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Physico-chemical properties and biological effects of diesel and biomass particles☆

Eleonora Longhin [a, *, 1], Maurizio Gualtieri [b, **, 1], Laura Capasso [a], Rossella Bengalli [a], Steen Mollerup [c], Jørn A. Holme [d], Johan Øvrevik [d], Simone Casadei [e], Cristiano Di Benedetto [a, 2], Paolo Parenti [a], Marina Camatini [a]

[a] Polaris Research Centre, Dept. of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza, 1, 20126, Milan, Italy
[b] Italian National Agency for New Technologies, Energy and Sustainable Economic Development — ENEA-SSPT-MET-INAT, Strada per Crescentino 41, 13040, Saluggia, Vercelli, Italy
[c] Dept. of Biological and Chemical Working Environment, National Institute of Occupational Health, N-0033, Oslo, Norway
[d] Domain for Infection Control and Environmental Health, Norwegian Institute of Public Health, N-0403 Oslo, Norway
[e] Innovhub-SSI Fuels Division, Via Galileo Galilei, 1, 20097, San Donato Milanese, Milan, Italy

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A B S T R A C T

Diesel combustion and solid biomass burning are the major sources of ultrafine particles (UFP) in urbanized areas. Cardiovascular and pulmonary diseases, including lung cancer, are possible outcomes of combustion particles exposure, but differences in particles properties seem to influence their biological effects.

Here the physico-chemical properties and biological effects of diesel and biomass particles, produced under controlled laboratory conditions, have been characterized. Diesel UFP were sampled from a Euro 4 light duty vehicle without DPF fuelled by commercial diesel and run over a chassis dyno. Biomass UFP were collected from a modern automatic 25 kW boiler propelled by prime quality spruce pellet. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images of both diesel and biomass samples showed aggregates of soot particles, but in biomass samples ash particles were also present. Chemical characterization showed that metals and PAHs total content was higher in diesel samples compared to biomass ones.

Human bronchial epithelial (HBEC3) cells were exposed to particles for up to 2 weeks. Changes in the expression of genes involved in xenobiotic metabolism were observed after exposure to both UFP already after 24 h. However, only diesel particles modulated the expression of genes involved in inflammation, oxidative stress and epithelial-to-mesenchymal transition (EMT), increased the release of inflammatory mediators and caused phenotypical alterations, mostly after two weeks of exposure.

These results show that diesel UFP affected cellular processes involved in lung and cardiovascular diseases and cancer. Biomass particles exerted low biological activity compared to diesel UFP. This evidence emphasizes that the study of different emission sources contribution to ambient PM toxicity may have a fundamental role in the development of more effective strategies for air quality improvement.

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* Corresponding author.
** Corresponding author. Present address: Université du Littoral Côte d'OpaleUnité de Chimie Environnementale et Interactions sur le Vivant UCEIV, 189A, avenue Maurice Schumann, 59140, Dunkerque, France.
E-mail addresses: eleonora.longhin@unimib.it (E. Longhin), maurizio.gualtieri@univ-littoral.fr, maurizio.gualtieri@enea.it (M. Gualtieri), cristiano.dibenedetto@kaust.edu.sa (C. Di Benedetto).
[1] Equal contributors.
[2] Present address: King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering (BESE), Thuwal, 23955-6900, Saudi Arabia.

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1. Introduction

Ambient particulate matter (PM) is a major air pollutant whose impact on human health is of great concern. A recent WHO report linked 7 million annual worldwide premature deaths to air pollution; among them 90% were attributed to cardiovascular diseases and 6% to lung cancer (WHO, 2014). In an effort to improve ambient air quality, awareness is now paid to the need to re-evaluate the environmental quality standards, aiming not only at lower PM concentration thresholds, but also focusing on specific reduction of the particles toxicity (Cassee et al., 2013). Much attention has been posed on the PM ultrafine component (UFP, particles that are less than 100 nm in diameter), due to its high reactivity and small size, resulting in higher lung deposition, reduced clearing efficiency, and capability to translocate through biological barriers (Schmid et al., 2009).

In the Lombardy Region, North of Italy, diesel combustion used for private and public transport and solid biomass burning for residential heating are the major sources of fine particles (PM2.5, particles that are less than 2.5 μm in diameter) emission, respectively accounting for 20 and 56% to PM2.5 concentration (ARPA INEMAR, 2012). However, these sources mainly produce particles of 15–30 nm in diameter, often aggregated, thus constituting the most relevant contribution to primary UFP emissions (Zheng et al., 2007; Maricq, 2007). Concerning many large cities and highly populated areas around the world, the issue of diesel PM pollution has been extensively addressed in the past decades (Schwarze et al., 2013; Pierdominici et al., 2014; Chuang et al., 2013). Although new combustion technologies have been developed, and the emissions from engines significantly reduced, the question remains whether these changes resulted in an equivalent decrease in harmful properties of the emitted particles.

During the last years the use of biomass burning for residential heating has increased in Northern Italy and elsewhere in Europe due to the lower price of solid biomass fuels when compared to the traditional fossil fuels (gas or electricity), combined with the political actions aiming at reducing greenhouse gases (EU Directive, 2009/287/EC). This determined an increased contribution of biomass burning emissions to outdoor and indoor air pollution (Sigsgaard et al., 2015; Sarigiannis et al., 2015). The relatively high PM emission from traditional domestic biomass burning is due to the general low combustion efficiency of appliances and boilers (Vicente et al., 2015b), compared to modern appliances with decreased emissions determined by improved combustion technologies (Sigsgaard et al., 2015).

Exposure to combustion particles has been related to the onset of cardiovascular and pulmonary diseases, including lung cancer (Lewtas, 2007). Oxidative stress and inflammation are known contributors to the initiation and development of these outcomes, and have been largely associated with particles exposure (Schwarze et al., 2013; Sarigiannis et al., 2015; Ristovski et al., 2012). Human inhalation studies on both diesel particles and wood smoke have been associated with increased pulmonary influx of inflammatory cells (Larsson et al., 2007), IL-6 and IL-8 cytokines in lavage fluid (Stenfors et al., 2004) and alveolar nitric oxide (NO), suggesting inflammation in the airways distal parts (Barregard et al., 2006). With regard to lung cancer progression, epithelial-to-mesenchymal transition (EMT) is regarded as a crucial step, involving morphological and phenotypic cellular changes (Xiao and He, 2010). Despite till now this aspect has been poorly investigated, a recent study highlighted alterations in cell migration and invasion, and in EMT markers expression in A549 cells exposed to urban PM (Yue et al., 2015).

Considerable variation in the responses to both diesel and wood/biomass particles has been observed in toxicological studies. For example, in an *in vivo* study different particles have been reported to modulate different inflammatory mediators; diesel particles induced high levels of MIP-2 (macrophage inflammatory protein 2-alpha), which is involved in immunoregulatory and inflammatory processes, whereas biomass particles increased the levels of tumor necrosis factor TNF-α, a multifunctional proinflammatory cytokine involved in cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation (Seagrave et al., 2005). Kocbach et al. (2008) reported that, in the monocytic cell line THP-1, traffic-derived particles induced a higher inflammatory effect, consisting in cytokines release, whereas wood smoke particles reduced cell proliferation and viability. In another *in vitro* study both inflammation as well as cytotoxicity were increased following diesel PM exposure, when compared to biomass particles (Totlandsdal et al., 2014). These observations highlight the complexity of studying combustion particles toxicity, illustrating that differences in fuel properties and specific combustion conditions modify the particles chemical content, thereby affecting their biological properties (Shen et al., 2014; Li et al., 2010).

Here diesel and biomass particles, produced under controlled laboratory conditions, have been characterized with regard to their physico-chemical properties and related biological effects. The content of PAHs and metals has been determined, and particles morphology described. Human bronchial epithelial cells (HBEC3) have been exposed to particles from 24 h up to 2 weeks, to investigate biological responses that may require longer time to develop. The modulation of genes involved in pathways related to xenobiotic metabolism, inflammatory response, oxidative stress and EMT have been evaluated. The release of inflammatory mediators and the cells phenotypical alterations have also been analyzed. PM2.5, sampled in Milan during winter, has been used to evaluate the effects of an ambient particulate in comparison with the diesel and biomass UFP, and to investigate the relative contribution of the selected sources to outdoor pollution. The results highlight the importance of separately evaluating the different emission sources in order to select more effective strategies for air quality retraining.

2. Materials and methods

2.1. Particles sampling

The fuels (commercial diesel and prime quality spruce pellet) were physically and chemically characterized (Supplementary data 1). Diesel exhaust emissions were sampled from a Euro 4 light duty vehicle without DPF run over a chassis dyno according to the “URBAN” Artemis Driving Cycle representatives of real average stop & go driving conditions typical of a European city urban context. Several URBAN cycles were performed in order to collect a suitable mass of particles for biological and chemical analyses. UFP were collected on Teflon filters (Whatman pure teflon filters), using a DGI-1570 (Dekati Gravimetric Impactor, Finland) to remove bigger aggregates. Teflon filters were inserted in the last stage of the impactor and secured by a custom design support. Biomass burning particulate emissions from a modern automatic 25 kW pellet boiler were sampled on Teflon filters after dilution of flue gases with clean air in order to improve volatile organic compounds condensation. The dilution rate was evaluated a posteriori considering the CO_2 content in the flue gases and in the sampling gases. A multi parametric gas analyzer Horiba PG250 (Horiba, Japan) was used to characterize the levels of gaseous emissions. Filters from diesel and biomass combustion, were kept at −20 °C immediately after sampling and until chemical characterization or UFP extraction for biological tests.

Winter PM2.5 (wPM2.5) sampled in Milan was available from previous studies (Gualtieri et al., 2012). The sampling site, Torre