

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.

Yongchun Liu et al.

liuyc@mail.buct.edu.cn

Received and published: 25 April 2019

Referee #2

In this work, the authors study the DTT and cytotoxicity response of several carbon nanomaterials and correlate them to their morphology and chemical composition. The main finding is that the epoxide content of graphene oxide is particularly high and also results in high apparent oxidative potential. This specificity is confirmed with thermal treatment of this substance to reduce the epoxide abundance (though also accompa-

C1

nied by morphological changes in the process). The manuscript is generally well written and addresses a current topic to interest of many researchers. The measurements appear technically sound, though further comments below could be addressed.

Response: Thank you for your positive comments.

General comments.

First question is regarding the XPS measurements: 1) How do the authors go from counts per second to oxygen content in (%) in Figure 5? If no calibration is performed, then is it possible to state absolute differences among functional groups or only C-O-C content among different materials? 2) How are epoxides distinguished from ethers? 3) It's not clear that these functional group characterizations are representative of the overall OC that is separately measured given the small probing depth of XPS. Can the authors comment on this?

Response: Thank you for your instructive suggestions. 1) About the XPS measurements, the instrument directly outputs the signal of O1s or C1s in cps, which means the number of electrons that escape from surface of the material being analyzed. When calculating the surface atom contents (%), we scaled the peak areas of each element according to the relative sensitivity factors. However, the relative sensitivity factors for each oxygen-containing species in the envelope of O1s are unavailable at the present time. We simply assumed all these oxygen-containing species in O1s having the same sensitivity factors. We agree with you that this might lead to additional uncertainty, while this method is usually used to calculate the relative content of oxygen-containing species (Chen et al., 2017) and absolute oxygen content of each species (Schuster et al., 2011) when comparing among different samples. Therefore, we calculated the relative fraction of each oxygen-containing species, then converted them into oxygen content.

On the other hand, Wepasnick et al. (2011) measured the surface oxygen-containing species in MWCNTs based on chemical derivation techniques in conjunction with XPS.

C2

The oxygen content of COOH, C=O and C-OH in oxidized MWCNTs were ($\sim 3.0\%$, $\sim 1.3\%$ and $\sim 1.0\%$), respectively. Using the method based on peak fitting in this work, we calculated the oxygen contents of COOH, C=O and C-OH that had been identified in the MWCNTs after oxidized by 70% HNO₃. These values were $\sim 3.9\%$, $\sim 2.0\%$ and $\sim 1.1\%$ and comparable with those measured with chemical derivation (Wepasnick et al., 2011). Therefore, we think the estimated absolute oxygen content in Fig. 5 should be reliable to semi-quantitatively discuss the influence of oxygen-containing species on the DTT decay rates although we agree with you that this might introduce additional uncertainty. In the revised manuscript (lines 447-453), we added the discussion about the possible uncertainty as “At the present time, the relative sensitivity factors for each oxygen-containing species are unavailable. Similar to the method used in the literatures (Chen et al., 2017; Schuster et al., 2011), we simply assumed all these oxygen-containing species in the envelope of O1s having the same relative sensitivity factors. It should be reliable when semi-quantitatively comparing the contents of oxygen-containing species among different samples although additional uncertainties might be introduced for the calculated oxygen content”.

2) If other ethers present in the carbon nanomaterials, it should also contribute to the O1s band which might be closed to that of epoxide. However, at the present time, it has been recognized that oxygen species including epoxide, hydroxyl, carbonyl and carboxylic groups present in graphene layer (Inagaki and Kang, 2014; Hunt et al., 2012). Epoxide should dominate the band at 532.6 eV compared with ethers (Hunt et al., 2012). In particular, the TGA results also supported the high content of epoxide in graphene oxide. For other samples, other ethers might overestimate their contents of epoxide. However, this should not have influence on our conclusion that epoxides are related to the high oxidation potential of graphene oxide. This discussion has been added in the revised manuscript (lines 520-529) as “It should be noted that if other ethers present in the carbon nanomaterials, they should also contribute to the O1s band which might be closed to that of epoxide. However, at the present time, it has been recognized that oxygen-containing species including epoxide, hydroxyl, carbonyl

C3

and carboxylic groups present in graphene layer (Inagaki and Kang, 2014; Hunt et al., 2012). Epoxide should dominate the band at 532.6 eV compared with ethers (Hunt et al., 2012). In particular, the TGA results also supported the high content of epoxide in graphene oxide. For other samples in this work, other ethers might overestimate their contents of epoxide. However, this should not have influence on our conclusion that epoxides are related to the high oxidation potential of graphene oxide”.

3) These oxygen-containing species measured using XPS are not representative of the overall OC because the probe depth of XPS is around 10 nm. On the other hand, OC includes not only the oxygen-containing species but also the hydrocarbons without oxygen atoms. Thus, XPS results only reflect the relative element ratio on the surface. However, the surface property should be very important to understand the toxicity of nanoparticles from the point view of particle-cell interaction (Cedervall et al., 2007). In the revised manuscript (lines 343-346), we added sentence to clarify this point as “It should be noted that XPS results only represent the surface atom ratios, which are different from the OC content representing the bulk composition. However, the surface property of particle should be very important to understand the toxicity of nanoparticles from the point view of particle-cell interaction (Cedervall et al., 2007)”.

The oxidation of SO₂ by epoxides 2016 is cited as support for ROS generation observed mechanism that is different from the mechanism by which oxidative potential of ROS is meant to be measured by DTT. The authors may wish to clarify this point as this may also be related to the discrepancy with the lack of difference in apparent cytotoxicity.

Response: Thank you for your instructive suggestions. We agree with you that the mechanism of SO₂ oxidation by epoxide might be different from that of DTT oxidation. Here we cited the oxidation of SO₂ by epoxides to support the oxidative property of graphene oxide. In fact, DTT is a stronger reducer than SO₂. Both direct oxidation by epoxides and indirect oxidation by ROS generated on the particle surface contribute to the consumption of DTT. Therefore, DTT decay rate should include a part of oxidation

C4

reactivity which can be explained by SO₂ oxidation. It has also been found that ozone oxidized carbon nanomaterials showed decreased DTT decay rates after treated by SO₂ compared with the pristine particles (Xu et al., 2015). In the revised manuscript (lines 517-519), we clarified this point as “This result is also well consistent with the previous founding that epoxides in graphene oxide can oxidize SO₂ to sulfate (He and He, 2016) although their oxidation mechanism might be different.”

The discrepancy of the observed strong oxidation potential of graphene oxide with the lack of difference in apparent cytotoxicity among these particles may also related to the different interaction mechanism between DTT assay and in vitro assays. In the revised manuscript (lines 400-405), it has been pointed out as “The interaction between target cells and particles should be much complicated than that between DTT and particles. As discussed above, the cytotoxicity of nanoparticles relied on not only the mode of action but also the chemical nature of particles. Therefore, the different responses of the oxidation potential and the cytotoxicity to the epoxide content in these carbon materials might be accounted for by different mechanisms of toxicity among these assays”.

As with the other reviewer I agree that the connection to atmospheric soot particles is quite tenuous; due to my delay in response I already see that the authors have proposed changes in this regard (which makes the work less relevant for ACP?).

Response: Thank you for your instructive suggestions. According to your suggestions, we removed the connection to atmospheric soot particles. We think this work is still atmospheric relevant because these carbon nanomaterials can be emitted into the atmosphere from different sources. The results of this work should be still interesting and important enough without the atmospheric extrapolation as commented by reviewer 1. In the revised manuscript (lines 53-56), we added a sentence to emphasize the importance of this work as “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”.

C5

One additional point on this is that the authors refer to "BC" but perhaps "soot" is more suitable, and the "surface functionalization" of soot have been characterized previously (including ethers) - e.g., Cain et al. 2010, Vander Wal et al. 2011, Popovicheva et al. 2014. However, atmospheric aging not only includes surface functionalization but also condensation of co-emitted species and photochemical oxidation products which are particularly rapid under conditions of soot emissions (Johnson et al. 2005 and Adachi et al. 2010); it is unclear how much of the oxidation potential attributable to functional groups would be dependent on the carbon nanomaterial itself in the environmental context.

Response: Thank you for your instructive suggestions. In the literatures, soot and black carbon are usually exchangeable. In the revised manuscript (lines 47-50), we added a sentence “Soot, 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)”. We replaced the “BC” with “soot” in the revised manuscript according to your suggestion, such as in lines 86, 216, 438 and 549.

The references related to surface functionalization including ethers (Cain et al., 2010; Wal et al., 2011; Popovicheva et al., 2015) have been added in the revised manuscript (lines 97-98).

We agree with you that atmospheric aging not only includes oxidation but also condensation or coating of co-emitted species and secondary products from photochemical oxidation. The relative contributions of these two processes in toxicity changes of soot or CB particles to the oxidation potential are unclear at present time and might be dependent on the carbon nanomaterial. It has been found that the DTT decay rates of SWCNTs (Liu et al., 2015) and engineered nanoparticles (SiO₂) (Liu et al., 2019) decreased significantly as a function of exposure time of these pollutants. In the revised manuscript (lines 582-591), we discussed the uncertainty of this work as “Finally, condensation of co-emitted species and photo oxidation products is particularly rapid

C6

under conditions of soot emissions (Johnson et al., 2005; Adachi et al., 2010; Peng et al., 2016). In previous our work, it has been found that condensation process significantly decreased the oxidation potential of the SWCNTs (Liu et al., 2015). A recent work has also found that condensation of organic aerosol leads to decrease in oxidation potential on engineered nanoparticles (Liu et al., 2019). Therefore, the contribution of functional groups to the oxidation potential should be greatly influenced by condensation of co-emitted species and photo oxidation products in the atmosphere. This might be dependent on the carbon nanomaterial itself and needs to be investigated in the future”.

Minor comments:

The methods section is very sparse in citations except a few of the authors own work, but citations to primary sources would be relevant here.

Response: Thank you for your instructive suggestions. Several relevant references have been cited in the revised manuscript (lines: 143, 153-154, 168).

There are typographical and grammatical errors which can be corrected during the editorial process of Copernicus.

Response: Thank you for your suggestions. We carefully checked and corrected some typographical and grammatical errors.

Reference:

Adachi, K., Chung, S. H., and Buseck, P. R.: Shapes of soot aerosol particles and implications for their effects on climate, *Journal of Geophysical Research-Atmospheres*, 115, 9, 10.1029/2009jd012868, 2010.

Cain, J. P., Gassman, P. L., Wang, H., and Laskin, A.: Micro-FTIR study of soot chemical composition-evidence of aliphatic hydrocarbons on nascent soot surfaces, *Phys. Chem. Chem. Phys.*, 12, 5206-5218, 10.1039/b924344e, 2010.

C7

Cedervall, T., Lynch, I., Lindman, S., Berggård, T., Thulin, E., Nilsson, H., Dawson, K. A., and Linse, S.: Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *Proc. Natl. Acad. Sci. USA*, 104, 2050-2055, 10.1073/pnas.0608582104, 2007.

Chen, D., He, D., Lu, J., Zhong, L., Liu, F., Liu, J., Yu, J., Wan, G., He, S., and Luo, Y.: Investigation of the role of surface lattice oxygen and bulk lattice oxygen migration of cerium-based oxygen carriers: XPS and designed H₂-TPR characterization, *Appl. Catal. B: Environ.*, 218, 249-259, <https://doi.org/10.1016/j.apcatb.2017.06.053>, 2017.

He, G., and He, H.: DFT studies on the heterogeneous oxidation of SO₂ by oxygen functional groups on graphene, *Phys. Chem. Chem. Phys.*, 18, 31691-31697, 2016.

Helland, A., Wick, P., Koehler, A., Schmid, K., and Som, C.: Reviewing the environmental and human health knowledge base of carbon nanotubes, *Environ. Health Perspect.*, 115, 1125-1131, 2007.

Hunt, A., Dikin, D. A., Kurmaev, E. Z., Boyko, T. D., Bazylewski, P., Chang, G. S., and Moewes, A.: Epoxide speciation and functional group distribution in graphene oxide paper-like materials, *Adv. Funct. Mater.*, 22, 3950-3957, 10.1002/adfm.201200529, 2012.

Inagaki, M., and Kang, F.: Acknowledgments, Second ed., *Materials science and engineering of carbon: fundamentals (Second Edition)*, Butterworth-Heinemann, Oxford, 542 pp., 2014.

Johnson, K. S., Zuberi, B., Molina, L. T., Molina, M. J., Iedema, M. J., Cowin, J. P., Gaspar, D. J., Wang, C., and Laskin, A.: Processing of soot in an urban environment: case study from the Mexico City Metropolitan Area, *Atmos. Chem. Phys.*, 5, 3033-3043, 10.5194/acp-5-3033-2005, 2005.

Liu, Q., Liggio, J., Breznan, D., Thomson, E. M., Kumarathasan, P., Vincent, R., Li, K., and Li, S.-M.: Oxidative and toxicological evolution of engineered nanoparti-

C8

cles with atmospherically relevant coatings, *Environ. Sci. Technol.*, 53, 3058-3066, 10.1021/acs.est.8b06879, 2019.

Liu, Y., Liggio, J., Li, S.-M., Breznan, D., Vincent, R., Thomson, E. M., Kumarathasan, P., Das, D., Abbatt, J., Antñolo, M., and Russell, L.: Chemical and toxicological evolution of carbon nanotubes during atmospherically relevant aging processes, *Environ. Sci. Technol.*, 49, 2806-2814, 10.1021/es505298d, 2015.

Muckenhuber, H., and Grothe, H.: The heterogeneous reaction between soot and NO₂ at elevated temperature, *Carbon* 44, 546-559, 2006.

Peng, J., Hu, M., Guo, S., Du, Z., Zheng, J., Shang, D., Levy Zamora, M., Zeng, L., Shao, M., Wu, Y.-S., Zheng, J., Wang, Y., Glen, C. R., Collins, D. R., Molina, M. J., and Zhang, R.: Markedly enhanced absorption and direct radiative forcing of black carbon under polluted urban environments, *Proc. Natl. Acad. Sci. USA*, 113, 4266-4271, 10.1073/pnas.1602310113, 2016.

Popovicheva, O. B., Kireeva, E. D., Shonija, N. K., Vojtisek-Lom, M., and Schwarz, J.: FTIR analysis of surface functionalities on particulate matter produced by off-road diesel engines operating on diesel and biofuel, *Environ. Sci. Pollut. Res.*, 22, 4534-4544, 10.1007/s11356-014-3688-8, 2015.

Schuster, M. E., Hävecker, M., Arrigo, R., Blume, R., Knauer, M., Ivleva, N. P., Su, D. S., Niessner, R., and Schlögl, R.: Surface sensitive study to determine the reactivity of soot with the focus on the european emission standards IV and VI, *J. Phys. Chem. A.*, 115, 2568-2580, 10.1021/jp1088417, 2011.

Tiwari, A. J., and Marr, L. C.: The role of atmospheric transformations in determining environmental impacts of carbonaceous nanoparticles, *J. Environ. Qual.*, 39, 1883-1895, 2010.

Wal, R. L. V., Bryg, V. M., and Hays, M. D.: XPS analysis of combustion aerosols for chemical composition, surface chemistry, and carbon chemical state, *Anal. Chem.*, 83,

C9

1924-1930, 10.1021/ac102365s, 2011.

Wepasnick, K. A., Smith, B. A., Schrote, K. E., Wilson, H. K., Diegelmann, S. R., and Fairbrother, D. H.: Surface and structural characterization of multi-walled carbon nanotubes following different oxidative treatments, *Carbon*, 49, 24-36, 10.1016/j.carbon.2010.08.034, 2011.

Xu, W. W., Li, Q., Shang, J., Liu, J., Feng, X., and Zhu, T.: Heterogeneous oxidation of SO₂ by O₃-aged black carbon and its dithiothreitol oxidative potential, *J. Environ. Sci.*, 36, 56-62, 10.1016/j.jes.2015.02.014, 2015.

Please also note the supplement to this comment:

<https://www.atmos-chem-phys-discuss.net/acp-2018-1366/acp-2018-1366-AC2-supplement.pdf>

Interactive comment on *Atmos. Chem. Phys. Discuss.*, <https://doi.org/10.5194/acp-2018-1366>, 2019.