scientific reports

Check for updates

OPEN

Maximizing the safety and sustainability of MXenes

Steffen Foss Hansen[1]✉, Maria Bille Nielsen[1], Lars Michael Skjolding[1], Jasreen Kaur[2], Nindya Desivyana[3], Frida Hermansson[3,4], James Bird[5], Suelen Barg[6], Alexander Khort[7], Inger Odnevall[7], Bengt Fadeel[2] & Rickard Arvidsson[3]

Safe and Sustainable by Design (SSbD) is a new regulatory concept guiding chemical and material innovation. The European Commission has recommended a two-stage SSbD framework and plan to revise it based on stakeholder feedback. The framework involves establishing key (re)design SSbD principles and assessment of the final innovation, however the applicability of the framework to advanced materials remains to be addressed. Here, we applied the SSbD framework on $Ti_3C_2T_x$ MXenes, an emerging advanced material, by (1) reviewing health, environmental and safety information; (2) characterizing MXenes transformation/dissolution in different media; (3) conducting (eco)toxicological experiments, and (4) completing a prospective life-cycle assessment. Our analysis suggests that $Ti_3C_2T_x$ is safe and sustainable when applying the SSbD framework. Further research is needed regarding long-term hazardous effects of $Ti_3C_2T_x$ and the sustainable production of the titanium precursor. Guidance is also needed on how much weight one should assign to the different lines of evidence in the overall SSbD assessment.

Keywords Advanced materials, Life cycle assessment, MXenes, Safe and sustainable by design

The Safe and Sustainable by Design (SSbD) concept has gained traction in recent years. The European Commission published a recommendation in 2022 on the implementation of the SSbD framework for chemicals and materials across different phases of innovation, i.e., design, preparation, experimentation, and prototyping[1]. The SSbD framework consists of two stages. Stage 1 entails a series of guiding (re)design principles to consider before and during the development of a new chemical or material, e.g., "minimise the use of hazardous chemicals or materials" and to "consider the whole life cycle", whereas Stage 2 focuses on assessment and documentation of the safety and sustainability of an existing chemical or new material. Stage 2 is divided into four steps: (1) Hazard assessment based on intrinsic properties, (2) Human health and safety aspects of production, (3) Human health and environmental aspects in the final application, and (4) Environmental sustainability assessment. The European Commission underlines that the SSbD framework should not be based solely on data already legally required to be provided by manufacturers by EU legislation on chemicals, but also on data outside the scope of those requirements such as from New Approach Methodologies (NAMs). The European Commission launched a testing period with a voluntary reporting mechanism and plans to revise the framework based on feedback from stakeholders such as industry, academia, and European Member States[1].

So far, only a few attempts to apply the SSbD framework, using the approach suggested by the European Commission, on actual chemicals and materials has been reported on in the scientific literature. For instance, Hansen and Trier[2] examined whether earlier versions of the SSbD framework and reporting criteria are sufficient to address the specific challenges of emerging smart materials, in particular in relation to environmental sustainability. It was found that lack of reliable data on environmental, health and safety and access to information needed for LCA was major challenges when it comes to the application of the SSbD framework.

It remains to be understood whether the SSbD framework is fit-for-purpose with respect to advanced materials. Advanced materials are materials that are designed to have new or enhanced properties, and/or enhanced structural features, with the objective to achieve specific or improved functional performance[3]. The two-dimensional (2D) carbides and nitrides named MXenes serve as a relevant example of advanced materials.

[1]Department of Environmental and Resource Engineering, Technical University of Denmark, Kgs. Lyngby 2800, Denmark. [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm 171 77, Sweden. [3]Environmental Systems Analysis, Chalmers University of Technology, Gothenburg 412 96, Sweden. [4]IVL Swedish Environmental Research Institute, Aschebergsgatan 44, Gothenburg 411 33, Sweden. [5]Henry Royce Institute, Department of Materials, School of Natural Sciences, The University of Manchester, Manchester M13 9PL, UK. [6]Institute of Materials Resource Management (MRM), University of Augsburg, 86159 Augsburg, Germany. [7]Biotechnology and Health, Department of Chemistry, Division of Surface and Corrosion Science, KTH Royal Institute of Technology School of Engineering Sciences in Chemistry, Stockholm 100 44, Sweden. ✉email: sfha@dtu.dk

$Ti_3C_2T_x$ is the best-characterized and most-studied of the MXenes. Moreover, there is a growing interest in scaling up $Ti_3C_2T_x$production[4]. $Ti_3C_2T_x$ MXenes thus provide a case study to assess the SSbD framework.

In order to provide input on the use of the SSbD framework, we set out to (1) review existing health, environment and safety information about titanium carbide MXenes ($Ti_3C_2T_x$), (2) characterize transformation/dissolution of $Ti_3C_2T_x$ MXenes upon exposure in various media with and without natural organic matter (NOM), (3) complete (eco)toxicological experiments to fill knowledge gaps with regard to $Ti_3C_2T_x$ MXenes, and (4) complete a life cycle assessment (LCA) of $Ti_3C_2T_x$ production. We focused this analysis on the most common production route for $Ti_3C_2T_x$ MXenes i.e., the top-down approach wherein one element is selectively etched from the parental MAX phase (where M is an early transition metal, A is an A-group (mostly groups 13 and 14) element, and X is C and/or N), usually by etching in hydrogen fluoride (HF).

Characterization of Ti3C2Tx MXenes: a prerequisite for safety assessment

The evaluation of advanced materials such as $Ti_3C_2T_x$ from an SSbD perspective must begin with a thorough characterisation. The $Ti_3C_2T_x$ MXenes investigated herein were thus subjected to characterization as detailed in the methods. XRD (Fig. 1A) and XPS (Fig. 1B, C) of the dry powder confirmed high purity of the phase

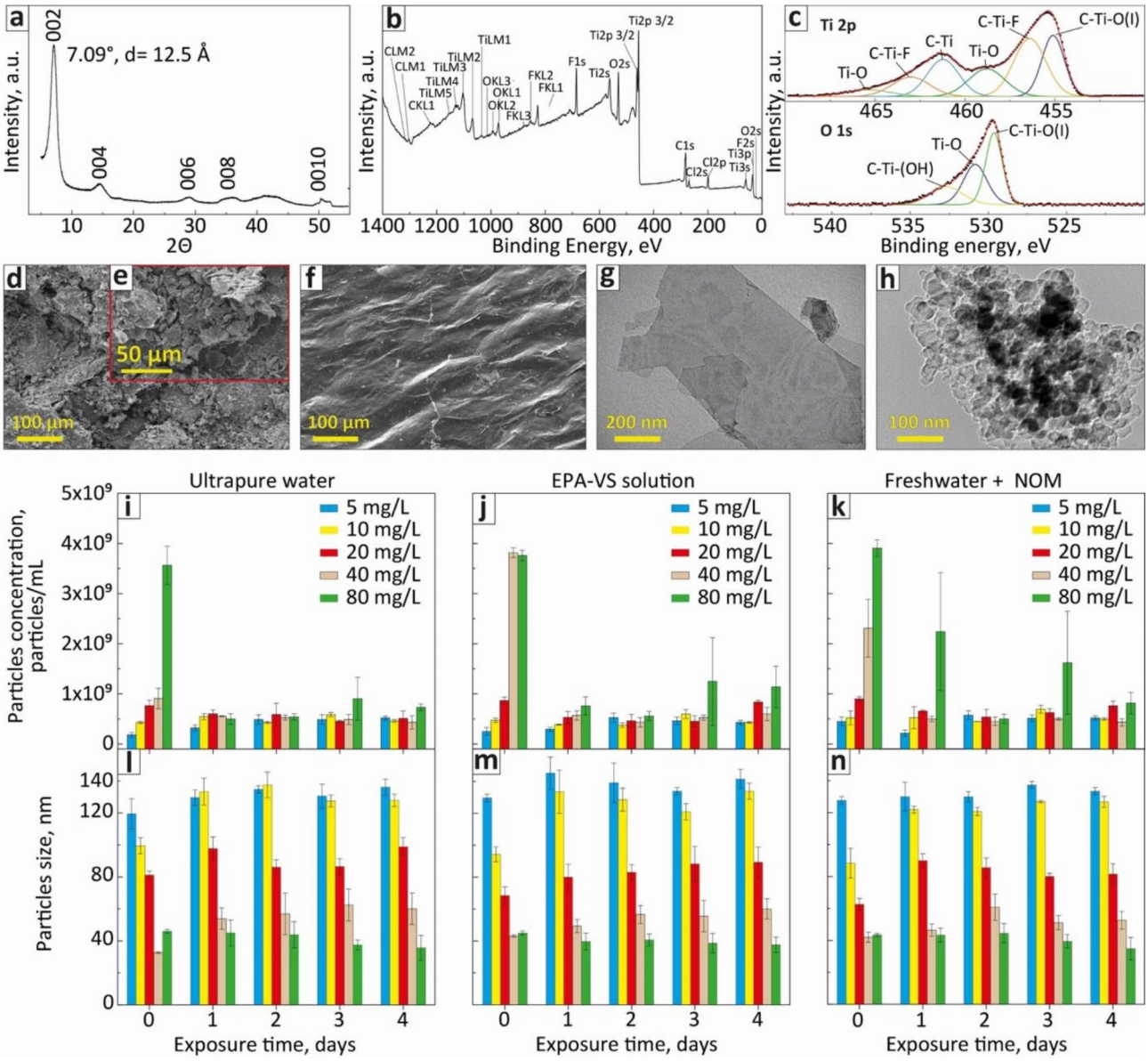

Fig. 1. Phase composition and physicochemical characteristics of Ti3C2Tx MXene. Typical (**a**) XRD profile and (**b**) survey XPS spectra of the MXene powder. (**c**) High-resolution XPS spectra in Ti 2p and O 1s regions. (**d**-**f**) SEM and (g-h) TEM images showing the typical MXene morphology and microstructure. (**i**-**k**) NTA particle concentration and (**l**-**n**) size over exposure MXene to (**i**, **l**) ultrapure water, (**j**, **m**) EPA-VS solutions, and (k, n) freshwater with NOM solution for up to 4 days.

composition of the investigated $Ti_3C_2T_x$ with the surface of the flakes being terminated with Cl^- and F^- functional groups. No significant amount of oxygen was observed, indicating that the material was non-oxidized. TEM and SEM (Fig. 1D-H) showed that the material consisted of exfoliated self-standing and stacked layers. Two types of morphological structures were observed, including thin large-sized films of several micrometres in size and small flakes of 50–150 nm in size. The flakes formed agglomerates of different shapes and sizes (Fig. 1I-N).

The investigated MXenes formed stable colloids in only some of the simulated natural media of relevance to the aquatic environment and eco-toxicity studies, including ultrapure water, freshwater with NOM (10 mg/L), and EPA-VS media. These media were hence chosen as the main experimental settings. In the other media investigated (freshwater, E3 media), components of the media were readily adsorbed to the MXene surface, mostly metal cations, forming large-sized aggregates up to several millimeters in diameter. These aggregates sedimented relatively rapidly. The results showed that the MXene-based colloids were stable in all investigated media for at least 4 days at nominal concentrations of 5–20 mg/L, with some concentration drop observed for suspensions with the highest nominal concentrations investigated (40 and 80 mg/L) after 1 and 2 days of exposure (Figure S1i-k). No significant changes in median particle sizes of the dispersed MXene powders were observed regardless of the sample nominal concentration (Figure S1l-n). Moreover, $Ti_3C_2T_x$ did not undergo dissolution in any of the media during the investigated time (42 days) as evidenced by the fact that the released amounts of Ti were below the limit of detection, i.e. <1 µg/L in the test media.

Step 1 investigation of intrinsic hazardous properties of MXenes

Step 1 of stage 2 of the SSbD framework requires an SSbD classification of the intrinsic hazardous properties of chemicals and materials that can have adverse effects on humans or the environment. The hazardous properties of MXenes remain largely unexplored[5]. Very few in vivo studies of the biocompatibility of MXenes exist. Some in vitro studies using primary human, or cell lines have pointed towards a potential cytotoxicity, though it should be noted that unrealistically high concentrations of MXenes were used[6,7]. It is therefore currently not possible to prepare a SSbD classification of $Ti_3C_2T_x$. This is due to the fact that three generic groups of hazardous chemicals are defined in the SSbD classification proposed by the European Commission, namely harmful substances such as carcinogens and reproductive toxicants category 1 (Group A), substances of concern such as carcinogens category 2 and chronic environmental toxicity (Group B) and substances that have other intrinsic hazards such as acute toxicity and acute environmental toxicity (Group C). These classifications are based on the hazard classes and categories established in the European Regulation on Classification and Labelling of Products (CLP Regulation) and in vitro studies alone are currently not considered adequate for such regulatory classification of chemicals[8].

Step 2 human health aspects of production and processing

Step 2 of the SSbD framework focuses on occupational health and safety during production and requires estimation of hazards, exposure, and efficiency of implemented risk management measures. Such estimates require information about the substances used in all production and processing steps (e.g., of raw chemicals or materials, processing aids), substances that may be produced during the processes (volatile organic compounds, by-products, etc.), operational conditions (closed/open processes and concentration), release potential (volatility, dustiness, fugacity, temperature, pressure) and the available risk management measures in place (e.g., local exhaust ventilation), as well as information on the most likely route of exposure (inhalation, dermal, and/or ingestion).

Depending on the route of synthesis of MXenes, several different kinds of hazards exist, including spontaneous combustion of reactants and handling of highly combustible powders like aluminium and titanium carbide or graphite, dust particle inhalation, combustion of the Ti_3AlC_2phase during drilling and explosion of ignited sample[3,9]. Several different etching routes also exist and the quality and yield of the produced $Ti_3C_2T_x$ material varies notably between these methods. Hazards during etching include exothermic reactions due to rapid addition of Ti_3AlC_2 to HF acid solution or solution with in-situ formed HF (mixture of LiF with HCl – MILD method), release of explosive gas (H_2) and exposure to HF[3]. Although there is an increasing effort to explore alternatives to HF, most synthesis routes use HF to remove the Al layer from Ti_3AlC_2[10]. After etching, unreacted HF and water-soluble salts are removed during the postprocessing treatment of the $Ti_3C_2T_x$ product slurry. The selection of post processing techniques depends on the applications of $Ti_3C_2T_x$.

Some approaches applied for delamination involves utilising alternative organic chemical etchants, such as dimethyl sulfoxide (DMSO), urea, tetrabutkylammonium hydroxide (TBAOH), choline hydroxide, and n-butylamine. These solvents range from being slightly to highly flammable. Primary hazards associated with DMSO and urea are irritation from inhalation, ingestion, or eye contact, and possible absorption through skin. Eye and skin exposure to solvents like TBAOH, choline hydroxide, and n-butylamine can cause severe burns and can prove fatal if inhaled[3].

Mainly three different classes of waste are produced during $Ti_3C_2T_x$ synthesis: acidic waste from HF/LiF/HCl (majority), metal waste of Ti and Al, and solid particulates such as C and under-/overetched Ti_3AlC_2 particles[3].

Risk management measures exist for all the hazards mentioned, e.g., minimisation of dust cloud formation to avoid spontaneous combustion of reactants, limit the amounts of toxic reactants and working in hoods to avoid dust particle inhalation during synthesis processes. For etching processes, risk management measures include, e.g., avoiding exothermic reactions by slowly adding Ti_3AlC_2 and applying external reactor cooling to minimise temperature increase and preventing explosive gas release by applying negative pressure hoods with ventilation and using isolated reactors with a proper exhaust system[3].

Step 3 human health and environmental effects: filling the data gaps

Step 3 of the SSbD framework requires an assessment of human health and environmental aspects considering the final application phase. The European Commission notes in its recommendation that information can be limited at the beginning of newly developed chemicals or materials and that it could be beneficial to characterize hazards as early as possible at the innovation stage (i.e., during the design of the chemical or material) by using, for example, NAMs to generate data and knowledge[1]. Limited information exists with respect to the potential ecotoxicicty of MXenes[3,4]. To address this data gap, we studied potential effects of $Ti_3C_2T_x$ on algae, crustaceans, and zebrafish embryos to provide data for a preliminary assessment of the ecotoxicological effects of $Ti_3C_2T_x$. Information about the properties and characteristics of the investigated $Ti_3C_2T_x$ and the tests performed on crustaceans (*D. magna*), algae (*R. subcapitata*) and zebrafish embryos (*D. rerio*) are provided in the supplementary information. The rationale behind the algae and daphnia testing is that these organisms are used in standardized tests and the results are therefore relevant in a regulatory setting. Moreover, they are representative of primary producers and primary consumers, respectively[11]. Zebrafish embryos were used as a model instead of adult fish in alignment with the EU's commitment to the 3Rs principle—Replacement, Reduction, and Refinement of animal use in scientific research (Directive 2010/63/EU). Zebrafish embryos are also amenable to high-throughput hazard-based screening of chemicals and materials[12,13].

The ecotoxicological tests for growth inhibition of algae and immobilization of daphnia with and without presence of NOM all showed minimal toxicity of $Ti_3C_2T_x$ with EC/LC_{50} values > 100 mg/L (Fig. 2).

The maximum tested concentration of 100 mg/L is used to support the classification and labelling scheme for when a compound is considered hazardous to the aquatic environment. Additionally, testing of higher concentration has been associated with physical effects that would not be transferable to effects occurring at environmentally realistic concentrations and thus cannot be translated using "assessment factors" as is customary in regulatory risk assessment[14].

Similarly to daphnids and algae, zebrafish embryos were exposed to different concentrations of $Ti_3C_2T_x$ with and without NOM up to 120 h post-fertilization (hpf) and were assessed for mortality or malformations every 24 h. No significant decrease in the survival rate of these embryos was observed even at the highest dose of 80 mg/L (Fig. 3A) and the addition of NOM did not have any significant effect (Fig. 3B).

The hatching rate was also recorded at 72 hpf, but significant effects on hatching were not observed in exposed or unexposed embryos. The embryos were assessed for malformations at 96 hpf and the embryos in both control and exposed group did not show any significant malformations (Figure S1).

Based on the studies performed here, it can be concluded that well-dispersed $Ti_3C_2T_x$ are not toxic in the size ranges studied at any of the tested concentrations and the presence of NOMs does not have any significant impact on the toxicity of $Ti_3C_2T_x$. It is noted that one study previously addressed the potential hazard using the zebrafish model, and no toxicity was observed[15]. However, MXenes formed large aggregates in seawater and E3 medium in the latter study (note that zebrafish are freshwater fish), which is why EPA VS medium was used here.

Step 4 Environmental sustainability assessment of Ti3C2Tx

As a final step of the SSbD evaluation, an environmental sustainability assessment is to be completed in the form of an LCA. We conducted an LCA of $Ti_3C_2T_x$ produced by means of HF etching of the MAX phase Ti_3AlC_2, according to the chemical reaction: $Ti_3AlC_2 + 3\ HF \rightarrow Ti_3C_2 + AlF_3 + 1.5\ H_2$. In addition to the use of HF and the Ti_3AlC_2 MAX phase, hydrochloric acid, as well as lithium chloride (LiCl) and water are also present in the etching reaction and delamination process, all in line with the currently most commercial $Ti_3C_2T_x$production route[16]. The functional unit of the study, to which all impacts are related, was the production of 1 kg of $Ti_3C_2T_x$ powder. Contrary to two previous LCA studies on Ti_3AlC_2MXenes[17,18], we applied prospective LCA to assess impacts at future large-scale production[19], in line with the suggestion from the European Commission Joint Research Centre[20]. Upscaling calculations and data for $Ti_3C_2T_x$ production (including its Ti_3AlC_2 MAX phase) are provided in the supplementary information, and the Environmental Footprint (EF) 3.1 method was used for the impact assessment.

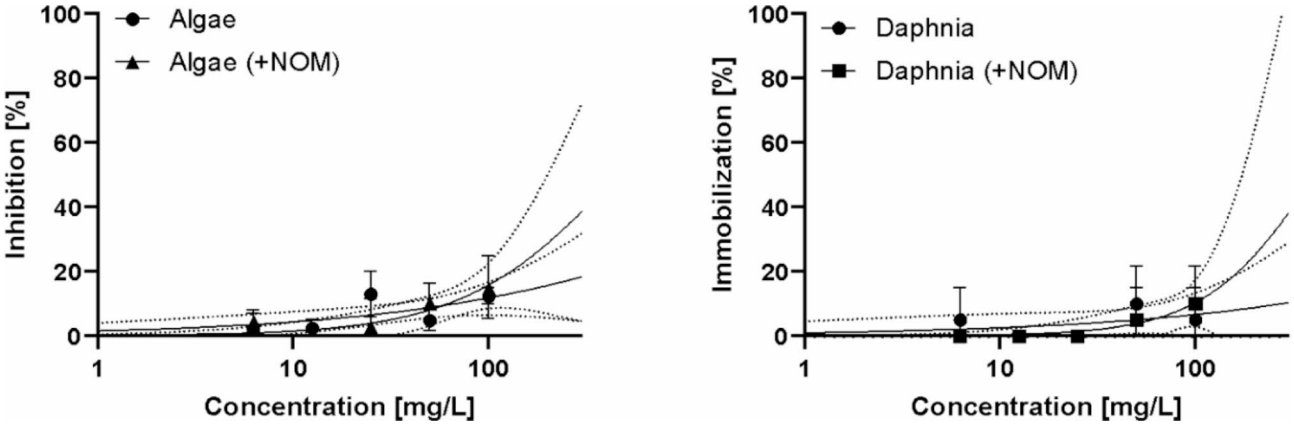

Fig. 2. Growth inhibition of algae and immobilization of daphnia with and without presence of NOM.

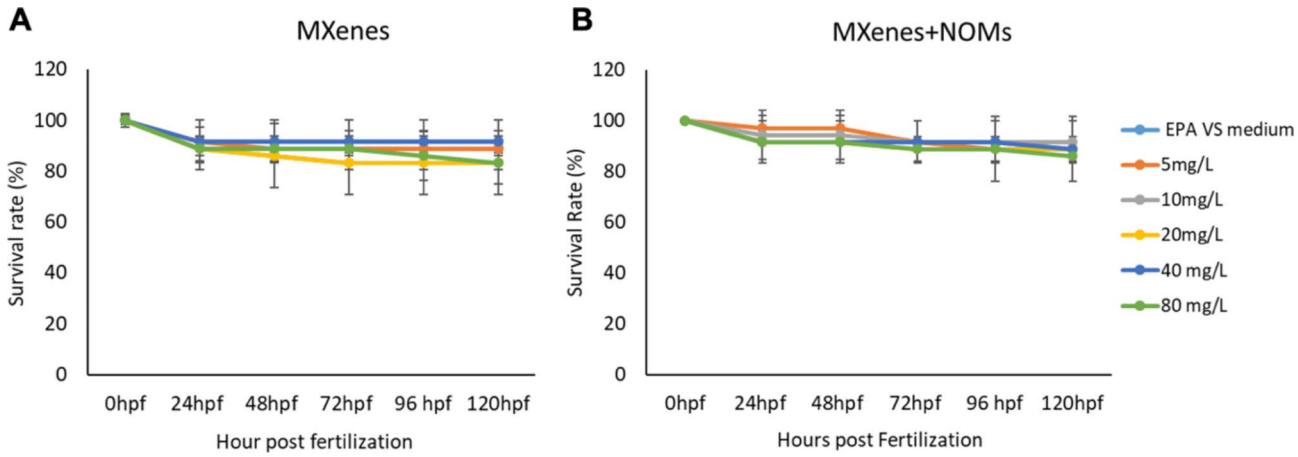

Fig. 3. Survival rate of zebrafish larvae exposed to MXenes in EPA VS medium at the indicated concentrations in the absence of NOM (**A**) and in the presence of 10 mg/L NOM (**B**). Data are expressed as mean ± S.D. from three independent experiments (30 embryos per experiment). Statistical comparisons between the control and specified exposure groups were analysed using two-way ANOVA ($p < 0.05$, $^{*}p < 0.01$, $^{**}p < 0.001$).

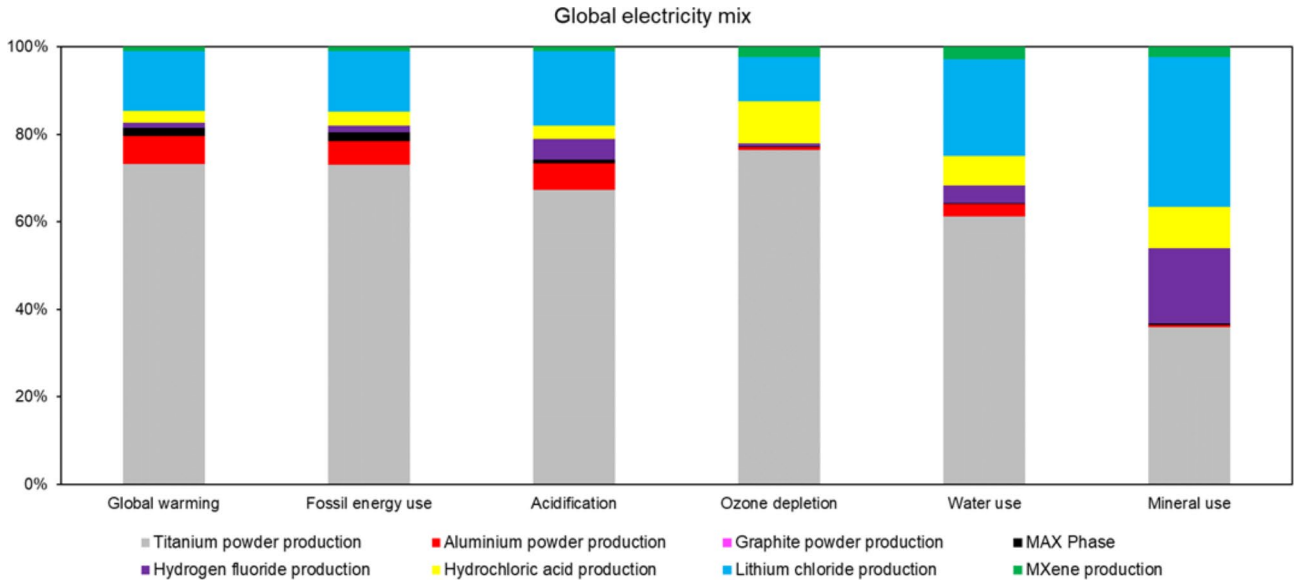

Fig. 4. Relative contribution to environmental impacts from producing 1 kg Ti_3C_2 using a global electricity mix.

Contribution results for global warming, fossil energy use, acidification, ozone depletion, water use, and mineral resource use are shown in Fig. 4 for a global electricity mix. For all impact categories, including global warming, the production of titanium powder is a clear "hotspot". This is primarily due to the high energy requirement of the titanium sponge production *via* the common Kroll process. The most important effort to reduce $Ti_3C_2T_x$ cradle-to-gate impacts is thus to source titanium powder produced in more sustainable ways[21,22]. Additionally, impacts could be reduced by increasing the share of recycled titanium further in future $Ti_3C_2T_x$ production. Results show that changing to a decarbonized electricity supply has a small but noticeable influence on impacts, although the main hotspot remains the same (Figure S2B). Detailed results for the impact categories are given in the supplementary information Table S8-S11. It is important to note that the assessment included the extraction of raw materials and processes until the $Ti_3C_2T_x$ factory gate (cradle-to-gate). Since future applications of $Ti_3C_2T_x$ and their end-of-life are currently unknown, no use phase or end-of-life scenarios were included. The two previous LCA studies highlighted energy use during $Ti_3C_2T_x$ production as the main "hotspot", which is likely due to the low throughput at the laboratory scale considered. One of the studies noted, in line with our results, that titanium had the largest impact of all input chemicals[17].

Conclusions

The almost parallel emergence and development of the SSbD concept and MXenes, brings an opportunity to apply the SSbD framework strategy to advanced materials at the development phase, addressing key questions such as whether MXenes can be argued to be safe and sustainable, whether the SSbD framework is applicable for novel materials such as MXenes and finally, if there are aspects that future material developers should consider when researching novel material applications.

In order to assess whether MXenes can be argued to be safe and sustainable, we used $Ti_3C_2T_x$ MXenes as a case study and addressed the four steps of the SSbD framework. Our analysis revealed that the intrinsic hazardous properties of MXenes are still largely unexplored (Step 1), that risk management measures can be implemented to address the different kinds of hazards that could be involved in the production of MXenes depending on the production route (Step 2), that $Ti_3C_2T_x$ MXenes are not toxic at any of the tested concentrations, and the presence of NOMs does not have any significant impact on the toxicity of $Ti_3C_2T_x$ (Step 3), and, finally, that from a life-cycle perspective, the production of titanium powder production is the biggest "hotspot" for all environmental impact categories (shared first place with lithium chloride production for mineral use) (Step 4).

Based on the available evidence, we tentatively conclude that $Ti_3C_2T_x$ MXenes are safe and sustainable when applying the SSbD framework, although further research is needed regarding the intrinsic hazards of $Ti_3C_2T_x$ MXenes (in particular, long-term studies) while efforts should also be made to source titanium powder produced in more sustainable ways. Regarding the intrinsic properties of MXenes, the non-solubility (i.e., no Ti dissolution) of the $Ti_3C_2T_x$ MXenes in the investigated aquatic media could be one factor that governs the lack of observed toxicity towards aquatic organisms. However, biotransformation of MXenes within a biological system should not be ignored. Furthermore, investigation into bioaccumulation, considering the potential tissue uptake of MXenes in higher trophic level pelagic organisms or sediment-dwellers, would be beneficial. Similarly, more chronic ecotoxicity tests evaluating the impact of the residual MXenes in the gut on e.g., energy budget of the organisms would facilitate a more comprehensive assessment of MXenes.

The LCA focused only on the production route of $Ti_3C_2T_x$, and we addressed only the currently most commercial production process for MXenes. In recent studies, other investigators reported on the environmental impact of $Ti_3C_2T_x$ MXenes produced through alternative routes, albeit at lab-scale rather than industrial scale[16,17] (see Table S12). Notwithstanding, use or end-of-life phase aspects can be considered by developers of specific $Ti_3C_2T_x$ MXene-enabled products in future LCAs, using the data generated in this study for upstream MXenes production. Furthermore, we have not considered all the impact categories recommended in the SSbD framework as data on e.g., toxic emissions in the MXene production are not currently available, and the toxicity impact assessment would consequently only reflect emissions in the background system, e.g., coal power and sulfuric acid production.

Our use of $Ti_3C_2T_x$ MXenes as a case study revealed that the SSbD framework can be used for novel materials and we have demonstrated how data on acute environmental toxicity can be generated and intetgrated into the SSbD framework proposed by the European Commission. Based on our analysis, we believe that the SSbD framework recommended by the European Commission has considerable potential for developing chemicals or advanced materials by industry and academic researchers.

Aspects that future material developers should consider when researching novel applications include how to overcome a situation when little or no information is available on, for instance, carcinogenicity or reproductive toxicity at the early stages of development of the material. Further guidance is needed by the Commission on how to address lack of environmental, health and safety data and information in the SSbD framework in general and specific recommendations are needed regarding the use of NAMs to generate data. Finally, italso remains unclear how much weight should be put on results from the different steps in the final SSbD assessment[1].

Methods

Ti3C2Tx MXenes

$Ti_3C_2T_x$ was synthesized using the initial MAX phase (Ti_3AlC_2) by applying the Minimally Intensive Layer Delamination (MILD) synthesis approach with subsequent washing, exfoliation, and freeze-drying.

The phase and chemical composition, surface state and morphology of the MXene were characterized using transmission and electron microscopy, x-ray photoelectron spectroscopy-XPS, X-ray diffraction-XRD and atomic absorption spectroscopy-AAS. Investigations of the colloidal stability and surface transformation of the MXenes during exposure in the experimental simulated media were conducted using in-situ dynamic reflective microscopy and spectroscopic methods.

The investigation was conducted with dry MXene powder and its suspensions in the selected simulated natural media with nominal concentrations of MXene in the range of 5–80 mg/L. The suspensions were prepared by means of tip sonication in ultra-pure water for 2–16 min with subsequent dilution in the specific media to achieve the target nominal concentration of MXenes.

Dissolution studies of the $Ti_3C_2T_x$ MXene in the simulated aquatic media were conducted for 42 days and the extent of Ti dissolution from the MXene was determined by means of AAS after separating non-dissolved $Ti_3C_2T_x$ by means of filtration using 20 nm Anatop membrane filter after specific time points.

Test organisms

The test organisms used for the toxicity tests of the MXenes were crustacean *Daphnia magna* and green algae *Raphidocelis subcapitata* (*R. subcapitata*). 12 adult daphnia were placed in a 1 L glass beaker with 800 mL of Elendt M7 medium. The medium was renewed two times a week. The daphnia were fed daily with *R. subcapitata* via pipette ($2.5 \cdot 10^5$ cells/mL). The temperature was 20 ± 1 °C and the light-dark cycle was 12:12 h.

*R. subcapitata*was continuously cultivated in algal medium from the ISO 8692[23] using 20 mL glass vials. These glass vials were fitted with a holed screw cap lid to ensure sufficient gas exchange with the atmosphere while simultaneously avoiding evaporation losses. To ensure proper growth conditions, the vials were kept on a shaking table with 300 rpm (IKA® Schütler MTS 4) and illuminated continuously by fluorescent tubes placed below (30 W/33; Philips Amsterdam, The Netherlands) at a light intensity of 100 ± 20 µmol m^{-2} s^{-1}. This was measured by the LI-189 Photometer (LI-COR, Nebraska, USA). Furthermore, the temperature was kept at 20 ± 2 °C.

Other chemicals

Suwannee River NOM (Product 2R101N) from the International Humic Substances Society (Saint Paul, Minnesota, USA) was used for the ecotoxicity tests. The NOM consists of organic compounds with approximately 85% fulvic acid and 15% humic acid[24].

MXene test solutions

The MXene stock solutions were prepared immediately prior to the test. Suspensions were sonicated with a probe sonicator (Branson Probe Ultrasonicator with 13 mm diameter tip) for 10 min in an ice bath at 66% (40 W). Test dilutions were prepared by adding the stock suspensions to a volumetric flask with either daphnia (Elendt M7 medium) or ISO 8692 algal medium. For the uptake and depuration tests with daphnia, the test dilutions were prepared prior to use by adding the appropriate amount of stock suspension to a volumetric flask containing the Elendt M7 medium[25].

Algal growth inhibition tests

The short-term algal growth inhibition tests were performed following ISO standard 8692 with a few modifications. Concentrations ranged from 0.3125 to 100 mg/L and was prepared diluting the stock suspension in 25 mL measuring flasks with ISO freshwater agal medium[22]. For tests with NOM, it was added in a concentration of 20 mg/L using the same stock suspension of 200 mg NOM/L MilliQ water. During preparation of test suspension, the flasks were shaken and it was ensured that pH-level did not deviate more than 1.5 pH-units from the algal medium with 0.1 M HCl and 0.1 M NaOH. Then, approximately 10,000 algae cells/mL was added to each measuring flask and 0.4 mL sample was extracted from each flask and transferred to a glass vial. A volume of 1.6 mL acetone was added to each glass vial, which were then stored in the dark at room temperature with closed lids until biomass measurement. From the remaining test suspensions, 3 replicates (5 for the control) were prepared for each test concentration with 4 mL test suspension in each test vial with perforated lids to enable transfer of CO_2. Vials were placed in racks on an orbital shaker with natural white light and incubated at 21 ± 2 °C. After 24, 48 and 72 h subsampling of 0.4 mL from each replicate and 1.6 mL acetone was added to glass vials. Glass vials were closed tightly and stored in the dark for at least 24 h before measurement of the fluorescence. Fluorescence of algal pigments was used as a biomass surrogate[26]. The fluorescence was measured in each glass vial on a fluorescence spectrophotometer (F-1000, Hitachi) with excitation wavelength of 420 nm and emission wavelength of 670 nm.

Acute immobilization of *D. Magna*

The acute toxicity tests followed the OECD 202 Guideline for acute immobilization tests with Daphnia sp.[24]. Neonates (< 24 hours) were used for all tests. The MXene stock suspensions were diluted with Elendt M7 medium to concentrations ranging from 6.25 to 100 mg/L with a factor of 2 between concentrations. A similar concentration series was prepared containing 20 mg/L NOM. pH was measured in the test suspension to ensure pH ranging from 7.5 to 8.5 using either 0.1 M HCl or 0.1 M NaOH. 5 organisms were placed in 25 mL glass beakers with 4 replicates for each test concentration and 6 replicates for the control (only M7 medium). The number of immobilised daphnia in each beaker were noted after 0, 24 and 48 h. Tests on the reference compound, potassium dichromate and pH-values were within the guidance validity criteria[24].

Animals were digested in 100 µL HF and 100 µL H_2O_2 at 80 °C and acidified with 1 mL HNO_3 (65%) prior to chemical analysis. The samples were diluted to a final acid concentration of 2% HNO_3 before titanium content of each sample was measured with inductively coupled plasma mass spectrometry on Perkin Elmer NexION 3500 (Waltham, Massachusetts, USA).

Growth rate/lethality calculations

Algal growth rates were calculated with Eq. 1, assuming exponential growth,

$$\mu = \frac{ln(N_n) - ln(N_0)}{t_d} \quad (1)$$

In Eq. 1, µ is the growth rate (d^{-1}), N_0 is the initial biomass measurement, N_n is the final biomass measurement and finally, t_d is the length of the test (d). Then, the relative inhibition can be calculated with Eq. 2, using the growth rate of the control replicate related to each individual growth rate exposure.

$$I_i = \left(1 - \frac{\mu_i}{\mu_c}\right) \cdot 100 \quad (2)$$

Here, I_i denotes the percent growth inhibition related to each concentration (i), μ_i is the average growth rate for each concentration while μ_c is the average growth rate of the control. Concerning the daphnia tests, the percent lethality of the test compound is calculated using the number of dead daphnia in each beaker and the total number of organisms in the beaker, where each beaker is a replicate. With the growth rate inhibitions and percent lethality data for the test organisms (respectively, *R. subcapitata* and *D. magna*), the estimated EC/LC-values, concentration-response curves and their accompanying 95% confidence intervals were calculated and plotted with statistical software R (loaded with the drc package) using a Weibull function[27].

Optimization of dispersions of $Ti_3C_2T_x$

MXenes are readily dispersed in dH_2O. However, we observed that MXenes agglomerated in E3 medium, a standard medium for studies using zebrafish (*Danio rerio*). We therefore evaluated the stability of the dispersions in various media (Table S1). MXenes formed aggregates in E3 medium likely due to the high salt concentrations, and DLS was not performed due to the presence of visible aggregates. Addition of NOM reduced the agglomeration to some extent, and the hydrodynamic diameter of the MXenes was found to be 2597 ± 57 nm (Table S2). Using other low salt media such as EPA S and EPA VS, we observed that the MXene dispersion was stable in EPA VS medium with a size range of 298 ± 7 nm with a PDI of 0.4 indicating that the particles were almost monodispersed. Moreover, no aggregation was observed after 24 h. Similar results were obtained for MXenes dispersed in EPA VS in the presence of NOM (Table S2). However, MXenes were found to aggregate in EPA S. As no mortality or malformations were observed when zebrafish embryos were maintained in EPA-VS medium for 120 h, this medium was selected for further studies.

$Ti_3C_2T_x$ for tests on zebrafish embryos

The stock solution of $Ti_3C_2T_x$ was prepared in Mill-Q® water at a concentration of 1 mg/mL and sonicated using probe sonication for 15 min with 1-minute intervals. Then, MXenes were dispersed in various media commonly applied in ecotoxicological studies, i.e., E3 medium, EPA VS medium, and EPA S medium, at a concentration of 5, 10, 20, 40 and 80 mg/L and sonicated for 2 min. Then, NOM at a concentration of 10 mg/L was added to the media. The stability of the various dispersions at the highest concentration, i.e. 80 mg/L was evaluated by dynamic light scattering (DLS) using a Malvern Zetasizer ZS.

The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines). Zebrafish experiments were performed in accordance with national ethical guidelines and were approved by the Regional Committee for Animal Experiments in Stockholm under ethical permit number 14,049 – 2019.

Exposure of zebrafish embryos to $Ti_3C_2T_x$

Wild-type zebrafish of the AB strain were maintained in a recirculating system following standard breeding protocols, which included a 14-h light and 10-h dark photoperiod at a stable temperature of 28 ± 0.5 °C. For embryo collection, adult zebrafish were allowed to spawn overnight in breeding tanks at a 1:1 male-to-female ratio. In our study, embryos with visible abnormalities, irregular cleavage, or signs of unfertilization were excluded to ensure uniformity, and healthy, normally developing embryos were randomly selected to minimize selection bias. Zebrafish embryos taken at 4 h post-fertilization (hpf) were then exposed to different concentrations of MXenes dispersed in EPA VS medium as described above. Embryos in the control group were maintained under identical conditions without exposure to any treatment. The survival rate of the embryos was assessed at 24, 48, 72, 96 and 120 hpf, and this was confirmed by four morphological endpoints: coagulation of the embryo, lack of somite formation, lack of heartbeat, and non-detachment of the tail, in accordance with OECD test guideline (TG) 236. Hatching was recorded daily in treatment and control groups from 48 hpf until 72 hpf. Embryos were observed for malformations at 96 hpf, and representative embryos were photographed using a Nikon SMZ25 microscope (Nikon, Japan). The experiment was conducted three times, with 10 embryos per sample exposed in each trial, and each experiment was performed in triplicate.

Life cycle assessment of MXenes

Modelling was done using the OpenLCA software coupled to the Ecoinvent database (version 3.9.1). Considering the currently unknown geographical locations of future MXene production sites, datasets representing global averages of most inputs were applied. The synthesis route for Ti_3C_2 MXenes followed the production process described by Shuck et al.[15] with the inputs upscaled linearly. The study combined 50 g Ti_3AlC_2 (MAX phase) powder with 50 mL hydrofluoric acid (HF), 300 mL hydrochloric acid (HCl), and 150 mL distilled water. A yield of 52% was assumed[15]and a 20% reduction of solvent use was applied assuming an increased efficiency at larger-scale production[28].

In the first step of the Ti_3C_2 MXene synthesis, HF, HCl, and distilled water are applied for etching. The calculated upscaled amounts of input and output materials are provided in Table S3. Upstream data for HF, HCl, and distilled water was obtained from the Ecoinvent database. For HF and HCl, this upstream data is based on 100% pure substances (i.e., not mixed with water), whereas the reported values in Shuck et al. (shown in Table S3) are for varying concentration. The upscaled values were multiplied with the corresponding concentration to correct this. Concentrations are assumed to be 49% for HF[3] and 37% for HCl in line with what Wyatt et al.[29] resulting in inputs of 1.1 kg of pure HF and 4.1 kg of pure HCl per kg Ti_3C_2 MXene powder.

According to Shuck et al., the HF, water, and HCl are added to the reactor. The Ti_3AlC_2 powder is added with a screw feeder uniformly over 5 min. The mixture is then stirred for 24 h with water constantly flowing through the reactor cooling jacket to maintain the temperature at 35 °C. The energy needed to keep the temperature at 35 °C is calculated using the following equation from Piccinno et al.:

$$Q_{react} = \frac{C_P \cdot m_{mix} \cdot (T_r - T_{out}) + 3.03\, W/K \cdot (T_r - T_{out}) \cdot t}{\eta_{heat}} \quad (3)$$

where C_p is the heat capacity, m_{mix} is the mass of the heated material, T_r is the reaction temperature (35 °C), T_{out} is the ambient temperature, and η_{heat} is the efficiency of the heating element. The ambient temperature is assumed at 25 °C and the heating time is 24 h (86 400 s). The heat capacity of water (4186 J/kg·K) is used as a proxy for the whole mixture. Equation 3 is applicable for a 1000 L reactor, which means that 1224 kg is being heated (see Table S1). This results in 72 MJ needed to heat 1000 L. However, the mass of the batch needed to produce 1 kg MXene is 19.8 kg (all upscaled inputs in Table S3), This results in 1.7 MJ to heat the mass needed to produce 1 kg MXene. The heat is assumed to come from steam in the chemical industry, with an energy content of 3.075 MJ/kg[30], which results in 0.380 kg steam. The energy requirement for stirring the mixture is calculated using another equation provided by Piccinno et al. for a 1000 L reactor:

$$E_{stir} = 0.018\, m^5 s^{-3} \cdot \rho_{mix} \cdot t \quad (4)$$

Values for the stirring time t, calculation of the density of the whole mixture (ρ_{mix}), and the calculated stirring energy requirement (E_{stir}) are provided in Table S1. Equation 2 is based on a 1000 L batch. We therefore normalize the energy consumption to 1 kg of mixture by dividing E_{Stir} with the mass of 1000 L (1224 kg). This results in 1.55×10^{-3} MJ/kg stirred mixture. To produce 1 kg, we need 19.8 kg mixture (see Table S1). This means that in total, 0.028 MJ (0.0077 kWh) are needed.

After the reaction is completed, the mixture was washed with deionized water by centrifugation. Wyatt, et al. writes that washing the output from etching 4 g of Ti_3AlC_2 required 250 mL of deionized water per washing cycle. This means that 120 L deionized water per washing cycle is needed in this case, given an input of 1 923 g Ti_3AlC_2 that is etched. 5 washing cycles are assumed, which results in 481 L water to wash 1 kg of produced Ti_3C_2 MXene powder, assuming also a 20% reduction of the washing water at large scale.

The energy needed for centrifugation is 10 kWh/tonne dry material (Piccinno et al.). Given 5 washing cycles and 5 rounds of centrifugation, this results in a total centrifugation energy consumption of 0.05 kWh to produce 1 kg of dry Ti_3C_2 MXene powder. The energy requirement for pumping liquids has been estimated at 55 J/kg pumped material (Piccinno et al.). Given the total inputs of liquids (17.9 kg) in the reaction step, and 480 kg deionized water used in the washing (for 5 washing cycles), the energy requirement for pumping becomes 0.027 MJ.

Finally, the produced Ti_3C_2 MXene undergoes a delamination step with LiCl. For producing 26 g delaminated Ti_3C_2, 50 g LiCl, and 1 L distilled water is required. The LiCl is the reactant, and a linear upscaling is thus applied, resulting in 1.9 kg per kg Ti_3C_2. For the distilled water, which acts as a solvent, a 20% reduction was again applied, resulting in 31 kg distilled water per kg Ti_3C_2. The solution is then stirred for 24 h (values shown in Table S2), resulting in a total of 0.052 MJ or 0.015 kWh is needed for stirring 33.7 kg of mixture. This is followed by the same washing and centrifugation procedure as in the step before, with a total energy requirement of 0.05 kWh for centrifugation as well as 0.028 MJ for pumping of 513 kg (inputs of LiCl, solvent, and washing water). After the washing and centrifugation, one final round of washing by centrifugation was done to make sure no multilayer powder remained, requiring 96 kg water, 0.01 kWh for centrifugation, and 0.0053 MJ for pumping. The final unit-process dataset for the Ti_3C_2 MXene production process can be found in Table S4.

Life-cycle assessment of MAX phase production

The corresponding MAX phase for the Ti_3C_2 MXene is Ti_3AlC_2, which can be produced by ball milling of titanium powder, aluminium powder, and graphite powder as described by Zhou and Barsoum[31]. Material inputs follow the stochiometric relationship between Ti: Al: C (3:1:2). The molar masses of titanium, aluminium, and carbon are 47.88 g/mol, 26.98 g/mol, and 12.01 g/mol, respectively. The molar mass of Ti_3AlC_2 is 194.60 g/mol. This means that to produce 194.60 g (i.e., 1 mol) Ti_3AlC_2, the stoichiometric inputs required are $3 \cdot 47.88 = 143.64$ g titanium, $1 \cdot 26.98 = 26.98$ g aluminium, and $2 \cdot 12.01 = 24.02$ g carbon. In addition, a material yield at 82.5 wt% is applied as reported by Pazniak et al.[32]. Ball milling is modelled as a grinding step, with energy requirements in the range of 8–16 kWh per tonne grinded material. The higher value is applied, as recommended by Piccinno et al. when the final particle size is unknown. Regarding the energy requirement for the sintering step, 1.8 kWh per kg finished product is assumed based on Kruzhanov and Arnhold and the energy requirement for the annealing step is assumed to be 3.6 MJ per kg finished product as reported by Azevedo et al.[33] for iron and steel powders. We assume that the energy carrier in the sintering and annealing processes is steam, resulting in 0.638 kg steam in total for both steps. Unit process for the Ti_3AlC_2 MAX phase production is presented in Table S5. The upstream data for the titanium powder production is obtained from Dolganov et al.[34]. Main input material is titanium sponge (so called due to its porous structure), which is produced in the Kroll process. The titanium sponge is then further processed using free fall gas atomization (FFGA), where the sponge is converted to molten phase. The molten titanium is then broken down into tiny droplets by pressurized argon gas. Unit-process data for the titanium powder production with FFGA can be found in Table S6. Unit-process data for the aluminium powder production is based on data from Huang et al.[35] where aluminium powder is produced from aluminium ingots by applying 1.9 kWh electricity per kg. A generic yield of 95% was assumed based on Wernet et al.[36]. Unit process for aluminium powder production is shown in the supplementary information Table S7.

To test the influence of a future decarbonized electricity supply, both a current global and a decarbonized electricity mix were applied in the MXene and MAX phase productions, the latter modelled as the current

Norwegian electricity mix (> 90% hydropower). In addition, as described in the supplementary information, the electricity supplies to the main upstream contributors to climate change were also changed to Norwegian electricity. The Environmental Footprint (EF) 3.1 midpoint method was used for converting emissions and resource use along the life cycle to impacts. In addition, the ReCiPe 2016 midpoint method with a hierarchical perspective was used for comparison, for which the results are found in the supplementary information.

Data availability

All the research data is provided in the manuscript and in the supplementary information.

Received: 27 September 2024; Accepted: 2 December 2024
Published online: 28 December 2024

References

1. COMMISSION RECOMMENDATION of 8. 12.2022 establishing a European assessment framework for 'safe and sustainable by design' chemicals and materials https://research-and-innovation.ec.europa.eu/news/all-research-and-innovation-news/recommendation-safe-and-sustainable-chemicals-published-2022-12-08_en
2. Hansen, S. F., Paulsen, F. & Trier, X. Smart materials and safe and sustainable-by-design - a feasibility and policy analysis. In M.-V. Florin (Ed.), Ensuring the environmental sustainability of emerging technologies (2 ed., pp. 73-89). Swiss Federal Institute of Technology Lausanne (EPFL). (2023). https://doi.org/10.5075/epfl-irgc-298445
3. OECD. Advanced Materials: Working Description Series on the Safety of Manufactured Nanomaterials No. 104. (2023). Organisation for Economic Co-operation and Development (OECD). (2023).
4. Lakhe, P. et al. Process Safety Analysis for Ti_3C_2Tx MXene Synthesis and Processing. *Ind. Eng. Chem. Res.* 58, 1570–1579 (2019). (2019).
5. Wu, J. et al. Safety Assessment of 2D MXenes: in Vitro and in vivo. *Nanomaterials* **12**, 828 (2022).
6. Jastrzębska, A. M. et al. *In vitro* studies on cytotoxicity of delaminated Ti_3C_2 MXene. *J. Hazard. Mater.* **5**, 1–8 (2017).
7. Wu, W. et al. Evaluating the cytotoxicity of Ti_3C_2 MXene to neural stem cells. *Chem. Res. Toxicol.* **33**, 2953–2962 (2020).
8. ECHA. Guidance on Information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. DOI: (2017). https://doi.org/10.2823/337352
9. Downes, M. et al. Comprehensive synthesis of $Ti_3C_2T_x$ from MAX phase to MXene. *Nat. Protoc.* **19**, 1807–1834 (2024).
10. Kamysbayev, Y. et al. Covalent surface modifications and superconductivity of two-dimensional metal carbide MXenes. *Science* **369**, 979–983 (2020).
11. Hjorth, R. et al. Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials. *NanoImpact* **8**, 28–37 (2017).
12. Wiley, D. S. et al. Chemical Screening in zebrafish for novel biological and therapeutic discovery. *Methods Cell. Biol.* **138**, 651–679 (2017).
13. Kaur, J. et al. Toxicity screening and ranking of diverse engineered nanomaterials using established hierarchical testing approaches with a complementary *in vivo* zebrafish model. *Environ. Sci. : Nano*. **9**, 2726–2749 (2022).
14. Skjolding, L. M. et al. Aquatic ecotoxicity testing of nanoparticles—the Quest to disclose Nanoparticle effects. *Angew Chem.* **55**, 15224–15239 (2016).
15. Nasrallah, G. K. et al. Ecotoxicological assessment of Ti_3C_2Tx (MXene) using a zebrafish embryo model. *Environ. Sci. Nano*. **5**, 1002–1011 (2018).
16. Shuck, C. E. et al. Scalable synthesis of Ti3C2Tx mxene. *Adv. Eng. Mater.* **22**, 1901241 (2020).
17. Ungureanu, A. et al. Systematic life cycle environmental impact comparison of alternative synthetic strategies for Ti_3C_2Tx MXene. *ACS Sustainable Chem. Eng.* **12**, 5893–5906 (2024).
18. Dadashi Firouzjaei, M. et al. Life-cycle assessment of Ti(3) C(2) T(x) MXene synthesis. *Adv. Mater.* **35**, e2300422 (2023).
19. Arvidsson, R. et al. Prospective life cycle assessment of graphene production by ultrasonication and chemical reduction. *Environ. Sci. Technol.* **48**, 4529–4536 (2014).
20. Caldeira, C. et al. *Safe and Sustainable by Design Chemicals and materials - Framework for the Definition of Criteria and Evaluation Procedure for Chemicals and Materials. EUR 31100 EN* (Publications Office of the European Union, 2022).
21. Gao, F. et al. Environmental impacts analysis of titanium sponge production using Kroll process in China. *J. Clean. Prod.* **174**, 771–779 (2018).
22. Feng, Q. et al. Research Progress of Titanium Sponge production: a review. *Metals* **13**, 408 (2023).
23. ISO. ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae (Edition 3). International Organization for Standardization. (2012).
24. Chang, T. et al. Effects of interactions between natural organic matter and aquatic organism degradation products on the transformation and dissolution of cobalt and nickel-based nanoparticles in synthetic freshwater. *J. Hazard. Mater.* **445**, 130586 (2023).
25. OECD. Test No. 202: Daphnia sp. Acute Immobilisation Test. Organisation for Economic Cooperation and Development. (2004).
26. Mayer, P., Cuhel, R. & Nyholm, N. A simple *in vitro* fluorescence method for biomass measurements in algal growth inhibition tests. *Water Res.* **31**, 2525–2531 (1997).
27. Ritz et al. *Dose-Response Anal. Using R PLoS ONE* **10**, e0146021 (2015).
28. Piccinno, F. et al. From laboratory to industrial scale: a scale-up framework for chemical processes in life cycle assessment studies. *J. Clean. Prod.* **135**, 1085–1097 (2016).
29. Wyatt, B. C. et al. High-temperature stability and phase transformations of titanium carbide (Ti3C2Tx) MXene. *J. Phys. : Condens. Matter*. **33**, 224002 (2021).
30. Nieuwlaar, E. et al. Final energy requirements of Steam for Use in Environmental Life Cycle Assessment. *J. Ind. Ecol.* **20**, 828–836 (2016).
31. Zhou, A. G. & Barsoum, M. W. Kinking nonlinear elastic deformation of Ti3AlC2, Ti2AlC, Ti3Al(C0.5,N0.5)2 and Ti2Al(C0.5,N0.5). *J. Alloys Compd.* **498**, 62–70 (2010).
32. Pazniak, A. et al. Ti3C2Tx MXene characterization produced from SHS-ground Ti3AlC2. *Mater. Design*. **183**, 108143 (2019).
33. Azevedo, J. M. C. et al. Energy and material efficiency of steel powder metallurgy. *Powder Technol.* **328**, 329–336 (2018).
34. Dolganov, A. et al. Environmental assessment of the near-net-shape electrochemical metallisation process and the Kroll-electron beam melting process for titanium manufacture. *Green Chem.* **22**, 1952–1967 (2020).
35. Huang, R. et al. Energy and emissions saving potential of additive manufacturing: the case of lightweight aircraft components. *J. Clean. Prod.* **135**, 1559–1570 (2016).
36. Wernet, G. et al. The ecoinvent database version 3 (part I): overview and methodology. *Int. J. Life Cycle Assess.* **21**, 1218–1230 (2016).

Acknowledgements
S.F.H., M.B.N., L.M.S., J.K., N.M.N.D., F.H., A.K., I.O., B.F. and B.F. would like to acknowledge that this work was supported by the Swedish Foundation for Strategic Environmental Research (Mistra) through the Mistra Environmental Nanosafety Programme. S.B. would like to acknowledge that this research was carried out in the framework of the AI production network Augsburg funded by the Bavarian State Ministry of Science and the Arts inside the High-Tech-Agenda Plus. Finally, J.B. would like to acknowledge the UK Engineering and Physical Sciences Research Council [EP/L01663X/1].

Author contributions
S.F.H. conceived and coordinated the study and drafted the manuscript; J.B. prepared the MXenes; A.K. performed material characterization; M.B.N. and L.M.S. performed ecotoxicological testing using algae and crustaceans; J.K. performed ecotoxicological testing using zebrafish embryos; B.F., I.O., and S.B. supervised the experimental work; N.M.N.D., F.H., M.B.N. and R.A. conducted the life cycle assessment of the MXenes. All authors contributed to the writing and editing of the manuscript, and approved the final version.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-82063-w.

Correspondence and requests for materials should be addressed to S.F.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.