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Atmospheric Chemistry and Physics
Discussions

Interactive comment on "Influence of functional groups on toxicity of carbon nanomaterials: implication for toxicological evolution during atmospheric relevant aging of soot" *by* Yongchun Liu et al.

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Referee #1 General Comments: This work deals with the very complex nature of how small particles affect human health. There is substantial evidence that shows that small particles do have adverse health effects, but it is still not clear what causes issues. The authors recognize this, and do a good job of presenting the problem and previous research (with one notable exception, which will be discussed below). They focus on different carbon-based nanoparticles, including engineered ones. Their results are generally in agreement with previous work, but their major new contribution is

the identification of epoxide groups on graphene oxide surfaces having a significantly larger effect on DTT decay rates.

Response: Thank you for your positive comments.

I have no major issues with how the experiments were performed (including the analytical methods used), and why the different nanomaterials were used. However, I have some issues with the interpretation and implications stated. The title states "implication for toxicological evolution during atmospheric relevant aging of soot" but most of the results are for engineered nanomaterials, not atmospheric soot, which is a very different type of particle. While it is important to learn about the toxicity of engineered nanomaterials, those results should not be applied to atmospheric soot particles (which have very few engineered nanoparticles). The results themselves are interesting and important enough without the atmospheric extrapolation, and I suggest revising the title and the Conclusions section.

Response: Thank you for your instructive suggestions. We revised the title as "Influence of functional groups on toxicity of carbon nanomaterials".

In the Conclusion section, we removed the sentences related to soot aging. For example, the following sentences have been deleted in the revised manuscript. "It is also a primary process in the atmosphere relating to chemical aging of particles including soot and CB particles", "This means that oxidation potential enhancement of CB particles is also possibly resulted from the formation of epoxide during chemical aging in the atmosphere", "On the other hand, it has been found that aging rate of BC particles under highly polluted urban environment is faster than that under clean conditions (Peng et al., 2016). In the future, much work should be performed on the toxicity evolution of CB or BC particles under real atmospheric conditions".

In the abstract section, the final sentence has been revised as "These results imply that epoxidation might enhance the oxidation potential of carbon nanomaterials". In the introduction section, the sentence "In the current study, both the cell-free toxicity and the cell cytotoxicity of carbon nanomaterials with different functionalities were evaluated to focus on the role of functionalization in their toxicities to understand the possible influence of different source or oxidation processes on the toxicity evolution of soot particles" has been revised as "In the current study, both the cell-free toxicity and the cell cytotoxicity of carbon nanomaterials with different functionalities were evaluated to focus on the role of functionalization in their toxicities".

A new sentence has been added into the conclusion to emphasis the toxicity of epoxide-containing carbon materials as "This means that exposure to epoxide-containing carbon materials should lead to high health risk regarding to oxidation potential" (lines 544-545 in the revised manuscript).

In the discussion section, reasons for the observed effects are given with very little evidence (though there are some references stated). For example, line 248 states: This means the cell membrane might be intact when exposed to SB4A. Another is line 293: For example, adhesions and/or covering on cells could be the main MOA for graphene/graphene oxide (2-D structure), while for carbon nanotubes (1-D structure), piercing and/or internalization by cells could be the main MOA. I suggest moving these types of sentences to the Discussion section and providing more references or information about these assumptions.

Response: Thank you for your suggestion. In the original manuscript, we did not separate the discussion from the results section. In the revised manuscript, we devided them into two parts. These sentences you mentioned have been moved to discussion section as "As shown in Fig. 2, all the carbon nanomaterials showed decreased ATP activities as a function of the dose. This means the carbon nanomaterials investigated in this work are toxic to murine J774 cell line. This is consistent with the previous results that CNT and Printex U are toxic to J774 cells (Kumarathasan et al., 2012) and graphene oxide can induce dose-dependent cell death in normal lung fibroblasts (HLF), macrophages (THP-1 and J744A), epithelial (BEAS-2B) cells, lung cancer cells (A549) etc. (Zhang et al., 2016;Li et al., 2018). At the same time, the BrdU activities

decreased as a function of the dose of carbon nanomaterials, which means they are inhibitor for cell proliferation of murine J744 (Cappella et al., 2015). In addition, except for SB4A, other carbon nanomaterials showed significant increases in LDH. This means that the integrity of cell membrane decreased when J774 cells were exposed to these engineered carbon nanomaterials, while the cell membrane might be intact when exposed to SB4A (Cho et al., 2008;Kumarathasan et al., 2015). This might be related to lipid peroxidation induced by these engineered particles (Li et al., 2018) and the non-sphere feature of these engineered particles as observed in Fig.S1. These results also consistent with the previous study that observed CNT cytotoxicity ranking was assay-dependent (Kumarathasan et al., 2015)". The corresponding references have been added in the revised manuscript as you suggested.

Specific Comments: As the authors correctly state, the particles studied have very different chemistry and morphology, making it almost impossible to discern the mechanisms or chemicals responsible for the observed results. While most of the particles have similar DTT decay rates, this could be a coincidence or the result of a similar mode of action. I do not think the authors have clearly identified which it is. The authors should reference the many works from the Prof. Barry Dellinger group at LSU (he is deceased, but the work continues). They have identified a new type of radical, called Environmentally Persistent Free Radicals, that produce cell damage in a catalytic cycle involving metals, nanoparticles, and quinones (they have many papers in ES&T). The catalytic cycle upsets the notion that it is simply the concentration of an active species that is important.

Response: Thank you for your instructive suggestion. We agree with you that the observed DTT activity could be a coincidence of the chemical composition, functional groups and morphology of these particles. This make it is difficult to clearly identify the crucial factor determining the toxicity. This is the reason why we mainly compared the toxicity among these particles with the similar morphology, in particular, between graphene and graphene oxide. On the other hand, we think the role of epoxide in the highest DTT activity of graphene oxide can be well supported by the different DTT activity between thermal treated graphene oxide and the pristine graphene oxide. In the revised manuscript, we emphasized this point as “Although the observed toxicity including DTT activity and cytotoxicity could be a coincidence of the chemical composition, functional groups and morphology of these particles, the above results at least imply that these physiochemical properties such as morphology, metal and OC content should not be crucial factors as for the toxicity of these carbon nanomaterials because it is difficult to observe an obvious dependence of the toxicity on these factors” (lines 483-488 in the revised manuscript).

As for the Environmentally Persistent Free Radicals, we added a paragraph in the revised manuscript (lines 514-520) as “Recently, environmentally persistent free radicals (EPFRs) (a kind of surface stabilized metal-radical complexes characterized by an oxygen-centered radical) (Dugas et al., 2016) have been identified in different source of particles including biomass/coal combustion, diesel and gasoline exhaust, ambient PM2.5 and polymer (Balakrishna et al., 2009;Truong et al., 2010;Dugas et al., 2016). However, it is unclear that whether epoxide in graphene oxide observed in this study contributes to the EPFRs formation. This is needed to be investigated in the future”.

Technical Corrections: The paper, in general, is well written. However, there are several awkward sentences, missing or unnecessary words that should be corrected (as a native English speaker, I cannot imagine writing a paper in a different language). These are all minor and did not affect my review.

Some examples are: Line 21 were investigated for understanding - change to "were investigated to understand"

Line 63 Change NO3 to NOx

Line 94 Functionalized does not to be capitalized

Line 348 Do you mean bonded?

Line 388 awkward sentence

Line 447 add "the"

Line 487 don’t need this sentence

Line 505 explain the difference between BC and CB. They are not the same type of particle.

Response: Thank you so much for your comments. We carefully corrected these errors.

Line 21 (line 19 in the revised manuscript): “were investigated for understanding” has been changed to "were investigated to understand”.

Line 63 (line 81 in the revised manuscript): “NO3” has been changed to "NOx”.

Line 94 (line 112 in the revised manuscript): “fMWCNTs” has been changed to "FMWCNTs”.

Line 348 (line 305 in the revised manuscript): This sentence has been revised as “This can be ascribed to desorption of surface adsorbents including bonded organics and trace water”.

Line 388 (line 333-338 in the revised manuscript): This sentence has been revised as “Several oxygen-containing species were observed as shown in Fig. 4A-F. Adsorbed oxygen was observed at 535.2 eV in the O1s spectra. Carbon-oxygen single bond in hydroxyl group (C-OH) and epoxide (C-O-C) were at 533.5 and 532.6 eV, respectively. Carbon-oxygen double bound (C=O) was observed at 531.8 eV, while highly conjugated form of carbonyl oxygen such as quinone groups was identified at 530.5 eV (Schuster et al., 2011)”.

Line 447 (line 494 in the revised manuscript): "the" has been added before C-O-C (epoxide).

Line 487: The sentence "The DTT decay rates of special black 4A (SB4A), graphene, graphene oxide, single wall carbon nanotubes (SWCNT), SWCNT-OH and SWCNT-COOH were 45.9ïĆś3.0, 58.5ïĆś6.6, 160.7ïĆś21.7, 38.9ïĆś8.9, 57.0ïĆś7.2 and 36.7ïĆś0.2 pmol min-1ïĄ■g-1, respectively. Epoxide has been for the first time identified as a highly active functional group in the carbon nanomaterials as far as the oxidation potential is considered." has been deleted in the revised manuscript.

Line 505: The definition of CB and BC was added in the revised manuscript (lines 36-56) as "Carbon nanomaterials are predominantly composed of carbon atoms, only one kind of element, but they have largely diverse structures characterized by different degrees of crystallinity and different macro- and micromorphology (Somiya, 2013). Their basic structure is that of graphite with planes of honeycomb-arranged carbon atoms. Carbon black (CB), which is produced from incomplete combustion of heavy petroleum materials under controlled conditions (Apicella et al., 2003), has been widely used in industrial products, such as inkjet printer ink, rubber and plastic products (Lee et al., 2016), electrically conductive plastics (Parant et al., 2017), paints, coatings and cosmetics (Sanders and Peeten, 2011) and so on. CB is a quasi-graphitic form of nearly pure element carbon (EC, consist of graphene layers). It is distinguished by its very low quantities of extractable organic compounds and total inorganics (Long et al., 2013) compared with soot or black carbon (BC) (Andreae and Gelencser, 2006). Soot or BC, which originates from incomplete combustion of biomasses, biofuels, fossil fuels and natural fires in reduced or anoxic environments, is a mixture of elemental carbon and organic carbon (OC) compounds (Muckenhuber and Grothe, 2006). In addition, as a class of engineering nanoparticles, carbon nanotubes (CNTs) and graphene materials are also a large group of carbon nanomaterials although their graphene sheets are arranged more regularly (Hu et al., 2010) than that in CB (Nienow and Roberts, 2006). During production and use of these consumer products, they are prone to enter into the environment and ultimately the human body (Helland et al., 2007;Tiwari and Marr, 2010), subsequently, to pose risk of adverse health effect".

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https://www.atmos-chem-phys-discuss.net/acp-2018-1366/acp-2018-1366-AC1-supplement.pdf

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