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Experimental approaches to data generation for REACH compliance of multi-walled carbon nanotubes: substance identification

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Applying regulatory-accepted, standardised test guidelines to multi-walled carbon nanotubes (MWCNTs) presents challenges. These challenges arise from the inherent physicochemical characteristics of MWCNTs to form entangled, highly cohesive bundles that agglomerate rapidly. Consequently, many test methods are not suitable for these materials. In this study, we evaluated the applicability of existing standardised analytical methods and explored potential alternatives suitable for MWCNTs. Our focus was on EU-REACH data requirements related to substance identification and physicochemical properties, particularly size, shape, and dustiness. We successfully established a non-invasive method to measure the length of individual carbon nanotubes within MWCNT bundle agglomerates that does not break the individual tubes.

Environmental significance

Generating mandatory data for nanomaterial identification under the REACH Regulation is challenging due to the lack of standardised test guidelines. Our research focusses on fulfilling data requirements for carbon-based nanomaterials, specifically multi-walled carbon nanotubes (MWCNTs), related to size, shape, and dustiness. We developed a non-invasive method to measure the length of individual carbon nanotubes within MWCNT agglomerates, addressing a key information gap. Our work is highly relevant given the increasing production and release of nanomaterials, and the need for reliable characterisation methods for a robust chemical risk assessment. By prioritising the protection of environmental and human health, our findings will ultimately contribute to assessing and managing risks to living organisms and their surroundings.

Introduction

In the European Union, manufactured or imported chemicals must be registered according to the provisions of the REACH Regulation (Regulation (EC) No. 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals). REACH Annexes VI to X specify the standard information requirements for registration purposes. In 2018, additional nano-specific data requirements were published for these annexes, implemented in Commission Regulation (EC) 2018/1881. Since January 2020, registrants of nanomaterials have been obliged to provide nano-specific information for compliant REACH registrations. According to Article 13(3) of the REACH Regulation, tests conducted to fulfil these information requirements '*shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the commission or the agency as being appropriate*'. Based on this provision, newly generated experimental data for REACH registration purposes are generally accepted only if standardised and validated test guidelines are followed, as defined in the EU Test Methods Regulation (Commission Regulation (EC) No. 440/2008), which refers to test guidelines (TGs) of the Organisation for Economic Co-operation and Development (OECD) for many of the REACH-required tests. To date, nano-specific test guidelines are often missing or have only limited applicability,[1–3] thus impeding the production of reliably accepted data. The situation is particularly challenging for purely carbonaceous nanomaterials, such as MWCNTs. Relevant guidance documents of the European Chemicals Agency (ECHA) address carbonaceous nanomaterials only to a limited extent and provide no guidance on how to consider the specific properties of MWCNTs, which cause severe problems in tests required by REACH.[4–6] Recognising these limitations, authorities have recently published calls for identifying information gaps related to nanomaterials. For example, in 2024 and

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2025, ECHA's European Union Observatory for Nanomaterials (EUON) issued calls for 'Closing nanomaterials' information gaps: EUON welcomes new study proposals' and 'EUON calls for study proposals to address nanomaterials knowledge gaps in the EU'.[7,8] In late 2024, EUON conducted a 'Survey on the state of the art of carbon-based nanomaterial detection and quantification in environmental and biological matrices' aimed at gathering insights into existing and novel analytical methods for identifying, characterising, and quantifying carbon-based nanomaterials in various matrices.[9] In June 2025, the ECHA-sponsored report on the results of the survey along with an extensive literature search for information on existing methods was published.[10,11] According to the authors, significant progress was made with regard to standardisation-readiness of available methods, and new ASTM, ISO, and OECD standardised guidelines applicable to carbon-based nanomaterials were published. Despite this, standardisation for carbon-based nanomaterial analysis remains challenging, *e.g.*, due to the variability of these materials, the lack of universally accepted protocols and reference materials, and the limitations of existing methodologies.[1,10] For instance, OECD TG 125 (ref. 12) is not applicable to the constituent particle size of high aspect ratio nanomaterials exceeding 20 μm in length. Furthermore, the inherent characteristics of MWCNTs, such as entanglement and high cohesion, make standard constituent particle size measurements according to this guideline technically unfeasible. These examples demonstrate the high priority of addressing shortcomings related to the generation of standardised experimental nanomaterial information.

In this study, we share the knowledge gained through the practical application of standardised test guidelines to fulfil REACH information requirements. Our work focusses on available, validated, and standardised guidelines for carbonaceous nanomaterials. Where standard methods were inapplicable but experimental data were necessary, we propose alternative methodologies. We showcase the technical and practical challenges of generating data for a real REACH registration dossier, highlighting failures and successes that we encountered while generating new data, along with feasible adaptations to test guidelines that we believe are acceptable to regulatory bodies. This study provides a realistic regulatory perspective that differs from studies conducted by research institutes or academia in several respects. For example, applying different analytical techniques is limited by availability, budget, and time constraints. These constraints generally also prevent the repetition of studies under refined experimental conditions after more experience is gained. While these issues may be perceived as limitations, this study provides real-world insights that are of interest for stakeholders involved in data generation for REACH registration purposes and the broader scientific community, interested in test methods for carbon-based nanomaterials. As is evident from the above, this work does not intend to validate TGs or analytical techniques for MWCNTs, nor does it aim to define new standard procedures; instead, it introduces new innovative experimental approaches to overcome challenges of generating data for specific, difficult-to-assess endpoints of MWCNTs in a REACH context.

In this paper, we present our novel approaches to overcome the burdens of non-compliance for endpoints related to substance identification and physicochemical properties under REACH Annexes VI and VII. These endpoints include size, *i.e.*, constituent particle length, shape, and dustiness for nanoforms. Challenges of data requirements related to environmental fate and human health are addressed in our companion articles.[13,14]

Shape, aspect ratio, and other morphological characterisation according to REACH Annex VI, section 2.4.4

Measuring the particle size and size distribution of nanomaterials is crucial for assessing potential health and environmental impacts, which is related to the specific shape-related toxicity of nanomaterials.[12,15] The REACH Regulation under Annex VI, section 2.4.4, requires the characterisation of shape and the calculation of the aspect ratio for nanomaterials. For elongated nanoparticles, such as MWCNTs, measuring the length of the constituent particles is an obligatory data requirement.[16] In the context of Annex VI, these constituent particles are defined as the individual carbon nanotubes. However, MWCNTs are commonly synthesised *via* chemical vapour deposition (CVD), which produces large agglomerated bundles consisting of numerous individual nanotubes.[19–22] These MWCNTs naturally exist in entangled, rope-like bundles and never as discrete individual tubes, making them inherently challenging to characterise. Common methods for measuring MWCNT length suggest untangling the individual tubes from the bundles. However, this often leads to tube breakage.[17,18] Hence, the available methods cannot accurately reflect the true length of an individual carbon nanotube and are prone to generate erroneous data of tube debris. To the best of our knowledge, no reproducible method currently exists that can disperse MWCNT bundles into single carbon nanotubes without breaking the individual tubes.

In 2023, OECD TG 125 'Nanomaterial Particle Size and Size Distribution of Nanomaterials' (ref. 12) was published providing standardised methods for nanomaterial particle size measurements, including high-aspect-ratio nanomaterials such as MWCNTs. The guideline covers a range of methods, including atomic force microscopy (AFM), dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and small angle X-ray scattering (SAXS) for primary particles and agglomerated nanomaterials, spanning from 1 nm to 1000 nm. OECD TG 125 and its respective validation report[23] use SEM as a method to measure MWCNTs, representative of a high-aspect nanomaterial, here defined as fibres based on

the sole criterion of an aspect ratio ≥3. Limitations arise because the maximum length of 20 μm clearly places K-Nanos MWCNTs outside the applicability domain of OECD TG 125. Yet, we had to characterise the constituent particle size of our MWCNT nanomaterial for a REACH-compliant registration. We measured the bundle length of K-Nanos MWCNTs by SEM, given that the sample powder observed in the electron micrographs appeared predominantly in agglomerated form, and the limited field of view at higher magnifications precluded the measurement of the full length of individual carbon nanotubes. With a novel approach, we measured the length of elongated constituent particles directly from SEM images by visually tracking the individual carbon nanotubes within the MWCNT agglomerates. We used images from early synthesis cycles, which provided shorter K-Nanos MWCNTs, to identify the individual MWCNTs within the agglomerates. These shorter K-Nanos MWCNTs could be fully captured in a single, high-magnification SEM image. We then derived the full constituent particle length by extrapolating these measurements to the complete growth cycle of K-Nanos MWCNTs synthesis.

Dustiness for nanoforms according to REACH Annex VII, section 7.14

Dustiness data are essential for robust risk management and workplace safety. In December 2024, a draft OECD 'Test Guideline on the Dustiness Determination of Manufacture Nanomaterials' (ref. 24) was made publicly available. This draft OECD TG was developed to address the need for standardised dustiness test methods applicable to powders containing granular and fibrous nano-objects, aggregates, and agglomerates (NOAAs). The draft OECD TG includes methodologies of test guideline EN 17199:2019 'Workplace exposure—Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles' (ref. 25), such as the rotating drum, small rotating drum, and continuous drop (CD) methods. In the present work, we applied the CD method to estimate workplace exposure to the respirable mass fraction of K-Nanos MWCNTs. This study was advised and analysed by the IGF Bochum (Institut für Gefahrstoff-Forschung, Bochum, Germany), an institute that participated in, and is referenced by, the respective OECD draft test guideline (ref. 24) for the CD method. Given the known physicochemical properties of MWCNTs, we expected the nanomaterial to diffuse poorly in air, agglomerate rapidly, and deposit quickly. Nevertheless, we aimed to produce meaningful results despite the anticipated challenges of generating measurable data.

Materials

Multi-walled carbon nanotube substance-specific information

K-Nanos. K-Nanos are elongated high-aspect-ratio MWCNTs that are aligned and entangled in bundle-type carbon agglomerates. Here, K-Nanos comprised three nanoforms, which were considered a 'set of similar nanoforms' based on REACH Annex VI, amended by Commission Regulation (EC) 2018/1881 and assessed according to the criteria of Janer *et al.*[26,27]: K-Nanos 100, K-Nanos 210, and K-Nanos 300. The purity of K-Nanos was >91%. The characteristics of constituent particles are as follows: the average individual carbon nanotube length and diameter ranged from 46.22–62.76 μm and 12.13–14.58 nm, respectively. Derived from these values, the aspect ratio was 3789 : 1–4304 : 1. The average bundle length of MWCNTs ranged from 42.00–54.85 μm, while the average bundle diameter ranged from 2.46–3.21 μm. The specific surface area and density at 20 °C were 200.8–247.7 $m^2\ g^{-1}$ and 1.76 $g\ cm^{-3}$, respectively. More details and nanoform-specific data can be found in SI Table S1.

Methods

Shape – MWCNT bundle length

SEM was employed to measure the length of pristine MWCNTs without sample modification but in air. Samples were prepared by mounting the MWCNT powder onto carbon tape affixed to a sample holder. The excess sample material was carefully removed using an air blower to ensure a clean observation surface. We acquired micrographs using a JEOL JSM-7500F scanning electron microscope, equipped with a field emission gun (FE-SEM). The instrument was operated with an accelerating voltage ranging from 0.1 kV to 30 kV and a probe current from 1 pA to 2 nA. The objective lens was of a semi-in-lens type, and the microscope provided a resolution of 1.0 nm at 15 kV and 0.4 nm at 1 kV, with a magnification range from ×25 to ×1 000 000. Tilting capabilities of −5° to +70° (X/Y) were available. The electron microscope was operated using the instrument's default calibration as provided by the manufacturer, and all images were obtained using the same SEM settings.

For quantitative analysis, we manually measured the vertical Feret diameter (bundle length) of randomly selected MWCNTs from end to end (Fig. 1), using the Digital Micrograph analysis program.

Size – individual MWCNT length

Since a bundle from a full synthesis cycle did not fit into a single SEM image at the required resolution, we produced

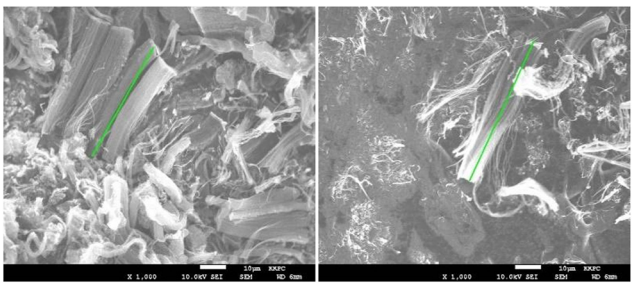

Fig. 1 K-Nanos 210 MWCNT bundle length (vertical Feret diameter, green line).†

K-Nanos 100, 210, and 300 MWCNTs at three different, controlled synthesis times: 1 min, 15 min, and 30 min. The MWCNT powders were mounted onto a sample holder with carbon tape. The excess sample was removed with an air blower. MWCNT images were captured with SEM (JEOL JSM-7500F) at ×5000 magnification (1.0 nm (15 kV), 0.4 nm (1 kV), 1 pA to 2 nA, WD 6.3–6.6 mm). The maximum length of a bundle that fitted in a single image at ×5000 magnification corresponded to approximately 30 min growth time. The single carbon nanotube length was measured digitally by tracking the entangled carbon nanotubes within the MWCNT bundle for each synthesis time (Fig. 2, SI S1–S3). The length of each bundle was also measured. Image analysis and processing were performed with Java ImageJ, version 1.54i.[28]

Subsequently, the individual tube length was extrapolated to the full growth time of 55 minutes, using linear regression analysis in Microsoft Excel™. We visually tracked and measured single carbon nanotubes (n = 9) within each bundle (n = 1) for growth times of 1, 15, and 30 minutes to derive the single tube length in μm for each time point. Statistical summary parameters were calculated for each time point in Microsoft Excel™, comprising the mean value, minimum, maximum, percentiles D10, D50 (median) and D90, standard deviation (SD), and coefficient of variation (CoV). Linear regression analysis of the individual tube length was performed across three time points (1, 15, and 30 min), *i.e.*, six equations were derived in total, one for each parameter (Mean, Min, Max, D10, D50, and D90). Measurement uncertainty was determined in accordance with principles outlined in JCGM guidance document 100:2008.[29] Information on measurement uncertainty was calculated for the individual tube length at growth times of 1, 15, and 30 minutes.

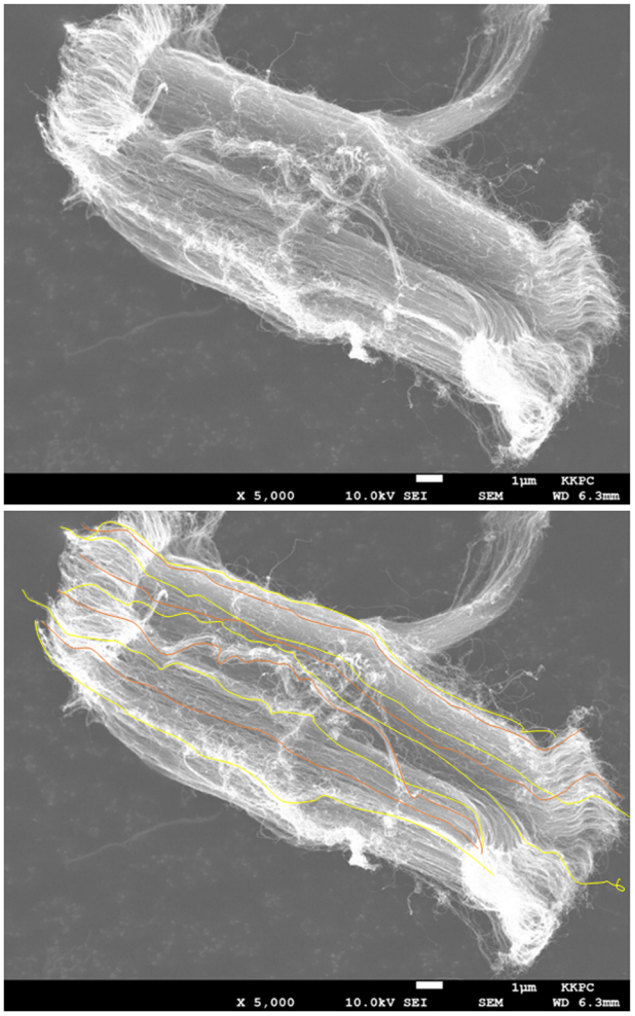

Fig. 2 K-Nanos 210 MWCNTs after 30 min growth time, with and without individual tube tracking using Java ImageJ (v1.54i); bundle length: 31.6 μm.†

Dustiness for nanoforms

We assessed nanomaterial dustiness for K-Nanos MWCNTs according to EN 17199-3:2019.[25] No pre-treatment of MWCNT samples was performed. The essential drop tube device for this test was developed and built by the testing facility in accordance with the guideline specifications (SI Fig. S7). This drop tube was specifically designed to detect airborne nanoparticles released from test materials, including submicron agglomerates from powders. A backflow pipe pumped purified air (generated using an ULPA filter, type U15) through the drop tube, creating a particle-free vertical air speed of 0.05 m s^{-1}, which corresponds to a controlled flow rate of 53 L min^{-1}. Before sample analysis, a blank measurement was conducted with an empty tube for 60 seconds to establish particle background levels. The main test was only initiated if the condensation particle count was below 20 particles per cm^3 over 60 seconds. The particle number concentration and size distribution specific to the test material were quantified in real time in triplicate (online sampling) over approx. 20 min using a scanning mobility particle sizer (SMPS), an aerodynamic particle sizer (APS), and a condensation particle counter (CPC). Dustiness indices were then calculated from the recorded data, where feasible.

Table 1 MWCNT bundle length [μm] (n = 30)

Parameter	K-Nanos 100	K-Nanos 210	K-Nanos 300
Mean	42.00	48.07	54.85
Standard deviation	13.41	21.08	19.34
Standard error	2.45	3.85	3.53
Min.	15.97	26.24	28.33
Max.	70.86	113.09	104.92
D10	24.47	28.44	35.06
D50	43.30	43.40	49.98
D90	61.82	79.39	93.14

D10: 10th percentile; D50: median; D90: 90th percentile.

† KPCC (2018, 2024).

Results

Shape – MWCNT bundle length

Summary statistics of the bundle length of K-Nanos MWCNTs measured by SEM are depicted in Table 1. The mean measured K-Nanos MWCNT bundle length was in the range of 42.00–54.85 μm (n = 30), with K-Nanos 100 at 42.00 μm, K-Nanos 210 at 48.07 μm, and K-Nanos 300 at 54.85 μm.

Size – individual MWCNT length

Summary statistics of the measured individual tube length for K-Nanos 210 MWCNTs at the three time points are depicted in Table 2, along with the extrapolated length data for the full 55 min growth time. The final extrapolated mean individual tube length after 55 min was 41.68 μm for K-Nanos 100, 60.54 μm for K-Nanos 210, and 95.94 μm for K-Nanos 300. Full data sets for K-Nanos 100 and K-Nanos 300 are provided in SI Tables S2–S4.

Fig. 3 depicts the corresponding linear regression lines and coefficients of correlation (R^2) for minimum, mean, and maximum individual carbon nanotube length of K-Nanos 210 MWCNTs. The complete data sets are provided in SI Table S6.

Dustiness for nanoforms

The measured particle numbers for K-Nanos MWCNTs in the nanoscale range of 1–100 nm fell between 14.8 and 22.4 particles per cm^3 (Table 3). The purified air was nearly particle-free, with a total particle count of 8.0 particles per cm^3 in the nanoscale range (1–100 nm). Thus, the background concentration was established at 8.0 particles per cm^3.

For assessing dustiness of nanoscale particulate materials, the significance criterion was established to be ≥1500 particles per cm^3 in agreement with the NanoCare[30] research project consortium. The number of particles specified in this criterion is for technical measurement purposes and is not relevant to health assessments. Consequently, the investigated MWCNTs were determined to have no significant dustiness (Tables 3 and SI S7). Particle release was also negligible in the μm range (SI Table S8). All three K-Nanos forms behaved congruently in terms of both behaviour and results. Scans collected from the measurement systems SMPS and APS lacked statistical significance because the determined values were, at most, only slightly above the lower detection limit of the devices. Scans for K-Nanos 100 were provided as representative examples (SI Fig. S8–S11). Visual observation indicated that K-Nanos MWCNTs had already coagulated before entering the drop tube, and the counter air flow was unable to separate the samples into primary particles (SI Fig. S12); instead, the investigated K-Nanos passed through the drop tube as agglomerates and deposited at the bottom of the device (SI Fig. S13). Due to the marginal release of particles across the entire observed size range, particularly in the nanoscale range (concentrations <50 particles per cm^3, SI Table S7), further result parameters mandated by EN 17199-3:2019 (ref. 25) were omitted. Specifically, these include the number-based emission rate (1 mg^{-1} s^{-1}), number-based dustiness index (DI_{number}, [1 mg^{-1}]), and respirable dustiness mass fraction (DI_{resp}, [mg kg^{-1}]). Under EN 17199-3:2019, the number-based dustiness index (DI_{number}) and the respirable dustiness mass fraction (DI_{resp}) cannot be identified or reported if results are not distinguishable from the background noise or below the limit of quantification (LOQ).

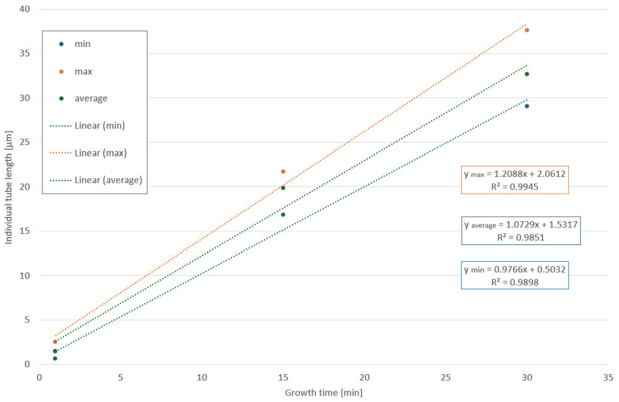

Fig. 3 Linear regression analysis of the individual tube length: K-Nanos 210 MWCNT minimum, mean, and maximum values over growth times of 1, 15, and 30 min.

Table 2 Summary statistics of measured individual K-Nanos 210 MWCNT length at time points 1, 15, and 30 min. The individual tube length at the completion of growth (55 min) was extrapolated using a linear regression of the measured individual tube lengths

		Individual K-Nanos 210 MWCNT length			
Parameter	Unit	1 min	15 min	30 min	55 min[a]
Mean	μm	1.46	19.83	32.65	60.54
SD	μm	0.66	1.66	2.49	—
CoV	%	45	8	8	—
Min.	μm	0.62	16.81	29.00	54.22
Max.	μm	2.49	21.71	37.60	68.55
D10	μm	0.62	16.81	29.00	54.22
D50	μm	1.19	20.74	31.95	59.67
D90	μm	2.49	21.71	37.60	68.55

SD: standard deviation; CoV: coefficient of variation; D10: 10th percentile; D50: median; D90: 90th percentile.[a] Based on linear regression.

Discussion

Shape, aspect ratio, and other morphological characterisation according to REACH Annex VI, section 2.4.4

K-Nanos MWCNTs are synthesised *via* a continuous growth process using chemical vapour deposition (CVD).[19–22] This process produces individual carbon nanotubes that form parallel-aligned bundles (SI Fig. S4).[21,22] Each bundle can contain 10^4–10^5 individual MWCNTs,[20] which are held together by the physical entanglement and van der Waals forces.[31–33] According to the OECD TG 125 definition, these

Table 3 K-Nanos MWCNT particle number concentrations [particles per cm^3] in the nanoscale range of 1–100 nm

Sample	Particle number concentration [particles per cm^3]	Blank background [particles per cm^3]	Dustiness [particles per cm^3]
K-Nanos 100	14.8	8.0	<1500; **non-dusty**
K-Nanos 210	18.2		
K-Nanos 300	22.40		

bundles are classified as agglomerates because the individual tubes are held together by weak physical interactions rather than chemical bonds, and the total external surface area of the bundle remains similar to the sum of the surface areas of the constituent tubes. Unlike typically weakly bound particles, MWCNT bundles resist separation into individual nanotubes. Even mild sonication is insufficient to overcome inter-tube van der Waals forces to effectively debundle the individual tubes (further addressed in our companion articles[13,14]). This difficulty prevents the accurate measurement of the true length of individual MWCNTs.[17,18]

The REACH Regulation Annex VI requires that nanomaterials must be characterised for shape, aspect ratio, and other morphological properties. For elongated particles such as MWCNTs, this includes measuring the length of the longest particle dimension, the length-to-width ratio (aspect ratio) and a description of rigidity (*e.g.*, entangled *vs.* straight particles).[16] Most common methods for measuring particle length suggest separating or untangling individual carbon nanotubes from their bundles, often using ultrasonication or surface acoustic wave irradiation.[17,18] While this disperses the bundles, it also shortens the individual nanotubes to only a few micrometres (SI Fig. S5 and S6). This effect significantly interferes with the accurate measurement and representation of the true length of individual MWCNTs.[17]

Furthermore, many reported methods for measuring MWCNT length are not validated or standardised and their results have not been evaluated for reproducibility.[10] Lastly, some instruments such as cryogenic transmission electron microscopy (cryo-TEM) are not broadly accessible, making them impractical for use as common analytical devices.

In the following section, we outline the major problems encountered when measuring the length of individual MWCNTs: (1) Sample preparation artefacts: Common imaging technologies such as atomic force microscopy (AFM), dynamic light scattering (DLS), and transmission electron microscopy (TEM) typically involve sonication during sample preparation, which is known to shorten the nanotubes; (2) Time-intensive and biased measurements: Estimating length *via* AFM requires measuring a large population of individual MWCNTs to achieve a statistically meaningful average. This process is time-intensive, and the physical deposition of long MWCNTs onto a substrate can introduce measurement bias.[17] Moreover, AFM has a much smaller maximum scan than SEM (typically limited to a lateral range of 10–15 μm) and, therefore, cannot measure objects of larger size. Most importantly, AFM measures topography, scanning the surface of an object; this does not allow for the tracking of individual tubes in their wavy alignment beneath the surface within a bundle. (3) Impracticality of specialised methods: A promising method published by Bengio *et al.*[17] involves untangling nanotubes, using stirring in a chlorosulfonic acid solution followed by cryo-TEM imaging. However, this method was only tested on shorter MWCNT samples (maximum 12.45 μm). In contrast, the maximum length of K-Nanos MWCNT bundles was approx. 113 μm (Table 1). Measuring a single strand of this size with cryo-TEM would be data-intensive and practically infeasible, requiring an excessive number of images to map the full length. Together, these issues drive the need for an alternative approach. It is important to note that AFM, DLS, TEM, and SEM are the methodologies now standardised in OECD TG 125. Furthermore, SEM, TEM, and DLS are also standardised for characterising MWCNTs' mesoscopic shape factors in ISO/TS 11888:2017.[34] Among these standardised methods, we considered SEM the most favourable because it does not require destructive sample preparation such as sonication. Samples are simply prepared in air, thus preserving the integrity of the MWCNT bundles. Moreover, excess sample material could be removed under vacuum, which was considered to have a lower mechanical impact compared to liquid-based sedimentation. The latter often requires solvents that can induce capillary-forced bundling during evaporation. Such liquid-based preparation is more invasive to the as-grown morphology than a dry air-blow approach. Regarding the potential impact of the electron beam on the fragile tube and bundle structures, the 10 kV beam used in this study operated at a medium intensity and lacked the threshold acceleration voltage required to cut or shorten the MWCNT length (>80 kV required).[35] While the beam may affect the inner tube structures or induce covalent 'welding' (stiffening the bundle), it does not provide the energy necessary for atomic knock-on damage.[35] Consequently, we considered dry sample preparation followed by SEM imaging the most effective method for preserving the as-grown morphology. This choice is supported by OECD TG 125 (ref. 12), which identifies electron microscopy as the only practicable method for measuring the length and diameter of high-aspect-ratio nanomaterials.

Our approach for particle length measurement. We employed SEM imaging to measure K-Nanos MWCNT bundle and constituent (individual) particle length. Since a bundle from a complete synthesis cycle did not fit into a single SEM image at the required resolution to identify the individual tubes, we devised a new approach. We achieved the measurement of individual carbon nanotubes

by visually tracking single tubes within a bundle. By using SEM images taken at different growth times, we were able to linearly extrapolate to the full length after a complete synthesis cycle. The steady-state linear growth kinetics of MWCNTs *via* CVD were extensively reported elsewhere, particularly for the active growth phase observed in this study.[19,22,36–38] However, case-by-case curve fitting should be performed, particularly if different synthesis conditions are employed. To assess the plausibility of the model, the final bundle length (at the full growth time of 55 min) was extrapolated from measurements at preceding time points and compared with the experimentally determined value (Table 4). The extrapolated bundle length showed good agreement with the experimental data. Moreover, we calculated the ratio of measured individual MWCNT length to bundle length. This ratio was greater than 1 in all cases, except for the minimum value originating from early-stage K-Nanos 210 MWCNT measurements (*i.e.*, 0.96; Table 4). This finding supports our assumption that the true individual tube length exceeds the bundle length. In this context, the observed ratio (1.04–1.28) can be interpreted as an indirect indicator of the degree of waviness and internal entanglement of nanotubes within a bundle (tortuosity). Here, the ratio was reported as a descriptive observation supporting the physical interpretation of a wavy nanotube arrangement, rather than a formal new material descriptor.

Table 4 Summary statistics for extrapolated MWCNT bundle and individual MWCNT lengths and ratios (individual MWCNT length/bundle length)

Bundle length [μm], extrapolated[a]			
	K-Nanos 100	K-Nanos 210	K-Nanos 300
—	37.9	56.4	83.9
Bundle length [μm], measured[b]			
D10	24.47	28.44	35.06
D50	43.30	43.40	49.98
D90	61.82	79.39	93.14
Individual MWCNT length[c] [μm]			
	K-Nanos 100	K-Nanos 210	K-Nanos 300
Mean	41.68	60.54	95.94
Min./D10	39.37	54.22	89.70
D50	40.75	59.67	92.97
D90	48.04	68.55	107.74
Max.	48.04	68.55	107.74
Ratio – individual MWCNT length ÷ bundle length, extrapolated[a]			
	K-Nanos 100	K-Nanos 210	K-Nanos 300
Mean	1.10	1.07	1.14
Min./D10	1.04	0.96	1.07
D50	1.08	1.06	1.11
D90	1.27	1.22	1.28
Max.	1.27	1.22	1.28

D10: 10th percentile; D50: median; D90: 90th percentile.[a] Based on linear regression (n = 1). [b] Measured data set (n = 30). [c] Based on linear regression (data set n = 9). Full data sets of individual MWCNT length and measured bundle length are provided in SI Tables S1 and S5.

Summary statistics for individual tube and bundle lengths were derived from a limited dataset, specifically from measurements of nine individual tubes within a single bundle. Validation of the proposed methodology will necessitate the analysis of more extensive datasets to ensure statistical robustness. However, the primary objective of this research was to introduce a novel methodological approach and an innovative concept for the length characterisation of MWCNTs. We argue that a sample size of n = 9 is sufficient for demonstrating the concept and the variation of individual tube length within a bundle, particularly when extrapolated to the full range of the measured bundle length (n = 30). For the measurement of bundle length, we contend that a dataset of n = 30 is representative; it employs a manual, non-automated technique that allows for precise differentiation between individual tubes and overlapping bundles, which automated systems often fail to resolve. A sample size of n = 30 is recognised as a significant threshold in classical statistics, where the sample mean distribution begins to approximate a normal distribution in accordance with the Central Limit Theorem. Following the logic of Masuda and Gotoh,[39] the required sample size is proportional to the desired precision. Given the high aspect ratio and complexity of MWCNTs, a higher admissible error is a standard compromise. A count of n = 30 is therefore considered sufficient to capture the primary variance in bundle lengths within a reasonable margin of error. Larger sample sizes should be targeted as methodologies become standardised (*e.g.*, the n = 200 recommendation for fibres in the OECD TG 125 validation report).[23] Nevertheless, the use of a high-quality, manually verified dataset ensures that the resulting characterisation provides a more accurate representation of the elongated K-Nanos MWCNT nanoforms than commonly available methods. We therefore successfully established a non-invasive approach for measuring individual carbon nanotube length without induced breakage, directly addressing a significant information gap. A limitation of this approach is the requirement for time-point length data, necessitating access to the production process. However, in the context of regulatory compliance, data are generally generated by or for manufacturers, making such access probable. Furthermore, for shorter commercial MWCNT bundles, the visual tracking method could be applied directly to SEM images without the need for extrapolation.

Finally, our extrapolated MWCNT constituent particle length consistently exceeded their respective MWCNT agglomerate length by 4–28%. If the relationship between the individual MWCNTs and their parent bundles during growth were better understood, measuring bundle length *via* SEM after a complete growth cycle and subsequently deriving the individual tube length (*e.g.*, by applying ratios as reported herein) would represent a highly effective approach. The methodology would avoid the need for elaborate time-point data and provide a fit-for-purpose means to derive individual

MWCNT length data based on the measured bundle length of standard-produced MWCNTs.

Effective particle size for robust risk assessment. Understanding the size and shape of constituent particles and their agglomerate state is crucial for hazard, exposure, and risk assessment of nanomaterials.[10,15,30] Generating standardised and comparable data is critical in a regulatory context.[2,3,10,30] Therefore, if primary particles cannot be easily separated from their agglomerates—as is the case with MWCNTs—the legally required characterisation and assessment should include the reporting of agglomerate properties alongside those of individual constituent particles. The effective particle size—the form most likely to interact with biological systems or the environment—must be considered as a relevant parameter for a reliable risk assessment. We consider the effective particle size under inhalation exposure scenarios to be closer to the size of bundles or agglomerates (mean approx. 42–55 μm; Table 1) rather than the length of individual nanotubes (mean approx. 46–63 μm; SI Table S1). While individual nanotubes may be longer than the corresponding bundles due to their wavy internal arrangement, they are not expected to exist as isolated entities during inhalation exposure. Instead, the material is most likely encountered as cohesive bundles and agglomerates, which then represent the physically relevant form for exposure considerations. Consequently, we believe that a rigorous evaluation of dustiness is essential and necessary.

Alignment with guidelines and applicability of OECD TG 125 to K-Nanos MWCNTs. For a comprehensive size and shape assessment, guideline methods should be designed to produce relevant data on nanomaterials as they exist in the real world (whether as individual particles or aggregates/agglomerates). This concept was successfully integrated into OECD TG 125 (ref. 12) and its validation report,[23] thereby addressing a key finding of the evaluation report on the state of the art of carbon-based nanomaterials.[10] Ultimately, test guidelines must fulfil their regulatory purpose by generating reliable and relevant data for meaningful chemical risk assessment (see also companion articles[13,14]).

The validation report (ref. 23) of OECD TG 125 aimed at covering nanomaterials of different sizes and shapes. With regard to elongated, high-aspect-ratio nanomaterials, SEM and TEM were identified as the only methods capable of measuring length and diameter, including agglomerates. However, the applicability domain for SEM covered diameters and lengths only up to 1000 nm and 20 μm, respectively.[12,23] In the OECD TG 125 validation report, MWCNT nanomaterials are defined as representative test materials of agglomerated fibres, specifically referring to issues related to the three-dimensional (3D) structure, leading to assessment bias toward shorter and thicker fibres. The OECD TG 125 validation report further provides hands-on guidance on how to treat visually complex situations that can appear in SEM images, proposing that several fibres can be traced individually for fibrous agglomerates. K-Nanos MWCNTs fall outside the applicability domain of OECD TG 125, as both their diameter and length exceed the specified limits. Nonetheless, we used the recommended SEM method to measure MWCNT bundle length in alignment with OECD TG 125. To overcome the limitation of image size, we developed the introduced approach of tracking the individual MWCNT fibres within the fibrous agglomerate derived from interrupted synthesis, using linear regression to extrapolate to the full length after a complete growth cycle. This procedure serves as a proposal for a new method that can be further evaluated, validated, and integrated to fill existing research and scientific gaps, as recommended elsewhere.[10]

Dustiness for nanoforms according to REACH Annex VII, section 7.14

MWCNTs can be characterised as black carbon powder, typically composed of low-density particles with high cohesive forces that lead to coagulation and/or agglomeration. While applying vibration for homogenising powder test materials is normally appropriate before measuring, it resulted in increased agglomeration in our test samples (SI Fig. S12 and S13). Typical for black carbon powders, the continuous drop (CD) system's counter-air flow was ineffective in separating these agglomerates due to their high cohesive forces. Thus, the visual appearance and behaviour of K-Nanos MWCNTs could be considered similar to other black carbon powders. Although not validated for fibrous nano-objects, aggregates, and agglomerates (NOAAs) in the respective OECD draft test guideline (ref. 24), a key advantage of the applied CD method is that it continuously introduces fresh sample materials throughout the test, simulating weak shear forces applied to the agglomerates.[30] This contrasts with other fibre-validated methods, such as the rotating drum method, which may 'denude' the initial powder of its fine part over time and generate new airborne particles in changing quantities. The permanent supply offers a more consistent assessment of dust emission during prolonged handling operations,[30] such as pouring or transferring of substances, which represents a relevant worker exposure scenario for the chemical safety assessment required by REACH. The CD method (*e.g.*, EN 15051-3:2014 (ref. 40) or EN 17199-3:2019 (ref. 25)) was further developed and adapted in research projects, such as the NanoCare[30] project, to enable the investigation of nanoscale products. These adaptations often included the integration of advanced aerosol measurement techniques, *e.g.*, scanning mobility particle sizer (SMPS), aerodynamic particle sizer (APS) and condensation particle counter (CPC), to accurately capture the nano-sized fraction of the emitted dust, as applied in our study.

Various dustiness methodologies exist, differing by their measurands and the particle type/exposure conditions being

assessed.[24,41–43] This variation complicates the comparison and interpretation of dustiness results. Since dustiness is crucial for potential inhalation toxicity, an in-depth evaluation is necessary to understand relevant exposure conditions. A limitation of the present assessment is the impracticability of reporting numerical values for the CD method. Reporting a zero-value or a value derived from background noise for the dustiness indices could lead to erroneous exposure predictions. In exposure modelling, a non-dusty material is handled fundamentally differently from one with a quantifiable index; therefore, precision in lower-limit measurements is vital. While the rotating drum (RD) method is frequently employed, its higher-energy nature may overestimate the breakage of bundles compared to real-world handling. As a potential alternative, the small rotating drum (SRD) method could be considered. The SRD method typically employs lower mechanical energy than the standard RD method, and thereby more accurately represents scenarios such as tipping, scooping, dropping, or mixing. Consequently, the SRD approach provides a more realistic assessment of the dustiness potential of MWCNT bundles without inducing the artificial structural breakage associated with more aggressive methods. Furthermore, the current draft OECD TG on dustiness (ref. 24) identifies the SRD method as validated for fibrous nanomaterials. Depending on the outcome, appropriate exposure conditions should be established during higher-tier toxicity testing to conduct a representative and robust hazard and risk assessment.

Shear forces and deagglomeration. Another relevant exposure consideration is the potential for nanomaterial agglomerates to break apart during application due to physical forces.[30,41] The deagglomeration of MWCNTs is strongly related to their tensile properties, which are technical characteristics for high-performance materials.[32,41] The NanoCare project proposed investigating the deagglomeration properties of nano-powders by applying shear forces during the dustiness assessment. Shear forces are an important parameter in a comprehensive assessment of MWCNT agglomerates in real-world scenarios. To the best of our knowledge, no official guidance or regulatory-accepted combined methodology currently exists to consider this important aspect of dustiness assessment.

Environmental and health concerns related to size, shape, and dustiness of elongated nanomaterials

The importance of considering the agglomerated or aggregated state of manufactured nanomaterials is highlighted by several official documents and related research when assessing exposure and conducting safety testing.[15,30,44] Both OECD TG 125 (ref. 12) and the draft OECD TG on dustiness (ref. 24) relate to the particle size of nanomaterials, including agglomerates, thereby demonstrating consideration for how nanomaterials exist in a realistic environment. Considering the realistic physical state of nanomaterials is highly relevant because the behaviour and biological effects of nanomaterials can be significantly altered by their state of agglomeration.[15,45,46] In fact, health concerns regarding nanomaterials are not solely related to the chemical entity of toxicity (commonly assessed for traditional (in)organic chemicals) but also encompass the physical entity of toxicity, such as shape-related toxicity.[15,47] Thus, the characterisation of size and shape is crucial for nanomaterials. Concerns are commonly related to elongated and rigid particles, which can form fibre-like structures that may cause toxicity *via* inhalation, similar to asbestos.[48–50] Although both materials, asbestos and MWCNTs, share a high aspect ratio, asbestos fibres are typically rigid and needle-like, often occurring as individual fibres which facilitates endocytosis. In contrast, pristine MWCNTs, such as K-Nanos, are characterised by flexible, wavy-entangled, and cohesive bundle structures. These differences in morphology, surface properties, and aggregation behaviour are expected to influence deposition, clearance, and biological interactions in the respiratory system. Overall, based on the currently available data, it remains impossible to draw a comprehensive conclusion regarding the relationship between the size, shape, and inherent toxicity of MWCNTs.[15,45,51–53]

The fibre paradigm in toxicology posits that the hazard of inhaled fibrous materials is strongly linked to the dimensions length and diameter, and biopersistence (resistance to dissolution or clearance in the lungs).[54,55] Rigidity plays a crucial role in biopersistence of high-aspect-ratio nanoparticles (HARNs) because flexible fibres may be cleared more easily by macrophages and are less likely to cause 'frustrated phagocytosis'.[15,48] While no universally agreed standalone rigidity standard exists, the regulatory landscape recognises the critical importance of this parameter for HARNs.[16,55] For example, ISO/TS 11888:2017 (ref. 34) provides the measurand static bending persistence length (SBPL) as a rigidity standard for its quantitative assessment in relation to safety. Rigidity is also a critical property acknowledged by both ECHA guidance[16] and the OECD draft validation report on dustiness,[56] recognising the importance of rigidity or flexibility as a key physicochemical property, which influences biopersistence and the potential for asbestos-like pathogenicity of fibrous nanomaterials. Instead of a direct rigidity threshold, the current regulatory focus for HARNs often centres on the combined criteria of length (>5 μm), diameter (<3 μm), aspect-ratio (>3 : 1), and biopersistence.[12,16,23,55,57]

K-Nanos MWCNTs are very long (*i.e.*, 42–55 μm; Table 1) and assessed as non-dusty according to the results of the continuous drop method according to test guideline EN 17199-3:2019. Given this information, the feasibility of conducting a reliable and adequate inhalation study must be carefully investigated before performing higher-tier

toxicity tests, particularly regarding the generation of relevant and stable exposure concentrations.[15,45] Based on experimental information from earlier toxicity studies performed with K-Nanos 100, *i.e.*, 28 day and 90 day repeated dose studies according to OECD TG 412 (ref. 58) and 413 (ref. 59) no adverse effects were observed.[60,61] In these experiments, it was impossible to generate stable aerosol concentrations higher than 1 mg m^{-3}. Based on practical experience, these concentration levels usually do not meet the requirements for a regulatory-accepted toxicity study unless at least mild effects were induced in the test specimen or the guideline's limit test concentration was reached. Kim *et al.*[62] performed a 28 day repeated dose inhalation toxicity study in rats equivalent to OECD TG 412, using a closely related elongated bundle-MWCNT test material, Jenotube 10B MWCNTs (for details on Jenotube MWCNTs see companion articles[13,14]), where higher test concentrations could be obtained. This study utilised an acoustic dry aerosol generator, in which the test material was dispersed using acoustic energy. MWCNT agglomerates were disaggregated/deagglomerated depending on the frequency and amplitude of the energy applied. The derived no observed adverse effect concentration (NOAEC) of 0.257 mg m^{-3} (low concentration) was based on adverse local effects observed in the animals' lungs at both higher concentrations tested (1.439 and 4.253 mg m^{-3}).[62] Notwithstanding, we anticipate major challenges to generate adequately high and stable exposure concentrations under relevant test conditions in any follow-up studies, such as an extended one-generation reproductive toxicity study (EOGRTS) according to OECD TG 443 (ref. 63)—an investigation that should target the route[64] and level of exposure most relevant for assessing MWCNT toxicity to humans.

In the search for new assessment approaches, the consideration of representative uses and exposure levels to predict potential harm is also set as a starting point for a more human-relevant, efficient, and ethical approach to chemical risk assessment, as exemplified by the concept of next generation risk assessment (NGRA).[65] Ultimately, information on a nanomaterial's effective particle size and shape, along with an in-depth understanding of its dustiness properties, is crucial for investigating relevant exposure scenarios and performing an accurate hazard assessment, thereby leading to a representative and protective chemical risk assessment.

Conclusion

We provided a comprehensive, state-of-the-art evaluation of the applicability, availability, and suitability of standard test guidelines for meeting regulatory requirements for carbon-based nanomaterials, derived from practical, material-specific experience. Our work (i) acknowledged the progress and limitations of the applied test methods, (ii) outlined potential novel approaches for future investigation, and (iii) provided the broader scientific community with insights from the practical, real-world REACH registration of a carbon-based nanomaterial.

The established non-invasive method for measuring individual and agglomerate MWCNT lengths offers an acceptable approach to characterise the size, shape, and aspect ratio of MWCNTs in accordance with the REACH Regulation. Findings regarding MWCNT dustiness suggest that K-Nanos MWCNTs tend to have rather low dustiness potential due to their strong cohesive forces and tendency to agglomerate. This information is vital for planning higher-tier inhalation toxicity studies by evaluating the properties critical to airborne exposure; this facilitates the generation of representative data for an accurate hazard assessment, leading to a proportionate and robust chemical risk assessment. This research supports compliance professionals, regulatory bodies, and researchers in their efforts to ensure the safe use of nanomaterials, thereby closing information gaps related to the physicochemical characterisation of elongated carbon-based nanomaterials with an agglomerated bundle shape.

Author contributions

Marie-Léonie Bohlen was responsible for conceptualisation, writing—original draft preparation, formal analysis, validation, visualisation, and supervision. She significantly contributed to writing—review & editing, and the methodology (concerning the formal analysis aspects of the individual MWCNT length). Hana Jo contributed to data curation, investigation (individual MWCNT length), writing—review & editing, and project administration. Yeojin Lee contributed to investigation (individual MWCNT length), writing—review, and project administration. Hyun Pyo Jeon contributed to conceptualisation, investigation (individual MWCNT length), and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI).

Supplementary information is available. See DOI: https://doi.org/10.1039/d5en01017a.

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Notes and references

1 W. Wohlleben, L. Ma-Hock, V. Boyko, G. Cox, H. Egenolf, H. Freiberger, B. Hinrichsen, S. Hirth and R. Landsiedel, Nanospecific guidance in REACH: a comparative physical-chemical characterization of 15 materials with methodical correlations, *J. Ceram. Sci. Technol.*, 2013, **4**, 93–104.
2 K. Rasmussen, H. Rauscher, P. Kearns, M. González and J. Riego Sintes, Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data, *Regul. Toxicol. Pharmacol.*, 2019, **104**, 74–83.
3 E. A. J. Bleeker, E. Swart, H. Braakhuis, M. L. Fernández Cruz, S. Friedrichs, I. Gosens, F. Herzberg, K. A. Jensen, F. von der Kammer, J. A. B. Kettelarij, J. M. Navas, K. Rasmussen, K. Schwirn and M. Visser, Towards harmonisation of testing of nanomaterials for EU regulatory requirements on chemical safety – A proposal for further actions, *Regul. Toxicol. Pharmacol.*, 2023, **139**, 105360.
4 ECHA, Guidance on Information Requirements and Chemical Safety Assessment, Appendix R7-1 for nanomaterials applicable to Chapter R7a - Endpoint specific guidance, Version 4.0, European Chemicals Agency, Helsinki, Finland, 2022.
5 ECHA, Guidance on Information Requirements and Chemical Safety Assessment, Appendix R7-1 for nanomaterials applicable to Chapter R7b Endpoint specific guidance, Version 2.0, European Chemicals Agency, Helsinki, Finland, 2017.
6 ECHA, Guidance on Information Requirements and Chemical Safety Assessment, Appendix R7-2 for nanomaterials applicable to Chapter R7c Endpoint specific guidance, Version 3.0, European Chemicals Agency, Helsinki, Finland, 2021.
7 Closing nanomaterials' information gaps: EUON welcomes new study proposals, https://euon.echa.europa.eu/view-article/-/journal_content/title/closing-nanomaterials-information-gaps-euon-welcomes-new-study-proposals, (accessed 9 September 2025).
8 EUON calls for study proposals to address nanomaterials knowledge gaps in the EU, https://euon.echa.europa.eu/view-article/-/journal_content/title/euon-calls-for-study-proposals-to-address-nanomaterials-knowledge-gaps-in-the-eu-1, (accessed 9 September 2025).
9 Participate in survey on carbon-based nanomaterials and analytical methods, https://euon.echa.europa.eu/view-article/-/journal_content/title/participate-in-survey-on-carbon-based-nanomaterials-and-analytical-methods, (accessed 9 September 2025).
10 NovaMechanics Ltd., Survey on state of the art of carbon-based nanomaterial detection and quantification in environmental and biological matrices, NovaMechanics Ltd., 2025.
11 A new study identifies challenges in detection and assessment of carbon-based nanomaterials, https://euon.echa.europa.eu/view-article/-/journal_content/title/url, (accessed 9 September 2025).
12 OECD, Test No. 125: Nanomaterial Particle Size and Size Distribution of Nanomaterials, OECD Publishing, Paris, 2023.
13 M.-L. Bohlen, S. Gartiser, H. P. Jeon, H. Jo, Y. Lee, J. Oltmanns and M. Schwarz, Experimental approaches to data generation for REACH compliance of multi-walled carbon nanotubes: environmental fate, *Environ. Sci.: Nano*, 2026, DOI: 10.1039/D5EN01018G.
14 M.-L. Bohlen, H. P. Jeon, H. Jo, Y. Lee and J. Oltmanns, Experimental approaches to data generation for REACH compliance of multi-walled carbon nanotubes: human health *in vitro/in chemico*, *Environ. Sci.: Nano*, 2026, DOI: 10.1039/D5EN01019E.
15 F. S. Bierkandt, L. Leibrock, S. Wagener, P. Laux and A. Luch, The impact of nanomaterial characteristics on inhalation toxicity, *Toxicol. Res.*, 2018, **7**, 321–346.
16 ECHA, Appendix for nanoforms to the guidance on registration and the guidance on substance identification: version 2.0 January 2022, European Chemicals Agency, Helsinki, Finland, 2022.
17 E. A. Bengio, D. E. Tsentalovich, N. Behabtu, O. Kleinerman, E. Kesselman, J. Schmidt, Y. Talmon and M. Pasquali, Statistical length measurement method by direct imaging of carbon nanotubes, *ACS Appl. Mater. Interfaces*, 2014, **6**, 6139–6146.
18 M. Miansari, A. Qi, L. Y. Yeo and J. R. Friend, Vibration-Induced Deagglomeration and Shear-Induced Alignment of Carbon Nanotubes in Air, *Adv. Funct. Mater.*, 2015, **25**, 1014–1023.
19 N. Zhao, C. He, Z. Jiang, J. Li and Y. Li, Fabrication and growth mechanism of carbon nanotubes by catalytic chemical vapor deposition, *Mater. Lett.*, 2006, **60**, 159–163.
20 Z. Wang, Q. Wu, F.-Y. Zhang and Y.-Y. Cui, Synthesis of multi-walled carbon nanotube bundles with uniform diameter, *Mater. Lett.*, 2007, **61**, 1955–1958.
21 H. Kim, K. S. Kim, J. Kang, Y. C. Park, K.-Y. Chun, J.-H. Boo, Y.-J. Kim, B. H. Hong and J.-B. Choi, Flow-dependent directional growth of carbon nanotube forests by chemical vapor deposition, *Nanotechnology*, 2011, **22**, 095303.
22 W. Cho, M. Schulz and V. Shanov, Growth and characterization of vertically aligned centimeter long CNT arrays, *Carbon*, 2014, **72**, 264–273.
23 OECD, Validation Report on Particle and Fibre Size Distribution Measurements of Nanomaterials. Supporting TG 125 on Particle Size and Particle Size Distribution of Nanomaterials Series on Testing and Assessment No. 352, OECD Publishing, Paris, 2022.
24 OECD, Test Guideline on the dustiness determination of manufactured nanomaterials DRAFT, OECD Publishing, Paris, 2024.
25 EN 17199-3:2019. Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles. Continuous drop method, DIN Media GmbH, 2019.
26 G. Janer, R. Landsiedel and W. Wohlleben, Rationale and decision rules behind the ECETOC NanoApp to support

registration of sets of similar nanoforms within REACH, *Nanotoxicology*, 2021, **15**, 145–166.
27 G. Janer, D. Ag-Seleci, J.-A. Sergent, R. Landsiedel and W. Wohlleben, Creating sets of similar nanoforms with the ECETOC NanoApp: real-life case studies, *Nanotoxicology*, 2021, **15**, 1016–1034.
28 T. Ferreira and W. Rasband, Java ImageJ v1.54i, Laboratory for Optical and Computational Instrumentation (LOCI), University of Wisconsin, USA, 2024.
29 JCGM, JCGM 100:2008 GUM 1995 with minor corrections. Evaluation of measurement data—Guide to the expression of uncertainty in measurement, Int. Organ. Stand. Geneva, 2008.
30 *NanoCare - Health related aspects of nanomaterials: final scientific report*, ed. T. A. J. Kuhlbusch, H. F. Krug and K. Nau, DECHEMA, Frankfurt am Main, 2009.
31 H. Hamasaki and K. Hirahara, The van der Waals cohesive force between two carbon nanotubes, *Appl. Phys. Express*, 2023, **16**, 035002.
32 H. Kim, M. Wang, S. K. Lee, J. Kang, J.-D. Nam, L. Ci and J. Suhr, Tensile properties of millimeter-long multi-walled carbon nanotubes, *Sci. Rep.*, 2017, **7**, 9512.
33 C. M. Long, M. A. Nascarella and P. A. Valberg, Carbon black vs. black carbon and other airborne materials containing elemental carbon: Physical and chemical distinctions, *Environ. Pollut.*, 2013, **181**, 271–286.
34 ISO/TS 11888:2017. Nanotechnologies — Characterization of multiwall carbon nanotubes — Mesoscopic shape factors, DIN Media GmbH, 2017.
35 B. W. Smith and D. E. Luzzi, Knock-on damage in single wall carbon nanotubes by electron irradiation, *AIP Conf. Proc.*, 1999, **486**, 360–363.
36 X. Cui, W. Wei and W. Chen, Study of Growth Kinetics of Ultra-Long Carbon Nanotube Arrays through Wall Number Statistical Investigation, *MRS Online Proc. Libr.*, 2010, **1204**, 501.
37 S. Hussain, J. C. Stallard, C. Jourdain, M. W. J. Glerum, J. Peden, R. Qiao and A. M. Boies, Measurement of High Carbon Nanotube Growth Rate, Mass Production, Agglomeration, and Length in a Floating Catalyst Chemical Vapor Deposition Reactor, *ACS Nano*, 2025, **19**, 8739–8752.
38 V. Pimonov, *PhD Thesis*, Université Montpellier, 2021.
39 H. Masuda and K. Gotoh, Study on the sample size required for the estimation of mean particle diameter, *Adv. Powder Technol.*, 1999, **10**, 159–173.
40 EN 15051-3:2014-03. Workplace exposure - Measurement of the dustiness of bulk materials - Part 3: Continuous drop method, DIN Media GmbH, 2014.
41 S. Marsh, Department of Design Sciences Faculty of Engineering LTH, Lund University, 2021.
42 G. Lidén, Dustiness Testing of Materials Handled at Workplaces, *Ann. Occup. Hyg.*, 2006, **50**, 437–439.
43 D. Broßell, E. Heunisch, A. Meyer-Plath, D. Bäger, V. Bachmann, K. Kämpf, N. Dziurowitz, C. Thim, D. Wenzlaff, J. Schumann and S. Plitzko, Assessment of nanofibre dustiness by means of vibro-fluidization, *Powder Technol.*, 2019, **342**, 491–508.
44 F. Henry, J. Bouillard, P. Marchal, A. Vignes, O. Dufaud and L. Perrin, Exploring a new method to study the agglomeration of powders: Application to nanopowders, *Powder Technol.*, 2013, **250**, 13–20.
45 C. Guo, M. D. Wright, A. Buckley, A. Laycock, T. Berthing, U. Vogel, F. Cosnier, L. Gaté, M. O. Leonard and R. Smith, Pulmonary Toxicity of Long, Thick MWCNT and Very Long, Thin Carboxylated MWCNT Aerosols Following 28 Days Whole-Body Exposure, *Toxics*, 2025, **13**, 401.
46 J. Pauluhn, Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures, *Toxicol. Sci.*, 2010, **113**, 226–242.
47 L. Ma-Hock, V. Strauss, S. Treumann, K. Küttler, W. Wohlleben, T. Hofmann, S. Gröters, K. Wiench, B. van Ravenzwaay and R. Landsiedel, Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black, *Part. Fibre Toxicol.*, 2013, **10**, 23.
48 M. S. P. Boyles, L. Young, D. M. Brown, L. MacCalman, H. Cowie, A. Moisala, F. Smail, P. J. W. Smith, L. Proudfoot, A. H. Windle and V. Stone, Multi-walled carbon nanotube induced frustrated phagocytosis, cytotoxicity and pro-inflammatory conditions in macrophages are length dependent and greater than that of asbestos, *Toxicol. In Vitro*, 2015, **29**, 1513–1528.
49 C. A. Poland, R. Duffin, I. Kinloch, A. Maynard, W. A. H. Wallace, A. Seaton, V. Stone, S. Brown, W. MacNee and K. Donaldson, Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study, *Nat. Nanotechnol.*, 2008, **3**, 423–428.
50 R. Fortini, A. Meyer-Plath, D. Kehren, U. Gernert, L. A. Jácome and H. Sturm, Measurement of Flexural Rigidity of Multi-Walled Carbon Nanotubes by Dynamic Scanning Electron Microscopy, *Fibers*, 2020, **8**, 31.
51 S. Sweeney, D. Berhanu, S. K. Misra, A. J. Thorley, E. Valsami-Jones and T. D. Tetley, Multi-walled carbon nanotube length as a critical determinant of bioreactivity with primary human pulmonary alveolar cells, *Carbon*, 2014, **78**, 26–37.
52 D. M. Saleh, W. T. Alexander, T. Numano, O. H. M. Ahmed, S. Gunasekaran, D. B. Alexander, M. Abdelgied, A. M. El-Gazzar, H. Takase, J. Xu, A. Naiki-Ito, S. Takahashi, A. Hirose, M. Ohnishi, J. Kanno and H. Tsuda, Comparative carcinogenicity study of a thick, straight-type and a thin, tangled-type multi-walled carbon nanotube administered by intra-tracheal instillation in the rat, *Part. Fibre Toxicol.*, 2020, **17**, 48.
53 H. Wolff and U. Vogel, Recent progress in the EU classification of the health hazards associated with certain multiwall carbon nanotubes (MWCNTs): what about the other MWCNTs?, *Nanotoxicology*, 2024, **18**, 119–121.
54 A. K. Madl and H. C. O'Neill, Fiber biodurability and biopersistence: historical toxicological perspective of synthetic vitreous fibers (SVFs), the long fiber paradigm, and

implications for advanced materials, *Crit. Rev. Toxicol.*, 2022, **52**, 811–866.
55 L. Tran, S. Hankin, B. Ross, R. Aitken, A. Jones, K. Donaldson, V. Stone and R. Tantra, An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres, 2008.
56 OECD, DRAFT Validation report on the dustiness determination of manufactured nanomaterials, OECD Publishing, Paris, 2024.
57 WHO, Determination of airborne fibre number concentrations, Geneva, Switzerland, 1997.
58 OECD, Test No. 412: Subacute Inhalation Toxicity: 28-Day Study, Paris, 2018.
59 OECD, Test No. 413: Subchronic Inhalation Toxicity: 90-day Study, OECD Publishing, Paris, 2018.
60 Multi-Walled Carbon Nanotubes (MWCNT), synthetic graphite in tubular shape 100.217.898 | Repeated dose toxicity: inhalation, 90-days, https://chem.echa.europa.eu/100.217.898/dossier-view/f90fef82-6eaf-4ca4-8bbb-bab07f2e93d4/93a556e2-833d-4886-9fc2-819d00335e65_2f39de9b-e86d-4c33-b7e1-b66f8af5b160?searchText=MWCNT, (accessed 5 August 2025).
61 Multi-Walled Carbon Nanotubes (MWCNT), synthetic graphite in tubular shape 100.217.898 | Repeated dose toxicity: inhalation, 28-days, https://chem.echa.europa.eu/100.217.898/dossier-view/f90fef82-6eaf-4ca4-8bbb-bab07f2e93d4/b5f67034-781a-4bb1-86c7-4417d12c79b0_2f39de9b-e86d-4c33-b7e1-b66f8af5b160?searchText=MWCNT, (accessed 5 August 2025).
62 J. K. Kim, M. S. Jo, Y. Kim, T. G. Kim, J. H. Shin, B. W. Kim, H. P. Kim, H. K. Lee, H. S. Kim, K. Ahn, S. M. Oh, W.-S. Cho and I. J. Yu, 28-Day inhalation toxicity study with evaluation of lung deposition and retention of tangled multi-walled carbon nanotubes, *Nanotoxicology*, 2020, **14**, 250–262.
63 OECD, Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Publishing, Paris, 2025.
64 M. S. P. Boyles, L. C. Stoehr, P. Schlinkert, M. Himly and A. Duschl, The Significance and Insignificance of Carbon Nanotube-Induced Inflammation, *Fibers*, 2014, **2**, 45–74.
65 F. Luo, J. Pu, Z. Su, S. Xing, Q. Wang and L. Sun, The research progress of next generation risk assessment in cosmetic ingredients and the implications for traditional Chinese medicine risk assessment, *Pharmacol. Res. - Mod. Chin. Med.*, 2023, **8**, 100282.