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Could combustion-generated nanoparticles induce cytotoxicity also at the extremely low doses typical of indoor environments?

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ABSTRACT. The presence of nanoparticles in the environment is mainly attributed to outdoor sources but sub-10 nm particles may also form indoor as effect of domestic activities such as cooking, heating, air freshening. Today, due to the COVID-19 pandemic, people are staying home for longer periods of times, thus being exposed to a poor indoor air quality. Due to elevated numerical concentration and large surface area, the health effect of sub-10 nm particles can go beyond what expected from their low mass concentration in the atmosphere.
The objective of this study is to find, based on analysis of recent in vitro studies, a dose-effect correlation based on particle size/surface more than on particle mass. Such a correlation cold be useful to assess the health effects of people exposed to very low mass doses of nanoparticles either indoor or outdoor.

Key words: *nanoparticles, indoor pollution, cytotoxicity, health effects, combustion.*

RIASSUNTO. Le nanoparticelle generate dalla combustione potrebbero avere effetti citotossici anche a dosi estremamente basse tipiche degli ambienti indoor?
La presenza di nanoparticelle nell'ambiente è principalmente attribuita a fonti di inquinamento esterne ma particelle con diametro nanometrico, qui definite sub-10 nm, possono essere formate anche negli ambienti confinati come risultato delle attività domestiche di riscaldamento, cottura dei cibi e profumazione degli ambienti. Attualmente, a causa della pandemia da COVID-19, le persone rimangono a casa per molto più tempo ed in casa svolgono la maggior parte delle loro attività e possono, quindi, essere maggiormente esposti ad una qualità dell'aria interna influenzata dalle attività domestiche. Le particelle con dimensioni nanometriche hanno una concentrazione in massa molto bassa ma questo non deve indurci a trascurare i possibili effetti dovuti all'elevata concentrazione numerica e all'ampia superficie specifica che hanno queste particelle.
L'obiettivo di questo lavoro è quello di valutare, sulla base dell'analisi di recenti studi *in vitro*, l'esistenza di una correlazione dose-effetto ottenuta in base alla dimensione/superficie delle particelle piuttosto che in base alla loro massa. Tale correlazione può essere utile per valutare gli effetti sulla salute dovuti a basse concentrazioni in massa delle nanoparticelle sia negli ambienti indoor che in quelli outdoor.

Parole chiave: nanoparticelle, inquinamento indoor, citotossicità, effetti sulla salute, combustione.

Introduction

PM10 is the mass concentration of the aerosol of particles suspended in the air with sizes below 10 micron. It comprises all particles with sizes down to few nanometers, and contains either primary emitted particles and those formed in the atmosphere due to photochemical transformation of precursor gaseous compounds. PM10 is undesirable in the context of aerosol/climate science and for its dangerous effects on human health.

Human activities produce particles which are emitted directly in the atmosphere; among all, combustion processes are the major sources of such particles, particularly those which sizes fall below 2.5 μm (PM2.5 or fine fraction). The fine fraction of PM is of particular concern because it is known to have significant negative health impacts (1,2).

Great attention is today given also to the ultrafine fraction of PM (UFPs), i.e., the particles with an aerodynamic diameter below 100 nm (or PM0.1), because they are believed to have even stronger effects on human health than PM2.5 (3). Among UFPs, the fraction of particles with sizes below 10 nm, here named sub-10 nm particles, is of particular relevance. These particles are emitted in huge number concentrations but they have mostly insignificant mass if compared to the largest size particles. Moreover, they are very difficult to be measured and very sophisticated diagnostic methods, not of common use, are needed for their characterization.

The fact that these nano-sized particles are not easily measured at the exhausts and in the atmosphere does not mean that they can be overlooked, particularly for those persons who live in environments in which nanoparticles are present in huge number concentrations. Sub-10 nm have up to orders of magnitude higher surface area to mass ratios compared to larger particles, have surfaces covered with adsorbed volatile and semi-volatile organic species or even are constituted by such semi-volatile species when the sizes is in the 2-5 nm range. In this context it is important to note that particle surface area is in general thought to scale best with the surface reactivity, bioactivity and toxicity of particles (4,5).

Due to elevated numerical concentration and large surface area, the health effect of sub-10 nm particles can go beyond what expected from their low mass concentration in the atmosphere.

The presence of nanoparticles in the environment is mainly attributed to outdoor sources, such as heavy traffic conditions, industrial emissions, incineration plants, and many other human activities. Sub-10 nm particles, to a lesser extent, may also form indoor. Among indoor sources, domestic heating boilers, cook-stoves, scented candles, domestic fireplaces, cigarette smoking, electrical appliances, and biogenic substances are the dominant sources. Today, do due to the COVID-19 pandemic, people are staying home for longer periods of times, often cooking and setting close to the fireplace, thus being exposed to a poor indoor air quality (6). Consequently they may get diseases, in particular respiratory and skin diseases (7), due to indoor produced nanoparticles more than to outdoor pollutants.

The assessment of the health effects of sub-10 nm particles is challenging and has been poorly documented. The fundamental objective of this study is to summarize recent literature studies on sub-10 nm particles produces indoor and outdoor and to compare the *in vitro* cytotoxicity when cells exposed to different doses of nanoparticles formed by domestic cooktop burner, gas-fueled heating boilers, bluish flames and internal combustion engines. Our aim is to find a dose-effect correlation based on particle size/surface more than on particle mass. Such a correlation cold be useful to assess the health effects of people exposed to very low mass doses of nanoparticles either indoor during domestic activities or outdoor at crossroad of heavy polluted cities.

Literature data review

In this paragraph, we summarize recent literature studies on sub-10 nm particles produces indoor and outdoor with particular emphasis to those studies that analyzed the health effects of the formed nanoparticles besides particle concentrations, morphology and chemical characteristics.

There is evidence that the smallest nanoparticles readily cross the blood-brain barrier in mammals (8) causing an inflammatory response and impacting development (9). *In vitro* toxicological studies have shown that once in the organs, sub-10 nm particles are taken up by cells causing toxicity and mutagenicity (3,10,11). Various studies have also found a significant amount of oxygen on the nanoparticles surfaces (12-14) which is correlated to their hydrophilic character (11,15). This hydrophilic character leads to partial water solubility, which is cause for concern in human health.

Nano-sized particles (sub-10 nm) are emitted by combustion systems, including vehicles, industrial burners and indoor combustion sources like cookstoves/heaters, which are a significant source of airborne nanoparticles (16-20,21). Particularly concerning is that many devices operating with a blue flame, such as natural gas domestic burners or cookstoves, are producing these nanoparticles (3,20). The emission of these nanoparticles can be explained by the decreasing physical interactions between nanoparticles below 5 nm leading to low coagulation efficiencies at flame temperatures (22). Preliminary work shows that after treatment systems are only able to remove 40-50% of particles below 10 nm (23) which are thus emitted in the atmosphere in huge number concentration.

Nanoparticles are also detected indoor. They are constituted by a mix of ambient particles that have infiltrated indoors, particles emitted indoors, and particles formed indoors through reactions of gas-phase precursors originating from both indoor and outdoor sources (24). Short-term studies including residential indoor ultrafine particle measurements have been carried out by several investigators (25-27). This studies were carried out during cooking events and provided mean peak volume concentrations of particles between 10 and 500 nm. Wallace (28) monitored UFPs and accumulation mode (0.1-1 μm) particles in an occupied suburban house at 5-minute intervals for 37 consecutive months. Number concentrations for 126 particle sizes from 9.8-947 nm were measured. Of the many indoor activities, some were chosen for detailed analysis. These included cooking with a gas stove, toasting with electric toasters and toaster ovens, burning candles and incense, and using a gas-powered clothes dryer. The average duration of elevated particle concentrations ranged from 20 minutes to 3 hours. Combustion of natural gas (boiling water, gas clothes dryer) showed number peaks near 10 nm, while the electric toaster and toaster oven had peaks close to 30 nm. More complex cooking (burners plus gas oven) produced peaks in the 35-50 nm range. Burning candles and incense resulted in peaks in the 60-nm range. Finally, outdoor sources peaked at nearly 70 nm, indicating the influence of aging in shifting size modes to higher diameters.

A study conducted in nine Boston-area homes showed that indoor particle events tend to be brief, intermittent, and highly variable. In addition to dramatic source events, data demonstrate that the impacts of indoor activities are especially pronounced in the UFP and coarse (PM10-2.5) modes. Furthermore, chemical analysis suggested that organic carbon is a major constituent of particles emitted during indoor source events (29).

In a recent study (30), the effects of outdoor particles and occupant activities on indoor particle concentrations were evaluated for apartment houses in South Korea. Measurement was performed in winter when the outdoor particle concentration is relatively high. The results showed that the inflow of outdoor particles is not significant because the recently built apartment houses have excellent airtightness, and the ventilation frequency is not high in winter. Cooking, in particular frying, has the greatest impact on indoor small particle concentrations, that rapidly dispersed to the living room.

Minutolo et al. (20) performed an experimental study of the emissions of new burners used for home appliances with the aim of evaluating the effect of burner configurations and operating excess air on the emissions of gaseous pollutants and organic carbon. Pollutant emissions from these burners were compared with those of typical stove-top burners. Measurements showed that particulate matter with size in the 1-10 nm size range is formed in all the examined conditions. The emitted mass concentration of these particles was very low, but they were emitted in huge number concentrations. In the stove-top burner, a larger amount of organic aerosol was emitted. In both indoor combustion systems, soot particles were not formed, showing that also bluish flames, those not forming soot or black carbon, may produce huge amounts of sub-10nm particle. The biological reactivity of such combustion-formed sub-10nm particles was tested in vitro (10) and compared with sub-10nm particles collected from Diesel vehicle exhausts. A significant cytotoxic response was measured above a critical dose in mouse embryo fibroblasts NIH3T3 cells along with possible evidence of cellular uptake by optical and confocal microscopy.

Toxicological assays (31,32) showed that nanoparticles collected from bluish flames and vehicle exhausts effectively interacted *in vitro* with both prokaryotic and eukaryotic cells. Subsequent studies on the flame formed nanoparticles analyzed their possible effect on epithelial (HaCaT) and endothelian cells (EC) cells growth and production of proinflammatory lipid mediators. Results indicated a dose and time-dependent reduction in cell viability. Cells treated with nanoparticles showed a cell proliferation index significantly lower than that of control cells and an increased apoptotic cell death. The annexin assay confirmed the increased apoptotic cell death. Moreover, nanoparticles also induced a time-dependent increase of proinflammatory lysophospholipid production.

Pedata et al. (33) analyzed also the inflammatory potential of sub-10nm particles from gas cooking appliances on immortalized human keratinocyte cell line (HaCaT cells). Particles collection was performed on a mid-range cooktop burner fed with network natural gas which contains methane and ethane, lower percentages of higher alkanes and about 20ppm of sulfur. Tests were performed at the exhaust of a free flame and by putting a pot on the burner in order to simulate operating conditions closer to the domestic ones. Nanoparticles in the size range between 2.5 and 20 nm were removed from the combustion exhausts and isolated from low molecular weight gas-phase organic product. The chemical and morphological characterization of the collected material showed that carbon nanoparticles were collected together with some nitrate and sulfate particles formed during exhaust cooling, these latter also having a nanometric size. Cell viability was evaluated by crystal violet and showed a negligible reduction in cell number, except for a little positive effect in increasing cell number at the lowest particle concentrations.

Pedata and co-workers (34) also demonstrated that nanoparticles produced by biodiesel and diesel oils induced a significant increase of mortality in cells lines. The cell number of HaCaT cells and A549 cells (human alveolar epithelial-like cells) treated with different concentrations for 24 and 48 h decreased in a concentration-dependent manner, with some differences in the two cell lines and depending on the origin of the nanoparticles. A clear evidence was found that the particle from biodiesel oil induced less cytotoxicity compared to diesel ones, on both cell models used. Although the results are limited to an *in vitro* study, they clearly shown that the dermal way of exposure is more sensitive than the inhalant way, as a demonstration that it is important to promote the culture of prevention also for the dermal way in particularly in exposed workers.

Results and Discussion

The combustion conditions examined over the years by our research group produced essentially UFPs with a bimodal number size distribution. The size distribution presents a nucleation mode particles with sizes smaller than 10 nm - mostly constituted by organic carbon and eventually nitrates, and sulphates if the fuel contains sulphur -, and a larger accumulation mode mainly peaked at 20-50 nm - composed of more graphitic soot particles with an elemental carbon structure. Only when the number of nucleated particles increases for effect of the increased local flame richness, aggregation of the soot particles and incorporation of nucleation mode particles and semi-volatile condensates on their surfaces lead to the formation of chain-like aggregates with sizes between 100 nm and 1 μm, combustion conditions not analysed in this paper.

The relative abundance of sub-10 nm mode particles and soot particles depends on the operating conditions used and on the fuel. Slightly fuel-rich conditions promotes the formation of macromolecules which aggregates in forms of stacks of few aromatic molecules held together by van der Waals interactions, thus forming sub-10 nm particles. Increasing flame richness favours coagulation of the nucleated particles and the formation of more graphitic soot particles with sizes around 20-40nm.

Over the years, we collected combustion-generated particles from laboratory flames, cookstove exhausts, domestic heating boilers and diesel engines by using a water-based sampling method which is able to condense combustion water out of the exhaust flow. A stainless steel suction probe draws out combustion products, which are then bubbled through a condenser containing bi-distilled laboratory grade water cooled in an ice bath. All volatile components of the sampled species produced in flame and collected in water were removed by rotary evaporation before sample characterization and toxicological testing. Evaporation was performed by reducing the pressure slowly at ambient temperature to avoid boiling until a steady state was observed; then, the sample was submersed in a water-bath held at 40 °C and evaporation continued at 50 mbar until water evaporation was observed, typically 20-30 min.

Cell viability was determined by MTT assay, Crystal Violet staining and Trypan Blue staining after 24 h and 48 h of treatment with nanoparticles and confirmed by quantification of apoptosis, measured by flow cytometry. Details of the experimental procedures are reported in previous publications (31-34).

Figure 1 reports the percentage of apoptotic cells as a function of the exposed dose of nanoparticles. Data have been divided, when possible, by separating sub-10 nm particles from the larger soot particles. In the cases the total amount of particles have been collected together and not separated before, the cytotoxicity is reported for the total UFPs. Data are relative to particles sampled in two zones of laboratory premixed flames: the bluish zone (nucleation region - Lab Flame Bluish zone), where essentially sub-10 nm are formed, and the yellow zone, where larger soot particles are produced (Lab Flame Yellow zone), and at the exhausts of Diesel engines operating with different fuels and loads.

Data clearly show a dose-dependent effect on cell viability which is stronger for sub-10 nm particles than for soot particles (dose data are reported on logarithmic scale). The effect of soot particle is even negligible if purely elemental, 20-50 nm carbon particle are considered. UFPs show an intermediate behaviour suggesting that for these particles it is the nanometric fraction having the stronger effect.

Based on the available size distribution of the particles, we have calculated the specific surface area of the particles considering them as spheres and using a unit mass density for the sub-10 nm particles and a density of 1.8 g/cm^3 for the larger soot particles. **Figure 2** reports the percentage of apoptotic cells as a function of the exposed specific surface area. Clearly, sub-10 nm particles contribute massively to the total specific surface area of the particles; their presence in the sample dominating the effects on cells. For this reason, data obtained at the exhaust of Diesel engines well correlate with data obtained for sub-10 nm particles in bluish flame suggesting that also for outdoor pollution great attention has to be devoted to such class of particles.

These data clearly show a stronger effect of the surface area on cell viability than the mass of the particle. The results are in agreement with suggestion of Oberdörster et al. (4) that show how particle surface area is a more appropriate dose metric than particle mass or particle number when evaluating dose-response relationships of nanoparticle-induced inflammation. Indeed, the number of surface molecules exposed to cells increases as the inverse of the diameter of the particles, therefore small nanoparticles, those comprising sub-10 nm particles, have most of their molecules on the surface and are those molecules that have interaction with cells causing a reduction of their viability.

It is important to remind that *in vitro* studies are limited to the analysis of the interactions of nanoparticles with cells and do not include their capacity to translocate into and in between cells, reaching target organs. *In vivo*-studies are necessary to definitely asses the cytotoxicity of such small nanoparticles.

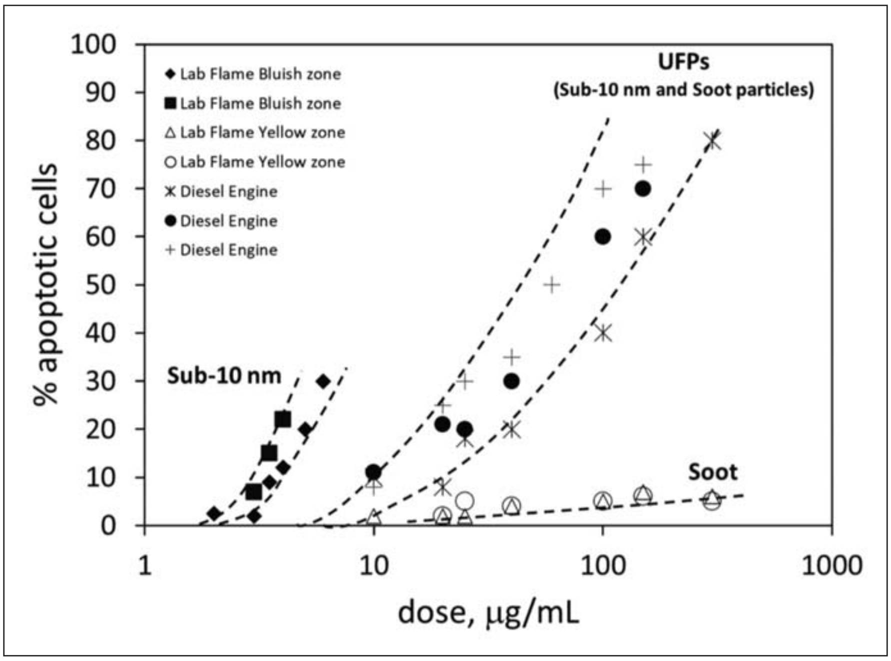

Figure 1. ***Percentage of apoptotic cells as a function of exposed nanoparticle doses. Data from (31-34)***

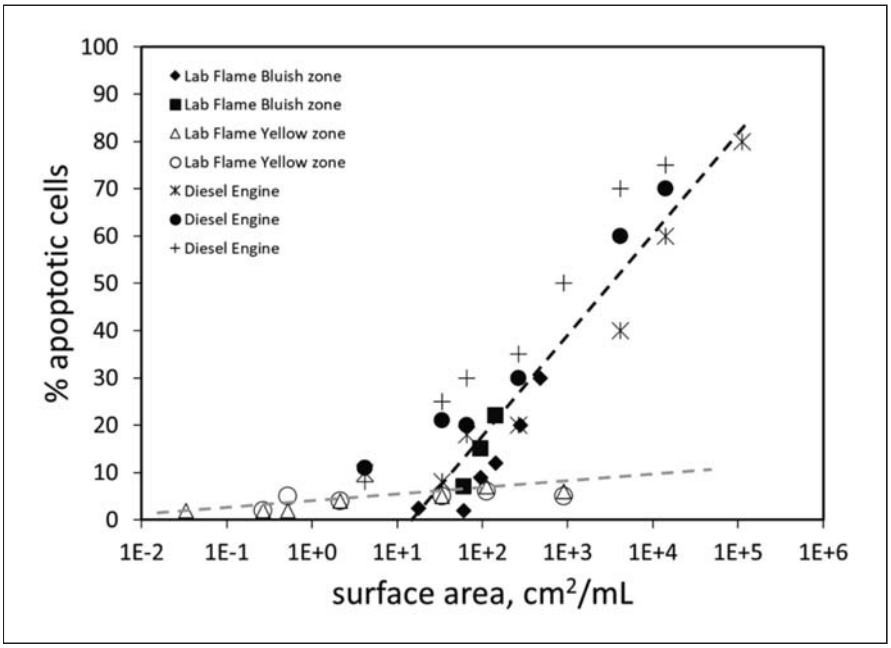

Figure 2. ***Percentage of apoptotic cells as a function of exposed nanoparticle surface. Data from (31-34)***

Anyhow, the results clearly demonstrate that also low doses of sub-10 nm particles might induce apoptosis for a large number of cells and thus our attention has to turn to this class of combustion-generated nanoparticles. Today sub-10 nm particles effects have been overlooked; these particles might be the future major constituents of air pollution, not only outdoor but also indoor where these particles may survive for long time before being removed from the environment.

Conclusion

Sub-10 nm nanoparticles are formed by new technologies combustion systems and even indoor and are largely emitted into the atmosphere. The fundamental properties of these particles - low coagulation efficiency, hydrophilic character - and their capacity to be easily transported in vulnerable regions of the human body need our attention.

Although our results clearly demonstrate that also low doses of sub-10 nm particles might induce apoptosis for a large number of cells, sub-10 nm particles effects on health are actually overlooked. Sub-10 nm particles might be the future major health issue, particularly for indoor occupants because these particles may survive for long time before being removed from the ambient air. Therefore, there is an urgent need to establish indoor exposure limits capturing the complex properties of combustion nanoparticles and determine the health and biological effects in addition to PM2.5.

References

1) Atkinson RW, Carey IM, Kent AJ, Van Staa TP, Anderson HR, Cook DG. Long-term exposure to outdoor air pollution and incidence of cardiovascular diseases. Epidemiology 2013; 24(1): 44-53.
2) Gold DR, Litonjua A, Schwartz J, Lovett E, Larson A, Nearing B, Allen G, Verrier M, Cherry R, Verrier R. Ambient pollution and heart rate variability. Circulation 2000; 101: 1267-1273.
3) Pedata P, Stoeger T, Zimmermann R, Peters A, Oberdörster G, D'Anna A. Are we forgetting the smallest, sub 10 nm combustion generated particles? Particle and Fibre Toxicology 2015; 12. ISSN: 1743-8977.
4) Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005; 113: 823-39.
5) Rushton EK, Jiang J, Leonard SS, Eberly S, Castranova V, Biswas P, et al. Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics. J Toxicol Environ Health A 2010; 73: 445-461.
6) Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, Behar JV, Hern SC, Engelmann WH. The National Human Activity Pattern Survey (NHAPS): A resource for assessing exposure to environmental pollutants. J Expo Sci Environ Epidemiol 2001; 11: 231-252.
7) Samet JM. Indoor air pollution: a public health perspective. Indoor Air 1993; 3: 219-226.
8) Kang JH, Cho J, Ko YT. Investigation on the effect of nanoparticle size on the blood/brain tumour barrier permeability by in situ perfusion via internal carotid artery in mice. Journal of drug targeting 2019; 27(2952): 103-110.
9) Allen JL, Oberdorster G, Morris-Schaffer K, Wong C, Klocke C, Sobolewski M, Conrad K, Mayer-Proschel M, Cory-Slechta D. Developmental neurotoxicity of inhaled ambient ultrafine particle air pollution: parallels with neuropathological and behavioral features of autism and other neurodevelopmental disorders. Neurotoxicology 2017; 59(3026): 140-154.
10) Sgro LA, Simonelli A, Pascarella L, Minutolo P, Guarnieri D, Sannolo N, Netti P, D'Anna A. Toxicological properties of nanoparticles of organic compounds (NOC) from flames and vehicle exhausts. Environmental science & technology 2009; (43): 2608-2613.
11) Sgro A. D'Anna, Minutolo P. On the characterization of nanoparticles emitted from combustion sources related to understanding their effects on health and climate. Journal of hazardous materials 2012; 420-426.
12) Irimiea C, Faccinetto A, Mercier X, Ortega IK, Nuns N, Therssen E, Desgroux P, Focsa C. Unveiling trends in soot nucleation and growth: when secondary ion mass spectrometry meets statistical analysis. Carbon 2019; 144: 815-830.
13) Cain JP, Gassman PL, Laskin A, Wang H. Micro-FTIR study of soot chemical composition: evidence of aliphatic hydrocarbons on nascent soot surfaces. Physical Chemistry Chemical Physics 2010; 12: 5206-5210.
14) Schenk M, Lieb S, Vieker H, Beyer A, Golzhauser A, Wang H, Kohse-Hoinghaus K. Morphology of nascent soot in ethylene flames. Proceedings of the Combustion Institute 2015; 35: 1879-1886.
15) Commodo M, De Falco G, Larciprete R, D'Anna A, Minutolo P. On the hydrophilic/hydrophobic character of carbonaceous nanoparticles formed in laminar premixed flames. Experimental Thermal and Fluid Science 2016; 73: 56-63.
16) Lighty JS, Veranth JM, Sarorofim AF. Combustion aerosols: factors governing their size and composition and implications to human health. Journal of the Air & Waste Management Association 2000; 50: 1565-1618.
17) Kittelson DB, Watts WF, Johnson GP. Nanoparticle emissions on Minnesota highways. Atmospheric Environment 2004; 38: 9-19.
18) Sirignano M, Conturso M, Magno A, Di Iorio S, Mancaruso E, Vaglieco BM, D'Anna A. Evidence of sub-10 nm particles emitted from a small-size diesel engine. Experimental Thermal and Fluid Science 2018; 95: 60-64.
19) Ronkko T, Virtanen A, Kannosto J, Keskinen J, Lappi M, Pirjola L. Nucleation mode particles with a nonvolatile core in the exhaust of a heavy duty diesel vehicle. Environmental Science and Technology 2007; 41: 6384-6389.
20) Minutolo P, D'Anna A, Commodo, M, Pagliara R, Toniato G, Accordini C. Emission of ultrafine particles from natural gas domestic burners. Environmental Engineering Science 2008; 25: 1357-1364.
21) Sgro LA, Basile G, Barone A, D'Anna A, Minutolo P, Borghese A, D'Alessio A. Detection of combustion formed nanoparticles. Chemosphere 2003; 51: 1079-1090.
22) Hou D, Zong D, Lindberg CS, Kraft M,You X. On the coagulation efficiency of carbonaceous nanoparticles. Journal of Aerosol Science 2020; 140.
23) Sirignano M, D'Anna A. Filtration and coagulation efficiency of sub-10 nm combustion-generated particles. Fuel 2018; 221: 298-302.
24) Morawska L, Ayoko GA, Bae GN, Buonanno G, Chao CYH, Clifford S, Fu SC, Hänninen O, He C, Isaxon C, Mazaheri M, Salthammer T, Waring MS, Wierzbicka A. Airborne particles in indoor environment of homes, schools, offices and aged care facilities: The main routes of exposure. Environment International 2017; 108: 75-83.
25) Abt E, Suh HH, Catalano P and Koutrakis P. Relative Contribution of Outdoor and Indoor Particle Sources to Indoor Concentrations. Environ Sci Tech 2000; 34: 3579-3587.
26) Long C M, Suh HH, Catalano P and Koutrakis, P. Using Time- and Size-Resolved Particulate Data to Quantify Indoor Penetration and Deposition Behavior. Environ Sci Tech 2001; 35: 2089-2099.
27) Dennekamp M, Howarth S, Dick CA, Cherrie JHW, Donaldson K and Seaton A. Ultrafine Particles and Nitrogen Oxides Generated by Gas and Electric Cooking. Occup Environ Med 2001; 58: 511-516.
28) Wallace L. Indoor sources of ultrafine and accumulation mode particles: size distributions, size-resolved concentrations, and source strengths. Aerosol Science and Technology 2006; 40: 348-360.

29) Long C, Suh HH, Koutrakis P. Characterization of Indoor Particle Sources Using Continuous Mass and Size Monitors. Journal of the Air & Waste Management Association 2000; 50(7): 1236-1250.
30) Hyungkeun K, Kyungmo K, Taeyeon K. Effect of occupant activity on indoor particle concentrations in Korean Residential buildings. 2020. Sustainability
31) Pedata P, Boccellino M, La Porta R, Napolitano M, Minutolo P, Sgro AL, Zei F, Sannolo N, Quagliuolo L. Interaction between combustion-generated organic nanoparticles and biological systems: in vitro study of cell toxicity and apoptosis in human keratinocytes. Nanotoxicology 2012; 6: 338-352.
32) Pedata P, Bergamasco N, D'Anna A, Minutolo P, Servillo L, Sannolo N, Balestrieri ML. Apoptotic and proinflammatory effect of combustion-generated organic nanoparticles in endothelial cells. Toxicology Letters 2013; 219: 307-314.
33) Pedata P, Malorni L, Sannolo N, Conturso M, Scantone S, Sirignano M, Ciajolo A, D'Anna A. Characterization and inflammatory potential of sub-10nm particles from gas cooking appliances. Chemical Engineering Transactions. 2016; 47: 433-438.
34) Malorni L, Guida V, Sirignano M, Genovese G, Petrarca C, Pedata P. Exposure to sub-10 nm particles emitted from a biodiesel-fueled diesel engine: In vitro toxicity and inflammatory potential. Toxicology Letters 2017; 270: 51-61.

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