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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Molecular characterization of water soluble organic nitrogen in marine rainwater by ultra-high resolution electrospray ionization mass spectrometry" *by* K. E. Altieri et al.

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We thank the reviewers for their careful review of our manuscript. Their suggestions have improved the clarity of the paper and made the results and conclusions of our study more useful. We have copied the reviewer comments below and reply in detail to their individual points.

Interactive comment on "Molecular characterization of water soluble organic nitrogen in

marine rainwater by ultra-high resolution electrospray ionization mass spectrometry" by K. E. Altieri et al. Anonymous Referee #1 Received and published: 17 December 2011 The paper intends to investigate chemical composition of WSON compound in a number of rainwater samples and assess possible distinctions with respect to geographic location and seasons. Chemical analysis is done by means of ultra high resolution mass spectrometry (UHR-MS) with electrospray ionization (ESI) that enables assignment elemental formulas to individual peaks based on the accurately measured m/z value. The topic of the work and the experimental approach are of substantial importance to the field and therefore have a great potential for publication. However, in the present submission, the UHR-MS data set is presented in the form that cannot be critically evaluated for its significance and accuracy. Moreover, my brief inspection of the proposed formula assignments reveals major problems with the data processing and interpretation that must be fully resolved before the atmospheric chemistry context of the paper can be reviewed and evaluated. My specific comments are listed below:

We thank the reviewer for his/her careful read. We have made changes in the data processing and interpretation of elemental formula assignments (see details below), and have created a more complete and comprehensive list of masses. This has improved the paper significantly in its accuracy in determining the types of organic compounds present. Overall, the changes in the total numbers of formulas in each compound class and the exclusion of some illegitimate formulas have not significantly changed the interpretation and conclusions of the manuscript. This is primarily because the relative numbers of formulas in each compound class, the average elemental ratios, and the van Krevelen diagrams have not changed significantly. In fact, in some cases the small changes have increased support for our conclusions and interpretations.

1) It is erroneous to assume that sodiation is the only mechanism of ionization in the positive ESI mode. N-containing species (especially with reduced N) often have high proton affinity and are commonly observed as protonated molecules (i.e. [M+H]+ ions). For instance, here I list few examples of m/z values, for which assignments based on

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protonation give more realistic and chemically meaningful results: e.g. m/z 343.113628 (assigned as C13H26O1N2S1P2-Na+ by the authors) is likely C14 H19N2O8-H+ (343.113592, +0.1 ppm); m/z 236.141111 (assigned as C15H19N1-Na+ by the authors) is likely C10H23N1O3P1-H+ (236.141007, +0.4 ppm). There are numerous examples of this sort and it would not be practical for me to revisit all the assignments presented in the paper. In addition, there is no chemical reason to believe that oxygen free CxHyNz species can yield sodiated peaks in (+)ESI spectra (i.e. group of peaks assigned as CxHyNz-Na+).

The data were re-processed allowing both mechanisms of ionization, sodiation and protonation. This, in conjunction with the necessary corrections in the way elemental formulas were assigned as plausible or not (described below), has led to a revised list of 4574 identified elemental formulas. Due to the possibility of both H+ and Na+, there were 1426 m/z's that had both a formula with H+ and one with Na+. This included the examples that the reviewer identified, where the H+ formula was more chemically meaningful, and also examples where the Na+ formula was more realistic (e.g., C15H30O2-Na+ vs. C9H27N7P1-H+). The following text was modified in the methods section on page 8 line 25: "Before calculating the elemental formulas, the measured m/z values were converted to neutral masses assuming addition of a sodium or hydrogen in the positive ion mode data; the mass of the electron was also considered." On page 9 line 20 "In some instances two formulas were possible for one m/z, one with a Na+ and one with H+. In this case the Kendrick mass defect was calculated (Hughey et al., 2001) and the longest homologous series of the simplest formula was retained. This, in addition to the use of 13C peaks as confirmation, allowed the assignment of one formula per m/z." was added to highlight for the reader that H+ and Na+ were both allowed in the elemental formula calculator, and to explain how the resulting multiple formula assignments were dealt with. Table 2 and Figures 3, 4, 6, S-2, and S-3 were updated to reflect the adjusted elemental formula list, the abstract, and any text pertaining to the number of formulas in a given compound class or their elemental ratios was also updated. The supplemental figures were also adjusted. The CHN-Na+ peaks

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were eliminated, and only CHN-H+ formulas were allowed.

2) Many of the assigned formulas are not chemically legitimate, i.e. they don't obey valence rules. For instance, many of the proposed assignments (e.g. C4H3O2N3P1, C5H7O1N1S1P1, C5H10O1N2S1P1, etc) cannot be associated with even hypothetically drawn molecular structures.

We thank the reviewer for identifying this error. The formulas were not properly screened for double bond equivalent values which allowed formulas with non-integer DBE values, and the "N rule" was not applied to all compound classes resulting in a subset of chemically illegitimate formulas. This has been rectified and all of the tables and figures have been updated to reflect the proper formulas, as described above. The methods text was changed to reflect this on page 9 line 16: "The "N rule" was also applied to elemental formulas whereby neutral molecules containing an odd number of 14N atoms must always exhibit an odd nominal mass, and conversely neutral molecules containing an even number of 14N atoms always exhibit an even mass as 14N has an odd number of valences and an even mass (Koch et al., 2005). The DBE must be a non-negative integer for chemically legitimate elemental formulas."

3) Presentation of the UHR-MS results as tabulated data is not sufficient for critical evaluation by both reviewers and general readers. There are numerous tools developed by the HR-MS community that must be applied for data reduction and visualization. To provide a solid ground for the discussion context of this manuscript, the UHR-MS data must be presented in a reader-friendly, assessable way that should include: * Presentation of the original UHR-MS spectra (either in the manuscript itself or in the SI file), along with the relevant statistics of assigned vs unassigned peaks.

Figure S-1 in the supplemental was added to show the peak heights and m/z's detected in all 7 rainwater samples. The statistics of assigned vs. unassigned peaks was added to the methods section on page 8 line 15 "There were 14924 m/z's detected across the seven rainwater samples." And on page 9 line 28 "There were 4574 elemental formulas

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assigned, with 2281 containing at least one N atom."

* Assignments must consider ionization on both sodium and proton, and perhaps on other metal cations too

* The authors should make sure that their peak assignments generate reasonable molecular formulas that conserve valence rules. They also have to explain how they dealt with redundant (multiple possible) assignments.

* Indication of specific fractions of N-containing species out of the total analyzed and identified peaks.

The text above reflects the three concerns on the consideration of both sodium and a proton for ionization, the presence of erroneous formulas, and the way multiple assignments were dealt with. In addition, text added on the specific fractions of N containing species of the total analyzed and identified peaks is also quoted above.

* Data reduction tools such as Kendrick analysis must be used to group and present chemical species in homologous groups. For instance, brief processing of the tabulated data with CH2-kendick analysis places _50% of the peaks in a number of homologous series containing between 3 to 17 individual peaks. Additional grouping (data reduction) might be obtained using different Kendrick bases and their combinations.

Kendrick analysis of the N containing compounds was used to identify homologous series of compounds that differ by CH2. A figure showing the Kendrick mass defect as a function of the Kendrick nominal mass for the NO, NO2, NO3, NO4, NO5, and N2O2 series was added to the Supplemental Information, Figure S-5. Text was added on page 14 line 2 describing the utility of plotting elemental formulas as Kendrick series "One way to identify these series of related compounds is the Kendrick mass defect (KMD). Compounds with the same KMD have the same number and type of heteroatoms and DBE but differ in the number of CH2 groups (Hughey et al., 2001). Of all the N containing compounds, 49% of the masses were in a Kendrick series ranging

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from 3-19 formulas. The longest homologous series was in the N1O5 family which went from C5H7O5N1 to C23H43O5N1. When the Kendrick mass defect is plotted as a function of the nominal Kendrick mass these series of compounds fall on a horizontal line separated by 14 Da (Figure S-5). This makes identifying compounds of different classes visually easier as they are displaced vertically. The contribution of amino acids to total WSON may only range from 2-25%, but since the CHON+ compound class dominates in number, and there are regular patterns in mass differences it is likely that there are numerous reaction products and thus their quantitative contribution to WSON could be much higher."

* Also, relative intensities of the individual peaks need to be indicated to ensure that peak assignments covers at least most abundant peaks.

The relative intensities are a measure of both concentration and ionization efficiency. In mixed standards of compounds with similar structures at the same concentration, the peak heights can vary well over an order of magnitude. Since we cannot ascertain structural information from the elemental formula we do not know the relative ionization efficiencies of the molecules, therefore we feel it is more appropriate to treat the peak heights as an indicator of presence/absence only.

* All items above require graphical presentation that needs to be included at least in the SI file. In addition to the changes in the text that are described above, two additional figures were added to the Supplemental Information, Figure S-1 displaying all measured m/z's and their respective peak heights, and Figure S-5 which shows the Kendrick series .

Interactive comment on Atmos. Chem. Phys. Discuss., 11, 31283, 2011.