NanoRelease: Pilot interlaboratory comparison of a weathering protocol applied to resilient and labile polymers with and without embedded carbon nanotubes

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Abstract

A major use of multi-walled carbon nanotubes (MWCNTs) is as functional fillers embedded in a solid matrix, such as plastics or coatings. Weathering and abrasion of the solid matrix during use can lead to environmental releases of the MWCNTs. Here we focus on a protocol to identify and quantify the primary release induced by weathering, and assess reproducibility, transferability, and sensitivity towards different materials and uses. We prepared 132 specimens of two polymer-MWCNT composites containing the same grade of MWCNTs used in earlier OECD hazard assessments but without UV stabilizer. We report on a pilot inter-laboratory comparison (ILC) with four labs (two US and two EU) aging by UV and rain, then shipping for analysis. Two labs (one US and one EU) conducted the release sampling and analysis by Transmission Electron Microscopy (TEM), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), UltravioleteVisible Spectroscopy (UVeVis), Analytical Ultracentrifugation (AUC), and Asymmetric Flow Field Flow Fractionation (AF4). We compare results between aging labs.
between analysis labs and between materials. Surprisingly, we found quantitative agreement between analysis labs for TEM, ICP-MS, UVeVis; low variation between aging labs by all methods; and consistent rankings of release between TEM, ICP-MS, UVeVis, AUC. Significant disagreement was related primarily to differences in aging, but even these cases remained within a factor of two.

1. Introduction

Any potential benefits of multi-walled carbon nanotubes (MWCNTs), such as weight reduction in automotive parts, enhanced strength of defense components, and flame retardancy in consumer products, are compromised if concerns about environmental releases during the use phase cannot be assessed by quantitative methods that are mutually accepted by producers, regulators, and consumers. Release assessment considers detachment of a fragment of a larger whole, such as a consumer product during use, and includes the release mechanism, form of released entity, release scenario, probability of release, and lifecycle simulation, if relevant [1]. In the case of a nanocomposite material of MWCNTs embedded in a solid matrix, a release event might include discharge of MWCNTs. Release from MWCNT-composite materials is considered a rather uncontrollable source of emission into the environment, especially by aging and/or wear-and-tear during the potentially decade-long, possible outdoor use phase of the nanocomposite [2].

Several reviews focus on the characteristics of release in the sense of identity and shape of released entities. Polydispersed sizes of fragments of the nanocomposite material are released by mechanical energy input and only exceptional cases report release of free carbon nanotubes (CNTs) [3–5]. In the case of MWCNTs with a matrix that is cross-linked or brittle, mechanically disrupted fragments show protrusions of the CNTs with only a few percentage’s elongation at break [6,7]. In contrast, the degradation of the matrix material has the potential to liberate the embedded MWCNTs [1,2,8,9].

The “materials task group” of the international NanoRelease initiative [10] speculated that with weathering, specifically, the matrix should have a strong impact on the release, and also extrapolated from the known photodegradation susceptibility that the greatest release should be observed from epoxy composites, and least from polyethylene (PE) composites [4]. Intermediate probabilities of release were predicted for polyurethane (PU), polyamide (PA) and polycarbonate (PC) composites [4]. Abundant experimental results – specifically on MWCNT composites of epoxy obtained by the unique high-intensity cold UV aging system at National Institute of Standards and Technology (NIST)- suggested MWCNT-epoxy as labile material [6,9,11–20]. Further aging and release results specifically on MWCNT composites of PA suggested MWCNT-PA as more resilient [21].

The methodology to assess releases by weathering lacked standardization, however, and various laboratories only came to the qualitative agreement that released entities are a heterogeneous, polydisperse mixture of fragments of degraded matrix with embedded or surface-protruding MWCNTs, agglomerates of MWCNTs with some degradation debris attached, and occasionally free MWCNTs [6,9,11–20]. These projects were exploratory in scope and without standardization of any of the elements of a release protocol: aging,
sampling and analysis. In a pilot interlaboratory comparison (ILC) of weathering-induced releases from SiO$_2$ in PA, aging and sampling conditions were identified as the primary sources of inconsistency [20].

Accordingly, this report examines potential sources of variation by four aging labs (with identical UV/rain conditions) that supplied aged specimens to three analysis labs, of which one analyzed the aged specimens (without release sampling), and two employed the same two release sampling conditions and five release analysis methods. We prepared 132 specimens of two materials containing the same grade of MWCNTs but without UV stabilizer, and expected to find more severe aging and release from MWCNT-epoxy than MWCNT-polyamide. Non-aged specimens served as negative controls, and pure CNT suspensions served as positive controls.

2. Experimental

2.1. Organization of ILC

Because previous multi-laboratory studies on the release from aging nanocomposites have demonstrated a considerable amount of variation in results [20], this study was designed to control to the extent possible for variations in weathering equipment and techniques, shipment, and analysis. Before initiating of the study, participating labs detailed all procedures and equipment to be used. As specified in the Experimental section, samples were handled, prepared, and placed in chambers using consistent procedures. Once weathering was completed, all samples were handled and shipped using consistent procedures to minimize variability. Sample placement in the aging instruments is shown in Fig. S1_1. Fig. 1 provides the overall schematic for sample origination, weathering labs and sample analysis. All results were acquired in duplicate for each combination of specimen, aging lab, analysis lab, sampling condition, analysis method. Each analysis lab analyzed the specimens aged in-house as well as those aged at the other three labs. Sample codes are described in Table S1_2. For readability and clarity of the present report, text and figures use abbreviated acronyms:

- P4 = PA with 4% MWCNT = MWCNT-PA.
- E4 = epoxy with 0.25% MWCNT = MWCNT-epoxy.
- P0 and E0 designate the control materials without MWCNTs, respectively.

The 2000 h of accelerated aging, or nearly 3 months in the laboratory simulator, accumulated 432 MJ/m$^2$ UV energy, which corresponded to roughly two years under mid-European external conditions. Aging was performed in parallel with only a few days’ difference in the four labs. Between the end of aging and beginning of sampling and analysis, the aged specimens were shipped and stored in the dark for about four months. The entire sampling and analysis program was executed over a span of six weeks at each of the analysis laboratory.
2.2. Materials

Epoxy-MWCNT modified resin was prepared from a master batch supplied by Nanocyl (EPOCYL NC R128–05). It is based on a liquid Bisphenol-A (Bis-A) epoxy resin and contains a high concentration of NC 7000 multi-wall carbon nanotubes (MWCNTs). The neat resin Epikote 828 was used to dilute the master batch in epoxy. The hardener is dicyandiamide Aradur 5021 from Huntsman. Samples at 0.1 and 0.25 wt% of CNTs were produced by diluting a master batch with neat resin under high shear mixer 1500 rpm for 10 min. The recommended ratio of hardener is added to the blend and additional 10 min of mixing was applied to homogenize all the ingredients. The mixture is poured into rectangular mold and then placed in an oven. The curing cycle is 1 h at 80 °C and 2 h at 120 °C. The plates with 6 mm of thickness were cut into rectangular dimensions.

All polyamide 6/MWCNT formulations investigated in this work was produced by dilution of a masterbatch containing 15 wt% MWCNT (Plasticyl tradename PA1503, manufactured by Nanocyl S.A. Belgium). The masterbatch was compounded with the same polyamide6 as used in the masterbatch using a Leistritz ZSK-MAXX L/D-48 co-rotating twin-screw extruder. The screw was composed of one plasticizing and three individually optimized mixing regimes. The operating temperature of the twin-screw extruder is close to 260 °C. By this melt dilution procedure, concentrations of 2 and 4 wt% MWCNT in polyamide6 were obtained. Test specimens (60 × 60 mm, thickness 2 mm) were produced with an Engel Victory 80 ton injection molding machine. The melt temperature during injection molding was kept at 260 °C for all formulations (virgin PA6, PA6/2 wt% MWCNT and PA6/4 wt% MWCNT).

The MWCNTs are of same grade (not same batch) as the “NM400” material of the OECD sponsorship program [22,23]. The results obtained from the ICP-MS analysis of the pure MWCNT (mg/kg±s.d. x 10^4) were: Al 2.21 ± 0.84; Co 0.183 ± 0.071; Fe 0.522 ± 0.16) after digestion using aqua regia (see Table SI_1 for results by alternative digestion protocols). These values are in line with results obtained in the SUN project on “Sustainable Nanotechnologies” [24]. The metallic components are known to represent the catalyst used during the MWCNT production, and are also observable in the TEM scan.

The TEM scan of the positive control (Fig. 2A) shows a characteristic fibrous structure with a clearly resolved hollow core, outer diameter of 7 nm - 20 nm, with non-straight (bent) shape, as figis well known for this specific type of MWCNT from its OECD batch investigated globally as “NM400”. UVeVis spectroscopy clearly resolves the 1 ppm positive control from the baseline of pure immersion fluid (Fig. 2B), but saturates at 10 ppm MWCNT content (Fig. 2B, see also Fig. SI_11). AUC detects 1 ppm MWCNT and quantifies 10 ppm and 100 ppm MWCNT correctly (Fig. 2C, cumulative distributions) as validated earlier on the same type of CNT [25]. The colloidal size between 10 nm and 30 nm is close to the diameter as expected, and well resolved against the pure immersion fluid background.

The MWCNTs were embedded in two matrices in concentrations relevant for commercial use as polymers with enhanced strength and electrical conductivity; however, the polymers deliberately contained no UV stabilizer that would be standard practice for any intended
outdoor use. Test specimens were made in epoxy with 0.25% MWCNT content and polyamide (PA) with 4% MWCNT content respectively, each with control specimens at 0% MWCNT (see terminology in Section 2.3). Epoxy specimens had outer dimensions of 50 mm × 50 mm × 5.5 mm, made by curing in moulds. PA specimens had outer dimensions of 60 mm × 60 mm × 2 mm. The bulk epoxy matrix (0% MWCNT) had very low Co content, while the bulk PA (0% MWCNT) had considerable Co content, making the quantification of MWCNT release from PA problematic (Table SI_1).

2.3. Protocol and methods

Aging was performed by ISO-standardized conditions with 60 W/cm² UV intensity (integrated across the wavelength range 300–400 nm) with water spray intervals under continued UV irradiation for 18 min after 102 min of UV only [26]. All four labs used the same equipment, Atlas SUNTEST XLS+. Weathering was interrupted after 1500 h to change the Xe-arc lamp, a precaution to ensure constant irradiation throughout since the manufacturer advised that the typical lifetime of the lamp is 1500 h. The total irradiance level is automatically maintained by the weathering system but spectral distribution of the light changes slowly over time. The actual UV dose after 2000 h was 99.8% of the expected dose. The spray water was de-ionized and not recycled. Run-off water was discarded, because a) earlier studies had established that MWCNTs do not release spontaneously in water [9], and b) earlier attempts to collect, freeze-dry and analyze the solids in polyamide run-off suffered from severe background issues due to the excessive engineered nanomaterial (ENM) dilution [21].

Since water spray is significantly weaker at the back of the chamber of the SUNTEST XLS+, all labs arranged specimens in the same order and location and fixed their positions by a 4 mm-mesh grid and screws of stainless steel (see Fig. SI_1); mesh, screws, and containers were sourced centrally and distributed to all four labs. After 2000h of aging, the specimens were shipped to the analysis labs with each packed in LDPE bags including photo documentation, which protected the aged surface from mechanical and chemical contact.

Characterization of aged wafer surfaces without immersion. The four aging labs produced a total of 32 wafers for SEM analysis – visible as the thinner wafers at the left in the aging chambers (Fig. SI_1.) These were shipped to NRC, where double-sided copper foil tape, commonly used for mounting samples for SEM imaging, lifted-off the material from the wafer surfaces. Small pieces of copper tape (3M #1182 HD) were firmly adhered to a standard 12.7 mm aluminum SEM stub. The protective film was removed from the second surface of the tape, and the exposed adhesive was lightly pressed repeatedly across the entire weathered surface of the wafer. The stub was then mounted into the SEM (Hitachi S-4800 FE-SEM). SEM images were collected at a working distance of 4 mm using 1.5 kV accelerating voltage, 5 μA beam current and vacuum below $10^{-3}$ Pa. Multiple SEM images were acquired at different levels of magnification for each sample. Energy dispersive X-ray spectra (EDX) were also obtained for each sample to assess observable elements.

Sampling of release by immersion was stepwise. Each specimen was immersed in 10 mL of the leaching fluid described in EPA Method 1311 (Toxicity Characteristic Leaching Procedure (TCLP Extraction Fluid #1). This solution can be made by adding 5.7 mL glacial
acetic acid to 500 mL of reagent water, then adding 64.3 mL of 1 N NaOH, and diluting to a volume of 1 L. NaOH was added to adjust the pH to 4.93 ± 0.05. The containers used at both analytical labs were the same diameter and the same amount of extraction fluid (10 mL) was used for immersion. The upper face of the weathered samples was placed facing downward into the immersion fluid to maximize liquid-to-surface contact at both labs.

The containers and shakers were not completely standardized. EPA, Athens Georgia, U.S.A. (EPA-Ath) used cylindrical 16 oz wide-mouth glass containers with 9 cm diameter and flat bottom purchased from Thermo-Scientific, shaken in a temperature-controlled (25 °C) incubator shaker at approximately 2 Hz. After shaking, samples of the fluid were taken, and the same specimens in the same immersion containers were then sonicated using a Cole-Parmer 8852. BASF used cylindrical PP containers of 9 cm diameter with a flat bottom and horizontal shaking on an IKA VibraX VXR with 1.8 Hz frequency and about 5 mm total amplitude, performed in an air-conditioned lab at 22 °C (Fig. 3). The height of the PP containers of 11 cm (Fig. 3) is more than required, but the specific containers were preferred due to their flat bottom. After sampling the fluid the same specimens in the same immersion containers were then sonicated using a Bandelin Sonorex Digital 10P. In both labs, each sample from the immersion fluid was 4 mL volume by a pipette: first sampling after 24 h shaking, and a second sampling after an additional 1 h bath sonication (Fig. 3). Immersion fluid was not replenished between samplings.

Analysis of released fragments used five different methods, of which three were performed in both the EPA-Ath and BASF labs:

- TEM to identify the form of release in four categories.
- ICP-MS to quantify by chemical selectivity the content of ENM across all release categories.
- UVeVis/turbidity to semi-quantitatively rank the release quantity vs. a control specimen.
- AUC (at BASF) or AF4 (at EPA-Ath) to quantify the free ENM category by size-selectivity.

In all methods, one useful control was the aged matrix without embedded ENM; and another was the non-aged matrix with embedded ENM. As a negative control, we used the pure immersion fluid. As positive controls we used pure MWCNT suspensions in the immersion fluid with concentrations of 1 ppm, 10 ppm, and 100 ppm (ppm = mg/L). All controls and samples underwent identical sample preparation steps described for each method below.

A 0.5 mL aliquot of the sonicated extract was used specifically for TEM studies. TEM analysis at EPA-Ath was performed at the Georgia Electron Microscopy (GEM) lab at the University of Georgia using an FEI Tenca 200 kV TEM for imaging. The protocol involved placing the copper mesh grid on a 5 cm × 5 cm piece of parafilm in a clean petri dish. A sample volume of 20 μL from the aliquot was diluted ten-fold by nanopure water. The resulting mixture was well mixed by vortexing then 1 drop was pipetted onto the TEM mesh grid and allowed to dry overnight in the sealed petri dish. The TEM images were obtained and, if the sample was poorly resolved because the particles were so concentrated that the
TEM beam could not penetrate sufficiently, another 20 μL from the aliquot was diluted 100-fold by nanopure water and mixed well. A single drop of this mixture was placed on a copper mesh grid on a 5 cm × 5 cm piece of parafilm in a clean petri dish. After drying overnight in the sealed petri dish, the TEM images were obtained. Generally, well-resolved images were obtained after the 10–100 × dilutions.

The TEM analysis at BASF first removed excessive salts in the EPA leaching fluid. This procedure is not advised for non-particulate releases (such as ionic leachates) but was critical for obtaining high-quality TEM scans with low background interferences. The aliquot was filled to 35 mL to dilute salts before being centrifuged at 30,000 rpm for 4 h; this step was crucial, since small fragments of free ENM might be lost by insufficient centrifugation. Compared to the experimentally determined sedimentation speed of free MWCNT (and in line with a theoretical calculation based on 9.5 nm diameter), all free MWCNT had completely sedimented by this time, so that they were not lost by this procedure – a fact further confirmed by the positive controls. The bottom pellet with 1 mL remaining water was sent to the TEM lab. The super-natant containing salts of the leaching fluid was discarded.

The pellet was sonicated (bath sonication, 3 min), and transferred to a C-coated TEM carrier grid. The carrier grid was placed in a petri dish on para-film. One drop of sample was placed on the grid. The petri dish was then covered and the sample dried at room temperature for 24 h. Samples were investigated on a Tecnai G2-F20ST machine (FEI Company, Hillsboro, USA) operated at 200 keV, equipped with a 2K Gatan CCD camera (Gatan, Pleasanton, USA, version 2.11). For each sample, a representative overview image (5.9 × 5.9 mm$^2$) and a detail image (784 × 784 nm$^2$) were acquired under bright-field conditions for documentation. All images were archived.

More than 400 TEM images were evaluated manually, using a ruleset to assess fibrillar structures; these were identified as MWCNTs, if all three rules were in line with the MWCNT positive control: observed hollow core, matching diameter between 5 nm and 20 nm, matching length (not longer than 2 μm). The operator checked yes or no if either of the four categories (see schematic in Fig. 7A) was observed in any scans relating to a specific specimen and sampling condition:

- „matrix fragments, possibly ENM embedded“ (= F)
- „matrix fragments, ENM protruding“ (= G)
- „ENM agglomerates, possibly with other debris attached“ (= “J”)
- „free ENM“ (=H)

Three of these categories are visualized as forms F-G-H in the systematization of release phenomena by Harper et al. 2015 [1], to which the experimental reality added the “J, dirty agglomerate” category. The decision was based on a screening of large sample areas. Representative images were additionally taken for documentation. At BASF, a single operator performed all analyses. At EPA-Ath, two operators evaluated the scans and cross-confirmed interpretation of results. In contrast to the TEM preparation, we used the original immersion fluid (no pelleting) for all other analyses.

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Specifically for ICP-MS, 2 mL of the 4 mL was used for analysis; however, separate digestion methods were performed due to availability of and preference for equipment.

For ICP-MS at EPA-Ath, Standard Method 3050 B was modified slightly, and used to further digest the particles in leachate from the weathered epoxy samples following their initial analysis without digestion. This step was performed because the epoxy samples showed greater indications of MWCNT release (i.e. more released Co and UV-absorbing particles) than PA samples. The samples were digested in an Enviromental Express Hotblock using 50 mL digestion vessels by taking 0.5 mL of the sample followed by adding 10 mL of HNO₃: H₂O (volume ratio 50:50) and then heating for 15 min to digest the organic matrix. The sample was added to the cooled digestion mixture and 5 mL of HNO₃ was added. The sample was heated to evaporate and concentrate to 5 mL. 2 mL H₂O and 3 mL H₂O₂ were added and the resulting mixture was heated until effervescence subsided, followed by adding 10 mL HCl and heating until dryness. The vial was then filled with water to 10 mL.

The samples were analyzed using a Perkin Elmer NexION 300D ICP-MS equipped with an ESI SeaFAST autosampler following digestion. Only 0.5 mL of leachate was used for further digestion as opposed to 1.0 mL because the digestion technique was added as a result of recommendations from BASF during the analysis phase of the project.

For ICP-MS at BASF, all handling of samples and immersion fluids is in plastic containers and Eppendorf tubes, avoiding glass or metal in order to make the protocol easily transferable to release studies of e.g. SiO₂ or Fe₂O₃ containing nanocomposites. An automated robot system performed the digestion protocol in quartz vessels by taking 1 mL of sample and adding 4 mL of H₂SO₄:HNO₃ (volume ratio 39:1). An additional 6 mL of HClO₄:H₂SO₄:HNO₃ (volume ratio 1:1:2) was added for mineralization. The water content was evaporated to dryness by IR heaters, followed by dissolving the dry sample in 13 mL HCL (5% by vol). The sample was then heated to boiling for 1 min to ensure homogenization, and the volume was adjusted to 10 mL with additional water.

The resulting solution was injected and measured by ICP-MS (Agilent 7500a) or ICP-AES (Varian 720). Samples were introduced via a Scott-type spray chamber with Meinhardt nebulizer (plasma power 1300 W, plasma gas 16.5 L/min, auxiliary gas 1.5 L/min). As internal standards, ⁴⁵Sc (ICP-MS) and Ar at 420.070 nm (ICP-AES) were used. Final results include the entire sample preparation procedures and reported the content of tracer metals (Al, Fe, Co) per volume of leaching fluid.

Before UV–Vis and AUC/AF4 analysis, the remaining 1.5 mL of the 4 mL were spiked with SDS to reach a final SDS concentration of 10 g/L; spiked samples were then bath-sonicated for 1 h to deagglomerate the samples. This ensured that any nanoscale releases were sufficiently dispersed to be detected, where the hydrodynamic diameter is – as expected for tubes – closer to the cross-section than to the length, as demonstrated by the positive control of a 1 ppm pure CNT suspension in Fig. 2C.

UV–Vis at BASF was performed on the spiked and sonicated sample by a simple Ocean-Optics USB2000 spectrometer. Full spectra 200 nm–800 nm were acquired. For evaluation, the absorption at 450 nm was reported and additionally the wavelength at which the
absorption crossed one optical density was reported. All raw spectra are archived at the NanoRelease@NIST database.

UV–Vis at EPA-Ath was performed on the spiked, sonicated, and additionally diluted sample using a Perkin Elmer Lambda 35 UV–Vis Spectrometer; recording absorbance at 260 nm. A sample volume of 1 mL was diluted 30 × in 1% SDS to increase the sample transmittance at 260 nm (1.00 cm pathlength) to > 0.1 to insure that the best analytical results were obtained. All raw spectra were archived at the NIST Materials Data repository.

In Analytical Ultracentrifugation (AUC-UV), a radially scanning UV detector is synchronized to the rotation of a centrifuge, so that one observes the colloidal speed of migration during centrifugal separation. Here we used a Beckman XL centrifuge at 8000 rpm with detection at 450 nm. MWCNT traces to 1 ppm are quantitatively detected with size and concentration accuracy better than a 10% [25] and not worse than a 20% error in the presence of an excess background of micrometer-sized fragments [27]. The wavelength was chosen because MWCNT absorption is higher at shorter wavelengths, but UV–VIS still shows an SDS signal around 350 nm, which decreased with increasing wavelength and vanished at 400 nm (Fig. 2B). UV–Vis confirms no signal from SDS at 450 nm, although alternatively 260 nm could have been chosen for enhanced selectivity. The evaluation of the raw data absorption profiles resolved in radius and time used SedFit v14.1 with a density of 1.8 g/cm³; and with concentration readings calibrated by the positive controls, using a dense grid around the peak of freely dispersed MWCNTs, while still capturing larger agglomerates and fragments. We reported the average size, and the mass concentration observed in the size range 5 nm–100 nm, and the UV signal at 450 nm after removal of the larger fragments by the centrifugal process itself. All size distributions are archived at the NIST Materials Data repository.

Asymmetric Flow Field Flow Fractionation (AF4) was performed to potentially compare size distribution results obtained using AUC-UV. The AF4 method is well described elsewhere [28]. In short, colloids are fractionated in a flat channel (about 300 μm thin, about 2 cm wide, about 30 cm long), where part of the elution medium is withdrawn through the channel bottom via an ultrafiltration membrane. Diffusion, as dictated by hydrodynamic size, acts against this cross-flow so that smaller colloids travel more often in the faster flowing regions and exit first towards the detector. The program of cross-flow, injection and detector flow was optimized for the best possible combination of flow rates using an injection volume of 100 μL. Absorbance at 254 nm and hydrodynamic diameter were measured in tandem by UV–Vis (PN 3241, Postnova) and DLS (Nano ZetaSizer, Malvern) detectors, respectively.

3. Results and discussion

3.1. Observations made during the aging

The general approach used here was to weather epoxy and polyamide (PA) composites of MWCNT along with epoxy and PA with no added MWCNT using simulated solar radiation and rainfall defined in a widely-used plastics aging protocol [26]. The weathering was interrupted after 1500 h in order to change the Xe-arc lamp. This was a precaution to ensure.
constant irradiation throughout the weathering since the manufacturer advised that the useful lifetime of the lamp is 1500 h. The total irradiance level is automatically maintained by the system but the spectral distribution of the light does change slowly over time.

Irradiated MWCNT composites were black in color and little visible change was observed on PA composites throughout weathering. Epoxy composites showed clear signs of surface changes, including cracking during the latter stages of the weathering. The wafers of epoxy and PA with no added MWCNT were initially pale yellow but the yellow coloration deepened as weathering proceeded. During the later stages (after 1700–1800 h) of weathering the epoxy wafer became orange in color. This development of color likely involved UV-induced production of reactive transients such as hydrogen peroxide, peroxyl radicals, and hydroxyl radicals that react with the polymer matrix. As a result of reactions between the transients and the matrix (such as epoxy) the matrix reacts with oxygen and/or fragments to produce colored by-products, carbon dioxide, hydroperoxides and other products. At this stage, we noticed that a brownish-yellow residue had been deposited on 20% of the surface of the lamp cover and over approximately 10% of the sides of the weathering chamber (Fig. SI_2). The irradiation was interrupted at both the BASF and EPA-Ath facilities and the hydrophilic deposit was removed by rinsing with deionized water. A portion of the rinsate was analyzed at EPA-Ath by UVeVis and ICP-MS. The absorption spectrum was featureless with increasing absorbance at low wavelength; a slight inflection in absorbance was seen at 250e260 nm. One surprising result was the relatively high level of nickel by ICP-MS analysis in the rinsate with dissolved deposit (7.9 ppm Ni; other metals0.04 ppm Co, 0.35 ppm Al, 0.6 ppm Fe).

3.2. Dry sampling with SEM and EDX analysis

Analysis of the exposed surface features collected from the neat polymer specimen E0 and P0 without MWCNTs (Fig. SI_3) allowed us to assess the susceptibility of each polymer type towards degradation from weathering. Overall, the polyamide appeared to show less degradation than the epoxy. The weathered surfaces display relatively minimal features and have no prominent clusters, particulates, fractures or fibrous structures. Samples taken from specimens aged at each of the four weathering labs were consistent with features observed by SEM imaging. In contrast, all of the neat epoxy samples clearly showed particulate and fragment structures of varying dimensions. Samples from three of the four aging labs also showed fibrous structures in the SEM images; the samples generated by LEITAT were the only ones without visible fibrous structures.

Representative SEM images of the samples taken from MWCNT-containing P4 and E4 specimens from all four aging laboratories are shown in Fig. 4. All samples consistently show an abundance of nanotubes was collected from the surfaces of each weathered wafer; thick tangled mats of MWCNT are clearly visible in each image.

The PA4 samples (Fig. 4A–D) appear to show a thin layer of polymer covering most of the exposed MWCNT; that residual polymer coating is more evident in higher magnification images of the same samples, as shown in the Supporting Information (Fig. SI_4). Polymer can be seen coating and bridging the MWCNT bundles in all of these samples. Exposed nanotubes were also easily observed for the E4 composites (Fig. 4E–H) despite the much
less bulk loading (0.25%-wt); there is no visible residual polymer coating in the E4 samples. Based on SEM observations, no obvious differences were found in the samples taken from specimens aged by the different labs for either nanocomposite.

EDX spectra were also obtained for each weathered wafer samples to assess elemental composition, particularly trace metals that might be associated with catalysts that produced the MWCNTs. Observed elemental compositions organized by element and aging lab, are summarized in the Fig. SI_5 to SI_8.

The neat polymer samples had generally similar results. P0 samples from the four aging labs showed very consistent carbon and oxygen content, with carbon at around 75%-atom and oxygen at approximately 20%-atom (Fig. SI_7). E0 samples had more variation in the carbon and oxygen content, showing carbon at 60–80%-atom and oxygen at 15–30%-atom (Fig. SI_5). Both PA and epoxy samples from specimens aged by US EPA-Cincinnati (EPA-Cin) show elevated silicon content that was not observed in any other sample; this may be due to specimen contamination. The catalyst metal (Fe, Al, Co) signatures did not differentiate from unspecific contaminations (Si, Ca, Cu) for any of the polymer-CNT samples Catalyst metals Co and Fe were not observed within the detection limit of the measurement (3000 ppm by weight, or approximately 0.1 atom-%) while Al was observed but at levels generally less than those of contaminant elements.

Carbon and oxygen contents in the polyamide-CNT samples are less uniform across samples from different weathering labs than in neat polyamide samples, but their highest observed values are greater than those of the neat polymers (Fig. SI_8). Epoxy-CNT samples also showed carbon content slightly higher than was observed in neat resin (Fig. SI_6). This is thought to be a result of the additional carbon present in the CNT-enriched tangled mat on the aged surface. The epoxy-CNT samples prepared by EPA-Cin showed anomalously lower carbon and higher oxygen content than comparable samples from the other labs (Fig. SI_6). These samples also showed very high silicon content, similar to what was observed for the neat polymer samples produced by this lab, which influenced the reported carbon measurement.

The polyamide-CNT was the sole sample prepared by EPA-Cin that did not display highly elevated silicon content. One of the EPA-Ath PA-CNT samples shows elevated silicon. The PA-CNT samples from LEITAT show high levels of calcium that were not observed in other samples. The catalyst metal (Fe, Al, Co) signatures did not differ from the unspecific contaminations (Si, Ca, Cu) for any polymer-CNT samples.

On the surface of weathered samples, MWCNTs were clearly accessible and concentrated for both types of polymers, and easily collected by our sampling protocol. The tangled mat of concentrated MWCNTs is a very well known phenomenon for epoxy-CNT composites [9,17] and PA-CNT composites [13,21], and is attributed to the collapse of the network of MWCNTs as the UV-degradable matrix breaks down to volatile species while the UV-persistent filler remains. The CNT-epoxy materials showed higher abundances of exposed MWCNTs in the collected samples, but they were not coated by or strongly associated with the residual epoxy matrix, itself. For the CNT-PA samples, the observed CNT structures
were mainly coated by PA or still embedded at or slightly below the surface of the residual PA matrix, despite the much higher CNT loading in these mixtures. These results are consistent with observations that the neat epoxy resin showed greater susceptibility to degradation by weathering than PA. The presence of the CNT in each polymer type did not appear to completely alter its resistance to weathering.

From the perspective of assessing consistency and reproducibility of sample generation protocols across the multiple labs, the weathering protocol produced consistent results from all of them. In the SEM imaging data, similar physical features and tendencies towards CNT accessibility were observed across samples from each of the source labs. EDX data were less consistent, likely due to apparent sample contamination of a few of the weathered wafers. It is unknown whether this contamination occurred at the source, during shipping or as part of handling during analysis. EDX data were consistent in that the highly specific catalyst metal Co was not observed above the limit of detection of 0.1 atom% in any collected material; however, traces of Al were detectable at a concentration much lower than other contaminants, especially Ca and Si. There was not significant correlation between results from the different aging labs or between E0, P0 controls and E4 and P4 materials, respectively. The limited information depth of EDX of few nm and the presence of matrix degradation products prevents a more quantitative use of EDX for release assessment. In other studies, XPS was used and also found organic and inorganic contaminations [20], which could, in some cases, be resolved by line shape analysis [6].

3.3. Immersion sampling with ICP-MS analysis (by digestion protocol)

A key part of our analyses focused on the use of ICP-MS to detect and quantify release. This technique is not only universally applicable to inorganic materials, but also to MWCNTs, because synthesis of CNTs typically utilizes metals such as Mo, Ni, Co, and Y, as catalysts [29,30]. Consequently, residual metal catalyst particles frequently persist in the CNT structure after manufacture [29]. Even after acid purification to remove metal impurities, metals intercalated within CNTs can account for several percent of the particle mass [30]. Analyses of the MWCNT sample used in this study indicated that Al, Co, Fe and other less abundant metals were present (Fig. 2, Table SI_1). We ruled out Fe because it is ubiquitous in many environments and likely to interfere with a metals-based analysis of MWCNTs. Data on Al was acquired on all immersion fluids but was also not used because it can be an abundant constituent of some polymer matrices, such as specifically the PA utilized in this study (Table SI_1). Co was therefore chosen as the preferred metal tracer for MWCNT in this protocol and has been used for this purpose in previous studies [30].

Results of the four aging and two analytical laboratories were in satisfactory agreement (Figs. 5–8). Overall, intra-lab duplicates agreed much better than inter-lab comparison between aging labs; this was observed for both the MWCNT-PA and the MWCNT-epoxy specimens, and by both analysis labs at BASF or at EPA-Ath. This makes the sampling procedure as a source of the variability less probable; the variability is also probably not related to the ICP-MS analysis technique, since the same pattern of variation between aging labs was observed by UV–Vis (Fig. 8). Variability might instead stem from a remaining variability of UV/spray aging conditions despite the use of ISO-standardized equipment and
might specifically relate to differences within each SUNTEST chamber e.g. due to the brown deposit or inhomogeneous spray intensity. Finally, it could be related to the use of different shipping containers or accidental damage during shipping. We note, however, that even the maximum variability of any combination of material; aging lab; analysis lab did not exceed a factor of three. This is a significant reduction of the uncertainty, and enhancement of the reproducibility of release testing, compared to earlier uncertainties without standardized protocols [20]) that exceeded several orders of magnitude.

As expected, sonicated samples released significantly higher amounts of Co than shaken samples. Weathered MWCNT-epoxy composites released more Co than the corresponding MWCNT-PA composites. When averaged across all four aging labs, sonication sampling from aged MWCNT-PA produced an average of 0.011 ± 0.0086 ppm Co and shaker sampling averaged 0.00977 ± 0.0017 ppm Co. With the MWCNT-epoxy sonicated sampling the average was 0.0280 ± 0.0086 ppm Co and the shaken sampling only 0.00972 ± 0.0021 ppm Co.

Particle digestion by the extensive system at BASF and by EPA Method 3050 at EPA-Ath helped improve the observed release of Co particularly in the case of the abundant particles released from the MWCNT-epoxy composites. Higher release rates from epoxy composites were observed despite the fact that MWCNT concentration in the epoxy was 16 times lower than MWCNT concentration in the PA composite. The same values of absolute mass of catalyst tracer in the released fragments correspond to a 16-fold higher release from epoxy than from PA relative to the original MWCNT content. This also confirms that the release-controlling processes are mainly light driven in the top part of the composites. Previous studies have shown that weathering rates are influenced by factors such as UV-light screening and scavenging of free radicals that drive autoxidation of the composites [4,31–35].

3.4. Immersion sampling with TEM analysis

The immersion fluid was observed to contain a significant amount of salts obscuring potential nanoparticles in the images. It was therefore diluted (EPA-Ath) or washed (BASF) as described in the Experimental section. The influence of the washing step on image quality is shown in Fig. SI_9 in the SI where the amorphous background is significantly reduced. An overview of the sample matrix of E4 and P4 material containing MWCNTs, imaged under bright field conditions, is provided in Fig. 6. Rows in the figure represent the different aging labs while columns show different samples imaged at different labs. The images were taken from material aged in row 1 (1 L) for the BASF microscopy lab and in row 3 (3 L) for the EPA-Ath microscopy lab and are a selection of more than 400 representative images taken for documentation. Duplicates were acquired from lines 2L and 4L respectively. A corresponding image matrix of the material containing no MWCNTs is shown in the SI (Fig. SI_10). All TEM images in these figures originated from the immersion fluid with sonication sampling, but analogous images have been acquired from the immersion fluid with shaker sampling.

Manual evaluation of the samples was performed during microscopy and covered a significantly larger area as shown in representative images. The samples were evaluated
according to the four categories shown in Fig. 7A (described in Experimental), which match forms denoted as F-G-H in Harper et al. [1]. This would result in a binary statistics, if a specific form was observed in any TEM sample region of a specific combination of aging lab, material, or sampling condition. We did not evaluate how often each category appears on all of the TEM scans acquired. To provide exemplary categorization on the scans shown in Fig. 5, a “particulate fragment with protruding CNT” is recognized on the images “P4 EPA (Ath.), aged at EPA (Cin.),” “E4 BASF, aged at BASF”, “E4 BASF, aged at EPA (Ath)”. In contrast, the category “particulate fragment” is recognized on the images “P4 BASF, aged at EPA (Cin.)” and “P4 BASF, aged at LEITAT”. The category “CNT agglomerate with minor other component” is recognized on the image “E4 BASF, aged at LEITAT”.

The full analysis on the selected cases of P4 shaker and P4 sonication in Fig. 7B and C demonstrate that all categories are well recognized. Variation between analysis labs is higher for “fragment with protrusions” which may be influenced more by subjective decisions and resolution issues than other classes. TEM images in Fig. 6 can be directly compared to the evaluation results of the sonicated E4 samples shown in Fig. 7 (Fig. 7B and C). We note that MWCNTs can be distinguished from other tubular artifacts only by criteria listed in the Experimental section, which include characteristic contrasts of dark walls surrounding a brighter core and characteristic length and diameter – all derived from the positive control samples specified in the Experimental. Despite the seemingly chaotic and actually polydisperse forms, the agreement between the aging labs was very good based on data from both labs (Fig. 7). Between analysis labs, the agreement of “what” is released is much better than expected, with no variability on the identification of “free single CNT” category which is absent in all MWCNTPA samples with shaker sampling, but present in all MWCNT-epoxy samples with sonication sampling. Further, there is minimal variability on the “particulate fragment” and “CNT agglomerate” categories, but minor variability on the “particulate fragment with protruding CNT” category, between both aging labs and analysis labs.

3.5. Immersion sampling with UV–Vis analysis

The two analysis labs used the same protocol to spike and disperse samples by SDS and sonication (see Experimental). The EPA-Ath lab then further diluted the samples by a factor of 30, and measured UV–Vis absorption (Fig. 8). MWCNT positive control spectra showed a band from 240 nm to 280 nm wavelength (Fig. SI_11A); the maximum of absorbance at 260 nm was chosen by the EPA-Ath lab for quantitative comparison of samples, aiming at spectral selectivity.

Spectra identified the PA_CNT with shaker sampling as the sample with the overall lowest UVeVis MWCNT absorption, significantly lower than the CNT-free pure PA, but higher than the non-aged PA_CNT (Fig. SI_11A and B). Scatter between aging labs was low (Fig. 8A). The highest absorption values were measured for the epoxy_CNT with sonication sampling, and show high contrast against PA_CNT_shaker values. Moreover, although there was some variability in the UV–Vis coefficients (260 nm) for the sonicated, weathered diluted epoxy
CNT extracts these coefficients correlated closely with released cobalt concentrations measured by ICP-MS in the samples (Fig. SI_12).

This correlation indicates both the ICP-MS and absorption measurements are useful indicators of MWCNT release and that the lab-to-lab variability may be related to differences in weathering, probably due to variations in UV exposure. The variability was caused in part by production of the UV-absorbing brown precipitate that partially blocked the UV (Fig. SI_2), especially for samples positioned near the center back of the aging chamber in the EPA-Ath experiments. Spatial differences in sonication intensity within the sonication bath should average out over the relatively large dimensions of the wafers, but may have additionally contributed. In effect, the EPA-Ath samples generated fewer particulate fragments than those that were aged elsewhere, as indicated by matching UV–Vis and ICP-MS observations. A closer look at the UV–Vis spectra measured on immersion waters of CNT-free specimen (Fig. SI_11A) reveals that these spectra also show a band around 260 nm wavelength. This is true for both aged PA and aged epoxy, and indicates that this hump is partly matrix-related, as well as CNT-related.

The other analysis lab at BASF chose to measure UV–Vis without dilution, focusing more on turbidity from scattering by particulate fragments, and less on identifying specific spectral features. Accordingly, evaluation of UV–Vis spectra (Fig. SI_11 B) was adapted to cover an extreme dynamic range of turbidity, and read from the spectra the wavelength $\lambda_1$ OD (where absorption drops below 1), and then referenced this value against the pure fluid control. The results (Fig. 7B) allow an even more significant discrimination between the two datasets of lowest and highest release: PA_CNT with shaker sampling increases $\lambda_1$ OD by only few nm wavelength, but the duplicates are significantly above the control for three of four aging labs. Epoxy_CNT with sonication sampling increases $\lambda_1$ OD by more than 100 nm wavelength, which results in a logarithmic strong and highly reproducible contrast between cases of lowest and highest releases (Fig. 7B).

### 3.6. Immersion sampling with AUC or AF4 analysis

#### 3.6.1. AUC analysis

Selected AUC size distributions are presented in Fig. 9A together with positive and negative controls. Contrary to earlier investigations of weathering releases with the same technique [20,36], we focused here exclusively on the size range of the smallest fragments below 100 nm diameter, where free single MWCNTs would be identifiable by their characteristic size. None of the size distributions allowed a convincing identification (Fig. 9A). Size distributions measured by sonication sampling of aged specimens of epoxy, PA or PA_CNT all resembled each other as well as the negative control of sonication sampling from a non-aged epoxy CNT control. Only the size distribution of epoxy CNT deviated, both in size interval of the positive control peak from 8 nm to 25 nm and at larger sizes from 40 nm to 100 nm. By integrating the size range from 5 nm to 100 nm, AUC results on PA_CNT with shaker sampling remained, consistently at a noise level of 1 ppm for all duplicates and all aging labs (Fig. 9B). Applied to epoxy CNT sampled by sonication, the AUC results indicated significantly higher solid content in this size range for the three aging labs, with values ranging between 3 ppm and 7 ppm, but with considerable error bars from duplicate specimens.
At one aging lab (EPA-Cin), AUC values of less than 2 ppm deviated from the other aging labs and showed no significant increase when compared to noise level. This was the case for both duplicate specimens, indicating this finding is not a single outlier, but related to the lab. The same immersion waters (epoxy-CNT, aged at EPA-Cin, and sampled by sonication and analyzed at BASF), were outliers according to the other three analysis techniques: they had half the Co content in ICP-MS (Fig. 5B); showed no MWCNT agglomerates or fragments with protruding MWCNTs in TEM (Fig. 7B); and had slightly lower UV–Vis turbidity (Fig. 8B). Considering the EDX findings on specimens aged in parallel in the same instrument - that these were contaminated by some Si-containing compound (Fig. SI_6) - we conclude that aging or shipping contaminated the surface of EPA-Cin specimens, leading to lower releases overall and most sensitively to an absence of releases in the nano size range.

3.6.2. AF4 analysis—Two sonicated epoxy-CNT samples at EPA-Ath were chosen to apply AF4 analysis to evaluate particle size release of CNT from the matrix. Fig. SI_13A shows DLS size count readings vs. time where the size increase of the samples over time did not follow the trend of the standards. As documented by Fig. SI_13A, both size standards (60 and 300 nm polystyrene nanospheres) exhibited single peaks in the absorbance spectrum, so that the size fractionation worked well in this size range. DLS measurements for the fractionated samples of immersion fluids were not as expected, however. Both small and large particles were eluted from the channel at the same time (Fig. SI_14), a phenomenon in AF4 channels known as steric elution. The limited dynamic range of AF4 in a single measurement—at best a factor of 20 in size despite cross-flow ramps—is a known limitation of AF4 [37]. The limited size-selectivity made assignment difficult, so AUC-UV was preferred for this inter-laboratory comparison of aging labs.

3.7. Comparison of (nano)materials and sampling protocols
In addition to the two case studies (P4, shaker sampling) and (E4, sonication sampling) we acquired equally complete datasets on all four materials with both sampling intensities. In the above discussion, the reproducibility of aging labs was assessed on the two case studies that are illustrated in Fig. 10.

In an orthogonal perspective, Fig. 10 averages across aging labs, and compares (nano)materials and sampling protocols. Our extended dataset can be interpreted by three perspectives that are highly relevant for release assessment:

- Does the matrix (epoxy vs. PA) matter? Yes, both form and rate of release depend significantly on the matrix. TEM identifies few cases of MWCNT agglomerates or free MWCNT from PA_CNT, but a dominance of these forms from epoxy-CNT were observed (Fig. 10A–B). The difference is even more pronounced in the release rate assessed by UV–Vis (Fig. 10C), with significantly higher release rates from E4 than P4, regardless of sampling intensity. This relationship is again confirmed by both analysis labs in all three versions of UV–Vis evaluation. Finally, the element-specific release by ICP-MS is about equal for sonication sampling of E4 or P4 which indicates a roughly 16-fold greater probability for MWCNT to release from epoxy than PA (Fig. 10D, see also discussion in Section 3.3). The higher release rate observed with epoxy CNT is
likely related to its higher rate of weathering-induced cracking, observed here as well as earlier [4,9,17].

- Is there an MWCNT (EO vs E4, P0 vs P4) effect? Apart from the trivial effect that MWCNT-containing forms are not observed in fragments from P0 and E0 (Fig. 10A–B), there are significant rate effects – but with the opposite sign depending on matrix material. UV–Vis analysis is valuable in this regard, as it is sensitive to changes in the neat polymer, too (Fig. 10C). For PA, MWCNTs add effective photoprotection and possibly free radical scavenging [33] with significant reduction of release rates; this reduction is observed for both shaker or sonication sampling, and is consistent with Nguyen’s concept of a dense collapsed MWCNT network that passivates the surface by UV absorption and mechanical effects [4,9,14]. Such a mechanically entangled network is observed in SEM (Fig. 4, SI_4). For epoxy, the nano-composite E4 releases significantly more fragments than E0, if sampled by sonication, and about an equal number if sampled by shaker. We believe an increased rate of epoxy degradation, induced potentially by the higher temperature of the black P4, overcompensates mechanical passivation [17].

- Does the sampling intensity (shaker vs sonication) matter? Yes, both for form and rate of release. Reproducibly sonication sampling induces more complex forms of release, with a higher share of MWCNT agglomerates or free MWCNTs, if the matrix is photodegradable/labile such as the epoxy (Fig. 10A–B). Between shaker and sonication sampling, the rate of release shows only a weak increase for the neat polymers E0 and P0 (Fig. 10C), and a moderate increase for P4 (Fig. 10D), but a very pronounced increase for heavily degraded E4 material (Fig. 10CeD). There might be a tendency toward larger relative error bars for sampling by sonication, but the sensitivity for detecting release is nonetheless higher than with the more realistic shaker sampling.

On a final note about analysis methods, TEM categories of “particulate fragment” and “particulate fragment with MWCNT protrusions” remain slightly ambiguous with about 20% differing attributions between labs. The “particulate fragment with MWCNT protrusions” category is the only weak correlation between analysis labs. We attribute this to the remaining subjectivity of detecting and classifying a tubular protrusion. All other form categories show a high reproducibility, with a minimal ambiguity between “MWCNT agglomerate” and “free MWCNT”. The distinction of “particulate” categories (blue + orange) vs. “ENM” categories (grey + yellow) is strongly reproducible across all laboratories.

### 3.8. Optimization of the NanoRelease protocol

Compared to the protocol described in the Experimental section, and to harmonize the interpretations of the analysis labs, we summarize here further specifications for future inter-laboratory comparisons (also see the list in Supporting Information). Although identified as the main sources of differences, homogeneity, and reproducibility of aging conditions cannot be easily improved from the current ISO-standard. Rotation of the specimen’s location inside aging chambers could reduce sample heterogeneities. For optimal reproducibility one
might perform EDX on neighboring samples: if the specimen surface shows more than 1% of elements not originally contained on the surface or in the bulk of the specimen, consider it contaminated and remove it from further analysis. This specific threshold would have removed most outliers in our present dataset and led to significantly lower error bars on ICP-MS, UV–Vis and AUC results. The outer specimen dimensions, specifically their immersed surface area, should not be smaller than those investigated here. Further, ICP-MS analysis should use digestion of the matrix to capture all categories of the form of release. For the matrices investigated here, the less aggressive but less hazardous EPA digestion proved sufficient. TEM can be performed on complex immersion fluids, but TEM evaluation is less debatable on less complex immersion fluids (such as de-ionized water), or purified samples of complex immersion fluids (washed or diluted). UV–Vis should be performed on properly diluted immersion fluids to exploit wavelength selectivity. AF4 cannot be recommended currently. AUC and UV–Vis should be performed (as was done here) in de-agglomerated samples using surfactant spiking and sonication.

3.9. Outlook on the applicability of the protocol to generic nano-composites

The present protocol was evaluated on composites of PA or epoxy containing MWCNTs. Results on the form of release are in accord with earlier reports using different sampling protocols on MWCNT composites of the same two classes of polymer matrix [9,21]. Using the identical grade of MWCNTs in the present study, a subset of the present immersion protocol (only sonication sampling, only TEM and AUC analysis) was applied to further investigate release from composites of the same MWCNTs in: thermoplastic polyurethane, cross-linked polyurethane, and polyoxymethylene [36]. They differed in many relevant factors, such as ENM concentration in the matrix, ENM compatibility with the matrix, and susceptibility of the matrix for UV radiation and pH-related change. The rate of release was ranked and showed strong dependence on the polymer matrix: the present results with polyamide matrix ranked above polyurethane, below polyoxymethylene; and the present results with epoxy matrix ranked above polyoxymethylene. This confirms that the protocol is widely applicable to diverse MWCNT composites, and results in plausible release rankings in the exact order predicted by Kingston et al. [4]. The identical protocol was also applied to assess and quantify nanoscale releases from neat materials without nanoscale fillers, such as the E0 and P0 specimens of the present study, and from further matrices such as polyethylene, polyurethane, and cement, both as neat matrices and with various nanomaterial fillers such as metal oxides, organic pigments, carbon black, and silica [36]. There, the rate of release was found to be primarily determined by the matrix across five orders of magnitude, and modulated by the nanomaterial filler by less than one order of magnitude.

Depending on the specific filler and matrix, certain steps of the protocol may be irrelevant; e.g., sampling by sonication because the hydrophilic filler releases spontaneously, such as silica from polyamide [20]. In this case, one should switch from UV + rain to UV dry-aging in order not to lose released materials in spontaneous run-off. In other cases, sonication may be a stronger stress than UV degradation, such as for cement, which had a vanishing dependence of release on UV dose [36]. This suggests re-thinking parts of the present protocol to simulate specific scenarios of environmental degradation. For example, to mimic
secondary degradation processes of micro-plastics or car tire wear, a protocol might iterate mechanical stresses by sanding and sonication and environmental stresses by UV and hydrolysis [38]. There is also a growing body of information on nanomaterial aging and transformations; quantification of carbon nanomaterials; plastics degradation to micro-plastics; and releases from materials such as textiles that can improve the methods described here [32,39–43]. Further data on fate and effects of the released fragments are needed. But the low mass and potential surface contamination of weathering-induced fragments are a challenge for their ensuing characterization. To overcome that challenge, recently a strategy was proposed to provide materials in hundreds of gram quantities mimicking actual released materials from coatings and polymer nanocomposites by producing “fragmented products” [44].

Our protocol is not intended for investigation of release scenarios in which the matrix is more persistent than the ENM, such that release is dominated by transformation or dissolution of nano-Ag, Qdots or similar substances. These scenarios require dedicated leaching or washing protocols.

4. Conclusions

The protocol of aging + immersion sampling analysis proved successful across the globe– in generating quantitative results for the rate of release (UV–Vis, ICP-MS) that were in accord within a factor of two, and in many cases not differing significantly between aging or analysis labs. Statistical comparison of the qualitative results on the form of release (TEM, AUC) was equally in accord between aging and analysis labs. Remaining inconsistencies were traced back primarily to differences within each SUNTEST aging chamber (intensity of degradation) and between aging labs (contaminations).

Even the maximum variability of any combination of {material; aging labs; and analysis labs} did not exceed a factor of three, however. This is a significant reduction of uncertainty, and an enhancement of reproducibility of release testing compared to earlier challenges caused by the absence of standardized protocols. The present choice of analysis methods is recommended as it provides synergistic information and is universally applicable not only to MWCNT-composites but also to composites of inorganic or organic nanomaterials, and even to the assessment of particulate releases from neat materials without embedded nanomaterials.

All rankings were mutually consistent and consistent with the supporting evidence of an alternative dry sampling protocol (with SEM analysis). EDX was helpful in identifying a contaminated specimen and might be used in the future to enhance reproducibility. We propose one of the materials, MWCNT-epoxy without UV stabilizer, as a positive control for future laboratory validation, and MWCNT-PA without UV stabilizer as a low-release control. We propose the present protocol as an interim voluntary standard with a known level of reproducibility and a slightly refined protocol for pre-normative validation. Based on the effectiveness of conventional UV stabilizers to reduce polymer aging, we anticipate an equally significant reduction of releases by UV-stabilized nano-composites intended for outdoor use, and we call for experimental confirmation.

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All raw data were uploaded to the NIST Materials Data repository, materialsdata.nist.gov, where they are located in the NanoRelease community, and will become available with the acceptance of the present report for further analysis and as future benchmarks.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
ILC organization with 4 labs to perform the aging, and 2 labs to perform the wet immersion sampling and TEM/UVeVis/ICP-MS/AUC/AF4 analysis on specimen from all 4 aging labs, 2 labs to perform dry sampling and SEM/EDX analysis. (A colour version of this figure can be viewed online.)
Fig. 2.
Characterization of the positive control samples of pure MWCNT dispersed in the immersion fluid via sonication. A) TEM of 10 ppm MWCNT; B) UV-Vis and C) AUC each of immersion fluid with 0 ppm (grey line), 1 ppm (black solid line), 10 ppm (black dashed line) and 100 ppm (black dotted line) MWCNT. For C), the size is represented as cumulative distributions (thick lines, right axis), highlighting the match of nominal and measured concentration.
For each 4-mL aliquot apply the following analyses:

- **TEM** “check which structures are observed”
  - washing or dilution, sonicate, place drop on TEM grid, evaporate water.
- **ICP-MS** “tracer elements of ENM”, here Al and Co
  - with acid digestion of any released fragments
- De-agglomeration by addition of SDS to 10g/L, bath sonication 1h
- **UV-Vis** “absorption/turbidity of leaching fluid”
- **AUC or FFF** “characteristic size of free ENM” / “absorption in size range 5-100nm”

**Fig. 3.**
Protocol summary. The exact instrumentation and procedures in EPA-Ath and BASF labs are described in the text. Photographs were taken at the BASF sampling labs. As indicated in the figure, 4 mL aliquots each were taken from the pH 4.93 immersion fluid following mild agitation in the shaker, and then from the sonicated extract. Aliquots were then analyzed. The samples analyzed by UV–vis and AUC/AF4 were pretreated by adding 1% (v.v.) sodium dodecyl sulfonate (SDS) followed by an additional 1 h bath sonication to reduce sample agglomeration. (A colour version of this figure can be viewed online.)
Fig. 4.
Representative SEM images of samples collected from the weathered surfaces of the 4%-wt MWCNT-polyamide (PA4) specimens (A–D) and the 0.25%-wt MWCNT-epoxy (E4) specimens (E–H). Images A) and E) are of samples of specimens aged by BASF, B) and F) aged by LEITAT, C) and G) aged by EPA-Ath, and D) and H) aged by EPA-Cin.
Fig. 5.
ICP-MS comparison of aging labs on the samples with lowest release (P4 shaker) and of highest release (E4 sonication). A) sampled and analyzed at EPA-Ath (black); B) sampled and analyzed at BASF (red). (A colour version of this figure can be viewed online.)
Fig. 6.
Exemplary BF-TEM images of sonicated P4 and E4 samples aged at different labs (rows) and imaged at BASF or EPA-Ath (columns).
Fig. 7. TEM comparison of aging labs on the samples with lowest release (P4 shaker) and highest release (E4 sonication). A) schematic; B) sampled and analyzed at EPA; C) sampled and analyzed at BASF. The analysis is based on duplicate wafers that were each aged, sampled and analyzed. The units of the y-axis are “%”. (A colour version of this figure can be viewed online.)
Fig. 8.
UVeVis comparison of aging labs on the samples with lowest release (P4 shaker) and highest release (E4 sonication). A) absorption at 260 nm, measured at EPA on diluted samples; B) shift of the wavelength at which absorption drops below 1, vs pure fluid control, measured at BASF on undiluted samples. The error bars reflect duplicate wafers that were each aged, sampled and analyzed. A more conventional evaluation of the same spectra (absorption at 450 nm, undiluted) is correlated against the absorption at 260 nm (diluted) in the discussion. For original spectra see Fig. SI_11. (A colour version of this figure can be viewed online.)
Fig. 9.
AUC results from analysis at BASF. A) size distributions of positive control (1 ppm MWCNT, black line), negative control (pure fluid, grey line), E4 (red line), E0 (red dots), P4 (blue line), P0 (blue dots), all with sonication sampling, showing weak differentiation of samples from baseline, except E4; B) cumulative content in the size range 5 nm–100 nm, comparing aging labs on the samples with lowest release (P4 shaker) and highest release (E4 sonication). The error bars reflect duplicate wafers that were each aged, sampled and analyzed. (A colour version of this figure can be viewed online.)
Fig. 10.
Comparison of materials (epoxy vs. PA), including effect of added MWCNT (EO vs E4, P0 vs P4), and mechanical stress on release (shaker vs sonication). A) TEM at EPA-Ath; B) TEM at BASF, with color coding of the form of release as defined in Fig. 7A. C) UV-Vis analysis; EPA absorbance at 260 nm diluted (black), BASF absorbance at 450 nm undiluted (green), BASF shift of the wavelength at which absorbance equals 1 (red, right-hand axis). D) ICP-MS analysis; EPA (black), BASF (red). Lower values of P4 analyzed at EPA are rationalized by the omission of acid digestion here, in contrast to the analysis of E4 that used acid digestion in both analysis labs. Further, P4 suffers from Co contaminations (Table SI_1), making it less suitable for ICP-MS comparison. Data obtained from N = 8 specimens (duplicates from each of the four aging labs) have been averaged separately for each analysis lab. All data are represented, with no outliers removed. Error bars indicate one standard deviation. (A colour version of this figure can be viewed online.)