition: core: C, impurity: metalic
- Crystallinity: monocrystalline
- Morphology: elongated 100%
- Size: median diameter: 1.6nm, median length: 6000nm
- Specific surface area 300 m2/g

Extrinsic properties and characteristics of the considered nanoform

- Life cycle use and route of exposure/ emission routes: inhalation, dermal, soil, water, air, wastewater
- Dustiness: 1660 mg/kg

SI6. Functionality evaluations of the produced NEPs

The technical function of the PC-SWCNTs nanocomposites was to provide improved conductivity for ATEX environments. This has been tested at LATI facilities on printed parts using samples printed at different temperatures. The conductivity measurements were performed using an insulation resistance tester (applied voltage=100 V). The summary of the results indicates that the application of a SbD strategy that involves the reduction of the nozzle temperature resulted only in minor deterioration of the conductivity of the 3D-printed objects. Nevertheless, the final product still maintained antistatic properties (Table 4 SI6), which may lead to the conclusion that a product with sufficient technical functionalities may be produced in a safer (lower emissions in terms of particle number concentration) and sustainable (lower energy consumption) way by tuning specific process parameters.

Table 4 SI6. Resistivity measurement results on the 3D-printed products

	Before SbD	**After SbD strategy**		
	PC-SWCNT.290	PC-SWCNT.270	PC-CNT.270-50%	PC-SWCNT.250
Resistivity (Ohm)	10^8	10^9	10^9	10^9

However, these results represent preliminary resistivity evaluation of a 3D printing object for its specific application. Indeed, the producer of equipment or systems intended for use in potentially explosive atmosphere should follow DIRECTIVE 2014/34/EU (http://data.europa.eu/eli/dir/2014/34/oj, https://ec.europa.eu/docsroom/documents/52840/attachments/1/translations/en/renditions/native). An equipment should meet several requirements, and it is not clearly defined a simple threshold of surface resistivity for the material that would make the equipment acceptable or not. Plastic materials are considered as potential source of electrical discharge over 10^9-10^{12} Ohm, and different plastic suppliers -referring also to different norms and type of tests- may consider different surface resistivity thresholds. For LATI partners, values up to 10^{10} Ohm are acceptable, so all the results obtained in the case studies are considered acceptable. However, some of the results are “borderline” and only the manufacturer of the final equipment should in “real life” prove that the equipment is safe for use in ATEX applications.

SI7. Results obtained from the Platform after the addition of functionality data of PC-SWCNT case study

Here below in figure 3 SI7 the results obtained from the Platform once performance data are added for each SbD alternative: 100% functionality for the PC-SWCNT and 90% for all the other NEPs (i.e., PC-SWCNT.270, PC-SWCNT.250, PC-SWCNT.270-50%).

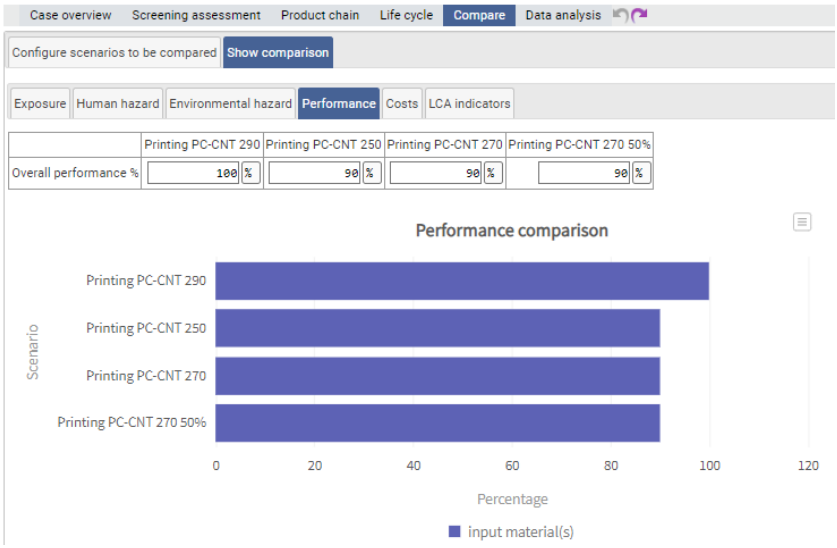


Figure 3 SI7. Comparison of the functionality evaluation results between SbD alternatives of PC-SWCNT.

SI 8 Sustainability and costs analysis inputs for the PC+SWCNT case study

It is assumed that the piece manufactured is used indoor, without exposure to weathering. The end-of-life scenario has been built considering a share of landfill, incineration and mechanical recycling processes. In thermal treatment processes (e.g., incineration with energy recovery) nanostructure destruction point is expected to be achieved (Ounoughene et al., 2017), leading to low SWCNT release, as established in previous literature (Bouillard et al., 2013; Holder et al., 2013; Organisation for Economic Co-operation and Development (OECD)-Working Party of Resource Productivity and Waste, 2015, p. 201).

Establishing a direct correlation between process temperature and energy consumption in FFF processes is a complex issue, given that the energy demand is determined by multiple parameters such as nozzle temperature, bed temperature, infill, characteristics of the machine, etc. (Bezzina and Refalo, 2023; Hopkins et al., 2021; Vidakis et al., 2023). However, the influence of nozzle temperature and bed temperature in the energy consumption is clear (Hopkins et al., 2021; Le Gentil et al., 2024; Napolitano et al., 2022). When printing a high-temperature material, the energy fractions for the nozzle and heated bed will increase, although the effect of other parameters must also be considered (Hopkins et al., 2021). In this analysis, in order to test the usability of the SAbyNA cost and sustainability case study, the potential reduction in energy consumption has been considered, linked to the lower nozzle temperature achievable when extruding a polymeric matrix with SWCNT through FDM. Although at this stage this data has not been empirically validated, different scenarios have been built, in order to check the potential influence of this reduction in the overall environmental profile. In this context, 3 scenarios have been modelled: the base scenario, a second scenario with a 5% reduction in energy consumption, and a third scenario with a 10% reduction in energy consumption.

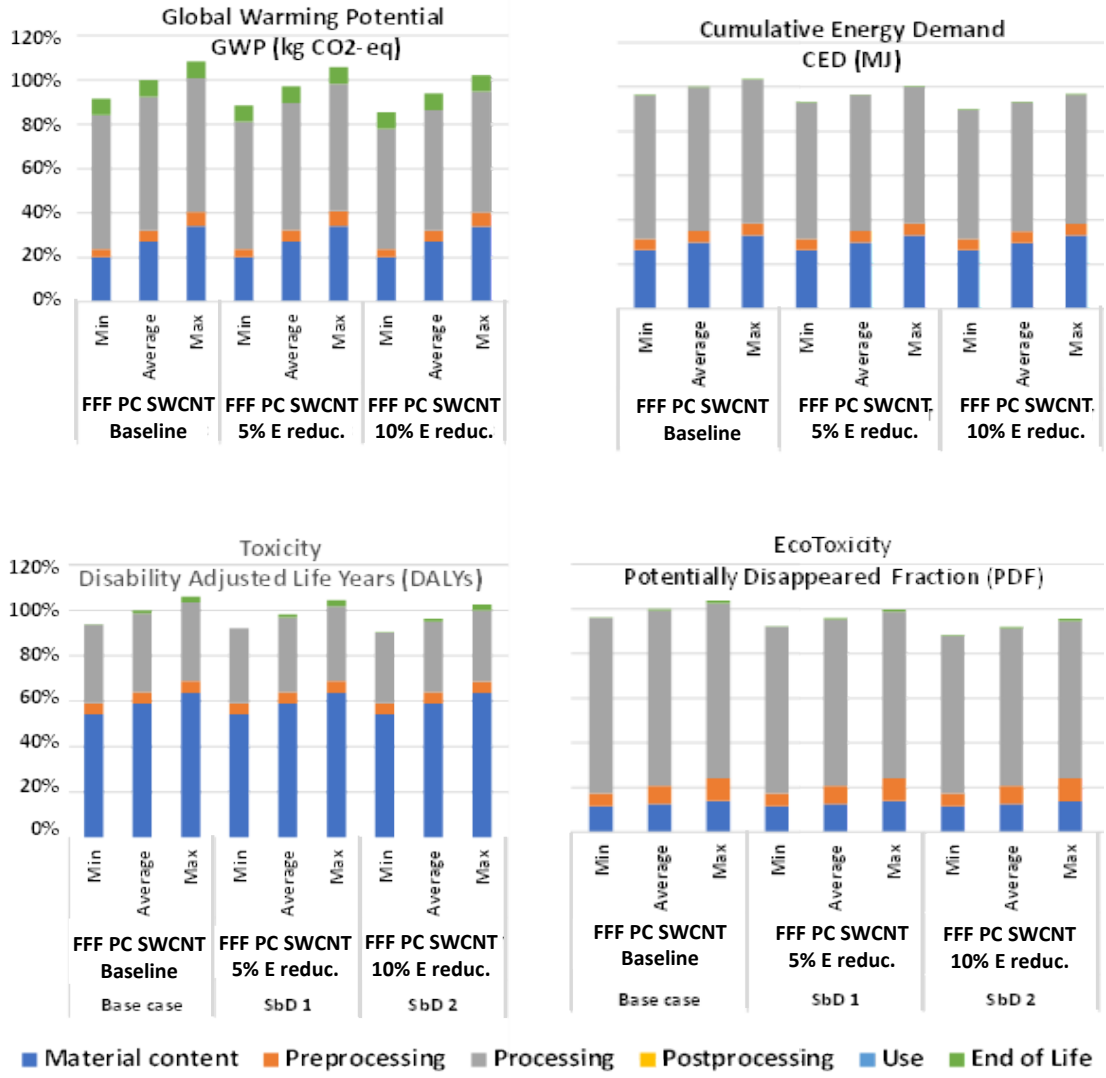


Figure 4 SI8. Results obtained of each of the four impact categories considered in the simplified LCA tool for the different SbD alternatives and the baseline case.

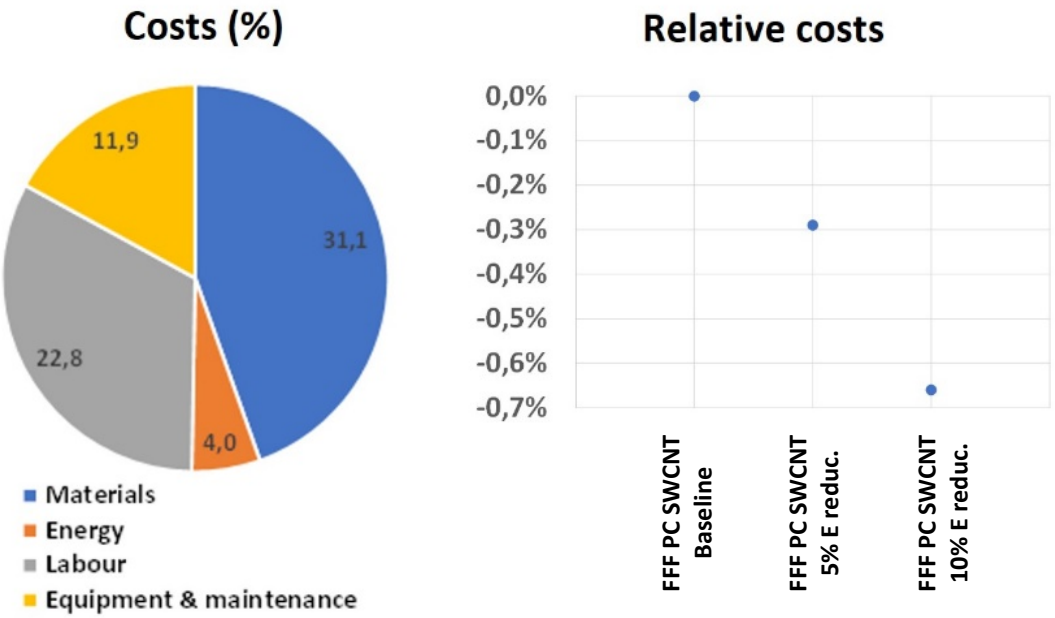
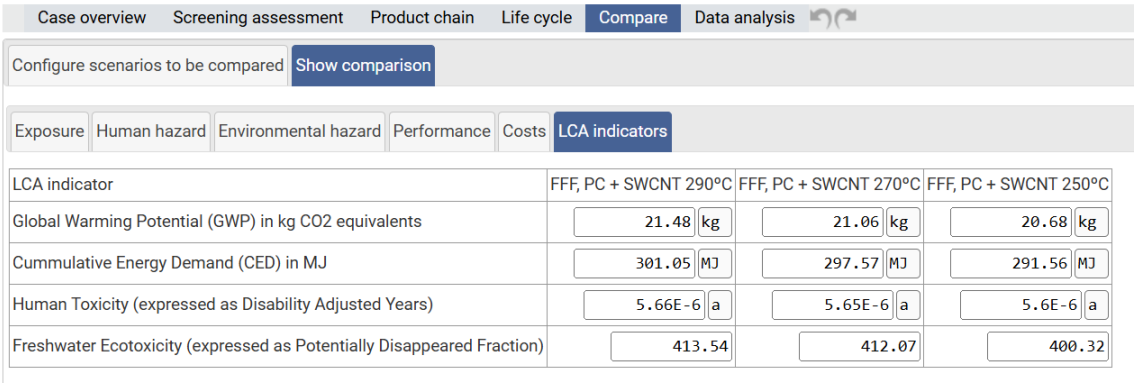


Figure 5 SI8. Costs (in %) for each category and relative costs (adimensional) considering PC-SWCNT.290 as baseline material.

Here below the results obtained from the Platform once performance data are added for each impact category and each SbD alternative.



LCA indicator	FFF, PC + SWCNT 290°C	FFF, PC + SWCNT 270°C	FFF, PC + SWCNT 250°C
Global Warming Potential (GWP) in kg CO2 equivalents	21.48 kg	21.06 kg	20.68 kg
Cummulative Energy Demand (CED) in MJ	301.05 MJ	297.57 MJ	291.56 MJ
Human Toxicity (expressed as Disability Adjusted Years)	5.66E-6 a	5.65E-6 a	5.6E-6 a
Freshwater Ecotoxicity (expressed as Potentially Disappeared Fraction)	413.54	412.07	400.32

Figure 6 SI8. LCA results obtained in the “Cost and sustainability assessment” section and manually added in the “Compare” section for the PC-SWCNT before and after the implementation of the SSbD strategies.

SI9. Particles emission monitoring at the manufacturing site

A monitoring campaign has been performed at the manufacturing site (LEITAT-3D HUB) by monitoring particles concentration emitted during the 3D printing process using the enclosed machine INTAMSYS to produce the NEPs.

The main focus was to study the effect of the adoption of the SbD interventions (i.e., variation of the process parameters: nozzle temperature and infill density) on the emissions during the 3D-printing of the NEP.

Table 5 SI9 shows the instruments used in the different monitoring locations. Two DiSCminis were placed in the two different monitoring locations to collect comparable results. NanoScan was used in the emissions source to provide timely resolved size distributions, while with SIOUTAS impactor size-segregated aerosols were collected on Teflon filters for their offline chemical analysis (ICP-MS). Also, samples were collected with SKC cassettes that were housing TEM grids for their offline electron microscopy analysis.

Table 5 SI9. Instruments used to measure particles emitted at the different locations.

Emission source	Worker area	Size/sampling range
DiSCmini	DiSCmini	10-700 nm
NanoScan-SMPS	10-420 nm	NanoScan-SMPS
SIOUTAS impactor	Cut-off: 0.25, 0.5, 1.0, 2.5 µm	SIOUTAS impactor
Sampling cassettes (TEM grid)	Total suspended particles	Sampling cassettes (TEM grid)

The average particle number concentrations of emissions are presented in Figure 7 SI9, where a clear and straight forward effect was observed: with increased nozzle temperature the emissions in terms of particle number concentration were also increased. The highest emissions were monitored with the nozzle temperatures of 290°C, reaching a concentration above 106 particles/cm3 (PC-CNTs: 3.84E+06 cm-3; PC: 1.90E+06 cm-3). A twenty degrees reduction of the nozzle temperature at 270°C led to a reduction of almost one order of magnitude of the emitted particle number concentrations (PC-CNTs: 3.80E+05 cm-3, PC: 1.74E+05 cm-3). Further decrease of nozzle temperature to 250°C additionally reduced the emitted concentration by a factor of two during the 3D-printing of PC-CNTs filament (1.76E+05 cm-3) and above one order of magnitude for the PC filament (6.22E+03 cm-3).

Considering the changing on the second process parameter, the 50% reduction of the infill density led to a reduction by a factor of two in particles emitted (LI_PC-CNTs_270: 1.86E+05 cm-3) when compared to the 100% infill density (PC-CNTs_270: 3.80E+05 cm-3).

In addition, a clear difference between non-activity (background) and 3D-printing processes can be observed, indicating the 3D-printing process as the source of emissions.

The size distributions of the emissions are shown in the part below of Figure 7 SI9 for the different nozzle temperatures. It can be observed that the distribution patterns are similar for the same temperatures of the different filaments (conventional with dashed lines; NEPs with continuous lines), while the concentration intensity is always higher for the NEP filaments in comparison to their conventional counterparts for the same nozzle temperature. The former in

combination with the average emissions in terms of particle number might be an indication that the main driver of release is the nozzle temperature.

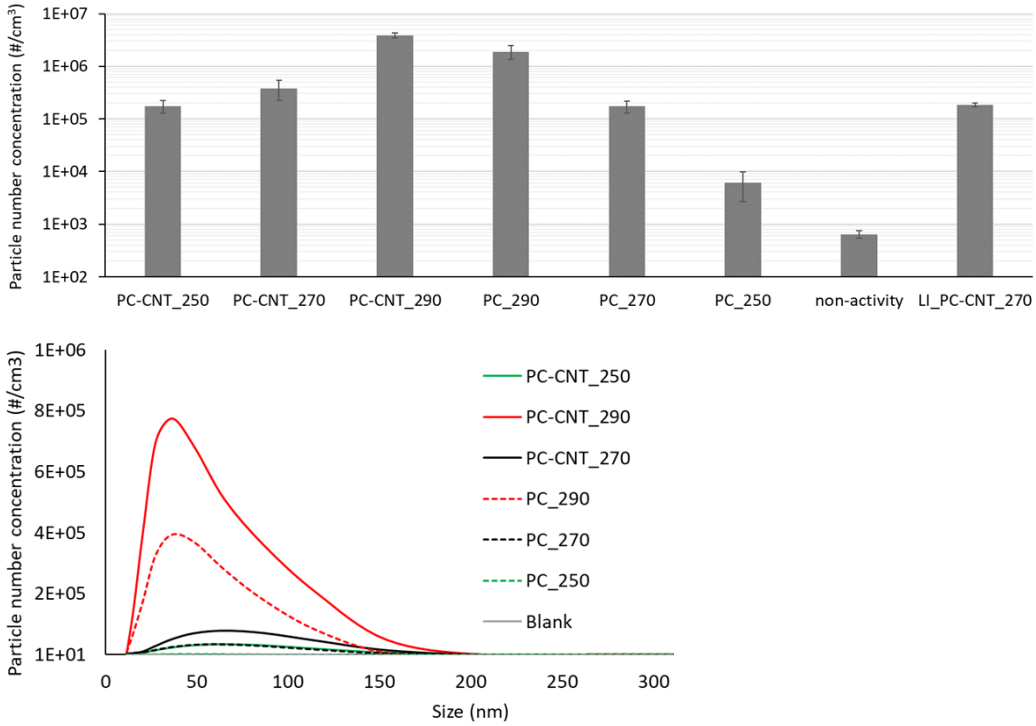


Figure 7 SI9. Average particle number concentrations (above) and particle size distribution (below) of the emissions in function of nozzle temperature variations and type of PC-based filaments.

Emitted aerosols have been sampled at the emission sources of the INTAMSYS 3D printer both directly on TEM grids for their offline morphological analysis (TEM/EDX) and on filters by cascade impactors for their offline size-segregated chemical analysis by ICP-MS of Fe impurities as indicator of the presence of SWCNTs. In addition, since SWCNTs are rather challenging to get identified via chemical analysis due to the high carbon background, in order to find an appropriate trace element for their identification, the pristine SWCNTs were characterized as a reference. It has been found that the pristine SWCNTs have a relatively high iron content (124054 ppm of Fe), while TEM analysis shown the presence of Fe impurities on clumps of agglomerated nanotubes with a primary diameter of approximately 2 nm (Figure 8 SI9). Hence, Fe was selected as the trace element of SWCNTs for the release studies and emissions characterization.

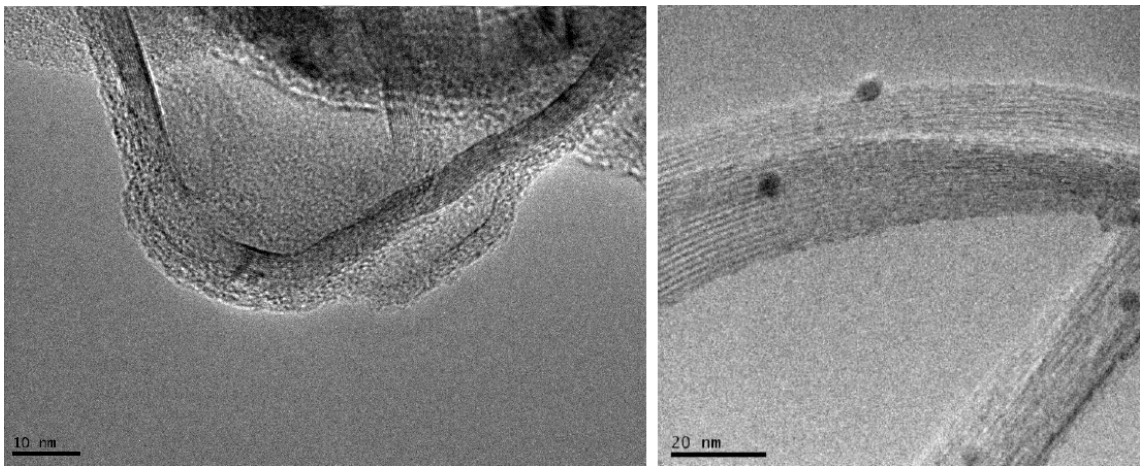


Figure 8 SI9. TEM images of airborne particles sampled during 3D-printing (left) and pristine SWCNTs (right).

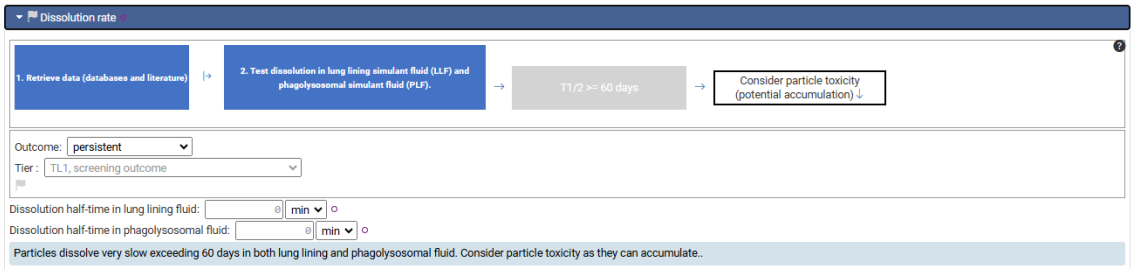
Results of TEM analysis of the aerosols released during the application of PC-CNTs filaments with the INTAMSYS machine, fiber-like structures have been identified that were protruding from larger particles (probably of polymeric-matrix composition). However, Fe were below the LoD of the ICP-MS of the filters used in air monitoring. Based on these results it may be concluded that the emissions of nanometric particles are not driven by the content of SWCNT, but the emitted aerosols are mainly process-generated.

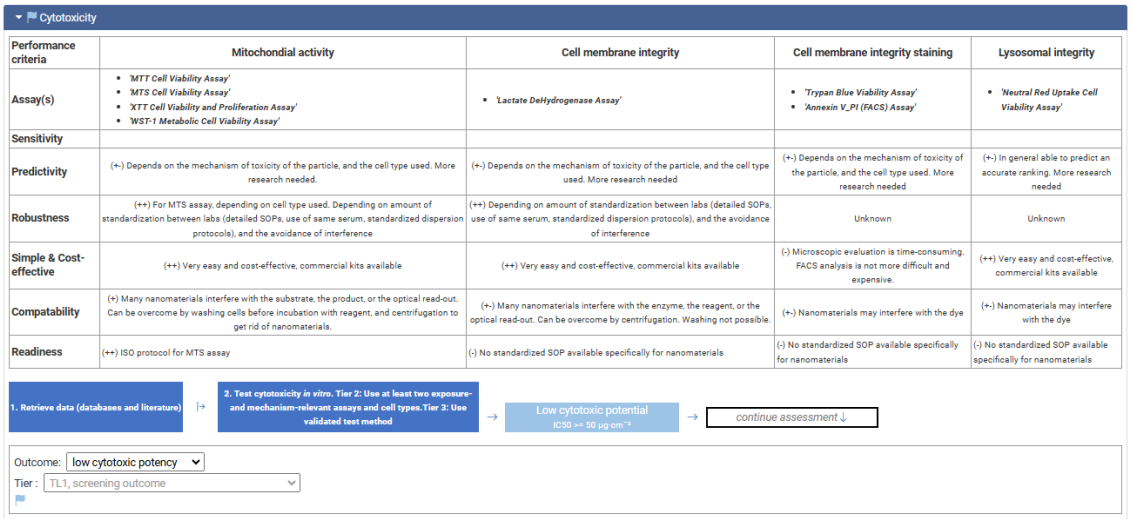
To perform air monitoring measurements, the user can find indications on how to characterise contaminant releases from unextruded plastics during fused filament fabrication 3-D printing using for example the following Usability Cards that can be found in the "SbD interventions towards Safer Processes" section of the Platform:

- "Three-dimensional printing with nano-enabled filaments releases polymer particles containing carbon nanotubes into air (2018 NIOSH)"
- "Three-dimensional printer emissions and employee exposures to ultrafine particles during the printing of thermoplastic filaments containing carbon nanotubes or carbon nanofibers (2020 NIOSH)"
- "Towards sustainable additive manufacturing: The need for awareness of particle and vapor releases during polymer recycling, making filament, and fused filament fabrication 3-D printing (2022 NIOSH)".

SI10. Detailed hazard assessment of PC-SWCNT

Results obtained from experimental tests performed in PC filaments and PC-SWCNT and literature review from SWCNT are reported through an IATA hazard strategy, using the following endpoints: dissolution, cytotoxicity, genotoxicity, ROS production, inflammation potential. The SWCNT was used as baseline material to compare then the different toxicological results (Figure 9 SI10) with the ones obtained for the PC (Figure 10 SI10) and the PC-SWCNT (Figure 11 SI10).





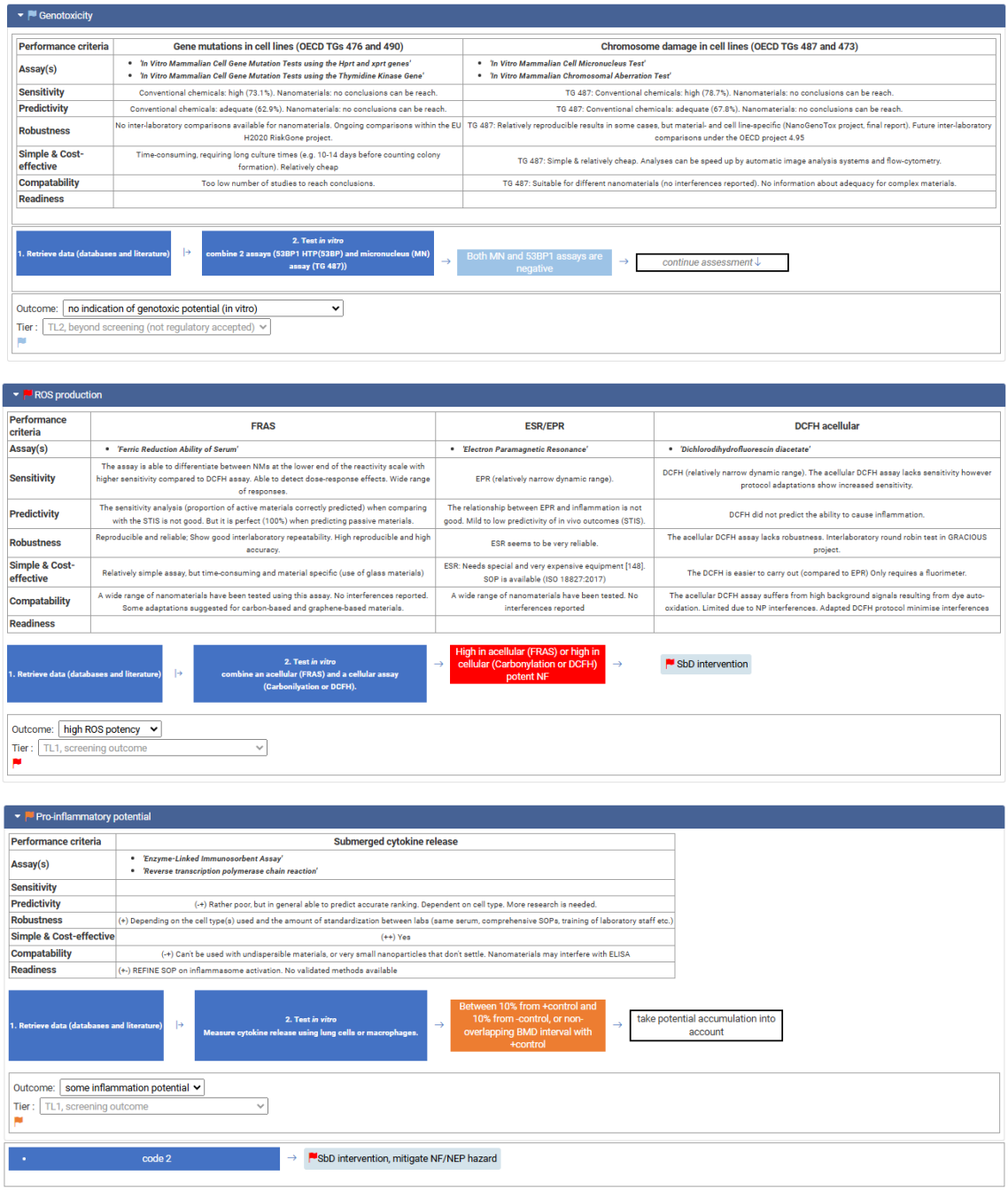
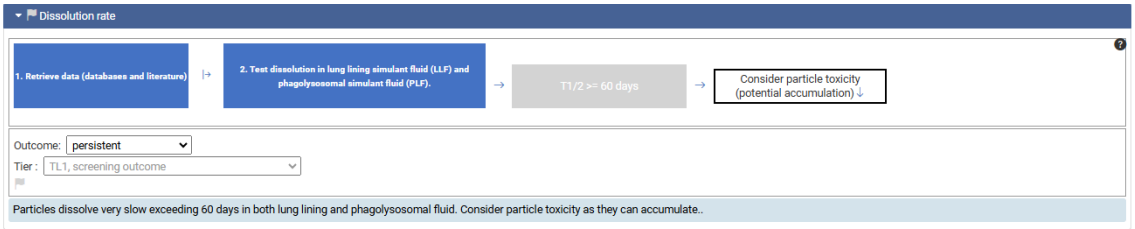


Figure 9 SI10. Results of the IATA for inhalation exposure for the SWCNT TUBALL.



Cytotoxicity

Performance criteria	Mitochondial activity	Cell membrane integrity	Cell membrane integrity staining	Lysosomal integrity
Assay(s)	• *'MTT Cell Viability Assay'* • *'MTS Cell Viability Assay'* • *'XTT Cell Viability and Proliferation Assay'* • *'WST-1 Metabolic Cell Viability Assay'*	• *'Lactate DeHydrogenase Assay'*	• *'Trypan Blue Viability Assay'* • *'Annexin V_PI (FACS) Assay'*	• *'Neutral Red Uptake Cell Viability Assay'*
Sensitivity				
Predictivity	(+-) Depends on the mechanism of toxicity of the particle, and the cell type used. More research needed.	(+-) Depends on the mechanism of toxicity of the particle, and the cell type used. More research needed	(+-) Depends on the mechanism of toxicity of the particle, and the cell type used. More research needed	(+-) In general able to predict an accurate ranking. More research needed
Robustness	(++) For MTS assay, depending on cell type used. Depending on amount of standardization between labs (detailed SOPs, use of same serum, standardized dispersion protocols), and the avoidance of interference	(++) Depending on amount of standardization between labs (detailed SOPs, use of same serum, standardized dispersion protocols), and the avoidance of interference	Unknown	Unknown
Simple & Cost-effective	(++) Very easy and cost-effective, commercial kits available	(++) Very easy and cost-effective, commercial kits available	(-) Microscopic evaluation is time-consuming. FACS analysis is not more difficult and expensive.	(++) Very easy and cost-effective, commercial kits available
Compatability	(+) Many nanomaterials interfere with the substrate, the product, or the optical read-out. Can be overcome by washing cells before incubation with reagent, and centrifugation to get rid of nanomaterials.	(+-) Many nanomaterials interfere with the enzyme, the reagent, or the optical read-out. Can be overcome by centrifugation. Washing not possible.	(+-) Nanomaterials may interfere with the dye	(+-) Nanomaterials may interfere with the dye
Readiness	(++) ISO protocol for MTS assay	(-) No standardized SOP available specifically for nanomaterials	(-) No standardized SOP available specifically for nanomaterials	(-) No standardized SOP available specifically for nanomaterials

Outcome: low cytotoxic potency

Tier : TL1, screening outcome

Genotoxicity

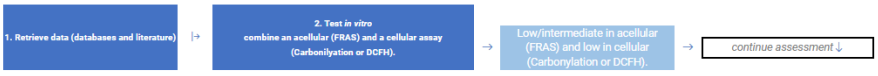
Performance criteria	Gene mutations in cell lines (OECD TGs 476 and 490)	Chromosome damage in cell lines (OECD TGs 487 and 473)
Assay(s)	• *'In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes'* • *'In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene'*	• *'In Vitro Mammalian Cell Micronucleus Test'* • *'In Vitro Mammalian Chromosomal Aberration Test'*
Sensitivity	Conventional chemicals: high (73.1%). Nanomaterials: no conclusions can be reach.	TG 487: Conventional chemicals: high (78.7%). Nanomaterials: no conclusions can be reach.
Predictivity	Conventional chemicals: adequate (62.9%). Nanomaterials: no conclusions can be reach.	TG 487: Conventional chemicals: adequate (67.8%). Nanomaterials: no conclusions can be reach.
Robustness	No inter-laboratory comparisons available for nanomaterials. Ongoing comparisons within the EU H2020 RiskGone project.	TG 487: Relatively reproducible results in some cases, but material- and cell line-specific (NanoGenoTox project, final report). Future inter-laboratory comparisons under the OECD project 4.95
Simple & Cost-effective	Time-consuming, requiring long culture times (e.g. 10-14 days before counting colony formation). Relatively cheap	TG 487: Simple & relatively cheap. Analyses can be speed up by automatic image analysis systems and flow-cytometry.
Compatability	Too low number of studies to reach conclusions.	TG 487: Suitable for different nanomaterials (no interferences reported). No information about adequacy for complex materials.
Readiness		

Outcome: no indication of genotoxic potential (in vitro)

Tier : TL3, regulatory accepted outcome

ROS production

Performance criteria	FRAS	ESR/EPR	DCFH acellular
Assay(s)	• *'Ferric Reduction Ability of Serum'*	• *'Electron Paramagnetic Resonance'*	• *'Dichlorodihydrofluorescin diacetate'*
Sensitivity	The assay is able to differentiate between NMs at the lower end of the reactivity scale with higher sensitivity compared to DCFH assay. Able to detect dose-response effects. Wide range of responses.	EPR (relatively narrow dynamic range).	DCFH (relatively narrow dynamic range). The acellular DCFH assay lacks sensitivity however protocol adaptations show increased sensitivity.
Predictivity	The sensitivity analysis (proportion of active materials correctly predicted) when comparing with the STIS is not good. But it is perfect (100%) when predicting passive materials.	The relationship between EPR and inflammation is not good. Mild to low predictivity of in vivo outcomes (STIS).	DCFH did not predict the ability to cause inflammation.
Robustness	Reproducible and reliable; Show good interlaboratory repeatability. High reproducible and high accuracy.	ESR seems to be very reliable.	The acellular DCFH assay lacks robustness. Interlaboratory round robin test in GRACIOUS project.
Simple & Cost-effective	Relatively simple assay, but time-consuming and material specific (use of glass materials)	ESR: Needs special and very expensive equipment [148]. SOP is available (ISO 18827:2017)	The DCFH is easier to carry out (compared to EPR) Only requires a fluorimeter.
Compatability	A wide range of nanomaterials have been tested using this assay. No interferences reported. Some adaptations suggested for carbon-based and graphene-based materials.	A wide range of nanomaterials have been tested. No interferences reported	The acellular DCFH assay suffers from high background signals resulting from dye auto-oxidation. Limited due to NP interferences. Adapted DCFH protocol minimise interferences
Readiness			



Outcome: low ROS potency

Tier : TL1, screening outcome

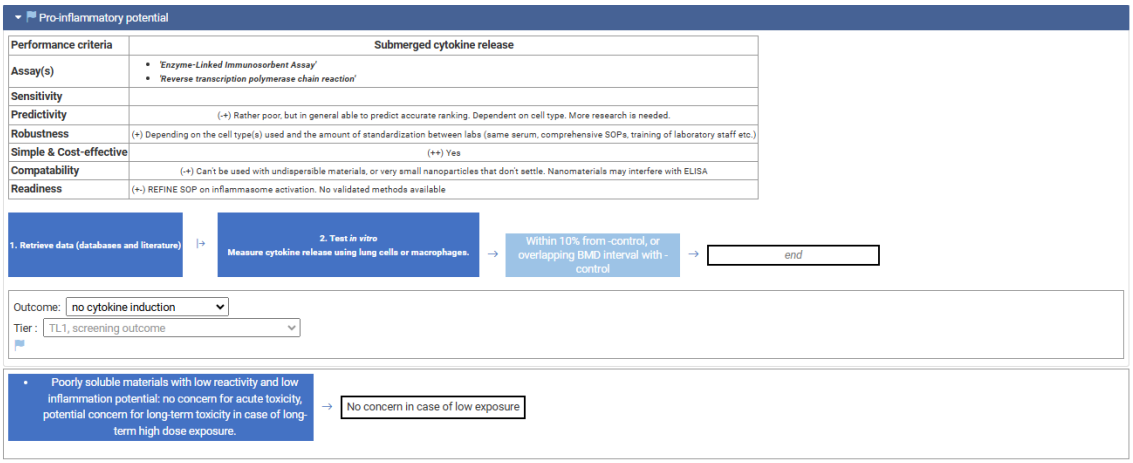
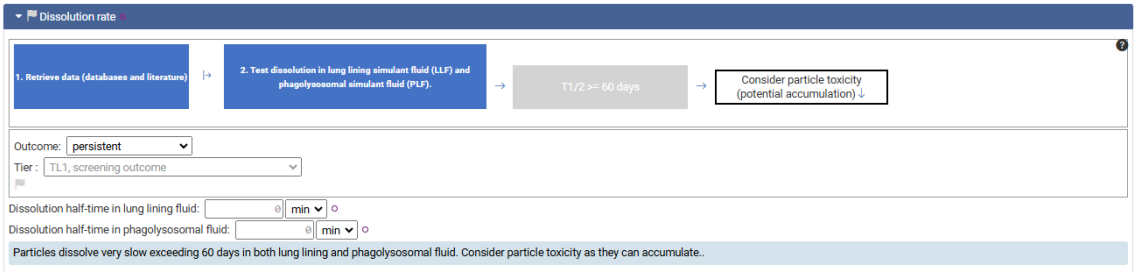
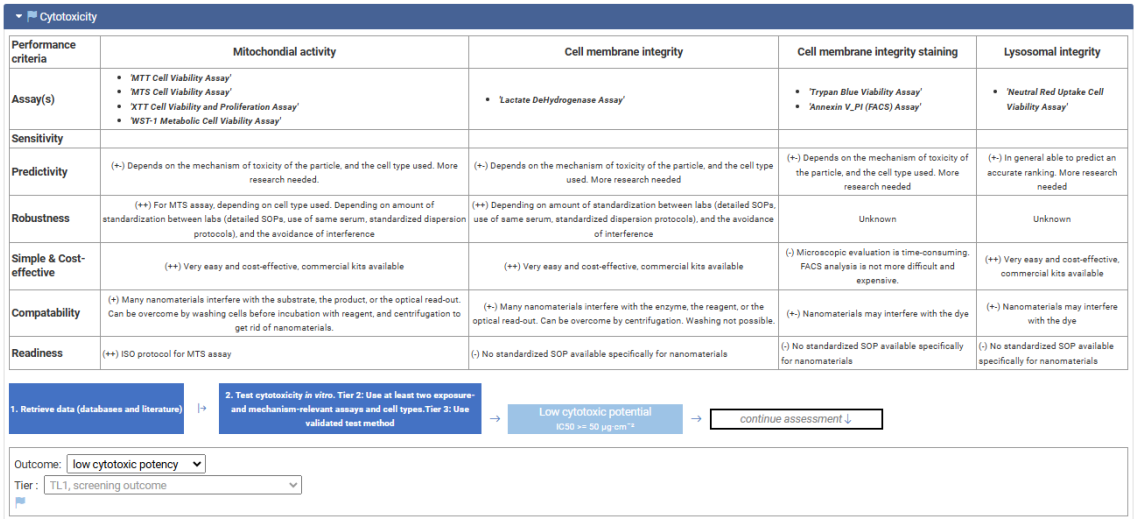
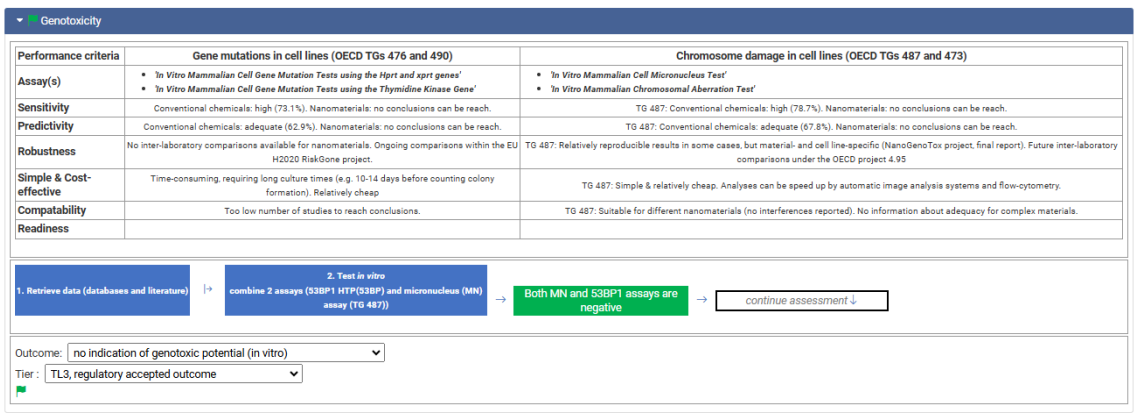


Figure 10 SI10. Results of the IATA for inhalation exposure for the PC material.







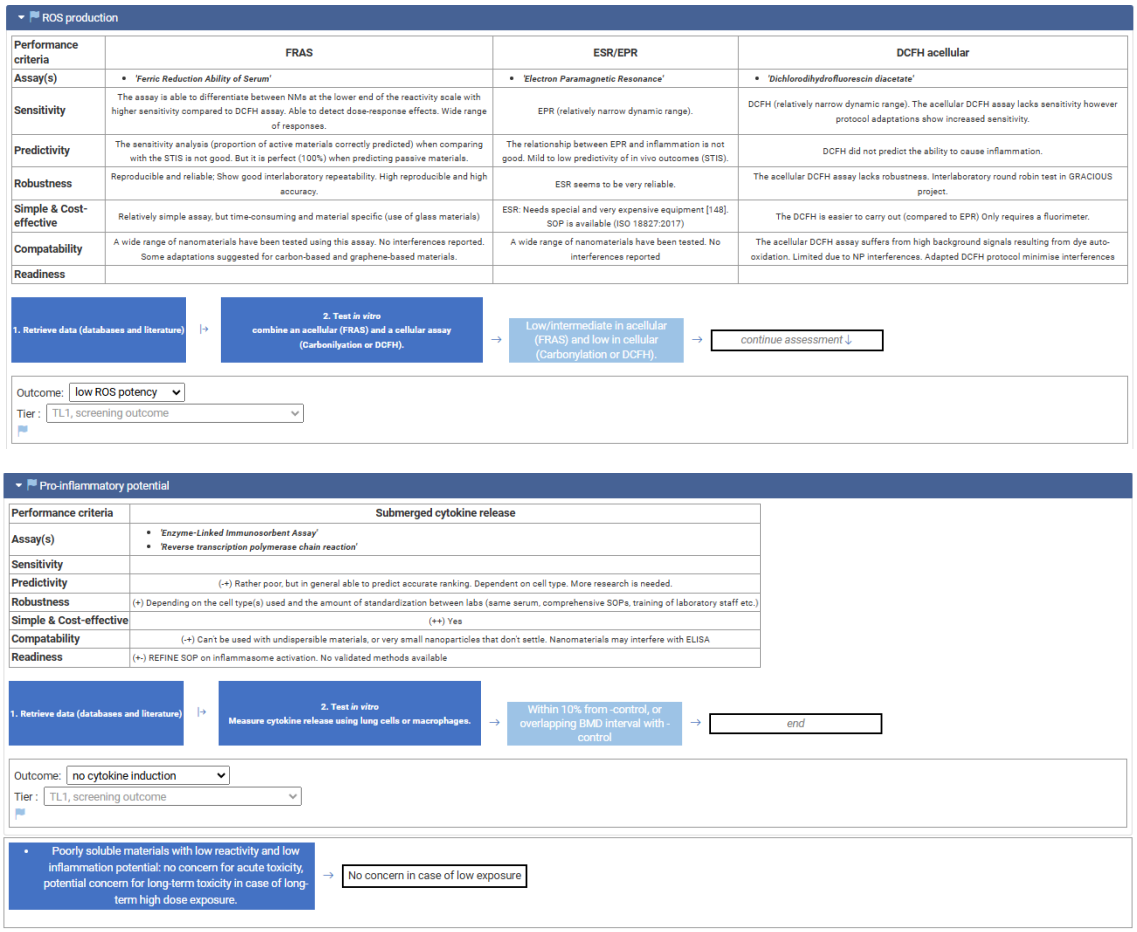


Figure 11 SI10. Results of the IATA for inhalation exposure for the PC-SWCNT material.

The decision tree was made to help interpret in vitro data; how this can be used in a SbD context to inform on potential risks. In the case of particles that have a low dissolution rate, low cytotoxicity, low reactivity, and low cytokine release, poorly soluble low toxicity particles (PSLT) can be considered. For these particles, there is no concern for acute toxicity (direct effects after a single exposure). There is, however, a concern that these particles might accumulate over time. In rats, PSLT can cause impaired clearance after long-term exposure to (very) high concentrations. The assumption is that the clearance capacity of the lungs can deal with lower concentrations. The effects observed in rats are related to impaired clearance which only occurs at high exposures that exceed the clearance capacity of the lungs (Bos et al. 2019). It is still under debate whether this could also occur in humans. Nevertheless, an orange flag is placed to be aware that in case of long-term (chronic) high dose exposure (in real life) this accumulation might occur which might lead to effects on the lungs.

References

Baumers, M., Tuck, C., Wildman, R., Ashcroft, I., Hague, R., 2011. Energy inputs to additive manufacturing: does capacity utilization matter?

Bezzina, C.M., Refalo, P., 2023. Fused Filament Fabrication and Injection Moulding of Plastic Packaging: An Environmental and Financial Comparative Assessment. Machines 11. https://doi.org/10.3390/machines11060634

Bouillard, J.X., R'Mili, B., Moranviller, D., Vignes, A., Le Bihan, O., Ustache, A., Bomfim, J.A.S., Frejafon, E., Fleury, D., 2013. Nanosafety by design: risks from nanocomposite/nanowaste combustion. J. Nanoparticle Res. 15, 1519. https://doi.org/10.1007/s11051-013-1519-3

Caldeira, C., Farcal, L., Garmendia Aguirre, I., Mancini, L., Tosches, D., Amelio, A., Rasmussen, K., Rauscher, H., Riego Sintes, J., Sala, S., 2022. Safe and Sustainable by Design chemicals and materials - Framework for the definition of criteria and evaluation procedure for chemicals and materials. EUR 31100 EN Publ. Off. Eur. Union Luxemb. ISBN 978-92-76-53264-4.

Enemuoh, E.U., Menta, V.G., Abutunis, A., O'Brien, S., Kaya, L.I., Rapinac, J., 2021. Energy and Eco-Impact Evaluation of Fused Deposition Modeling and Injection Molding of Polylactic Acid. Sustainability 13. https://doi.org/10.3390/su13041875

Faludi, J., Baumers, M., Maskery, I., Hague, R., 2017. Environmental Impacts of Selective Laser Melting: Do Printer, Powder, Or Power Dominate? J. Ind. Ecol. 21, S144–S156. https://doi.org/10.1111/jiec.12528

Garcia, F.L., Nunes, A.O., Martins, M.G., Belli, M.C., Saavedra, Y.M.B., Silva, D.A.L., Moris, V.A. da S., 2021. Comparative LCA of conventional manufacturing vs. additive manufacturing: the case of injection moulding for recycled polymers. Int. J. Sustain. Eng. 14, 1604–1622. https://doi.org/10.1080/19397038.2021.1990435

Hernandez Korner, M.E., Lamban, M.P., Albajez, J.A., Santolaria, J., Ng Corrales, L. del C., Royo, J., 2024. Cost Model Framework for Pieces Additively Manufactured in Fused Deposition Modeling for Low to Medium Batches. 3D Print. Addit. Manuf. 11, 287–298. https://doi.org/10.1089/3dp.2022.0044

Holder, A.L., Vejerano, E.P., Zhou, X., Marr, L.C., 2013. Nanomaterial disposal by incineration. Env. Sci Process. Impacts 15, 1652–1664. https://doi.org/10.1039/C3EM00224A

Hopkins, N., Jiang, L., Brooks, H., 2021. Energy consumption of common desktop additive manufacturing technologies. Clean. Eng. Technol. 2, 100068. https://doi.org/10.1016/j.clet.2021.100068

Ingarao, G., Priarone, P.C., 2020. A comparative assessment of energy demand and life cycle costs for additive- and subtractive-based manufacturing approaches. J. Manuf. Process. 56, 1219–1229. https://doi.org/10.1016/j.jmapro.2020.06.009

Jiang, J., Xiong, Y., Zhang, Z., Rosen, D.W., 2022. Machine learning integrated design for additive manufacturing. J. Intell. Manuf. 33, 1073–1086. https://doi.org/10.1007/s10845-020-01715-6

Kellens, K., Mertens, R., Paraskevas, D., Dewulf, W., Duflou, J.R., 2017. Environmental Impact of Additive Manufacturing Processes: Does AM Contribute to a More Sustainable Way of Part Manufacturing? 24th CIRP Conf. Life Cycle Eng. 61, 582–587. https://doi.org/10.1016/j.procir.2016.11.153

Kellens, K., Yasa, E., Renaldi, Dewulf, W., Kruth, J.P., Duflou, J.R., 2011. Energy and Resource Efficiency of SLS/SLM Processes.

Kokare, S., Oliveira, J.P., Godina, R., 2023. Life cycle assessment of additive manufacturing processes: A review. J. Manuf. Syst. 68, 536–559. https://doi.org/10.1016/j.jmsy.2023.05.007

Kwon, J., Kim, N., Ma, J., 2020. Environmental sustainability evaluation of additive manufacturing using the NIST test artifact. J. Mech. Sci. Technol. 34, 1265–1274. https://doi.org/10.1007/s12206-020-0225-1

Le Gentil, T., Therriault, D., Kerbrat, O., 2024. A comprehensive methodology to support decision-making for additive manufacturing of short carbon-fiber reinforced polyamide 12 from energy, cost and mechanical perspectives. Int. J. Adv. Manuf. Technol. 131, 611–622. https://doi.org/10.1007/s00170-023-11161-2

Le, V.T., Paris, H., 2018. A life cycle assessment-based approach for evaluating the influence of total build height and batch size on the environmental performance of electron beam melting. Int. J. Adv. Manuf. Technol. 98, 275–288. https://doi.org/10.1007/s00170-018-2264-7

Lyons, R., Newell, A., Ghadimi, P., Papakostas, N., 2021. Environmental impacts of conventional and additive manufacturing for the production of Ti-6Al-4V knee implant: a life cycle approach. Int. J. Adv. Manuf. Technol. 112, 787–801. https://doi.org/10.1007/s00170-020-06367-7

Ma, H., Zhang, Y., Jiao, Z., Yang, W., He, X., Xie, G., Li, H., 2021. Comprehensive Assessment of the Environmental Impact of Fused Filament Fabrication Products Produced Under Various Performance Requirements. J. Inst. Eng. India Ser. C 102, 59–73. https://doi.org/10.1007/s40032-020-00637-9

Malshe, H., Nagarajan, H., Pan, Y., Haapala, K., 2015. Profile of Sustainability in Additive Manufacturing and Environmental Assessment of a Novel Stereolithography Process, in: MSEC2015. Volume 2: Materials; Biomanufacturing; Properties, Applications and Systems; Sustainable Manufacturing. https://doi.org/10.1115/MSEC2015-9371

Napolitano, F., Cozzolino, E., Papa, I., Astarita, A., Squillace, A., 2022. Experimental integrated approach for mechanical characteristic optimization of FDM-printed PLA in an energy-saving perspective. Int. J. Adv. Manuf. Technol. 121, 3551–3565. https://doi.org/10.1007/s00170-022-09535-z

Organisation for Economic Co-operation and Development (OECD)-Working Party of Resource Productivity and Waste, 2015. Incineration of waste containing nanomaterials ENV/EPOC/WPRPW(2013)3/FINAL.

Ounoughene, G., LeBihan, O., Debray, B., Chivas-Joly, C., Longuet, C., Joubert, A., Lopez-Cuesta, J.-M., Le Coq, L., 2017. Thermal disposal of waste containing nanomaterials: first investigations on a methodology for risk management. J. Phys. Conf. Ser. 838, 012024. https://doi.org/10.1088/1742-6596/838/1/012024

Peng, T., Lv, J., Majeed, A., Liang, X., 2021. An experimental investigation on energy-effective additive manufacturing of aluminum parts via process parameter selection. J. Clean. Prod. 279, 123609. https://doi.org/10.1016/j.jclepro.2020.123609

Peng, T., Wang, Y., Zhu, Y., Yang, Yang, Yang, Yiran, Tang, R., 2020. Life cycle assessment of selective-laser-melting-produced hydraulic valve body with integrated design and

manufacturing optimization: A cradle-to-gate study. Addit. Manuf. 36, 101530. https://doi.org/10.1016/j.addma.2020.101530

Priarone, P.C., Ingarao, G., 2017. Towards criteria for sustainable process selection: On the modelling of pure subtractive versus additive/subtractive integrated manufacturing approaches. J. Clean. Prod. 144, 57–68. https://doi.org/10.1016/j.jclepro.2016.12.165

Priarone, P.C., Lunetto, V., Atzeni, E., Salmi, A., 2018. Laser powder bed fusion (L-PBF) additive manufacturing: On the correlation between design choices and process sustainability. 6th CIRP Glob. Web Conf. – Envisaging Future Manuf. Des. Technol. Syst. Innov. Era CIRPe 2018 78, 85–90. https://doi.org/10.1016/j.procir.2018.09.058

Ulkir, O., 2023. Energy-Consumption-Based Life Cycle Assessment of Additive-Manufactured Product with Different Types of Materials. Polymers 15. https://doi.org/10.3390/polym15061466

Vidakis, N., Kechagias, J.D., Petousis, M., Vakouftsi, F., Mountakis, N., 2023. The effects of FFF 3D printing parameters on energy consumption. Mater. Manuf. Process. 38, 915–932. https://doi.org/10.1080/10426914.2022.2105882

Yosofi, M., Kerbrat ,Olivier, and Mognol, P., 2018. Energy and material flow modelling of additive manufacturing processes. Virtual Phys. Prototyp. 13, 83–96. https://doi.org/10.1080/17452759.2017.1418900

Zakaria, S., Mativenga, P., Cseke, A., 2022. Energy Consumption and Scope 2 Emissions for Fused Deposition Modelling.