

REPLY TO REFEREE 1

We thank the Referee for the useful comments. We agree that the current version of the manuscripts perhaps lacks a thorough recognition of previous studies on atmospheric aerosol health effects. We answer to his/her specific comments (reported here in *italics*) below:

“This paper involves an analysis of existing data bases to infer possible toxic components of ambient SOA. The analysis reported is not comprehensive, serving mainly as a proof of concept. The idea is interesting, worthy of publication, but would be more appropriate for this journal if linked to existing knowledge on aerosol chemistry and health effects. My main suggestion with this paper is that a more substantial discussion should be added to put the work in context with known aerosol toxicity, source apportionment, and epidemiology studies. There is a substantial body of published studies that have identified various aerosol sources that are strongly linked to adverse health outcomes, such as incomplete combustion; eg, vehicle tail pipe emissions and biomass burning. These produce SOA and many of these compounds seem to be found in the main factors of this study with high toxicity. In contrast, from other studies biogenic SOA tends to be less clearly associated with health effects (check out the published literature). So why not link Fig 2 in at least a qualitative way with published health studies through a more comprehensive discussion, possibly identifying factors or toxic compounds in this study linked to SOA from incomplete combustion, biogenic VOC SOA etc. Putting these results in a large context of published work would significantly increase the impact of these findings, at least for the readers of this journal.”

REPLY: The Reviewer is right: there is massive amount of literature results for anthropogenic and natural aerosol health effects that is simply not referenced in our manuscript. We have addressed this, in part, by adding a range of references to cover studies highlighting distinct aerosol source contributions. On the other hand, we would like to note that much fewer studies have focused on SOA than on combustion aerosols, and mutagenicity is only one in the wide range of the possible endpoints explored in the field of aerosol toxicology. Moreover, a substantial fraction of recent studies focused on properties, such as ROS content or ROS expression, which cannot be considered strictly toxicological endpoints but more specifically refer to the mode of action of toxic agents. Even fewer studies performed source apportionment of the observed toxicity burden of the aerosol. To the best of our knowledge, the only study attempting source apportionment of ambient aerosol mutagenicity is the paper by Hannigan et al. (2005) which is based on a chemical mass balance (CMB) approach employing source profiles uniquely for primary organic aerosol, therefore providing no information on the potential contributions from SOA. The main body of the existing literature on role of SOA on aerosol mutagenic activity refers mostly to nitro-PAHs (and to a lesser extent, to oxo- and hydroxyl-PAHs) (e.g., Enya et al., 1997). References to compounds other than polyaromatics is quite sparse. To go back to the original point raised by the Reviewer, we thus provide below a list a papers dealing with mutagenic effects of SOA but not specifically or uniquely from polyaromatic compounds. We also included some of the recent studies of SOA toxicity targeting ROS expression, because this can be linked to the mechanistic profiles of some classes of oxygenated compounds predicted to be mutagenic by our QSAR approach. The overall picture certainly goes in the direction mentioned by the Reviewer: there is a more consistent evidence of the health effects for the products of anthropogenic combustion

emissions than for other sources of organic aerosols (Delfino et al., 2009), although biogenic SOA systems have certainly been subjected to lesser extent. We will summarize these literature findings in a new dedicated paragraph to append to Section 3.3 and, in compact, form in the Conclusions.

Selected literature citations:

| Citation | Selected results: | Implications for the present study |
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| Kamens et al., Environ. Sci. Technol., 18, 523, 1984. | - Mutagenicity in aged wood smoke increased of a factor of 2 to 10 with respect to fresh smoke. | - Experimental evidence of the mutagenic effects of SOA from combustion sources. |
| Alves et al., Environmental and Molecular Mutagenesis, 57, 41-50, 2016. | - Episodes of high mutagenicity in the Sao Paulo State (Brazil) seem to occur when the air mass from the rural area of sugar cane production is mixed with air in the region impacted by anthropogenic activities. | - Secondary organic reactions concomitant to both anthropogenic and biogenic SOA formation affect observed mutagenicity of ambient PM in rural areas. |
| Hannigan et al., Environ Health Perspect., 104, 428–436, 1996. | - Report stresses the importance of proximity to sources of direct emissions of bacterial mutagens but also implies that if ‘important mutagen-forming atmospheric reactions occur, they likely occur in the winter and spring seasons as well as the photochemically more active summer and early fall periods.’ | - Seasonal variation in mutagenicity, according to different atmospheric reactive conditions |
| Filep et al., Aerosol and Air Quality Research, 15: 2325–2331, 2015. | - Eco toxicity parameters (cyto and geno) are strongly emission source dependent; the higher the ratio of the biomass burning related carbonaceous aerosol the higher the cytotoxicity and the higher the ratio of the fossil fuel related carbonaceous aerosol the higher the genotoxicity. | - These results, obtained on biomass burning samples, indicate that specific mutagenic effects could change within one PM type – supports potential for composition dependency. |

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| <p>Kroflič et al., Scientific Reports 5, Article number: 8859, 2015.</p> | <ul style="list-style-type: none"> - assessment of the impact of low- and semi-volatile aromatic pollutants on the environment due to their atmospheric aqueous phase aging. It reveals that remote biotopes might be the most damaged by wet urban guaiacol-containing biomass burning aerosols. It is shown that only after the primary pollutant guaiacol has been consumed, its probably most toxic nitroaromatic product is largely formed. | <ul style="list-style-type: none"> - The study suggests pyrolysis of the polymer lignin during biomass burning leads to semi-volatile organic compounds (SVOC), such as guaiacol (GUA), which then produce nitro-aromatic derivatives. |
| <p>Palacio et al., Mutation Research, 812, 1–11, 2016; Gutiérrez-Castillo et al., Environmental and Molecular Mutagenesis, 47, 199-211, 2006.</p> | <ul style="list-style-type: none"> - Both organic and water-soluble associated compounds with particulate matter can produce genotoxic effects at concentrations commonly found in urban areas around the world | <ul style="list-style-type: none"> - Polar organic compounds accounting for SOA composition can contribute to the mutagenic activity of ambient PM. |
| <p>Barale et al., Environ. Health Perspect., 102 (Suppl. 4), 67–73, 1994.</p> | <ul style="list-style-type: none"> - Chemical fractionation of aerosol extracts showed that mutagenicity was contributed mostly by polar compounds not PAHs. | <ul style="list-style-type: none"> - Polar organic compounds accounting for SOA composition can contribute to the mutagenic activity of ambient PM. |
| <p>Fu et al., Journal of Environmental Science and Health, Part C, 30, 1–41, 2012.</p> | <ul style="list-style-type: none"> - Photo-oxidation products of polyaromatic compounds lead to the formation of epoxides, endoperoxides and quinones which eventually lead to ROS and DNA damage. | <ul style="list-style-type: none"> - Evidence for the mutagenic effect of aromatic oxygenated compounds including epoxides, endoperoxides and quinones as in several clusters of compounds predicted to be mutagenic in our paper. |
| <p>Risom et al., Mutation Research, 592, 119–137, 2005; Oh et al., Mutation Research, 723, 142– 151, 2011.</p> | <ul style="list-style-type: none"> - Oxidative stress caused by PM pollution is genotoxic; - Ambient air PM induces oxidative DNA damage in in vitro systems. | <ul style="list-style-type: none"> - Experimental evidence of PM-bound ROS-generating organic compounds (not limited to polyaromatic compounds) can be associated to mutagenicity. |

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| Valavanidis et al., Journal of Environmental Science and Health Part C, 26, 339–362, 2008. | | |
| Rattanavaraha et al., Atmospheric Environment, 45, 3848-3855, 2011 | <ul style="list-style-type: none"> - ROS expression in aged diesel emissions increased by a factor of 2-4 over fresh diesel particles. - The highest ROS potentials are found when including secondary organic aerosols from an α-pinene, + toluene + an urban HC mixture. | <ul style="list-style-type: none"> - Several classes of compounds predicted to be mutagenic in our study are characterized by ROS expression among the alerts. Reaction chamber experiments have confirmed the production of redox-active secondary organic compounds in both biogenic and anthropogenic systems. |
| Tuet et al., Atmos. Chem. Phys., 17, 839–853, 2017. | <ul style="list-style-type: none"> - Redox-active compounds (measured by the DTT assay) are produced in negligible amounts in isoprene, caryophyllene and pentadecane SOA, in only moderate amounts in α-pinene and monoaromatic SOA, and in much greater amounts in naphthalene SOA. | <ul style="list-style-type: none"> - Measured ROS expression in SOA qualitatively agree with the predicted mutagenicity of SOA tracers (Figure 2 of our paper), where isoprene, sesquiterpenes, <i>n</i>-alkane SOA compounds exhibit no mutagenic effects, while monoterpenes SOA include some mutagenic species and mostly non-mutagenic ones, while low MW PAHs SOA account for the largest share of mutagenic species. These results reinforce the link between ROS expression and mutagenicity. |
| Verma et al., Environ. Sci. Technol., 49, 4646–4656, 2015. | <ul style="list-style-type: none"> - Linear regression analysis between ROS generating capacity and OA fractions by AMS-PMF shows that in the SE USA, ROS expression of isoprene SOA is very small while biomass burning OA shows the greatest contribution. | <ul style="list-style-type: none"> - These results also agree qualitatively with the mutagenic activity of isoprene SOA compounds and of aromatic secondary species presented in our study. |

“Finally, it is exposure that determines health effects, that is toxicity times concentration of a given species. A discussion on this would also be very helpful. It would be useful if the authors could provide some idea of typical concentrations of these identified toxic species (or groups), maybe for a range of sites. For example, maybe only a small fraction of the biogenic SOA leads to a toxic substance, but maybe the concentration of these species are very high (or low) making it a potentially important (or not) species.”

REPLY: Most of the SOA studies quoted in Table S1 focus on the identification of specific organic compounds on the basis of mass spectrometric analyses. However, the paucity of authentic standards makes quantitation challenging. As a result, the literature provides rather sparse information on the actual abundance of SOA markers in atmospheric samples. Most consistent data refer to well-studied systems such as α -pinene SOA and isoprene SOA. For instance, Kristensen et al. (2014) found that the most common α -pinene SOA tracers, including pinonic, hydroxyl-pinonic and pinic acids (mtr_05, mtr_06 and mtr_07 in Table S1), terpenyllic, terebic and diaterpenyllic acids (mtr_11, mtr_12 and mtr_13), 3-methyl-1,2,3-butanetricarboxylic (MBTCA) (mtr_17), together with their dimers (mtr_15, mtr_16), overall account for 10 to 15% of SOA mass. Similarly, Lin et al (2013) showed that isoprene SOA tracers can represent 12 – 14% of ambient particulate organic matter in an environment characterized by strong isoprene emissions. The contributions of the diverse isoprene SOA species varied a lot, with much greater shares from methyl-tetrols (iso_01 in Table S1), C5-alkene triols (iso_04) and their sulfate esters (iso_02) than from 2-methyl-glyceric acid and its derivatives (iso_06, iso_07). No concentration data are available for the majority of the compounds listed in Table S1, making any attempts of exposure assessment impossible for them. We would like to clarify, however, that the toxicity predictions conducted in our study are useful mainly for *hazard identification*, which is only the first step of risk assessment. Clearly, additional information on concentrations and exposure, as well as compound-specific dose-response functions are required to characterize the health risk associated with the SOA compounds predicted to be mutagenic in this study. On the other hand, hazard identification is necessary to guide the subsequent steps of risk assessment, including the development of adequate analytical techniques to determine the concentrations of specific chemical compounds according to a priority list.

“Minor comment. The format is a bit strange: Why two Introduction sections? Table 1 has no caption.”

REPLY: That is an error: Section 2 is in fact “Methods” not again “Introduction”. It is also true that Table 1 has only footnotes not a proper caption. The table reports a summary of the observed mutagenic properties of the 13 organic compounds for which experimental data could be found in the literature.

REFERENCES:

- Delfino et al., Air Pollution Exposures and Circulating Biomarkers of Effect in a Susceptible Population: Clues to Potential Causal Component Mixtures and Mechanisms, *Environ. Health. Perspect.*, 117, 152-156, 2009.
- Enya et al., 3-Nitrobenzanthrone, a Powerful Bacterial Mutagen and Suspected Human Carcinogen Found in Diesel Exhaust and Airborne Particulates, *Environ. Sci. Technol.*, 31 (10), 2772–2776, 1997.

Hannigan et al., Source Contributions to the Mutagenicity of Urban Particulate Air Pollution, *J. Air & Waste Manage. Assoc.* 55, 399-410, 2005.

Kristensen et al., Dimers in α -pinene secondary organic aerosol: effect of hydroxyl radical, ozone, relative humidity and aerosol acidity, *Atmos. Chem. Phys.*, 14, 4201–4218, 2014.

Lin et al., Investigating the influences of SO₂ and NH₃ levels on isoprene-derived secondary organic aerosol formation using conditional sampling approaches, *Atmos. Chem. Phys.* 13, 8457–8470, 2013.

LIST OF RELEVANT CHANGES TO THE MANUSCRIPT IN RESPONSE TO REFEREE 1'S COMMENTS:

Section 2.1 Organic compounds:

“...The list in Table S1 includes compounds of varying degrees of “identification factor” (“I factor”, (Nozière et al., 2015)): for only a fraction of them a chromatographic separation, confirmation with standards and quantification in real samples could be achieved. These include, for instance, most common α -pinene SOA tracers, including pinonic, hydroxyl-pinonic and pinic acids (mtr_05, mtr_06 and mtr_07 in Table S1), terpenyllic, terebic and diaterpenyllic acids (mtr_11, mtr_12 and mtr_13), 3-methyl-1,2,3-butanetricarboxylic (MBTCA) (mtr_17), together with their dimers (mtr_15, mtr_16), which can add up 10 to 15% of SOA mass (Kristensen et al.; 2014). Other well-known SOA compounds included in the list account for the isoprene chemistry: methyl-tetrols (iso_01 in Table S1), C5-alkene triols (iso_04) and their sulfate esters (iso_02) are common constituents of organic aerosols in rural areas, where they can account for 12 – 14% of ambient particulate organic matter (Lin et al., 2013). Concentration data for anthropogenic compounds, such as quinones, oxo- and nitro-PAHs are also available in the literature (Chung et al., 2006; Huang et al., 2014). For most of the other organic compounds in the list, concentration data are simply not available. These include molecular species of uncertain identification, and whose molecular structures were inferred solely from the interpretation of mass spectra....”

Section 2.2. Toxicity endpoints:

“...Past studies have attempted to apportion total PM mutagenicity among distinct primary organic aerosol sources (Hannigan et al., 2005). No such exercise has been carried out yet for SOA sources, in spite of the fact the mutagenicity of SOA have been demonstrated at least 30 years ago (Kamens et al., 1984). ...”

And “...It should be noted that screening tests performed on strains of prokaryotes, such as the Ames test, are not meant to mimic exposure conditions for humans. They are used instead in the preliminary phases of hazard assessment, and especially for hazard identification. ...”

Section 3.3. Cluster analysis:

“...These clusters encompass organic compounds with clear source fingerprints (from PAHs and amine oxidation and reaction with NO_x) and that are found in polluted environments in concentrations ranging from 10⁻³ to 10⁰ ng m⁻³ (Chung et al., 2006; Huang et al., 2014; Farren et al. 2015) (Table S10). Nitro- and oxo-aromatic compounds are by far the best studied mutagenic components of SOA (e.g., Enya et al., 1997; Di Filippo et al. 2007; Huang et al., 2014). They can form upon oxidation of

primary organic compounds which can be also mutagenic (PAHs) or that are just non toxic, such as methoxy-phenols in biomass burning emissions (Kroflíč et al., 2015).

The other three clusters encompass oxygenated organic compounds that, in our knowledge, have been ignored so far in mutagenicity studies of atmospheric aerosol compounds. They could contribute to explain the yet unknown composition of the most polar fraction of mutagenic compounds in atmospheric aerosol (Barale et al., 1994; Gutiérrez-Castillo et al. 2006; Palacio et al., 2016). First, 5-membered lactones formed by the oxidation of monoterpenes are alkylating agent and possibly mutagenic (Cluster 0). These include terpenyllic acid, which occur in concentrations of 6 to 15 ng m⁻³ in polluted rural areas (Kristensen et al., 2013; Brüggemann et al., 2017). [...] No experimental data are available for the ambient concentrations of organic compounds belonging to Clusters 7 or 8. However, SOA modelling studies indicate that they can account for a significant fraction of SOA mass formed by substituted benzene oxidation (Ruggeri et al., 2016), which translates into ambient concentrations of several ng m⁻³ in the very polluted environments (such as in Yuan et al., 2013) (Table S10). The formation of endoperoxides and epoxides and of other reactive oxygenated organic compounds such as those in Clusters 7 and 8 was hypothesized by Fu et al. (2012) to explain the DNA damage caused by photodegraded aromatic compounds. Moreover, the finding of biogenic SOA components with potential mutagenic properties (Cluster 0, and some compounds in Clusters 7 and 8) is in agreement with the results of Alves et al. (2016), showing that mutagenicity of the aerosol is enhanced when pollution get mixed with emissions in rural areas. The overall picture, however, is that a greater proportion of mutagenic compounds was predicted to occur in the anthropogenic SOA classes than in the biogenic ones (Figure 2). Exceptions are alkane SOA which were predicted to be non-mutagenic, and some of the monoterpene SOA which instead showed mutagenic activity on the basis of our QSAR approach. These results show a very good, qualitative overlap with the findings of Verma et al. (2015) and Tuet et al. (2017) about the redox potentials of specific classes of SOA. Although ROS expression is only one of the possible mechanisms of action responsible for mutagenicity, it characterizes several of the compounds predicted to be mutagenic in our study, especially in Clusters 1, 3, 7 and 8. The formation of ROS in atmospheric SOA and the genotoxic effect of oxidative stress caused to PM exposure have been documented by a number of studies (e.g., Risom et al., 2005; Valavanidis et al. 2008; Rattanavaraha et al., 2011; Oh et al., 2011; Fu et al., 2012). ...”

Section 4 Conclusions

“...The results, based on a list of ~100 individual organic compounds representative of the most important anthropogenic and biogenic SOA classes, indicate that diverse groupings of molecular species exerting specific genotoxic effects might be of important, and among these are classes of SOA compounds previously unexplored for their mutagenic potential. These includes 5-membered lactones and a heterogeneous class of oxygenated compounds with epoxide, peroxide functionalities and promoting ROS expression. Since, in our list of 104 compounds, redox-active species with mutagenic properties are more frequently found in SOA formed by the degradation of aromatic VOCs, the apportionment of genotoxic compounds among SOA classes shows an overall dominance of anthropogenic over biogenic species (Figure 2). Therefore these results, based on a first application of *in silico* methods on a limited number of molecular species, are in qualitative agreement with the existing

general knowledge of aerosol health effects indicating a more robust association of combustion generated aerosols with adverse outcomes with respect to other aerosol sources (Delfino et al., 2009)....”

REPLY TO REFEREE'S 2 COMMENTS

We thank the Referee for his/her useful comments which give us the opportunity to clarify some aspects of the methodology.

COMMENT: Have the compounds which were tested previously been measured in atmospheric SOA, and if they have at what concentrations and which types of SOA (e.g. anthropogenic, biogenic, etc)? If they haven't been specifically measured can the authors give a rough estimation of the concentrations that could be expected in different types of SOA? Is there a trend of more of the toxic compounds being likely to be present in a specific type of SOA?

REPLY: We have screened the existing literature and compiled a new table (Table S10, in attach to the present document, and to be included in the Supplementary Information of the revised paper) with concentration data observed for a subset of SOA compounds from the most relevant clusters of mutagenic species in our list. When direct observations are not available, we provided a rough estimate of ambient concentrations on the basis of observed/predicted yields in laboratory setups and on source apportionment results for ambient organic aerosols. We found that the new SOA compounds predicted to be mutagenic can occur in ambient air in concentrations of $10E-2$ to $10E1$ ng m⁻³. In respect to the Referee's question about possible trends in the abundance of toxic compounds between different classes of SOA, we think that Figure 2 of the present version of the manuscript already addresses this. The figure shows that a greater fraction of species predicted to be mutagenic is found in anthropogenic SOA systems than in the natural ones. As observed by Referee 1, this finding - though limited to the pool of compounds considered in our study - is qualitatively in line with the current evidence of clearer toxicological and epidemiological effects of anthropogenic combustion-related aerosol with respect to biogenic particles. We will add a note about this in the Discussion section of the revised version of the manuscript.

COMMENT: Are the concentrations which would be expected in the respiratory tract upon inhalation of atmospheric SOA similar to the concentrations used during the Ames test and assumed in the models? When both positive and negative Ames tests results are reported due to testing conditions (Page 7, Line 2) are there any trends that have been observed for the range of results (e.g. a dependence on concentration, or a different experimental condition) and could some of the experimental results be discarded due to those conditions being unlikely to occur in the respiratory tract/ human body after inhalation?

REPLY: We are happy to re-iterate and further clarify the role of presented data in our study using three key points related to the questions posed.

- 1) First of all, it is worth re-iterating that mutagenicity is often related to long-term, chronic exposures in environmental toxicology and that, therefore, even trace air concentrations of mutagens cumulate to mg-level doses during a lifetime exposure. That is why the WHO air quality guidelines recommend sub-ng/m³ threshold values for the concentrations of benzo[a]pyrene (WHO, 2000). The concentrations of the new SOA compounds predicted to be mutagenic in the Ames test on the basis of our QSAR approach are not always known. The ranges of concentrations of the compounds predicted to be mutagenic in our study (new Table S10, in attach to the present document) compare well with those observed for known atmospheric organic mutagens such as PAHs and nitro-PAHs (Alves et al., 2017). In conclusion,

the newly identified mutagenic compounds can occur in ambient air in appreciable concentrations and be inhaled in mg amounts during a lifetime exposure. We therefore recommend them for *in vitro* toxicological screening for confirmation of their mutagenic effect and for determination of dose-response functions.

- 2) As far as the comparison with the doses used in the Ames test is concerned, we would like to emphasize that the Ames test (or bacterial reverse mutation test) is only a screening *in vitro* test performed to support a preliminary hazard assessment of chemicals, which are screened for their mutagenic potential. In more detail, the Ames test uses amino-acid requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations (OECD, 1997). Such mutations lead to revertant bacteria in which the functional capability to synthesize the essential amino acid is restored. Revertant bacteria are then detected by their ability to grow in absence of the amino acid necessary for the growth of the parent test strain. A wide range of concentrations are tested, with an upper limit which mainly depends on the solubility and cytotoxicity of the test compound in the final treatment mixture. The tested concentrations are expressed in either $\mu\text{g}/\text{plate}$ or $\mu\text{L}/\text{plate}$, where “plates” are samples of bacterial strains, which makes a direct comparison with exposure concentrations for humans not properly feasible. It is important to emphasize that screening tests, such as the Ames test, are supposed to be used in the preliminary phases of hazard assessment, and especially for hazard identification, and not to mimic exposure conditions. We will add this to the text of the new version of the manuscript. Finally, as for the appropriateness of conditions used in models to replicate conditions in the respiratory tract, this is not within scope of the paper. Indeed, as we iterate, the driver for this work is to demonstrate the efficacy of a methodology to highlight potential hazardous compounds that might be measured in future epidemiological studies.
- 3) In addition to the inability to strictly link to concentrations expected in the human body noted in the previous points, the variation of “testing conditions” mentioned in the manuscript include factors such as the testing methodology (e.g., plate incorporation method or preincubation method), the tested strains of *Salmonella typhimurium* and *Escherichia coli* (at least five different strains are required, as specified in the OECD guideline, in order to detect different mutations), presence or absence of metabolic activation, exposure concentration. No specific trends have been observed among positive and negative results. The Ames test is an *in vitro* test using prokaryotic cells and, as such, it does not consider important processes in organisms of higher clades (e.g., rodent *in vivo* toxicological studies). Therefore, there are no experimental conditions that can be considered representative for the mechanisms of uptake of the test substance by humans (e.g., absorption through the respiratory tract), and, as a corollary, no specific testing conditions used in the Ames test could be discarded.

COMMENT: Are there any limitations to the two models used (the ACD/Impurity Profiling model and the Vega/CAESER model), for example, can they reliably predict the toxicity of any compound? What are the differences between them which leads to one giving a positive result whilst the other gives a negative result in some cases (as shown in Table 2)?

REPLY: This is a good point and we are happy to clarify here. All QSAR models are characterized by a defined applicability domain which means that no QSAR model can be guaranteed to provide a reliable prediction for any compound, regardless of the endpoint. In this specific case, both the ACD/Impurity Profiling QSAR model and Vega/CAESER model are supported with specific parameters providing information on prediction reliability, including applicability domain considerations. As described in the Supplementary Information (Table S4), ACD/Percepta predictions are supplemented with a Reliability Index (RI), which ranges from 0 to 1, and gives an evaluation of whether a submitted compound falls within the Model Applicability Domain. In particular: RI < 0.3 (Not Reliable), RI in range 0.3-0.5 (Borderline Reliability), RI in range 0.5-0.75 (Moderate Reliability), RI >= 0.75 (High Reliability). Estimation of the RI takes into account two main aspects: similarity of the tested compound to the training set and the consistency of experimental values for similar compounds. Similarly, Vega/CAESAR predictions are provided with an Applicability Domain Index (ADI), which ranges from 0 to 1, and gives an evaluation of whether a submitted compound falls within the Model Applicability Domain. In particular: ADI > 0.9 means that the predicted substance is into the AD of the model; ADI < 0.7 means that the predicted substance is out of the AD of the model; ADI in range 0.7-0.9 means that the predicted substance could be out of the AD of the model and further considerations are needed. The ADI is calculated by grouping several other indices, each one taking into account a particular issue, such as: training/test set similar molecules with known experimental value, concordance for similar molecules, accuracy of prediction for similar molecules, atom Centered Fragments similarity check and model descriptors range check.

Predictions obtained by the two QSARs were combined by taking into account the applicability domain of each model (prediction reliability) and consistency between predictions. In case of conflicting reliable predictions, the most conservative outcome, i.e. positive prediction, was assigned. We will add the following text to the revised version of the manuscript:

“Overall, the predictions generated by the two QSAR models were in agreement for the majority of compounds (not consistent predictions obtained for only 4 compounds out of the 53 reliably predicted by both QSAR models) and the combination of the two tools resulted successful in covering a wider chemical space (59% of compounds reliably predicted by ACD/Percepta alone, 74% of compounds reliably predicted by Vega/CAESAR alone, 82% of compounds reliably predicted combining the two QSAR models). As far the 4 compounds with opposite predictions are concerned, this is mainly due to issues related to the different applicability domain of the two QSAR models.”

Minor comments (1): There are a lot of abbreviations throughout the text which should be written out in full for clarity (e.g. PaDEL, nHBDon, nHBAcc, KNIME).

REPLY: These includes names of softwares (PaDEL-Descriptor, and KNIME) for which specific references are already reported in the manuscript. We will instead report explanations for the following acronyms: nHBDon (= number of donor atoms for hydrogen bonds) and nHBAcc (= number of acceptor atoms for hydrogen bonds).

Minor comments (2): There - Section 2 should be renamed from ‘Introduction’ to ‘Methods’.

REPLY: Yes, we thank the Referee for noticing this mistake.

REFERENCES:

- 1) Alves et al., “Polycyclic aromatic hydrocarbons and their derivatives (nitro-PAHs, oxygenated PAHs, and azaarenes) in PM_{2.5} from Southern European cities”, *Science of the Total Environment* 595 (2017) 494–504.
- 2) OECD GUIDELINE FOR TESTING OF CHEMICALS n. 471. Bacterial Reverse Mutation Test. Available at: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en.

WHO (2000) Air Quality guidelines for Europe, 2nd edition: WHO Regional Office for Europe (WHO Regional Publications, European Series, No. 91).

LIST OF RELEVANT CHANGES TO THE MANUSCRIPT IN RESPONSE TO REFEREE 2’S COMMENTS:

Section 2.2 Toxicity endpoints:

“...It is worth reminding that mutagenicity is only one of the possible toxicological endpoints for atmospheric particulate organic compounds, and that the same classes of compounds can be more or less toxic depending on the specific endpoints (e.g., mutagenicity vs. cytotoxicity, etc.) (Filep et al., 2015).”

And “.. It should be noted that screening tests performed on strains of prokaryotes, such as the Ames test, are not meant to mimic exposure conditions for humans. They are used instead in the preliminary phases of hazard assessment, and especially for hazard identification.”

3.2 Screening for genotoxicity – QSAR predicted data:

“... Overall, the predictions generated by the two QSAR models were in agreement for the majority of compounds (not consistent predictions obtained for only 4 compounds out of the 53 reliably predicted by both QSAR models) and the combination of the two tools resulted successful in covering a wider chemical space (59% of compounds reliably predicted by ACD/Percepta alone, 74% of compounds reliably predicted by Vega/CAESAR alone, 82% of compounds reliably predicted combining the two QSAR models). As far the 4 compounds with opposite predictions are concerned, this is mainly due to issues related to the different applicability domain of the two QSAR models. Based on the combined predictions, 53 compounds were predicted as negative, 32 as positive (including four compounds with conflicting reliable predictions), while for 19 compounds QSAR predictions were assessed as “indeterminate” (i.e., not reliable or equivocal predictions) (Table 2).”

Section 3.3. Cluster analysis:

“...These clusters encompass organic compounds with clear source fingerprints (from PAHs and amine oxidation and reaction with NO_x) and that are found in polluted environments in concentrations ranging from 10⁻³ to 10⁰ ng m⁻³ (Chung et al., 2006; Huang et al., 2014; Farren et al. 2015) (Table S10). Nitro- and oxo-aromatic compounds are by far the best studied mutagenic components of SOA (e.g., Enya et al., 1997; Di Filippo et al. 2007; Huang et al., 2014). They can form upon oxidation of primary organic compounds which can be also mutagenic (PAHs) or that are just non toxic, such as methoxy-phenols in biomass burning emissions (Kroflíč et al., 2015). The other three clusters encompass oxygenated organic compounds that, in our knowledge, have been ignored so far in mutagenicity studies of atmospheric aerosol compounds. They could contribute to

explain the yet unknown composition of the most polar fraction of mutagenic compounds in atmospheric aerosol (Barale et al., 1994; Gutiérrez-Castillo et al. 2006; Palacio et al., 2016). First, 5-membered lactones formed by the oxidation of monoterpenes are alkylating agent and possibly mutagenic (Cluster 0). These include terpenyllic acid, which occur in concentrations of 6 to 15 ng m⁻³ in polluted rural areas (Kristensen et al.,2013; Brüggemann et al., 2017). [...] No experimental data are available for the ambient concentrations of organic compounds belonging to Clusters 7 or 8. However, SOA modelling studies indicate that they can account for a significant fraction of SOA mass formed by substituted benzene oxidation (Ruggeri et al., 2016), which translates into ambient concentrations of several ng m⁻³ in the very polluted environments (such as in Yuan et al., 2013) (Table S10). ...”

Finally, a new table (Table S10) is now included in the Supplementary Information.

Evaluating the mutagenic potential of aerosol organic compounds using informatics based screening

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10 **Abstract.** Whilst general policy objectives to reduce airborne particulate matter (PM) health effects are to reduce exposure to PM as a whole, emerging evidence suggests more detailed metrics associating impacts with different aerosol components might be needed. Since it is impossible to conduct toxicological screening on all possible molecular species expected to occur in aerosol, in this study we perform a proof of concept evaluation on the information retrieved from *in silico* toxicological predictions, in which a subset (N = 104) of secondary organic aerosol (SOA) compounds were screened for their mutagenicity
15 potential. An extensive database search showed that experimental data are available for 13% of the compounds while reliable predictions were obtained for 82%. A multivariate statistical analysis of the compounds based on their physico-chemical, structural and mechanistic properties showed that 80% of the compounds predicted as mutagenic were grouped into six clusters, three of which (5-membered lactones from monoterpene oxidation, oxygenated multifunctional compounds from substituted benzene oxidation, and hydroperoxides from several precursors) represent new candidate groups of compounds for
20 future toxicological screenings. These results demonstrate that coupling model-generated compositions to *in silico* toxicological screening might enable more comprehensive exploration of the mutagenic potential of specific SOA components.

1 Introduction

Ambient air pollution was ranked as the 7th highest risk factor for human health (Lim et al. 2012), being responsible for almost three billions deaths per year globally. Evidence for air pollution impacts on life expectancy and for cardiovascular and
25 respiratory illnesses has grown considerably in the last two decades (Beelen et al., 2014), and the ongoing global demographic and societal changes (ageing, urbanization) are projected to exacerbate atmospheric pollution health effects. Evidence from both short and long-term epidemiological effects of particulate matter with particle diameter below 10 μm or 2.5 μm (PM10 or PM2.5, respectively) is robust, with a range of possible policies aiming to mitigate PM health effects reflected by the possible sources from which PM arises.

Since at least the early 2000's, metrics for PM chemical composition and sources have been incorporated along with PM₁₀, PM_{2.5} or PM_{0.1} (ultrafine) in epidemiological studies. Recently, black carbon (BC), which is a proxy for primary combustion particles, was associated with an increased risk of mortality two times greater than for total PM (Janssen et al., 2011). Recent findings highlight that more detailed air quality metrics may be valuable in evaluating the health risks by specifically distinguishing between e.g. black carbon, secondary organic and inorganic aerosols (Cassee et al., 2013). A study in London, UK, suggested that certain particle components might be more important to specific diseases (Atkinson et al., 2010), some toxicological studies suggesting pulmonary and vascular inflammation as the relevant biological response mechanism (WHO 2013; HEI 2010). Despite this, the overarching developments of policy objectives remain to substantially reduce population exposure to PM_{2.5} as a whole. Stanek et al. (2011) provided a compilation and meta-analysis of 29 epidemiological and in vivo toxicological studies that explicitly investigated the statistical relationship between PM chemical composition and adverse health effects. They noted that, from a mechanistic perspective, it is highly plausible that the chemical composition of particulate matter (PM) would better predict health effects than other characteristics, such as PM mass or size (Stanek et al., 2011). However, their conclusion is that no consistent relationships have emerged so far: there is little evidence of a systematic association of specific groups of compounds with adverse effects across all studies. Possible reasons for the inconsistencies between epidemiological results are the paucity of studies, non-additive (synergic) effects of pollutants and study design issues: differences in the exposed populations, differences in pollution levels between environments, and differences in the air quality networks. In particular, the sets of PM chemical compounds measured by different monitoring networks overlap for only a few species: metals, inorganic ions, BC, possibly organic carbon (OC), with little or null information of OC molecular composition. West et al. (2016) note that epidemiologic studies should consider other air pollutants suspected to be important for health, such as polycyclic aromatic hydrocarbons (PAHs), metals, reactive oxygen species (ROS), and other chemical components of PM. These include classes of compounds defined by their reactivity (ROS) with unclear overlaps with the other compounds in the list (Zhang et al., 2008). More importantly, guidelines should be provided for the identification of possible targets (the compounds suspected to be important for health). A group of substances commonly monitored (PAHs and metals) has been extensively characterized by the toxicological research on combustion and industrial aerosols. On the other hand, the recent advances in the chemical characterization of ambient organic particles, including secondary organic aerosols (SOA) (Hallquist et al., 2009), have been exploited in PM toxicology only to a limited extent (Verma et al., 2015; Lakey et al., 2016). Given emerging evidence points towards the need for more information on associations between health impact potential and separate PM fractions, it is worthwhile evaluating the role of emerging technologies in extracting this information.

At present, only information on bulk SOA in vivo and in vitro biological effects is available (Delfino et al., 2008; Chen et al., 2011; Rohr et al., 2013; Saffari et al., 2015; Künzi et al., 2015). The lack of actual molecular targets for the SOA fraction responsible for its toxicity might pose limitations to the design of epidemiological studies incorporating specific SOA tracers. Moreover, while molecular identification of SOA compounds is normally achieved through the analysis of exact molecular masses and the interpretation of mass fragmentation spectra, the synthesis of authentic standards has been conducted to a very limited extent and for a few compounds. The lack of standards is critical for the determination of toxicity endpoints via classical

toxicological assays. Estimation of such endpoints can be pursued, however, on the basis of the sole chemical composition by means of *in silico* approaches, such as quantitative structure-activity relationships (QSARs). *In silico* toxicology is widely used in drug chemistry, especially as a screening before *in vitro* and *in vivo* assays. In particular, QSARs are theoretical models relying on a mathematical relationship (often a statistical correlation) between one or more quantitative parameters derived from chemical structure and a quantitative measure of a property or activity (e.g. a toxicological endpoint). The reliability of a QSAR prediction highly depends on the scientific validity of the employed QSAR model, which should fulfill the internationally recognized principles for QSAR validation as defined by OECD (OECD 2004, 2006, 2007), and the applicability domain of the QSAR model, i.e. whether the compound of interest is sufficiently similar to the compounds used to train the model (Netzeva et al., 2005; Sushko et al., 2010; Sahigara et al. 2012). So far, as noted previously, the use of *in silico* methods for the toxicological assessment of aerosol organic compounds has been limited and restricted to specific chemical classes like PAHs (Papa et al., 2008; Schwarz et al., 2014). In this study, we perform a proof of concept evaluation on the information retrieved from *in silico* methods specifically applied to a subset of measured compounds attributed to multiple sources of SOA. These compounds have been largely unexplored with regards to their toxicological properties, and the general evaluation of available *in silico* methods has hitherto not been carried out. The potential of such methods is considerable as, in principle, toxicity endpoints of aerosols can be generated directly from molecular compositions that can either be measured directly or predicted using state-of-the-art explicit chemistry models.

This evaluation is timely. In the past decade, the rapid development of aerosol mass spectrometric techniques of increasing resolution has boosted the molecular identification of SOA compounds (Laskin et al., 2012; Nozière et al., 2015). At the same time, mechanistic models predict the chemical composition of SOA with an increasing degree of sophistication by accounting for the movement of compounds between the gaseous and condensed phases at a molecular level. Despite uncertainties in fundamental parameters leading to variable mass and composition predictions (McVay et al., 2016), the amount of information available from both measurement and modelling technologies might be combined to start associating health impact potential with distinct groups of compounds. This is the driver for the study presented here.

2 Methods

A list of ~100 SOA compounds was identified for subsequent toxicity predictions using *in silico* (i.e. QSAR) methods. First, *in vitro* genotoxicity data as bacterial mutagenicity (Ames test) available for these compounds were searched for in several international databases. Following this, QSAR predictions for bacterial mutagenicity were performed for all the species, and the results were compared to the toxicity endpoints determined by *in vitro* methods (for the subset of compounds for which *in vitro* data could be found). Finally, the results of the toxicity predictions were interpreted using a cluster analysis of the compounds based on a suite of molecular descriptors: structural, mechanistic (in relation to express toxicity on humans), etc., for understanding inter and intra -class variability.

2.1 Organic compounds

A set of 104 compounds speciated according to biogenic and anthropogenic SOA was compiled for this proof of concept study (Table S1). They are organized in chemical classes defined as products originating from specific volatile organic compounds (VOCs). In order to keep the total number of compounds to ~100, a number of isomeric species have been filtered out. The list as a whole is an attempt to sample the greatly diverse chemical space of SOA species that can be derived from the vast literature on this subject. To create this list, exemplar molecular species were chosen to represent all relevant classes of SOA treated in recent review studies (Hallsquit et al., 2009; Nozière et al., 2015), which include the products of oxidation of isoprene, monoterpenes (α -pinene, β -pinene, Δ^3 -carene, *d*-limonene), sesquiterpenes, as well as of anthropogenic VOCs: substituted benzenes, alkenes and amines. SOA compounds from intermediate-volatility organic precursors (“IVOCs”: low-molecular weight PAHs, and C₁₂-C₁₆ *n*-alkanes) and examples of the products of heterogeneous (particle-phase) reactions of small carbonyls (glyoxal, methyl-glyoxal, pyruvic acid) are also included. Finally, highly-reactive compounds forming from radical chemistry, such as secondary ozonides, adducts of carbonyls on Criegee Intermediates (CI), and polyperoxides (“highly oxygenated organic molecules”, HOMs) are accounted for. A range of secondary organic compounds missing an unequivocal source assignment, such as oxalic or succinic acid, were excluded.

The list in Table S1 includes compounds of varying degrees of “identification factor” (“I factor”, (Nozière et al., 2015)): for only a fraction of them a chromatographic separation, confirmation with standards and quantification in real samples could be achieved. These include, for instance, most common α -pinene SOA tracers, including pinonic, hydroxyl-pinonic and pinic acids (mtr_05, mtr_06 and mtr_07 in Table S1), terpenyllic, terebic and diaterpenyllic acids (mtr_11, mtr_12 and mtr_13), 3-methyl-1,2,3-butanetricarboxylic (MBTCA) (mtr_17), together with their dimers (mtr_15, mtr_16), which can add up to 15% of SOA mass (Kristensen et al.; 2014). Other well-known SOA compounds included in the list account for the isoprene chemistry: methyl-tetrols (iso_01 in Table S1), C5-alkene triols (iso_04) and their sulfate esters (iso_02) are common constituents of organic aerosols in rural areas, where they can account for 12 – 14% of ambient particulate organic matter (Lin et al., 2013). Concentration data for anthropogenic compounds, such as quinones, oxo- and nitro-PAHs are also available in the literature (Chung et al., 2006; Huang et al., 2014). For most of the other organic compounds in the list, concentration data are simply not available. These include molecular species of uncertain identification, and whose molecular structures were inferred solely from the interpretation of mass spectra. It should be emphasized that this compound list was similarly formulated in order to maximize the diversity between molecular structures. As a consequence, the chemical classes that correspond to the most extensively studied laboratory SOA systems have more representative compounds than the SOA systems for which only a limited sets of conditions (e.g., photochemical) have been explored. Overall, the list of compounds was not designed to reproduce the entire molecular composition of SOA in any particular environment. In this sense, and to re-iterate the rationale behind this study, we use this list of compounds to demonstrate the information that can be extracted from an *in silico* assessment of the mutagenic activity of the main classes of anthropogenic and biogenic atmospheric SOA.

The computational workflow set up for this proof of concept can be further extended to even more chemical classes and toxicological endpoints.

2.2 Toxicity endpoints

Standard *in silico* methods employed in pharmaceuticals as well as in chemical risk assessment enable the prediction of a variety of toxicological and eco-toxicological endpoints, such as skin and eye irritation, acute toxicity, genotoxicity, reproductive toxicity, aquatic toxicity etc. (Worth et al., 2011; Benfenati et al., 2016; ECHA 2016). The present study focuses only on genotoxicity, because a) it can be associated to any kind of exposure, b) it can be induced also at very small doses, c) it leads to adverse health effects on humans upon chronic exposures (and the increase in mortality risk for PM is much higher for chronic than for short-term exposures (Pope et al. 2011)), d) it is a well-studied endpoint with defined mechanisms of action (Worth et al., 2011; Schultz et al., 2015). Most importantly, genotoxicity has been characterized in real PM samples and it is subject to monitoring in several countries (e.g., Cassoni et al., 2004). **Past studies have attempted to apportion total PM mutagenicity among distinct primary organic aerosol sources (Hannigan et al., 2005). No such exercise has been carried out yet for SOA sources, in spite of the fact the mutagenicity of SOA have been demonstrated at least 30 years ago (Kamens et al., 1984). It is worth reminding that mutagenicity is only one of the possible toxicological endpoints for atmospheric particulate organic compounds, and that the same classes of compounds can be more or less toxic depending on the specific endpoints (e.g., mutagenicity vs. cytotoxicity, etc.) (Filep et al., 2015).**

In this study, QSAR predictions focus on Salmonella *in vitro* mutagenicity (Ames test). **It should be noted that screening tests performed on strains of prokaryotes, such as the Ames test, are not meant to mimic exposure conditions for humans. They are used instead in the preliminary phases of hazard assessment, and especially for hazard identification.**

20 2.3 Collection of experimental *in vitro* genotoxicity data

In this study, a search for *in vitro* mutagenicity (Ames test) data available for the 104 aerosol compounds was performed with the support of the OECD QSAR Toolbox (OECD 2009, 2013, 2015; detailed description of the tool is provided in SI - Table S2). The Toolbox is connected to a variety of toxicity databases and allows for gathering experimental data for the target compounds. The following databases were enquired for *in vitro* mutagenicity (Ames test) data: Bacterial mutagenicity ISSSTY (by Istituto Superiore di Sanità), which includes TOXNET databases, Carcinogenic Potency Database (CPDB), ECHA CHEM, Toxicity Japan MHLW (EXCHEM), Genotoxicity OASIS, Carcinogenicity & Mutagenicity ISSCAN. A brief explanation of the screened databases is provided as Supporting Information (Table S3).

2.4 QSAR prediction of *in vitro* genotoxicity

The 104 aerosol compounds were then screened for their mutagenic potential by means of QSAR predictions. QSAR predictions for Salmonella *in vitro* mutagenicity (Ames test) were generated by employing two models: i) ACD/Impurity Profiling model for Salmonella composite, implemented in the commercial software ACD/Labs Percepta (ACD/Percepta,

release 2015, www.acdlabs.com), and ii) Vega/CAESAR Mutagenicity model, implemented in the open platform VegaNIC (VegaNIC Application ver. 1.1.0, <http://www.vega-qsar.eu/download.html>). The two models, which fulfill the OECD principles for QSAR scientific validity, provide qualitative predictions for genotoxicity as Ames test (e.g., positive/negative) supported with specific parameters providing information on prediction reliability. Detailed information on the employed models are provided as Supporting Information (Table S4). The two QSAR models were used in order to complement each other. In case of conflicting reliable predictions, the most conservative health impact potential, i.e. positive prediction, was assigned.

2.5 Cluster analysis

A workflow for clustering the 104 aerosol compounds was designed with the aim to group compounds into clusters sharing similar physico-chemical, structural and mechanistic profile (Figure S1). The workflow was automatized using the KNIME Analytics Platform (Berthold et al., 2007) and is described below:

Step 1. Descriptors accounting for physico-chemical, molecular and reactivity properties: **LogP (Log octanol-water partition coefficient)**, **WS (water solubility)**, **MW (molecular weight)**, **TPSA (total polar surface area)**, **nHBDon (number of donor atoms for hydrogen bonds)**, **nHBAcc (number of acceptor atoms for hydrogen bonds)**, **HOMO (highest occupied molecular orbital)**, **LUMO (lowest unoccupied molecular orbital)**, etc.... These were calculated using PaDEL-Descriptors (Yap et al., 2011), ACD/Percepta, and Schrödinger software (Schrödinger Suite 2014 Update 3 Release, Maestro v9.9.013 - Sep 2014, evaluation copy). Bidimensional molecular descriptors (e.g., constitutional, topological, shape descriptors) were also calculated using PaDEL-Descriptors. Descriptors providing mechanistic information for genotoxic activity were derived using five genotoxicity profilers implemented in the OECD QSAR Toolbox (Table S2). In particular, the employed profilers identify in the target chemicals the presence of structural alerts for DNA binding or mutagenicity, with or without the associated MoA (mechanism of action) responsible for genotoxic activity. The outcome of the profilers was used to derive the mechanistic descriptors, which consisted in binary variables indicating the presence (1) or absence (0) of a structural alert (with or without MoA information). A total of 138 descriptors, including 18 physico-chemical descriptors, 70 structural descriptors and 50 mechanistic descriptors, was calculated. The complete list of calculated descriptors and their description is provided in Supporting Information (Table S5).

Step 2. To reduce redundant and non-useful information, constant descriptors (i.e., descriptors having the same value for all the compounds) and pair-correlated descriptors (i.e., descriptors with pair-wise correlation >0.8) among the physico-chemical and structural descriptors were removed. The initial set of 138 descriptors was reduced to 75 variables, including all 50 mechanistic descriptors, 8 PC descriptors and 17 structural descriptors (Table S5). Mechanistic descriptors were binary variables (0,1), while physico-chemical and structural descriptors were continuous variables. Thus, to analyse the three groups of descriptors in combination, continuous variables (i.e., physico-chemical and structural descriptors) were normalised within [0-1] range.

Step 3. A Principal Component Analysis (PCA) was performed on the final set of 75 descriptors to extract important information encoded by physico-chemical, structural and mechanistic properties and to reduce the number of input variables used for cluster analysis. The PCA was carried out in KNIME employing specific nodes for PCA (i.e., PCA compute and PCA apply), based on covariance matrix implementation method. The first 13 principal components (PCs) of the PCA were maintained, which preserved 85% of the information encoded in the original variables.

Step 4. Hierarchical clustering of the 104 compounds was performed using as input variables the first 13 principal components (PCs) of the PCA (see Step 3). An agglomerative clustering was performed employing the Hierarchical clustering KNIME node (metric: Euclidean distance; linkage criterion: average linkage). The number of clusters was set a priori ($n = 10$).

10 3 Results

3.1 Screening for genotoxicity – experimental data

Experimental data for Salmonella in vitro mutagenicity (Ames test) was available for 13 out of 104 aerosol compounds (i.e., $\approx 13\%$). These compounds include aromatic oxygenated compounds (found among the PAH-SOA constituents) and N-nitrosamines (formed by amine oxidation and NO addition). A summary of retrieved data is provided in Table 1. Detailed information on the retrieved experimental data are reported in Supporting Information (Table S6). Overall, it was noted that consistent negative experimental Ames test data (conducted on different Salmonella strains, with and without metabolic activation) are available for four compounds (two substituted benzene SOA compounds, and two from PAH-SOA). In addition, consistent positive experimental Ames test data (different Salmonella strains, with and without metabolic activation) are available for two compounds (one PAH-SOA compound, and one amine-SOA), while for the remaining seven compounds, both positive and negative Ames test results are reported, based on the Salmonella strain and testing conditions used.

3.2 Screening for genotoxicity – QSAR predicted data

In silico predictions for Salmonella in vitro mutagenicity were generated for the 104 studied aerosols employing two QSAR models, i.e. ACD/Percepta Impurity (Salmonella composite) model and Vega/CAESAR Mutagenicity model. Predictions obtained by the two QSARs were combined taking into account the applicability domain of each model and consistency between predictions (Table S7). Predictions were assessed for their reliability and different levels of confidence were assigned (“not”, “borderline”, “moderate” and “high” reliable predictions). Overall, the predictions generated by the two QSAR models were in agreement for the majority of compounds (not consistent predictions obtained for only 4 compounds out of the 53 reliably predicted by both QSAR models) and the combination of the two tools resulted successful in covering a wider chemical space (59% of compounds reliably predicted by ACD/Percepta alone, 74% of compounds reliably predicted by Vega/CAESAR alone, 82% of compounds reliably predicted combining the two QSAR models). As far the 4 compounds with opposite predictions are concerned, this is mainly due to issues related to the different applicability domain of the two QSAR models.

Based on the combined predictions, 53 compounds were predicted as negative, 32 as positive (including four compounds with conflicting reliable predictions), while for 19 compounds QSAR predictions were assessed as “indeterminate” (i.e., not reliable or equivocal predictions) (Table 2).

It is worth noting that 30% of the compounds were screened as genotoxic by at least one of the two QSAR models employed in this study. These genotoxic SOA components included all five N-nitrosamines from amine atmospheric oxidation, but none of the compounds chosen to represent isoprene-SOA, sesquiterpene-SOA or (among the anthropogenic) alkane photo-oxidation SOA. On the other hand, the relative large classes of compounds in the list correspondent to monoterpene-SOA, substituted benzene SOA and PAH-SOA included both several genotoxic and non-genotoxic species. In the following section, we provide clues for understanding inter- and intra-class variability.

10 3.3 Cluster analysis

The use of cluster analysis is two fold: 1) Identify genotoxicity trends within clusters, and 2) Identify gaps in experimental and *in silico* genotoxicity data. Hierarchical clustering initially resulted in 10 clusters, each characterised by a different composition in terms of number of compounds belonging to each cluster and the SOA class of each compound. Due to the high number of the members included in Cluster no. 9, an additional hierarchical clustering was performed on this cluster, which leads to four sub-clusters which are reported as such in proceeding analysis. Therefore, a total of 13 clusters were generated, as illustrated in Table S9. The 104 compounds are also visualised in the PCA score plots (Figure 1), where compounds are displayed in the space described by the first two principal components (PCs) and marked with different colours based on SOA classes and on clusters. The corresponding loading plot can be found in Figure S2.

The figure shows that SOA classes with compounds located in confined regions of the PCA score plots often correspond to individual clusters (e.g., amine-SOA with Cluster 2), while monoterpene-, substituted benzene- and PAH-SOA intercept more clusters and show diverse behaviours in the space described by the first two PCs. The distribution of compounds in the score plots of the first two PCs, which explain 35% of the total variance, is primarily based on the mechanistic descriptors. In particular, compounds with no or few structural alerts for genotoxicity (e.g., compounds belonging to cluster 9) are displayed in the bottom-right part of the plots (Figure S2), while the remaining compounds exhibit different alerts and mechanisms for genotoxicity. In particular, typical descriptors for the clusters characterized by high values of the second PC (clusters #7 and #8) are: radical reactivity via ROS formation, occurrence of hydroperoxides and of “H-acceptor-path3-H-acceptor” substructures (= two hydrogen acceptors at a distance of three atoms) (Figure S2). The role of other relevant descriptors (e.g., lipophilicity, electronic descriptors etc.) in the distribution of compounds among different clusters becomes more evident when analyzing the other principal components.

Within each cluster, SOA compounds were analysed in terms of structural and physico-chemical properties, mechanistic profile and mutagenic potential. The analysis and results for each cluster are provided in Supporting Information (Table S8a,b) but we present the overarching results in the following. In general, SOA classes (defined on the nature of precursors) show a variable degree of overlap with the clusters: there are some well populated clusters that include multiple SOA classes, in

particular sub cluster 9-3, including many aliphatic compounds from isoprene, dicarbonyls, monoterpene and substituted benzene oxidation, and which are mostly non mutagenic. On the other hand, there are distinct clusters of mutagenic or non-mutagenic compounds that are associated to specific SOA systems (Figure 2) (e.g., Cluster 1: mutagenic quinones from PAH oxidation, and sub Cluster 9-1: alkane-SOA, all non-mutagenic). By combining the information on compounds and properties from the clusters (encoding physico-chemical, structural and mechanistic descriptors of the compounds) with that of sources (specific SOA systems) (Table S9), we can list the following groups of mutagenic compounds (with characteristics of their mechanistic profile provided in parenthesis):

- Cluster 0: 5-membered-lactones in monoterpene SOA (alkylation via Michael-type addition of 5-membered-lactone as well as via ring opening SN2 reaction)
- Cluster 1: quinones from low MW PAHs oxidation (DNA intercalation, radical reaction mechanism via ROS formation, Michael addition to quinoid structure)
- Cluster 2: N-nitrosamines (nucleophilic attack after nitrenium or carbenium ion formation)
- Cluster 3: hydroxy- and nitro-PAHs (several mechanisms, including DNA intercalation, ROS formation, and nucleophilic attack after reduction and nitrenium ion formation). The same cluster includes one glyoxal-imidazole, for which no reliable QSAR prediction was obtained. For this compound experimental testing for mutagenicity is recommended.
- Cluster 7: non-aromatic products of substituted benzene oxidation (several mechanisms including ROS formation, alkylation by epoxides, and reactions involving a H-acceptor-path3-H-acceptor). This cluster also includes a formaldehyde -CI adduct formed by monoterpene ozonolysis..
- Cluster 8 (only 5/13 mutagenic): alkyl hydroperoxides and peroxyacids from substituted benzene, alkene and monoterpene oxidation (ROS generation, H-acceptor-path3-H-acceptor reactions), and one 5-membered-lactone in substituted benzene SOA (alkylation via Michael-type addition of 5-membered-lactone).

Other classes of mutagenic compounds were found within sub Cluster 9-2 (nitro- and oxo-PAHs) and sub Cluster 9-3 (only three compounds with heterogeneous descriptors). The abundance of mutagenic species in each cluster is graphically represented in Fig. 2 together with examples of molecular structures of representative mutagenic compounds in each of the major clusters and SOA types.

Note that not all compounds carrying genotoxicity alerts were predicted to be genotoxic from the QSARs. For instance, monoterpene-SOA dimers bearing ozonide bonds (Cluster 6) are likely precursors of ROS, but were predicted to be non-genotoxic with a moderate to high reliability. It is worth remembering that the mechanistic profile (e.g., the presence of reactive functional groups in a molecule) must be considered a collection of alerts, and that the mutagenicity depends on all the characteristics of the specific compound, which affect and modulate the actual expression of the alerts.

The six clusters of compounds listed above, accounting for 26 of the 32 compounds predicted mutagenic by the QSAR analysis, include three clusters representing groups of compounds with well-known toxicological properties: N-nitrosamines (Cluster 2), hydroxy- and nitro-PAHs (Cluster 3) and quinones (Cluster 1). **These clusters encompass organic compounds with clear**

source fingerprints (from PAHs and amine oxidation and reaction with NO_x) and that are found in polluted environments in concentrations ranging from 10⁻³ to 10⁰ ng m⁻³ (Chung et al., 2006; Huang et al., 2014; Farren et al. 2015) (Table S10). Nitro- and oxo-aromatic compounds are by far the best studied mutagenic components of SOA (e.g., Enya et al., 1997; Di Filippo et al. 2007; Huang et al., 2014). They can form upon oxidation of primary organic compounds which can be also mutagenic (PAHs) or that are just non toxic, such as methoxy-phenols in biomass burning emissions (Kroflíč et al., 2015).

The other three clusters encompass oxygenated organic compounds that, in our knowledge, have been ignored so far in mutagenicity studies of atmospheric aerosol compounds. They could contribute to explain the yet unknown composition of the most polar fraction of mutagenic compounds in atmospheric aerosol (Barale et al., 1994; Gutiérrez-Castillo et al. 2006; Palacio et al., 2016). First, 5-membered lactones formed by the oxidation of monoterpenes are alkylating agent and possibly mutagenic (Cluster 0). These include terpenyllic acid, which occur in concentrations of 6 to 15 ng m⁻³ in polluted rural areas (Kristensen et al., 2013; Brüggemann et al., 2017). A second class (Cluster 7) of mutagenic compounds encompass multi-functional oxygenated non-aromatic species (including epoxides) formed from the oxidation of substituted benzenes as well as the products of addition of formaldehyde to monoterpene Criegee Intermediates. Finally, a more heterogeneous class of SOA compounds originating from both biogenic (monoterpenes) and anthropogenic VOCs (substituted benzenes, alkenes) and characterized by the presence of certain functionalities like hydroperoxy-, peroxy-acidic groups or H-acceptor-path3-acceptor structures account for mutagenic compounds in Cluster 8. No experimental data are available for the ambient concentrations of organic compounds belonging to Clusters 7 or 8. However, SOA modelling studies indicate that they can account for a significant fraction of SOA mass formed by substituted benzene oxidation (Ruggeri et al., 2016), which translates into ambient concentrations of several ng m⁻³ in the very polluted environments (such as in Yuan et al., 2013) (Table S10). The formation of endoperoxides and epoxides and of other reactive oxygenated organic compounds such as those in Clusters 7 and 8 was hypothesized by Fu et al. (2012) to explain the DNA damage caused by photodegraded aromatic compounds. Moreover, the finding of biogenic SOA components with potential mutagenic properties (Cluster 0, and some compounds in Clusters 7 and 8) is in agreement with the results of Alves et al. (2016), showing that mutagenicity of the aerosol is enhanced when pollution get mixed with emissions in rural areas. The overall picture, however, is that a greater proportion of mutagenic compounds was predicted to occur in the anthropogenic SOA classes than in the biogenic ones (Figure 2). Exceptions are alkane SOA which were predicted to be non-mutagenic, and some of the monoterpene SOA which instead showed mutagenic activity on the basis of our QSAR approach. These results show a very good, qualitative overlap with the findings of Verma et al. (2015) and Tuet et al. (2017) about the redox potentials of specific classes of SOA. Although ROS expression is only one of the possible mechanisms of action responsible for mutagenicity, it characterizes several of the compounds predicted to be mutagenic in our study, especially in Clusters 1, 3, 7 and 8. The formation of ROS in atmospheric SOA and the genotoxic effect of oxidative stress caused to PM exposure have been documented by a number of studies (e.g., Risom et al., 2005; Valavanidis et al. 2008; Rattanavaraha et al., 2011; Oh et al., 2011; Fu et al., 2012).

As a final remark, it should be noticed that the vast sets of molecular structures emerging from the laboratory experiments on the best-studied SOA systems (such as monoterpene and substituted benzene SOA) include compounds of very diverse

chemical and biological activity. As a corollary, it is possible that expanding the chemical characterization of the SOA components from other VOC precursors will lead to the identification of new potential mutagens. In summary, the identification of new classes of compounds with predicted mutagenic activity calls for more research on the identification and quantification of such compounds in ambient aerosol samples, as well as on the toxicological properties of the specific SOA systems, and to precautions in the laboratories involved in the synthesis of SOA tracer compounds (Nozière et al., 2015).

4 Conclusions

The present study represents a hitherto unexplored proof-of-concept investigation that *in silico* methods might be successfully employed to support safety assessment of atmospheric aerosol organic compounds through the screening and prioritization of the most hazardous ones based solely on the information of the chemical structure. In addition, the clustering of chemicals into small groups sharing common structural features, physico-chemical properties and mechanistic profiles, set the basis for the application of the read-across approach, where few representative compounds are tested and the outcome is extrapolated to similar compounds. The results, based on a list of ~100 individual organic compounds representative of the most important anthropogenic and biogenic SOA classes, indicate that diverse groupings of molecular species exerting specific genotoxic effects might be of important, and among these are classes of SOA compounds previously unexplored for their mutagenic potential. These includes 5-membered lactones and a heterogeneous class of oxygenated compounds with epoxide, peroxide functionalities and promoting ROS expression. Since, in our list of 104 compounds, redox-active species with mutagenic properties are more frequently found in SOA formed by the degradation of aromatic VOCs, the apportionment of genotoxic compounds among SOA classes shows an overall dominance of anthropogenic over biogenic species (Figure 2). Therefore these results, based on a first application of *in silico* methods on a limited number of molecular species, are in qualitative agreement with the existing general knowledge of aerosol health effects indicating a more robust association of combustion generated aerosols with adverse outcomes with respect to other aerosol sources (Delfino et al., 2009).

The usefulness of *in silico* methods stands in their ability in screening vast sets of aerosol organic substances. In principle this would include a much larger set than the 104 compounds considered here, whilst at the same time providing directions for implementing targeted *in vitro* (and *in vivo*) toxicological assessments on specific compounds. As already noted, there have been significant advances in atmospheric aerosol modelling and instrumentation. We cannot measure every compound in atmospheric aerosol. As mechanistic models have the potential to predict concentrations of many millions of compounds (McVat et al., 2016), even with uncertain process descriptions, the challenge is how to use this growing amount of information in a meaningful way. As we continually identify and hypothesize new processes and compounds deemed important for better understanding aerosol transformation and impacts, employing automated *in silico* methodologies offers the opportunity to replicate this proof of concept study with many simulations of a changing environment.

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References

- Alves, D. K. M.; Kummrow, F.; Cardoso, A. A.; Morales, D. A.; Umbuzeiro, G. A., Mutagenicity Profile of Atmospheric Particulate Matter in a Small Urban Center Subjected to Airborne Emission From Vehicle Traffic and Sugar Cane Burning, *Environmental and Molecular Mutagenesis*, 57, 41-50, 2016.
- Atkinson, R.W.; Fuller, G.W.; Anderson, H.R.; Harrison, R.M.; Armstrong, B. Urban ambient particle metrics and health: a time-series analysis. *Epidemiology*, 21, 501–11, 2010.
- Barale, R.; Giromini, L.; Del Ry, S.; Barnini, B.; Bulleri, M.; Barrai, I.; Valerio, F.; Pala, M.; and He, J., Chemical and Mutagenic Patterns of Airborne Particulate Matter Collected in 17 Italian Towns, *Environ. Health Perspect.*, 102 (Suppl. 4), 67- 73, 1994.
- Beelen, R.; Raaschou-Nielsen, O.; Stafoggia, M.; Andersen, Z.J.; Weinmayr, G.; Hoffmann, B.; Wolf, K.; Samoli, E.; Fischer, P.; Nieuwenhuijsen, M.; Vineis, P.; Xun, W.W.; Katsouyanni, K.; Dimakopoulou, K.; Oudin, A.; Forsberg, B.; Modig, L.; Havulinna, A.S.; Lanki, T.; Turunen, A.; Oftedal, B.; Nystad, W.; Nafstad, P.; De Faire, U.; Pedersen, N. L.; Östenson, C. G.; Fratiglioni, L.; Penell, J.; Korek, M.; Pershagen, G.; Eriksen, K. T.; Overvad, K.; Ellermann, T.; Eeftens, M.; Peeters, P. H.; Meliefste, K.; Wang, M.; Bueno-de-Mesquita, B.; Sugiri, D.; Krämer, U.; Heinrich, J.; de Hoogh, K.; Key, T.; Peters, A.; Hampel, R.; Concin, H.; Nagel, G.; Ineichen, A.; Schaffner, E.; Probst-Hensch, N.; Künzli, N.; Schindler, C.; Schikowski, T.; Adam, M.; Phuleria, H.; Vilier, A.; Clavel-Chapelon, F.; Declercq, C.; Grioni, S.; Krogh, V.; Tsai, M. Y.; Ricceri, F.; Sacerdote, C.; Galassi, C.; Migliore, E.; Ranzi, A.; Cesaroni, G.; Badaloni, C.; Forastiere, F.; Tamayo, I.; Amiano, P.; Dorronsoro, M.; Katsoulis, M.; Trichopoulou, A.; Brunekreef, B.; Hoek, G. Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project. *Lancet*, 383, 785–795, 2014.
- Benfenati, E. *In Silico* Methods for Predicting Drug Toxicity; In: *Methods in Molecular Biology*; Vol 1425. Humana press - Springer Science+Business Media: New York; 2016.
- Berthold, M. R.; Cebon, N.; Dill, F.; Gabriel, T.R.; Kötter, T.; Meinl, T.; Ohl, P.; Sieb, C.; Thiel, K.; Wiswedel, B. KNIME: The Konstanz Information Miner. In *Studies in Classification; Data Analysis; and Knowledge Organization (GfKL 2007)*; Springer.
- Brüggemann, M.; Poulain, L.; Held, A.; Stelzer, T.; Zuth, C.; Richters, S.; Mutzel, A.; van Pinxteren, D.; Iinuma, Y.; Katkevica, S.; Rabe, R.; Herrmann, H.; and Hoffmann, T., Real-time detection of highly oxidized organosulfates and BSOA marker compounds during the F-BEACH 2014 field study, *Atmos. Chem. Phys.*, 17, 1453–1469, 2017.
- Cassee, F. R.; Héroux, M.-E.; Gerlofs-Nijland, M. E.; Kelly, F. J. Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission. *Inhal Toxicol.*, 25, 802 – 812, 2013.

- Cassoni, F.; Bocchi, C.; Martino, A.; Pinto, G.; Fontana, F.; Buschini, A. The Salmonella mutagenicity of urban airborne particulate matter (PM_{2.5}) from eight sites of the Emilia-Romagna regional monitoring network (Italy). *Sci. Tot. Environ.*, 324, 79-90, 2004.
- Chen, X.; Hopke, P. K.; Carter, W. P. L. Secondary organic aerosol from ozonolysis of biogenic volatile organic compounds: chamber studies of particle and reactive oxygen species formation. *Environ. Sci. Technol.*, 45, 276–282, 2011.
- Chung, M. Y.; Lazaro, R. A.; Lim, D.; Jackson, J.; Lyon, J.; Rendulic, D., and Hasson, A. S., Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts, *Environ. Sci. Technol.*, 40, 4880-4886. 2006.
- Delfino, R.J.; Staimer, N.; Tjoa, T.; Polidori, A.; Arhami, M.; Gillen, D. et al. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with urban air pollution in elderly subjects with a history of coronary artery disease. *Environ. Health Perspect.*, 116, 898–906, 2008.
- Delfino, R. J.; Staimer, N.; Tjoa, T.; Gillen, D.L.; Polidori, A.; Arhami, M.; Kleinman, M. T.; Vaziri, N. D.; Longhurst, J.; and Sioutas, C.; Air Pollution Exposures and Circulating Biomarkers of Effect in a Susceptible Population: Clues to Potential Causal Component Mixtures and Mechanisms, *Environ. Health. Perspect.*, 117, 152-156, 2009.
- Di Filippo, P.; Riccardi, C.; Gariazzo, C.; Incoronato, F.; Pomata, D.; Spicaglia, S.; and Cecinato, A. , Air pollutants and the characterization of the organic content of aerosol particles in a mixed industrial/semi-rural area in central Italy, *J. Environ. Monit.*, 9, 275-282, 2007.
- ECHA - European Chemicals Agency (2016) Practical Guide 5: How to use and report (Q)SARs. ECHA-16-B-09-EN. DOI 10.2823/81818. Available at: https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf
- Enya, T.; Suzuki, H.; Watanabe, T.; Hirayama, T.; and Hisamatsu, Y., 3-Nitrobenzanthrone, a Powerful Bacterial Mutagen and Suspected Human Carcinogen Found in Diesel Exhaust and Airborne Particulates, *Environ. Sci. Technol.*, 31 (10), 2772–2776, 1997.
- Farren, N. J.; Ramirez, N., Lee, J. D.; Finessi, E.; Lewis, A. C.; and Hamilton, J. F., Estimated exposure risks from carcinogenic nitrosamines in urban airborne particulate matter, *Environ. Sci. Technol.*, 49, 9648-9656, 2015.
- Filep, A.; Drinovec, L., Palagyi, A.; Manczinger, L.; Vagvolgyi, C.; Bozoki, Z.; Hitzengerger, R.; and Szabo, G., Source Specific Cyto- and Genotoxicity of Atmospheric Aerosol Samples , *Aerosol and Air Quality Research*, 15: 2325–2331, 2015.
- Fu, P. P.; Xia, Q.; Sun, X.; and Yu, H., Phototoxicity and Environmental Transformation of Polycyclic Aromatic Hydrocarbons (PAHs)—Light-Induced Reactive Oxygen Species, Lipid Peroxidation, and DNA Damage, *Journal of Environmental Science, Journal of Environmental Science and Health, Part C*, 30, 1-41, 2012.
- Gutiérrez-Castillo, M. E.; Roubicek, D. A.; Cebrian-Garcia, M. E.; De Vizcaya-Ruiz, A.; Sordo-Cedeno, M.; and Ostrosky-Wegman, P., Effect of Chemical Composition on the Induction of DNA Damage by Urban Airborne Particulate Matter, *Environmental and Molecular Mutagenesis*, 47, 199-211, 2006.
- Hallquist, M. ; Wenger, J. C.; Baltensperger, U.; Rudich, Y.; Simpson, D.; Claeys, M.; Dommen, J.; Donahue, N. M.; George, C.; Goldstein, A. H.; Hamilton, J. F.; Herrmann, H.; Hoffmann, T.; Iinuma, Y.; Jang, M.; Jenkin, M. E.; Jimenez, J. L.; Kiendler-Scharr, A.; Maenhaut, W.; McFiggans, G.; Mentel, T. F.; Monod, A.; Prévôt, A. S. H.; Seinfeld, J. H.; Surratt, J. D.;

- Szmigielski, R.; Wildt, J. The formation, properties and impact of secondary organic aerosol: current and emerging issues. *Atmos. Chem. Phys.*, 9, 5155-5236, 2009.
- Hannigan, M. P.; Rusby Jr., W. F.; Cass, G. R.; Source Contributions to the Mutagenicity of Urban Particulate Air Pollution *J. Air & Waste Manage. Assoc.* 55, 399-410, 2005.
- 5 HEI Panel on the Health Effects of Traffic-Related Air Pollution. Traffic-related air pollution: a critical review of the literature on emissions; exposure; and health effects. Boston: Health Effects Institute; 2010. Special Report 17.
- Huang, B.; Liu, M.; Bi, X.; Chaemfa, C.; Ren, Z.; Wang, X.; Sheng, G.; and Fu, J., Phase distribution, sources and risk assessment of PAHs, NPAHs and OPAHs in a rural site of Pearl River Delta region, China, *Atmos. Poll. Res.*, 5, 210 - 218, 2014.
- 10 Janssen, N. A.; Hoek, G.; Simic-Lawson, M.; Fischer, P.; van Bree, L.; ten Brink, H.; Keuken, M.; Atkinson, R. W.; Anderson, H. R.; Brunekreef, B.; Cassee, F. R. Black carbon as an additional indicator of the adverse health effects of airborne particles compared with PM10 and PM2.5. *Environ. Health Perspect.* 2011, 119, 1691–1699, 2011.
- Kamens, R. M.; Rives, G. D.; Perry, J. M.; Bell, D. A.; Paylor Jr. R. F.; Goodman, R. G.; and Claxton, L. D., Mutagenic changes in dilute wood smoke as it ages and reacts with ozone and nitrogen dioxide: an outdoor chamber study. *Environ. Sci. Technol.*, 18, 523 - 530, 1984.
- 15 Kristensen, K.; Enggrob, K. L.; King, S. M.; Worton, D. R.; Platt, S. M.; Mortensen, R.; Rosenoern, T.; Surratt, J. D.; Bilde, M.; Goldstein, A. H.; and Glasius, M., Formation and occurrence of dimer esters of pinene oxidation products in atmospheric aerosols, *Atmos. Chem. Phys.*, 13, 3763–3776, 2013.
- Kristensen, K.; Cui, T.; Zhang, H.; Gold, A.; Glasius, M., and Surratt, J. D.; Dimers in α -pinene secondary organic aerosol: effect of hydroxyl radical, ozone, relative humidity and aerosol acidity, *Atmos. Chem. Phys.*, 14, 4201–4218, 2014.
- 20 Kroflic, A.; Grilc, M.; and Grgic, I., Does toxicity of aromatic pollutants increase under remote atmospheric conditions?, *Scientific Reports* 5, Article number: 8859, 2015.
- Künzi, L.; Krapf, M.; Daher, N.; Dommen, J.; Jeannet, N.; Schneider, S.; Platt, S.; Slowik, J. G.; Baumlin, N.; Salathe, M.; Prévôt, A. S. H.; Kalberer, M.; Strähl, C.; Dümmbgen, L.; Sioutas, C.; Baltensperger, U.; Geiser, M. Toxicity of aged gasoline exhaust particles to normal and diseased airway epithelia. *Sci. Rep.*, 5, 11801, 2015.
- 25 Lakey, P. S. J.; Berkemeier, T.; Tong, H.; Arangio, A. M.; Lucas, K.; Pöschl, U.; Shiraiwa, M., Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract. *Sci. Rep.*, 6, 32916, 2016.
- Laskin, A.; Nizkorodov, S. A.; Laskin, J. Mass spectrometric approaches for chemical characterization of atmospheric aerosols: critical review of the most recent advances. *Environ. Chem.*, 9, 163–189, 2012.
- 30 Lim, S. S.; Vos, T.; Flaxman, A. D.; Danaei, G.; Shibuya, K.; Adair-Rohani, H.; AlMazroa, M. A.; Amann, M.; Anderson, H. R.; Andrews, K. G.; Aryee, M.; et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380, 2224-60, 2012.

- Lin, Y. -H.; Knipping, E. M.; Edgerton, E. S.; Shaw, S. L., and Surratt, J. D.; Investigating the influences of SO₂ and NH₃ levels on isoprene-derived secondary organic aerosol formation using conditional sampling approaches, *Atmos. Chem. Phys.* 13, 8457–8470, 2013.
- McVay, R. C.; Zhang, X.; Aumont, B.; Valorso, R.; Camredon, M.; La, Y. S.; Wennberg, P. O.; Seinfeld, J. H. SOA formation from the photooxidation of α -pinene: systematic exploration of the simulation of chamber data. *Atmos. Chem. Phys.*, 16, 2785-2802, 2016.
- Netzeva, T. I.; Worth, A. P.; Aldenberg, T.; Benigni, R.; Cronin, M. T. D.; Gramatica, P.; Jaworska, J. S.; Kahn, S.; Klopman, G.; Marchant, C. A.; Myatt, G.; Nikolova-Jeliazkova, N.; Patlewicz, G. Y.; Perkins, R.; Roberts, D.W.; Schultz, T. W.; Stanton, D. T.; van de Sandt, J. J.M.; Tong, W.; Veith, G.; Yang, C. Current status of methods for defining the applicability domain of (quantitative) structure-activity relationships. The report and recommendations of ECVAM Workshop 52. *Alternatives to Laboratory Animals (ATLA)*, 33, 155–173, 2005.
- Nozière, B.; Kalberer, M.; Claeys, M.; Allan, J.; D’Anna, B.; Decesari, S.; Finessi, E.; Glasius, M.; Grgić, I.; Hamilton, J. F.; Hoffmann, T.; Iinuma, Y.; Jaoui, M.; Kahnt, A.; Kampf, C. J.; Kourchev, I.; Maenhaut, W.; Marsden, N.; Saarikoski, S.; Schnelle-Kreis, J.; Surratt, J. D.; Szidat, S.; Szmigielski, R.; Wisthaler, A. The Molecular Identification of Organic Compounds in the Atmosphere: State of the Art and Challenges. *Chem. Rev.*, 115, 3919–3983, 2015.
- OECD: Organization for Economic Cooperation and Development (OECD) OECD series on testing and assessment. No. 49. The report from the expert group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the principles for the validation of (Q)SARs. ENV/JM/MONO(2004)24. 2nd Meeting of the ad hoc Expert Group on QSARs. OECD Headquarters; 20-21 September; 2004. Available at: [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono\(2004\)24](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2004)24)
- OECD - Organization for Economic Co-operation and Development (2006) Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals. OECD Environment Health and Safety Publications; Series on Testing and Assessment No. 58. ENV/JM/MONO, 2006, 25. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2006\)25&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2006)25&doclanguage=en). Accessed 11 May 2015
- OECD - Organization for Economic Co-operation and Development (2007). Guidance document on the Validation of (Quantitative) Structure Activity Relationship [(Q)SAR] Models. OECD Environment Health and Safety Publications; Series on Testing and Assessment No. 69. ENV/JM/MONO, 2007, 2. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2007\)2&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2007)2&doclanguage=en). Accessed 11 April 2016
- OECD: Organization for Economic Co-operation and Development (2009) Guidance document for using the OECD (Q)SAR Application Toolbox to develop chemical categories according to the OECD Guidance on Grouping of Chemicals. OECD

- Environment Health and Safety Publications; Series on Testing and Assessment No. 102. ENV/JM/MONO, 2009, 5.
[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono\(2009\)5](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2009)5).
- OECD: Organization for Economic Co-operation and Development (2013); User Manual based on QSAR Toolbox version 3.0. Strategies for grouping chemicals to fill data gaps to assess genetic toxicity and genotoxic carcinogenicity.
5 <http://www.oecd.org/env/ehs/risk-assessment/genetic%20toxicity.pdf>.
- OECD: (Q)SAR Application Toolbox; version 3.3.2 (2015) Organization for Economic Co-operation and Development.
<http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm>. Accessed 6 May 2015.
- Palacio, I. C.; Barros, S. B. M.; and Roubicek, D. A., Water-soluble and organic extracts of airborne particulate matter induce micronuclei in human lung epithelial A549 cells, *Mutation Research*, 812, 1-11, 2016.
- 10 Oh, S. M.; Kim, H. R.; Park, Y. J.; Lee, S. Y.; and Chung, K. H., Organic extracts of urban air pollution particulate matter (PM_{2.5})-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells), *Mutation Research*, 723, 142- 151, 2011.
- Papa, E.; Pilutti, P.; Gramatica, P. Prediction of PAH mutagenicity in human cells by QSAR classification. *SAR QSAR Environ Res.*, 19, 115-27, 2008.
- 15 Pope, C.; Brook, R.; Burnett, R.; Dockery, D. How is cardiovascular disease mortality risk affected by duration and intensity of fine particulate matter exposure? An integration of the epidemiologic evidence. *Air Qual. Atmos. Health*, 4, 5–14, 2011.
- Risom, L.; Moeller, P.; and Loft, S., Oxidative stress-induced DNA damage by particulate air pollution, *Mutation Research*, 592, 119-137, 2005.
- Rohr, A. C. The health significance of gas- and particle-phase terpene oxidation products: A review. *Environment*
20 *International*, 60, 145–162, 2013.
- Rattanavaraha, W.; Rosen, E.; Zhang, H.; Li, Q.; Pantong, K.; and Kamens, R. M., The reactive oxidant potential of different types of aged atmospheric particles: An outdoor chamber study, *Atmospheric Environment*, 45, 3848-3855, 2011.
- Ruggeri, G.; Bernhard, F. A.; Henderson, B. H.; and Takahama, S., Model-measurement comparison of functional group abundance in α -pinene and 1,3,5-trimethylbenzene secondary organic aerosol formation, *Atmos. Chem. Phys.*, 16, 8729-8747,
25 2016.
- Saffari, A.; Hasheminassab, S.; Wang, D.; Shafer, M. M.; Schauer, J. J.; Sioutas, C. Impact of primary and secondary organic sources on the oxidative potential of quasi-ultrafine particles (PM_{0.25}) at three contrasting locations in the Los Angeles Basin. *Atmos. Environ.*, 120, 286-296, 2015.
- Sahigara, F.; Mansouri, K.; Ballabio, D.; Mauri, A.; Consonni, V.; Todeschini, R. Comparison of Different Approaches to
30 Define the Applicability Domain of QSAR Models. *Molecules*, 17, 4791-4810, 2012.
- Schultz; T. W.; Amcoff, P.; Berggren, E.; Gautier, F.; Klaric, M.; Knight, D.J.; Mahony, C; Schwarz, M.; White, A.; Cronin, M.T.D. A strategy for structuring and reporting a read-across prediction of toxicity. *Regulatory Toxicology and Pharmacology*, 72, 586–601, 2015.

- Schwarz, M. A.; Behnke, A.; Brandt, M.; Eisenträger, A.; Hassauer, M.; Kalberlah, F.; Seidel, A. Semipolar polycyclic aromatic compounds: identification of 15 priority substances and the need for regulatory steps under REACH regulation. *Integr Environ Assess Manag.*, 10, 415-28, 2014.
- 5 Stanek, L. W.; Sacks, J. D.; Dutton, S.; Dubois, J.-J. B. Attributing health effects to apportioned components and sources of particulate matter: An evaluation of collective results. *Atmos. Environ.*, 45, 5655-5663, 2011.
- Sushko, I.; Novotarskyi, S.; Körner, R.; Pandey, A.K.; Kovalishyn, V.V.; Prokopenko, V.V.; Tetko, I.V. Applicability domain for *in silico* models to achieve accuracy of experimental measurements. *Journal of Chemometrics*, 24, 202-208, 2010.
- Tuet, W. Y.; Chen, Y.; Xu, L.; Fok, S.; Gao, D.; Weber, R. J.; and Ng, N. L., Chemical oxidative potential of secondary organic aerosol (SOA) generated from the photooxidation of biogenic and anthropogenic volatile organic compounds, *Atmos. Chem. Phys.*, 17, 839-853, 2017.
- 10 Valavanidis, A.; Fiotakis, K., and Vlachogianni, T., Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms, *Journal of Environmental Science and Health Part C*, 26, 339-362, 2008.
- Verma, V.; Fang, T.; Xu, L.; Peltier, R. E.; Russell, A. G.; Ng, N. L.; Weber, R. J., Organic Aerosols Associated with the 15 Generation of Reactive Oxygen Species (ROS) by Water-Soluble PM_{2.5}. *Environ. Sci. Technol.*, 2015, 49, 4646–4656, 2015.
- West, J. J.; Cohen, A.; Dentener, F.; Brunekreef, B.; Zhu, T.; Armstrong, B.; Bell, M. L.; Brauer, M.; Carmichael, G.; Costa, D. L.; Dockery, D. W.; Kleeman, M.; Krzyzanowski, M.; Künzli, N.; Liou, C.; Lung, S.-C C.; Martin, R. V.; Pöschl, U.; Pope, C. A.; Roberts, J. M.; Russell, A. G.; Wiedinmyer, C. What We Breathe Impacts Our Health: Improving Understanding of the Link between Air Pollution and Health. *Environ. Sci. Technol.*, 2016, 50, 4895–4904, 2016.
- 20 WHO. Review of evidence on health aspects of air pollution—REVIHAAP Project. Technical Report. Copenhagen; Denmark: WHO Publications; WHO Regional Office for Europe; 2013.
- Worth, A.; Fuat-Gatnik, M.; Lapenna, S.; Lo Piparo, E.; Mostrag-Szlichtyng, A.; Serafimova, R. The Use of Computational Methods in the Toxicological Assessment of Chemicals in Food: Current Status and Future Prospects. *JRC Scientific and Technical Reports.*, 2011, EUR 24748 EN. DOI 10.2788/6234.
- 25 Yap, C. W. PaDEL-Descriptor: An open source software to calculate molecular descriptors and fingerprints. *Journal of Computational Chemistry*, 32, 1466-1474, 2011.
- Yuan, B.; Hu, W. W.; Shao, M.; Wang, M.; Chen, W. T.; Lu, S. H.; Zeng, L. M.; and Hu, M., VOC emissions, evolutions and contributions to SOA formation at a receptor site in eastern China, *Atmos. Chem. Phys.*, 13, 8815-8832, 2013.
- Zhang, Y.; Schauer, J. J.; Shafer, M. M.; Hannigan, M. P.; Dutton, S. J. Source apportionment of *in vitro* reactive oxygen 30 species bioassay activity from atmospheric particulate matter. *Environ. Sci. Technol.*, 42, 7502-7509, 2008.

Table 1: Experimental data for Salmonella in vitro mutagenicity

| Compound name | No. studies | Test Calls | Overall outcome ^c |
|---|-------------------|------------------------------|------------------------------|
| 2-nitrophenol | 23 ^{a;b} | - (23) | - |
| 2-methylphenol | 20 ^{a;b} | - (20) | - |
| naphthol | 11 ^{a;b} | - (9), + (2) | + |
| naphthoquinone | 17 ^{a;b} | - (13), + (3), equivocal (1) | + |
| dihydroxy-phthalic acid | 27 ^{a;b} | - (27) | - |
| phenanthrene-9,10-dione | 6 ^{a;b} | - (6) | inconclusive |
| phenanthren-9-ol | 3 ^{a;b} | - (1), + (2) | + |
| 9-nitrophenanthrene | 16 ^{a;b} | - (2), + (14) | + |
| 4-nitro-6H-dibenzo[b,d]pyran-6-one | 6 ^{a;b} | - (5), + (1) | + |
| 1-nitro-pyrene | 25 ^{a;b} | - (1), + (19), equivocal (3) | + |
| N'-nitrosornicotine | 18 ^{a;b} | - (13), + (4), equivocal (1) | + |
| 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone | 7 ^{a;b} | - (3), + (3), equivocal (1) | + |
| 4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanol | 1 ^a | + (1) | + |

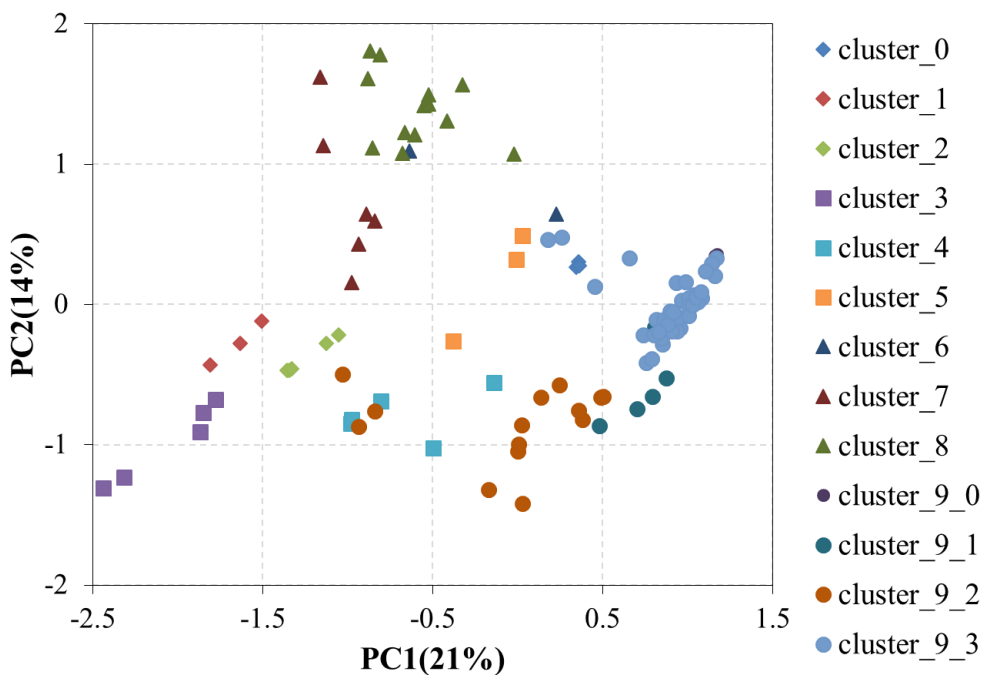
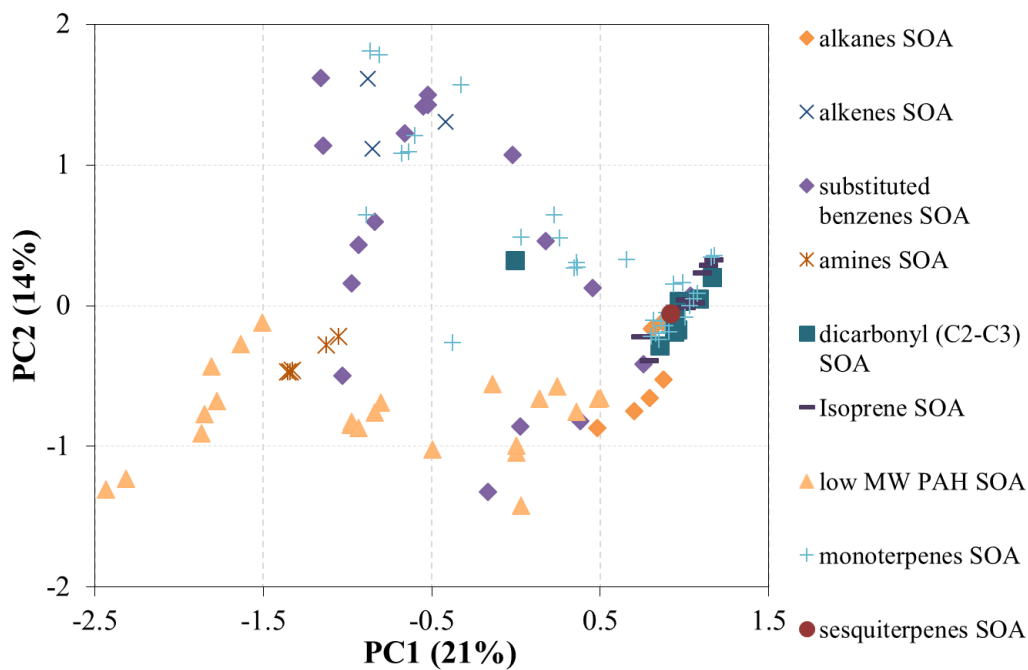
a Bacterial mutagenicity ISSSTY; b Genotoxicity OASIS; c Overall outcome according to Bacterial mutagenicity ISSSTY assessment: positive (“+”) (at least one strain is positive (with or without Metabolic activation)); “equivocal” (no strain is positive, and at least one equivocal result is present in one of the following strains (with or without Metabolic activation): TA1535, TA100, TA98, TA1538, TA1535, TA97); negative (“-”) (no positive or equivocal results are present in any strain, and negative outcomes exist for: a) at least one strain from among TA1535 or TA100 or TA97 (with and without Metabolic activation); and b) at least one strain from among TA1538 or TA98 or TA1537 (with and without Metabolic activation)); “inconclusive” (if none of the above criteria is fulfilled). Full information on genotoxicity observation data (strains, metabolic activation, etc.) with references can be found in Table S6.

Table 2: Results of the QSAR genotoxicity predictions from VEGA/Caesar and ACD/Percepta models.

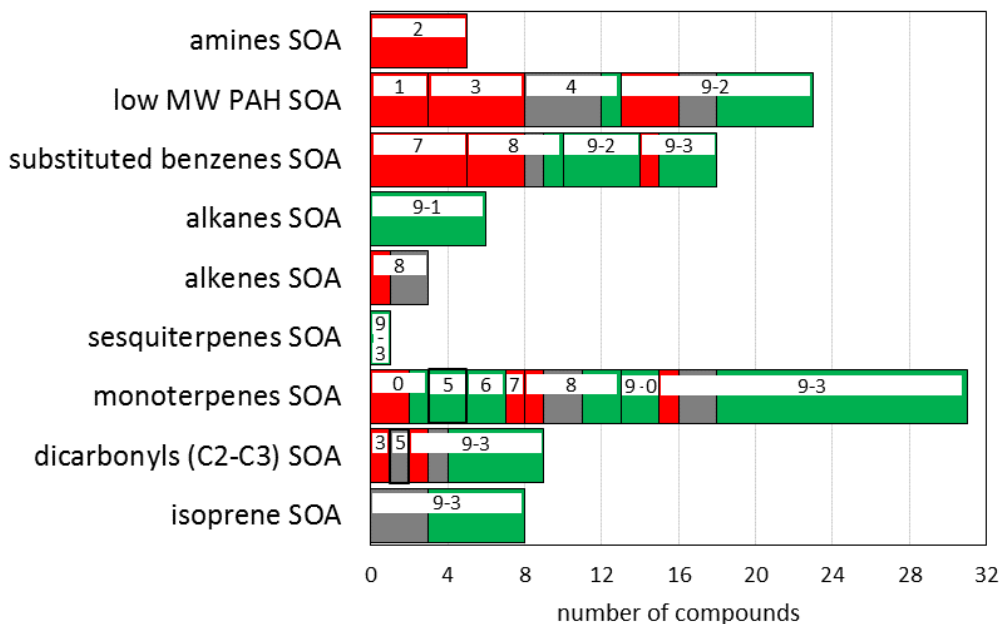
| | | VEGA/Caesar | | | | tot |
|--------------|-----------|-------------|----------|-----|-----|-----|
| | | Negative | Positive | out | n/a | |
| ACD/Percepta | Negative | 36 | 3 | 7 | - | 46 |
| | Positive | 1 | 13 | - | 1 | 15 |
| | Equivocal | - | 2 | 4 | - | 6 |
| | out | 10 | 12 | 14 | 1 | 37 |
| tot | | 47 | 30 | 25 | 2 | 104 |

Combining predictions obtained by the two models, the total number of compounds classified as genotoxic from either VEGA/Caesar or ACD/Percepta amounts to 32 species (red); the number of compounds determined as non-genotoxic by one model and that is not classified as genotoxic by the other is 53 (green); the number of compounds classified as indeterminate (i.e. “equivocal” or “not reliable” prediction) (grey) by both methods is 19.

5

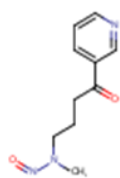


5 **Figure 1: PCA score plot of 104 aerosol compounds: compounds marked according to different SOA classes (top); compounds marked according to different clusters (bottom).**

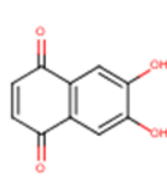


amine SOA

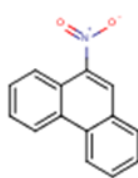
low MW PAH SOA



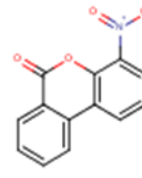
(cluster 2)



(cluster 1)



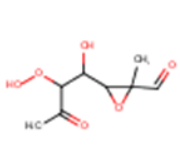
(cluster 3)



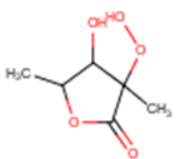
(cluster 9-2)

substituted benzene SOA

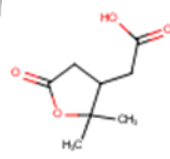
monoterpene SOA



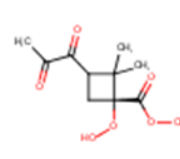
(cluster 7)



(cluster 8)



(cluster 0)



(cluster 8)

Figure 2: (top) Distribution of mutagenic (red); non-mutagenic (green) and indeterminate (grey) compounds between clusters (labels in white boxes) and SOA classes. (bottom) Examples of molecular structures of compounds predicted to be mutagenic.