Chemical and Toxicological Evolution of Carbon Nanotubes During Atmospherically Relevant Aging Processes

Yongchun Liu,^{†,‡} John Liggio,^{*,†} Shao-Meng Li,[†] Dalibor Breznán,[‡] Renaud Vincent,[‡] Errol M. Thomson,[‡] Premkumari Kumarathasan,[§] Dharani Das,[§] Jonathan Abbatt,^{||} María Antiñolo,^{||} and Lynn Russell[⊥]

[†]Atmospheric Science and Technology Directorate, Science and Technology Branch, Environment Canada, 4905 Dufferin Street, Toronto, Ontario M3H 5T4, Canada

[‡]Inhalation Toxicology Laboratory, Healthy Environments and Consumer Safety Branch, Health Canada, 0803C Tunney's Pasture, Ottawa, Ontario K1A 0K9, Canada

[§]Analytical Biochemistry and Proteomics Laboratory, Healthy Environments and Consumer Safety Branch, Health Canada, 0803C Tunney's Pasture, Ottawa, Ontario K1A 0K9, Canada

^{||}Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario M5S 3H6, Canada

[⊥]Scripps Institute of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92037, United States

^{*}Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, China

Supporting Information

ABSTRACT: The toxicity of carbon nanotubes (CNTs) has received significant attention due to their usage in a wide range of commercial applications. While numerous studies exist on their impacts in water and soil ecosystems, there is a lack of information on the exposure to CNTs from the atmosphere. The transformation of CNTs in the atmosphere, resulting in their functionalization, may significantly alter their toxicity. In the current study, the chemical modification of single wall carbon nanotubes (SWCNTs) via ozone and OH radical oxidation is investigated through studies that simulate a range of expected tropospheric particulate matter (PM) lifetimes, in order to link their chemical evolution to toxicological changes. The results indicate that the oxidation favors carboxylic acid functionalization, but significantly less than other studies performed under nonatmospheric conditions. Despite evidence of functionalization, neither O₃ nor OH radical oxidation resulted in a change in redox activity (potentially giving rise to oxidative stress) or in cytotoxic end points. Conversely, both the redox activity and cytotoxicity of SWCNTs significantly decreased when exposed to ambient urban air, likely due to the adsorption of organic carbon vapors. These results suggest that the effect of gas–particle partitioning of organics in the atmosphere on the toxicity of SWCNTs should be investigated further.

INTRODUCTION

Carbon nanotubes (CNTs) are expected to be widely used in multiple commercial applications due to their outstanding properties, such as low mass density, high mechanical strength, electron/hole mobility, and thermal conductivity.¹ Numerous consumer products currently exist that contain CNTs, including textiles, automobiles, electronics, X-ray tubes, and batteries.^{2,3} The global commercial production capacity of CNTs has increased from ~300 tons/year in 2006² to ~4500 tons/year in 2011^{1,3} and is expected to increase even further.

As part of the life cycle of CNT-containing products, CNTs are expected to enter into the environment (water, soil, and air) and ultimately the human body.^{4,5} This may be cause for concern, as CNTs are biologically active due to their large specific surface area⁴ and lipophilicity.⁶ In this regard, several publications have reviewed their toxicities.^{4,7–9} Poland et al.¹⁰

demonstrated that multiwall carbon nanotubes (MWCNTs) induced the formation of granulomas in mice 7 days after their administration, while Takanashi et al.¹¹ found that MWCNTs in mice did not demonstrate a significant carcinogenicity evaluated at 26 weeks postexposure. Although the carcinogenicity of CNTs is presently unclear, it is well-recognized that CNTs are redox active; are capable of causing oxidative stress; and result in inflammation, epitheloid granulomas, fibrosis, and biochemical changes in lungs.^{4,7} A negative effect associated with CNTs on the survival of invertebrates² and human

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epithelial cells^{9,12} has also been observed due to the formation of reactive oxygen species (ROS).^{2,9}

In addition, the toxicity of nanoparticles has been found to be dependent upon specific physiochemical factors.¹ For example, single wall carbon nanotubes (SWCNTs) modified by the addition of phenyl-(COOH)₂ functional groups has demonstrated a stronger cytotoxic effect than SWCNT-phenyl-SO₃H.¹³ Furthermore, COOH functionalized CNTs significantly induced autophagic cell death in human lung cells when compared to polyaminobenzenesulfonic acid (PABS) and polyethylene glycol (PEG) functionalized SWCNTs.¹⁴ These studies suggest that the transformation of CNTs in the environment, resulting in their functionalization, has the potential to alter their toxicity.

Currently, the majority of studies on the transformation of CNTs in the environment¹⁵ and their associated health impacts have focused on transformation processes in the water and/or soil environmental compartments.⁹ However, it is recognized that inhalation of manufactured nanoparticles (MNPs) including CNTs in ambient air will be an important pathway for population exposure in the future,¹⁶ as the MNPs are possibly emitted through the degradation of their end products or during their manufacture. They have also recently been found in diesel exhaust (MWCNTs)¹⁷ and other flame exhausts.¹⁸ Once emitted into the atmosphere, chemical modification of CNTs may occur through the oxidation by O₃, OH, and NO₃ radicals and by the reaction with, or condensation of, pre-existing ambient organic species. While perhaps qualitatively similar to functionalization processes during their manufacture,¹⁹ atmospheric oxidation/reactions are likely to result in differing and mixed proportions of CNT associated functional groups, formed in an uncontrolled manner, at rates which may be significantly different from that in aqueous or soil media, and whose effect on oxidative stress and/or other cytotoxic end points is highly uncertain. Finally, the eventual deposition of these transformed CNTs implies that some fraction of them found in water and soil will have originated from the atmospheric transformation process.

At the present time, it has been recognized that black carbon, transition metals, humic-like substances, and quinones in particulate matter (PM) are redox active and may be related to oxidative stress in humans.^{20,21} A number of studies have demonstrated that the redox activity of carbon materials including black carbon,²² flame soot,²³ and diesel soot^{24,25} increases as a result of ozone oxidation. However, the effect of atmospheric oxidation (by O₃ or OH radical) on the chemical evolution of CNTs leading to changes in redox activity and/or the ability to cause oxidative stress is unclear. The evolution of the cytotoxic properties of CNTs as a result of atmospherically relevant aging is also highly uncertain.

In the current study, the oxidation of SWCNTs by O₃ and OH radicals to simulate the aging process in the atmosphere (0.5–12 days) was investigated. The chemical functional group evolution during this oxidation was examined in an attempt to link the functionalization of SWCNT in the atmosphere to changes in toxicity. In addition, changes in cytotoxicity and redox activity of SWCNT as a result of urban ambient air exposures were also investigated. The results of this study will aid in the evaluation of the potential health risks associated with SWCNT that have been transformed by the atmospheric oxidation process.

■ EXPERIMENTAL SECTION

Chemicals. Two batches of SWCNTs (denoted as SWCNT-1 and SWCNT-2) with 1–2 nm o.d. and 0.5–2 μm length was supplied by Sun Innovation's Inc. The specific surface area and purity reported by the manufacturer is 405 m² g⁻¹ and >90%. Prior to the experiments, the SWCNT samples were heated overnight at 473 K and 70 kPa to remove adsorbed water and semivolatile organic compounds. As shown in Supporting Information Figure S1, the aerodynamic diameters measured with an Aerodynamic Particle Sizer (TSI, 3321) for both SWCNT-1 and SWCNT-2 are in the range 0.5–4.0 μm , with an aerodynamic mode diameter of 0.7 and 1.0 μm , respectively. Both O₃ oxidation and ambient exposure experiments were conducted using SWCNT-1 while SWCNT-2 was used for OH oxidation experiments. While there will be slight batch to batch variations in the physiochemical properties of SWCNT, the relatively large amount of sample required for these experiments (10–20 g) necessitated the use of multiple batches. Exposed SWCNT samples were stored at -4 °C to reduce the potential for chemical degradation.

SWCNT Aging Experiments. O₃ Oxidation. Atmospherically relevant ozone oxidation of SWCNT was performed in a dark flow tube reactor (to avoid the formation of OH radical), shown in Supporting Information Figure S2A. SWCNT powder was suspended into a flow tube via a Small Scale Powder Disperser (SSPD 3433, TSI), and exposed to O₃ (TG-10, Ozone Solutions). The temperature and relative humidity (RH) in the reactor was 295 $\pm$ 2 K and (37 $\pm$ 3)%. The total flow rate (ozone + particles) was 13 L min⁻¹ resulting in a residence time within the reactor of 6.6 min. The O₃ concentration was measured at the exit of the flow tube with an O₃ monitor (model 205, 2B Technologies). A qualitative measure of the organic and oxygenated content of fresh and aged SWCNT was obtained with an Aerodyne Compact Time-of-Flight Aerosol Mass Spectrometer (C-ToF-AMS). A description of the AMS and its principles of operation has been given elsewhere.²⁶ Although the AMS is intended to detect nonrefractory materials, a fraction of the refractory organic content of the SWCNT likely vaporizes at 600 °C. The fraction that is evolved can be used to investigate the oxidized nature of the SWCNT. Similar to the work of Lambe et al.,²⁷ the AMS data were analyzed using ToF-AMS analysis software (Pika 1.12) to estimate the oxygen to carbon (O/C) elemental ratio.²⁸

Oxidized particles were collected at the exit of the reactor (Supporting Information Figure S2A) on a Teflon filter, following a counterflow virtual impactor (CVI, BMI Inc.) to remove the gas-phase O₃ in the sample flow. The details of the CVI are described in the Supporting Information. The collected particles were used to perform a number of cytotoxicity assays, and redox activity analysis. The O₃ exposure was converted to an equivalent ambient exposure representing 1–7 days by assuming a 30 ppb global mean O₃ concentration in the troposphere.²⁹

OH Radical Oxidation. OH initialized oxidation of SWCNTs was carried out at (37 $\pm$ 3)% RH and 298 K in a photochemical flow reactor (7.3 cm i.d. $\times$ 25 cm L) (Supporting Information Figure S2B), described elsewhere³⁰ and in the Supporting Information. Briefly, SWCNT powder was suspended using a SSPD and OH radicals were produced by photolysis of O₃ and H₂O by a 254 nm UV light. OH concentration (0–1.1 $\times$ 10¹¹ molecules cm⁻³) was measured

with the decay of methanol using an Ionicon Analytik high resolution time-of-flight proton transfer reaction mass spectrometer (HR-ToF-PTR-MS).³¹ OH exposure was also converted to an equivalent ambient exposure representing 1–7 days assuming a global mean OH concentration³² of 1×10^6 molecules cm^{-3} . Oxidized SWCNTs were directly sampled with Teflon filter (without a CVI) as OH radicals will be lost to the exit walls and associated tubing immediately.

It should be noted that the suspended SWCNTs tend to form agglomerates due to their geometry and van der Waals forces, as supported by the aerodynamic size distribution (Supporting Information Figure S1) and previous measurements of CNTs collected directly from engine exhaust¹⁷ and other flame exhausts.¹⁸ While a kinetic limitation to the oxidation of internal SWCNTs in the agglomerates of the current experiments may occur, it is also likely to occur in the atmosphere.

Ambient Air Aging. SWCNTs were exposed to ambient air by evenly dispersing ~ 15 mg SWCNT on a Teflon filter and passing urban ambient air through the filter at a rate of 2.0 L min^{-1} . Particles in the ambient air were removed upstream using a Teflon filter. The sampling location was ~ 100 m from a highly traveled road and exposure experiments were performed in the first 2 weeks of December 2012.

Toxicity Testing. DTT Assay. The dithiothreitol (DTT) assay is an indirect chemical assay used for measuring the redox cycling capacity of PM. The rate at which DTT is oxidized in the presence of an analyte is correlated with the ability of that analyte to cause oxidative stress.^{21,33} A detailed description of this assay has been given elsewhere,²² and in the Supporting Information. Briefly, the SWCNT samples were suspended in phosphate buffer (0.1 M, pH 7.4), sonicated for 15 min, and diluted. The diluted extract was used in the DTT assay. The loss of DTT via a redox reaction was monitored as the decrease of absorption at 412 nm. The response to the DTT assay was also measured for the water-soluble components of SWCNT by filtering aliquots of the samples and measuring the activity of the solution without particles.

In Vitro Assays. All SWCNTs were dispersed with 0.025% Tween-80 in 0.19% NaCl solution using a Dounce glass homogenizer, followed by sonication. A visibly homogeneous and stable suspension of SWCNTs was obtained after the sonication process. Cytotoxicity assessment of the SWCNTs was carried out using the human A549 adenocarcinoma-derived alveolar epithelial cells and THP-1 leukemia-derived peripheral blood monocytes. Four different assays targeting distinct mechanisms of cellular metabolic perturbations were assessed simultaneously, including resazurin reduction (using Alamar Blue or CTB assays), ATP energy metabolism, LDH membrane integrity, and BrdU incorporation into DNA. Briefly, 2×10^5 A549 cells/mL and 1.2×10^6 THP-1 cells/mL were exposed to SWCNTs in 96-well plates for 24 h for all assays except the BrdU assay in which A549 only cells were seeded at 1×10^5 cells/mL. SWCNTs were dosed at 0, 10, 30, and $100 \mu\text{g}/\text{cm}^2$ in a final volume of 200 mL/well. Detailed descriptions of these assays are given elsewhere.^{8,9}

Functional Group Characterization of SWCNT. Functional groups were characterized before and after oxidant/ambient air exposures by Fourier transform infrared (FTIR) spectroscopy. Particle samples were manually removed from filters after collection, mixed with KBr (transparent in IR), and pressed to make pellets which were analyzed by the FTIR. The IR spectra for O_3 and OH oxidized SWCNTs were recorded with a

mercury cadmium telluride (MCT) detector at a resolution of 4 cm^{-1} for 200 scans in transmission mode, using an iS50 spectrometer (Nicolet), while it was performed using a Bruker Tensor 27 with the same parameters for ambient air aged SWCNTs. Peak fitting was performed after baseline correction with Peakfit 4.12 software.

RESULTS AND DISCUSSION

Chemical Evolution During Oxidation: Particle Mass Spectra. The particle mass spectra normalized to the total organic carbon (OC; measured by the AMS) of fresh and oxidized SWCNT-1 by O_3 are shown in Figure 1A and the

Figure 1. Particle mass spectra of unreacted and oxidized (A) SWCNT-1 and (B) SWCNT-2.

corresponding mass spectra of SWCNT-2 oxidized by OH in Figure 1B. As a refractory material, SWCNT is thermally stable up to 1073 K in a vacuum (10^{-7} Torr).³⁴ However, structural defects, which contain sp^2 - or sp^3 -hybridized carbons with H atoms during manufacturing, are always present in CNTs,^{35,36} and hence, the mass spectra shown here likely represent the evaporable fraction due to break down of the SWCNT at these defects at ~ 873 K.

As shown in Figure 1, both fresh (nonoxidized) SWCNT-1 and SWCNT-2 result in strong signals in m/z channels 43, 55, 73, 207, and 281 (blue lines). The peaks at m/z 207 ($\text{C}_{16}\text{H}_{15}^+$) and 281 ($\text{C}_{22}\text{H}_{17}^+$) are indicative of polycyclic aromatic hydrocarbon (PAHs) derivatives present in the evaporable components of SWCNT. Nonaromatic hydrocarbons fragments are also present in the SWCNT as supported by the signals at m/z 41 (C_3H_5^+), 43 (C_3H_7^+ , CH_3CO^+ or CH_2CHO^+), 55 (C_4H_7^+ , $\text{C}_3\text{H}_3\text{O}^+$), 57 (C_4H_9^+), and 73 ($\text{C}_3\text{H}_5\text{O}_2^+$).^{37–39} The trace oxygen may originate from postprocessing by the manufacturer. Despite having the same product ID, the relative abundances of these fragments are different in these two batches of SWCNTs (Figure 1). For example, the most prominent fragment is m/z 73 for SWCNT-1, while it is m/z 43 for SWCNT-2.

The exposure of SWCNT-1 to 3.6×10^{17} molecules cm^{-3} s of O_3 (approximately equivalent to 5.6 days of ambient exposure) resulted in a large increase in the signal intensity of m/z 44 and a relative decrease in the m/z fragments described above (Figure 1A). A similar phenomenon was observed for SWCNT-2 (Figure 1B), with the largest increase associated with m/z 44 when exposed to OH at an exposure of 6.1×10^{11} molecules cm^{-3} s, (approximately 5.7 days of ambient exposure). The fragment at m/z 44 (CO_2^+) is usually used as an indicator of a high oxidation state likely from carboxylic

Figure 2. Evolution of the m/z channels at 44, 43, 207, and the O/C as a function of (A) O_3 exposure and (B) OH exposure; evolution of mass normalized infrared absorbance of SWCNT as a function of (C) O_3 exposure and (D) OH exposure. SWCNT-1 was used for O_3 oxidation, and SWCNT-2 was for OH oxidation. The equivalent ambient exposures are calculated assuming a global mean concentrations of 30 ppb O_3 and 1×10^6 molecules cm^{-3} OH. The lines are guides for the eye only.

acids in organic particles, while m/z 43 is typical of organic aerosol (OA) with a lower oxidation state.⁴⁰ The change in normalized signal intensities for fragments at m/z 43, 44, and 207 (open squares, cycles, and triangles, respectively) as a function of oxidant exposure (O_3 vs OH radical) is shown in Figure 2A,B. The signals of m/z 207 and m/z 43 decreased, accompanied by an increase of m/z 44 when exposed to 3.3×10^{16} molecules cm^{-3} s (0.5 day) of O_3 . However, further decreases in these fragments were not observed with further oxidation, while m/z 44 increased gradually as a function of O_3 exposure (Figure 2A). This is in contrast to Figure 2B, where m/z 207 decreased (and m/z 44 increased) gradually across the entire range of OH exposures, with no change in m/z 43. This might be explained by differences in the oxidation mechanisms for OH and O_3 . On the other hand, both $C_3H_7^+$ and CH_3CO^+ or CH_2CHO^+ may contribute to the signal at m/z 43. If the production rate of CH_3CO^+ or CH_2CHO^+ is comparable to the consumption rate of $C_3H_7^+$, the signal at m/z 43 may not change. The estimated evolution of the O/C ratio (blue solid squares) for ozone and OH exposures is shown in Figure 2A,B. The O/C ratio of fresh SWCNT-1 is 0.44 ± 0.02 , which is significantly higher than that of fresh SWCNT-2 (0.08 ± 0.02). The lower O/C ratio is consistent with the higher fraction of m/z 43 in SWCNT-2. The exposure of SWCNT-1 to O_3 (0.5–5.6 days) resulted in an increase in the O/C ratio to 0.51 ± 0.01 , while OH exposure of SWCNT-2 increased the O/C to 0.21 ± 0.01 (1–5.7 days). Regardless, the results further suggest that SWCNTs are relatively stable under simulated atmospheric oxidative conditions, as the O/C increased by a factor of 2 at most, after an equivalent of ~ 6 days of exposure.

Functional Groups Analysis. While AMS measurements (above) are a qualitative measure of the oxygenation of SWCNTs, the exact functional group contribution was obtained through FTIR spectroscopy as a function of oxidant exposure. The baseline-corrected IR spectra of unreacted SWCNTs and oxidized SWCNTs in the range 700–1800 cm^{-1} is shown in Supporting Information Figure S4 with the peak assignments given in Supporting Information Table S1.

The absolute absorbance of SWCNT-2 was significantly weaker than that of SWCNT-1 (for the same sample mass) suggesting that the total functional group content of SWCNT-2 was less than that of SWCNT-1, which is consistent with the lower O/C of SWCNT-2 as discussed above.

As shown in Supporting Information Figure S4, carbonyl groups associated with carboxylic acids or esters (1720 cm^{-1});^{41–44} esters (1635 , 1565 , and 1540 cm^{-1})^{42,45,46} which are accompanied by C–O–C stretch vibrations at 1380 , 1200 , 1080 , and 1040 cm^{-1} ; C–O stretch vibrations of alcohols or phenols (1080 cm^{-1});⁴⁷ and ethers (1140 cm^{-1})⁴⁸ are all present in the unreacted SWCNTs (SWCNT-1 and SWCNT-2). These functional groups may be formed as a result of contamination or postprocessing by the manufacturer.⁴⁹

Upon exposure to O_3 and OH, the intensity of carboxylic acids or esters (1720 cm^{-1}) increased significantly (Supporting Information Figure S4). In addition, a new peak at 925 cm^{-1} , which is assigned to an epoxide,⁵⁰ was observable when SWCNT-1 was exposed to O_3 . The evolution of the normalized absorbance (normalized to sample mass) as a function of oxidant exposure is shown in Figure 2C,D. For many of the observed bands, a clear evolution as a function of oxidant exposure is not evident. However, the intensity of the band at 1720 cm^{-1} (carboxylic acid and/or esters) increases with O_3 or OH exposure (Figure 2C,D). This is consistent with the increase of m/z 44 and O/C as a function of oxidant exposure as measured by the ToF-AMS. It also suggests that carboxylic acids are likely the most prevalent products for both O_3 and OH oxidation of SWCNTs which is in agreement with X-ray photoelectron spectroscopy (XPS) studies.¹⁹ It should also be pointed out that Mawhinney et al.⁴³ observed increases of both esters (1040 , 1200 , and 1739 cm^{-1}) and quinones (1650 cm^{-1}) when SWCNTs were exposed to extremely high O_3 levels (0 to 1.29×10^5 ppm min; 0–3000 days ambient exposure). Simmons et al.⁵¹ also observed carbonyls and ethers formed by O_3 oxidation using XPS. Although the broad band at 1635 cm^{-1} in the current work is close to that of quinones (1650 cm^{-1}), it is more likely attributed to the scissoring mode of

water (1630 cm^{-1})⁵² since its intensity does not increase with oxidant exposure. Consequently, quinones are likely not an important product under realistic atmospheric conditions. The evolution of the particle mass spectra and functional groups determined by FTIR clearly demonstrates that oxidation by both O_3 and OH results in chemical modification of SWCNTs and increases in oxygen-containing functional groups in both cases. The results further highlight the fact that the functionalization of SWCNT in the atmosphere will likely be less significant than that reported for atmospherically non-relevant conditions.^{19,43} The effect of this SWCNT functionalization on various toxicity end points is described below.

Redox Activity Evolution During Oxidation. Oxidative stress refers to an imbalance of oxidants and antioxidants in biological systems, and is an important toxicological mechanism resulting from particulate matter (PM) inhalation.⁵³ The DTT assay indirectly measures the redox activity of PM,³³ with a higher DTT decay rate ($\text{pmol } \mu\text{g}^{-1} \text{ min}^{-1}$) indicating a stronger oxidation potential of PM. The DTT decay rates resulting from the exposure of SWCNTs to O_3 and OH are shown in Figure 3A,B. The solid squares indicate the DTT decay rates of a SWCNT suspension, and the open circles represent the decay rates for the particle filtered solution.

Figure 3. Evolution of DTT decay rates of SWCNT exposed to (A) O_3 , (B) OH at varying equivalent exposure times. RH, $37 \pm 2\%$; T , 295 K . 30 ppbv of O_3 and $1.0 \times 10^6\text{ molecules cm}^{-3}$ of OH concentration are assumed in ambient air; and (C) particle-free ambient air. SWCNT-1 was used for O_3 oxidation, and SWCNT-2 was for OH oxidation.

As shown in Figure 3, SWCNT suspensions were strongly redox active, while the filtered solution (containing soluble species) was inactive, suggesting that the redox activity originates from the particle surface but not from water-soluble substances. This is similar to diesel particles where 89–98% of the redox activity occurs at the black carbon surface,²⁵ and in contrast with secondary organic aerosol (SOA) where 90% of the redox activity occurs in the water-soluble fraction.²⁰

The DTT decay rates did not change as a result of exposure to O_3 or OH radicals within the experimental uncertainty (Figure 3A,B), despite O_3 and OH exposures on the order of approximately 2 weeks (~ 11.4 and 12.3 days). The DTT activity for ambient air exposed SWCNT (Figure 3C) will be discussed in the Cytotoxicity Evolution During Oxidation section. It should be noted that ~ 0.3 – 3.5 ppm of O_3 (used to generate OH) was present during OH oxidation experiments and not removed by the CVI during sampling. Therefore, 12.3 days of OH exposure was accompanied by ~ 6 days O_3

exposure. These results suggest that oxidation by both O_3 and OH has a negligible influence on the redox activity of SWCNTs in the atmosphere. Incidental UV irradiation (254 nm) of the SWCNTs during OH oxidation also resulted in a negligible change in the redox activity although it has been reported that CNTs may undergo photolysis forming ether, epoxy, and carbonate functionalities.⁵⁴

The current SWCNT DTT assay results are similar to the redox activity response of O_3 oxidized graphite,²² but are in contrast to that of black carbon,²² flame soot,²³ and diesel soot^{24,25} during O_3 aging. The differences may be attributed to differences in structure and organic carbon content among SWCNT, black carbon, flame and diesel soot, and differing oxidation conditions between experiments. First, SWCNTs consist of sp^2 -hybridized carbon atoms forming a hexagonal network that is itself arranged helically within a tubular motif.⁵⁵ The relative content of disordered carbon to graphene carbon (DC/GC) in soot and black carbon is much higher than that of CNTs, and it has been demonstrated that disordered carbon is significantly more reactive than graphene carbon toward O_3 ,^{56,57} leading to redox active species. Second, quinones, humic-like substances, transition metals, and black carbon have been identified as the redox active species in ambient PM.^{20,21,25,58} In particular, quinones are the most effective redox active component.⁶⁴ Flame soot or diesel soot both contain significant organic carbon (OC) including saturated and unsaturated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and partially oxidized organics.^{47,59–61} This OC component is readily oxidized,^{56,59} from which quinones have been identified during the oxidation of both soot⁵⁷ and PAHs by O_3 .^{20,62,63}

In contrast to flame or diesel soot, OC was removed from the SWCNTs prior to oxidation in this study (Chemical Evolution During Oxidation: Particle Mass Spectra section), and the most significant products for both atmospheric O_3 and OH oxidation of SWCNT were carboxylic acids or esters, both of which are not redox active. Furthermore, quinone functional groups, which are redox active, either did not exist, or did not increase during SWCNT oxidation by either O_3 or OH. Finally, the O_3 exposure time in this study is comparable to that for flame soot oxidation (~ 9 days),²³ but is significantly shorter than that of black carbon oxidation experiments (~ 9 – 270 days).²² In the work of Li et al.,²² the DDT decay rate of Printex U black carbon increased from 31 to $58\text{ pmol min}^{-1} \mu\text{g}^{-1}$ after exposure to O_3 of $9.2 \times 10^{14}\text{ molecules cm}^{-3} \text{ h}$ (equivalent to ~ 270 days in the atmosphere). However, the increase was much slower after an initial large increase, changing from 31 to $\sim 37\text{ pmol min}^{-1} \mu\text{g}^{-1}$ after ~ 9 days of exposure.²² This increase is within our experimental uncertainties.

As discussed above, both O_3 and OH oxidation produced surface carboxylic acids or esters, which lead to enhancement of the O/C ratio. Although the O/C ratio of fresh SWCNT-1 is significantly higher than that of fresh SWCNT-2 (F , 138.5 ; P , 3.50×10^{-7} for ANOVA statistical analysis) (Supporting Information Figure S5), the mean DTT decay rate of SWCNT-1 ($59.3 \pm 7.4\text{ pmol min}^{-1}$) is only slightly larger than that of SWCNT-2 ($48.1 \pm 7.3\text{ pmol min}^{-1}$). The $\Delta\text{O/C}$ for either SWCNT-1 (0.07) or SWCNT-2 (0.13) during oxidation is much lower than the difference in O/C between the fresh SWCNT-1 and SWCNT-2 (0.36). This suggests that changes in the DTT activity of SWCNTs during oxidation are likely less than that between fresh SWCNT-1 and SWCNT-2 and within the measurement uncertainty.

Figure 4. Cytotoxicity evolution of SWCNT-1 and relative changes in select functional groups on SWCNT-1 during ambient air exposure: (A and B) resazurin reduction assay; (C and D) ATP assay; (E and F) LDH assay for THP-1 human macrophage cells and A549 human lung epithelial cells, respectively; (G) BrdU incorporation assay for A549 human lung epithelial cells; (H) observed relative change of the area percentages for select IR bands (relative to the entire spectrum) as a function of ambient particle free air exposure. The red, green, blue, and black boxes indicate the 0, 10, 30, and 100 $\mu\text{g cm}^{-2}$ doses, respectively. The dashed lines are guides for the eye. Data for three independent experiments ($n = 3$), with duplicate wells within each experiment presented as fold-effect, relative to zero dose controls.

When all of the data in Figure 3A,B are taken into consideration, the mean DTT decay rate is $51.9 \pm 8.9 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ for the oxidized SWCNT, which is the same as that of the fresh SWCNT ($51.9 \pm 5.1 \text{ pmol min}^{-1} \mu\text{g}^{-1}$). This value is higher than those of black carbon,²² diesel exhaust particles (DEP),²⁵ and ambient PM,^{21,64} while much lower than that of naphthalene SOA ($118 \pm 5.1 \text{ pmol min}^{-1} \mu\text{g}^{-1}$).²⁰ For black carbon²² and ambient PM,^{21,64} the DTT assays were performed at 310 K, whereas it was performed at 298 K for SWCNT (this study), DEP,²⁵ and naphthalene SOA.²⁰ Consequently, the redox activities among the SWCNT, DEP, and naphthalene SOA can be compared directly. These results demonstrate that although fresh SWCNTs are highly redox active, the modification of surface structure of these SWCNTs under the relatively mild atmospheric oxidation conditions of this study will not result in significant increases in their redox activity.

Cytotoxicity Evolution During Oxidation. The influence of atmospherically relevant aging on the *in vitro* toxicity of SWCNTs was further assessed with several *in vitro* assays. The toxicities of SWCNTs in this study were compared using assays that assessed changes in metabolic activity (resazurin reduction assay and ATP), cell membrane permeability (LDH release), and DNA synthesis (BrdU). The evolutions of the cytotoxicities for O_3 and OH oxidized SWCNTs, respectively, are shown graphically in Supporting Information Figures S6 and S7. The results of a corresponding two-way ANOVA analysis of these data (with dose and exposure time as factors) are presented in Supporting Information Table S2, with a comparison of means performed using the Dunn–Sidak method. In these figures, EHC6082 is reference Ottawa urban dust and was used as a control. Dimethyl sulfoxide (DMSO), culture media, and PM buffer were assessed in blank experiments (not shown).

Over the dose range assessed, unreacted SWCNTs exhibited greater cytotoxic potency than the urban particles EHC6082, as

measured by decreased metabolic activity (resazurin reduction), reduced ATP content, and increased release of LDH in both A549 and THP-1 cells (Supporting Information Figure S6 and S7). The ANOVA analysis results for a given oxidant exposure level also indicate that there is a statistically significant difference in the mean toxicities as a function of dose. However, while broadly toxic relative to control samples, and as a function of dose, the differences in the mean toxicities as a function of O_3 exposure of SWCNT-1 are not statistically significant for both the A549 and THP-1 cell lines (Supporting Information Figure S6), regardless of the dosage (see Supporting Information Table S2). The same is also generally true for OH oxidized SWCNT-2 (Supporting Information Figure S7 and Table S2), although there is some indication of the potential for decreased toxicity at the highest exposure (THP-1, 10.9 days, Supporting Information Table S2). Regardless, there is not a statistically significant trend over the exposure time studied, and no increase in toxicity.

At the present time, there is a general consensus that CNTs are capable of producing inflammation, granulomas, fibrosis, and biochemical/toxicological changes in lungs regardless of the process by which they are synthesized.^{4,7} However, no studies have focused on the influence of atmospherically relevant aging on the toxicity of CNTs. Bottini et al.⁶⁵ found that MWCNTs oxidized by concentrated HNO_3 exhibited a stronger cytotoxic effect to human T lymphocytes than fresh MWCNTs. Kumarathasan et al.^{8,9} also observed an increase in cytotoxicity (A549 and J774 cells) for CNTs oxidized by a 3:1 (v/v) mixture of $\text{H}_2\text{SO}_4/\text{HNO}_3$. In those studies, the increase in toxicity was attributed to surface modification (carboxylic acids) which resulted in a transformation from hydrophobic to hydrophilic CNTs.^{9,65} Although carboxylic acids were formed in both O_3 and OH oxidation experiments, we did not observe an increase in cytotoxicity for these oxidized SWCNTs. However, it should be noted that the oxidation level (atomic

ratio of O/(O + C)) of MWCNTs resulting from O₃ oxidation in those studies was 4.2–5.1%, and 9.5–10.2% when using concentrated HNO₃ or H₂SO₄/HNO₃.¹⁹ In the current work, the oxidation by O₃ and OH was significantly milder in order to simulate relevant tropospheric conditions. For example, to achieve a CNT oxidation level of ~10 at. %, ¹⁹ an O₃ exposure ~420 times that of the current study was required, which is also comparable among the work of Bottini et al.,⁶⁵ Kumarathasan et al.,^{9,66} and Wepasnick et al.¹⁹ This suggests that the absolute carboxylic acid content formed by O₃ or OH oxidation in the current study was significantly lower than other studies, consequently having a negligible influence on the hydrophilicity and cytotoxicity of the SWCNTs.

Redox Activity and Cytotoxic Evolution During Ambient Exposure. While the oxidation of SWCNTs by O₃ and OH in this study had a negligible effect on both redox activity and cytotoxic end points, statistically significant effects were observed when SWCNTs were exposed to ambient air. As shown in Figure 3C, exposure to particle-free ambient air from 0 to 7 days resulted in a linear decrease in the DTT decay rate. The results of the cytotoxic assays for SWCNT-1 exposed to the same particle-free ambient air are shown in Figure 4A–G. In general, the cytotoxicity of SWCNT-1 was highest prior to ambient exposure, with potency decreasing with exposure time. This effect was observed for both A549 and THP-1 cell lines investigated, particularly at the 100 $\mu\text{g cm}^{-2}$ dosage, between 0 and 7 days of simulated exposure. The results of a two-way ANOVA analysis (Supporting Information Table S2) are also consistent with a decrease in potency as a function of ambient exposure, as comparison of means between 0 and 7 days of exposure indicates that they are statistically different.

Since the SWCNT samples used in this study have a very large specific surface area (405 m² g⁻¹), semivolatile organic compounds (SVOCs) in ambient air are likely adsorbed onto the surface. A high adsorption capacity of PAHs by CNTs has been observed in previous studies.⁶⁷ As a result, it is hypothesized that the observed decrease in the DTT decay rate may be related to adsorption of SVOCs with lower redox activity, whereby the total mass normalized redox activity is effectively reduced, or redox active sites are effectively blocked. This is supported by an observed increase in the substituted aromatic hydrocarbon band (790 cm⁻¹) and a decrease of the ester (1200 cm⁻¹) and carboxylic acid or ester (1540 cm⁻¹), after exposure to ambient air as shown in Figure 4H. The decrease in the bands at 1200 and 1540 cm⁻¹ may be due to the surface coverage of the associated molecules or interaction with other adsorbents (i.e., aromatic hydrocarbons above). These results further suggest that the adsorption of nonpolar organics has possibly decreased the hydrophilic nature of these SWCNTs under the current experimental conditions, rendering them less available to the cells during the in vitro assays. The fact that two independent measures of both direct (in vitro) and indirect (DTT assay) toxicity resulted in a similar decreasing trend supports the validity of these results. Furthermore, it implies that additional organics are not solubilized into the aqueous buffer used for the DTT assay, as the activity remains associated with the particle and not the soluble extract.

It should also be noted that particle size may have an influence on the toxicity of SWCNTs. In both DTT and in vitro assays, SWCNTs were dispersed in water. Agglomeration of SWCNTs in a water medium may lead to larger particle bundles, subsequently affecting the absolute toxicity, compared

to primary SWCNT particles. However, a possible size effect should be constant for both DTT and in vitro assays under the different aging conditions, as the same sample pretreatment method was used in both cases. Hence, the relative toxicological evolution of SWCNTs observed in this study as a function of the atmospheric aging conditions remains meaningful.

Implications. Atmospheric oxidation is a complex process for organic aerosols, leading to increases in oxygen-containing species,^{42,43} O/C ratio,⁶⁸ and structure modifications.⁶⁹ Previous redox^{22–25} and cytotoxicity assay results^{9,23,65} for oxidized elemental carbon-based materials demonstrated increases in redox activity and/or cytotoxicity with oxidation. However, the present results indicate that for realistic tropospheric lifetimes, the toxic properties of SWCNT remain unchanged by O₃ or OH oxidation, despite observed changes in chemical composition and oxygen content. Furthermore, the adsorption of a multitude of organic species from the atmosphere to SWCNTs may have the effect of reducing their ultimate toxicity as observed in this study. These results will improve the risk assessment of CNTs and the modeling of their atmospheric fate, and suggests that significant further study is warranted with respect to the modification of CNTs via organic adsorption/reaction. It should be pointed out that acute toxicity tests were performed in this study. Both O₃ and OH oxidation have increased –COOH functionality, yet as found in this study, the cytotoxicity and redox activity are seemingly not related to the functionalization of the SWCNT under mild atmospheric oxidation conditions. However, the chronic long-term exposure effect on human health with increased functionalization is unknown. As also noted in the Chemicals section, batch-to-batch variability in physiochemical properties is possible for SWCNTs, implying that the current results may not be generalized to all CNTs. Furthermore, in ambient air the concentrations of trace gases have high temporal and spatial variations which make a generalization of the effect of current ambient exposures difficult. Future work is required to identify the chemical species which impact the observed toxicity changes during ambient air aging.

■ ASSOCIATED CONTENT

● Supporting Information

Detailed descriptions of the counter-flow virtual impactor use, photochemical flowtube and techniques, the effect of particle phase diffusion, FTIR spectral assignments, and DTT and in vitro assay results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 1-416-739-4840; fax: 1-416-739-4281; e-mail: John.Liggio@ec.gc.ca.

Notes

The authors declare no competing financial interest.

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