Grouping all carbon nanotubes into a single substance category is scientifically unjustified

To the Editor — The International Chemical Secretariat (ChemSec) recently added carbon nanotubes (CNTs) to the so-called SIN ('Substitute It Now') list of chemicals that they believe should be restricted or banned in the EU[1]. CNTs are the first nanomaterials to be placed on the SIN list. Should this 'designation' concern us as scientists active in the areas of nanotoxicology and nanomedicine? Yes, as it implies that all CNTs can be considered as one material category, which is not the case. Grouping or categorization of chemicals is a valid approach in risk assessment provided that substances with similar properties are grouped together[2]. However, the key (scientific) question is whether all CNTs display the same properties.

Five years ago, the International Agency for Research on Cancer (IARC) classified a particular type of long and rigid CNT, designated as MWCNT-7, as possibly carcinogenic to humans on the basis of available animal studies, whereas all other CNTs were considered 'not classifiable' with regard to their carcinogenicity[3]. The findings of the original evaluation on the inadequate or limited evidence of carcinogenicity for most CNTs were confirmed in a thorough follow-up study a few years later[4]. Hence, while there is no doubt that long and rigid CNTs may cause considerable damage to the lungs following pulmonary exposure (especially when administered at high doses[5]), it is important to note that short and/or tangled CNTs are much less harmful[6,7]. Indeed, it has been demonstrated that the 'asbestos-like' pathogenicity of long CNTs can be alleviated through chemical functionalization, possibly as a result of the effective shortening of the CNTs through debundling or untangling[8]. Chemical functionalization may also impact the stiffness of CNTs, which is perhaps one of the most important parameters with regard to biological reactivity[9].

Most toxicological studies have focused on the length of CNTs owing to the fact that long (>15–20 μm) and biopersistent fibres are known to induce 'frustrated' phagocytosis[4]. However, the diameter and rigidity of CNTs are also important drivers of their biological effects. More specifically, the propensity of CNTs to induce damage to lysosomes — key organelles within the cell — as a function of their biological stiffness has been proposed as a general predictor of the pathogenicity of such materials[10]. Indeed, the rigidity of CNTs is strongly correlated with both acute and chronic inflammation[11]. The take-home message is that not all CNTs are created equal and specific properties including length, diameter and rigidity, as well as the degree of chemical functionalization, determine the biological reactivity or pathogenicity of these materials.

Biopersistence is another important factor that has to be considered. In a study published 10 years ago in this journal, short, single-walled CNTs were shown to be susceptible to degradation by primary human neutrophils[12]. In addition, macrophages have been shown to be capable of digesting multiwalled CNTs[13], and processing of CNTs in microglia — the resident macrophages of the brain — has also been documented[14,15]. Thus, CNTs are not necessarily biopersistent, although the rate of biodegradation may vary depending on the specific material properties. Further studies are needed to address this question.

We concede that the precautionary principle may be a reasonable approach in cases in which data are lacking[1]; however, there are plenty of data to show that CNTs should not be viewed as one material but instead as a class of materials with varying properties that may elicit distinct biological outcomes in vitro and in vivo. ❐

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Banning carbon nanotubes would be scientifically unjustified and damaging to innovation

To the Editor — In a recent correspondence, the Swedish non-profit organization ChemSec announced the addition of carbon nanotubes to the SIN ('Substitute It Now') list[1]. Carbon nanotubes were added as an entire material class that "should be restricted or banned in the EU." We believe that this recommendation confuses researchers and the public as it is based on evidence from a very narrow subset of data. Such a designation will likely hinder innovations that could lead to safe and effective applications of carbon nanotubes. Furthermore, this line of reasoning could damage other fields of science and technology, if applied similarly.

We have worked with carbon nanotubes since the 1990s, a time marked

by excitement and confusion about the promises and concerns of nanomaterials[2,3]. During this period, broad claims of toxicities were ascribed to carbon nanotubes, which were later found to apply only to a narrow subset of carbon nanotube preparations and/or exposure routes[4,5]. Numerous subsequent publications that reported more nuanced results were given much less attention[6–8]. Importantly, data showing a lack of toxicity are often not published, as they are usually considered 'negative' results[9]. Unfortunately, we are left with a one-sided story that damages research efforts. The recent report by the advocacy group ChemSec seems to have been confused by these issues.

The REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation (and the recent amendments to the Toxic Substances Control Act (TSCA) in the USA) places the burden of proof on producers and importers of chemicals to demonstrate safety. The nanotechnology field subscribes to this principle and routinely conducts tests on the biocompatibility and potential biotoxicity of nanomaterials that are under development for medical and non-medical applications. REACH registration has been attained for limited quantities of three classes of carbon nanotube materials (932-414-1, 943-098-9 and 701-160-0). The inclusion of all carbon nanotubes in the SIN list discourages research and investment in these materials that are being applied, for instance, to treat kidney disease[10], track viral outbreaks[11] and to investigate Parkinson's disease[12]. ChemSec should take special care to not inadvertently damage a research field by generalizing narrowly-applicable findings to a diverse family of materials, and to not misapply the solid precautionary principles on which REACH and TSCA are based.

Nanomaterial diversity leads to benefits and confusion

The problematic risk assessment of nanomaterials stems in part from the virtually infinite possible material variants and modifications[13], leading to a variety of physical, chemical, mechanical and biological properties[14]. Under the umbrella of 'carbon nanotubes', which includes cylindrical carbon-based structures, physical dimensions vary by many orders of magnitude[15]. Carbon nanotube diameters may range from several ångströms to hundreds of nanometres, with lengths from nanometres to metres, in different forms such as powders, sponges, freestanding films, on substrates and dispersed in solutions. Moreover, they can be covalently or non-covalently functionalized with nearly every class of chemical species[16], from rare earth metals to RNA. Nanotubes can be aggregated or organized into diverse microscopic or macroscopic structures with different strength and stiffness profiles. The resulting materials range from structures that resemble carbon fibres, to improve, for instance, the strength of building materials[17] or to restore myocardial conduction in arrhythmic hearts[18], to nanoscopic colloids that can interrogate the properties of living cells[19], augment stem cell differentiation[20], or deliver RNA[10]. Carbon nanotubes have also been precisely synthesized into centimetre-long fibres[21], while shorter, functionalized tubes can enter the lysosomes of cells for molecular imaging studies[22]. In applications such as nanobionics[23], gene delivery[24], image-guided surgery[25] and non-invasive disease monitoring[26], processed, functionalized carbon nanotubes have been successfully used without inducing toxicity in cells[27,28], small animals[29] or non-human primates[30].

Unfortunately, every broad claim of concern resulting from a study using one variant of carbon nanotubes reverberates throughout the entire research field. For example, studies using long, insoluble nanotube aggregates with large diameters, administered via instillation (that is, depositing a bolus in the animal), reported lung toxicity in mice[31,32]. As a result, measures have been in place since the early 2000s to prevent human exposure to airborne nanotubes. However, it was later reported that proper functionalization can abrogate lung toxicity[7]. Moreover, soluble, short nanotubes showed no toxicities in primates, as measured by blood chemistry, haematology and pathology[30]. Unfortunately, these results did not reach the prominence of the earlier publications and were apparently not considered in the ChemSec report[6].

Conclusion from the World Health Organization

Scrutiny from regulatory intergovernmental agencies has resulted in the recognition of nanomaterial diversity. In 2014, the International Agency for Research on Cancer (IARC) published a monograph evaluating the carcinogenic risks of carbon nanotubes[33]. The monograph concluded that 'single-walled carbon nanotubes are not classifiable as to their carcinogenicity to humans (Group 3)"[34]. A review published in the same year concluded that the majority of studies did not characterize the properties of the nanomaterials, which considerably reduced their significance[9]. Additionally, many of these earlier studies were performed with nanotubes that were long, improperly stabilized by excipients leading to aggregation, administered to animals in the microgram scale and/or contained metal catalysts. Both ChemSec and IARC monographs cite the 'suspected carcinogen' status of "Carbon Nanotube Single-walled (>55%) below 2 nm (diam.) and 5-15 micrometer length (EC no. 608-533-6)". However, ChemSec decided that data from a preparation with up to 45% impurities and with lengths above 5 micrometres could accurately reflect the carcinogenicity of all single-walled carbon nanotubes. The disagreement in the conclusions of the IARC and ChemSec stems from the decision of the IARC Working Group which stated: "CNT cannot be considered as a single well-defined substance but as families of different materials, the number of which is growing dramatically." In 2019, the Working Group recommended re-evaluation of multiwalled carbon nanotubes as a high priority due to the availability of new bioassays and mechanistic evidence[35]. Based on the body of recent evidence, single-walled carbon nanotubes were not recommended for re-evaluation[35].

A way forward

Human and environmental safety are a top priority; however, engineering of novel technologies progresses only through research and development. As our understanding of a material increases, so does our ability to safeguard against its harms by engineering it into safe formulations, such as silica[36] and iron oxide[34] — materials that can either pose inhalation hazards or be injected into humans for imaging[37]/therapeutic[38] applications. Nanotechnology researchers are well aware that the unique properties of nanomaterials, which hold the potential for technological advancements, can also lead to unique biological interactions[39]. To enable precise mapping of nanomaterial identity and biological interactions, a comprehensive set of standards governing material characterization, biological characterization and details of experimental protocols was proposed in 2018 and reported in *Nature Nanotechnology*[40]. Additionally, the multiple routes of potential exposure result in a different set of risk parameters and safety concerns. Although the nanomaterial community is becoming aware of the importance of using standardized and accepted characterization methods (for example, Organisation for Economic Co-operation and Development (OECD) guidelines), we are still at the early stages of defining distinct nanomaterial preparations related to specific toxicities. A standardized safety and material-handling procedure should be established for dispersed engineered nanomaterials; for example, those exposed

to easily aerosolizable materials should wear appropriate respiratory protection. As applications are realized, the entire life cycle of safety should be assessed, including production, manufacturing, shipping, use and end-of-life. These will be very different for carbon nanotubes used, for example, in drugs and medical devices (where each step of the supply and use chain is tightly controlled) versus consumer products such as batteries and sensors. The criteria used by ChemSec for toxicity are well-reasoned. However, guidelines must only be applied to the specific sub-classes of nanomaterials for which evidence is available. Such a precise approach to regulating individual nanomaterial preparations certainly requires more effort; however, conclusions of safety or toxicity have to be based on experimental data in the right context. We call on ChemSec to modify the record of carbon nanotubes in the SIN list, to remove the broad claims of toxicity for an entire material class, and to delineate the specific materials for which data actually exist. ❐

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