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**Combined effects of nanoparticles and other environmental contaminants on human
health - an issue often overlooked**

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Abstract

Air pollution is considered as a major public health issue worldwide. It consists of a complex mixture of pollutants including nanoparticles to which we are increasingly exposed to due to the dramatic development of the nanotechnologies and their incidental or intentional release in the environment. Consequently, some concerns have raised about the combined toxicity of air particulates and other air pollutants on human health. However, the interactions between the contaminants and their resulting combined toxicity are often overlooked. Indeed, the biological effects triggered by nanoparticles are usually assessed focusing on individual nanoparticles, while their interaction with co-contaminants can deeply impact, either positively or negatively, their biodistribution, fate in the organism and toxicological profile (additive, synergistic or antagonistic responses). This paper presents a bibliographic review on the combined toxicity of nanoparticles and co-pollutants and discusses the underlying mechanisms. It also highlights the scarcity of data in the current literature, arguing for an urgent need to take into account the mixture effects to be more representative of real-life conditions for a better and accurate human health risk assessment and management.

Key-words: inhaled particles; co-exposure; combined toxicity; human health; mixture of pollutants.

1. Introduction

Humans are constantly exposed to a wide variety of pollutants, including nanoparticles to which we are increasingly exposed to due to the tremendous rise of the nanotechnologies and their incidental or intentional release in the environment. Consequently, some concerns have raised about the combined toxicity of airborne nanoparticles and other air pollutants on human health. However, the interactions between the contaminants and their resulting combined toxicity are often overlooked. The aim of this paper is to propose a bibliographic review on the joint toxicity of nanoparticles and co-pollutants to illustrate the lack of data in the current literature, arguing for the urgent need to carefully consider this issue. The underlying mechanisms of the joint toxicity of nanoparticles and co-pollutants are also discussed.

1.1. Air pollution

Air pollution is considered as a major public health issue worldwide. It is estimated that it is responsible for millions of premature deaths per year (Miller et al., 2012; Miller and Newby, 2020; Son et al., 2020; WHO, 2014). Ambient (outdoor) air pollution represents the 5th biggest risk factor for all-cause mortality, above more well-recognized risk factors such as low exercise and poor diet, and the number one environmental risk factor (Miller and Newby, 2020).

Initially, the effects of air pollution on the respiratory system received much attention as lungs are directly and constantly exposed to airborne pollutants. In addition, correlations between episodes of high air pollution and a worsening of allergic and inflammatory conditions of the lung, such as asthma and chronic obstructive pulmonary disease have been clearly evidenced (Miller et al., 2012). However, more recent studies highlighted clear relationships between air pollution and cardiovascular diseases, even claiming that cardiovascular conditions accounted for 40–60% of the premature mortality from air pollution (Beelen et al., 2014; Brunekreef and Holgate, 2002; Dockery et al., 1993; Germanova and Kernozhitskaya, 2017; Greim, 2019;

Miller et al., 2012; Miller and Newby, 2020; Shah et al., 2013). Studies also suggested that childhood leukemia and diseases of the nervous system were related to air pollution (Amigou et al., 2011; Germanova and Kernozhitskaya, 2017).

Air pollution can be defined as a complex mixture of gaseous, volatile, semi-volatile and particulate matter (PM), containing organic and inorganic compounds (Guo et al., 2021; Miller et al., 2012; Miller and Newby, 2020). Methane, benzene, naphthalene, formaldehyde, polyaromatic hydrocarbons (PAHs), and alkanes are among the various semi-volatile organic chemicals of air pollution. The numerous gases found in ambient air include carbon dioxide (CO₂) and monoxide (CO), ozone (O₃), nitrogen dioxide (NO₂) and sulphur dioxide (SO₂) (Miller et al., 2012; Miller and Newby, 2020). While these gases can induce adverse effects on human health, epidemiological studies reported that the PM contributed even more importantly to air pollution toxicity (Miller et al., 2012). PM is divided into coarse (PM₁₀: particles with a diameter less than 10 µm), fine (PM_{2.5}: particles with a diameter less than 2.5 µm) and ultrafine (PM_{0.1}: particles with a diameter less than 100 nm) particles. Because of their small size and large reactive surface area, ultrafine fractions can penetrate deeply into the lungs, reaching the alveoli and also potentially translocating in the bloodstream. Thus, they are acknowledged to produce greater effects (Miller et al., 2012; Miller and Newby, 2020). Moreover, ultrafine particles can adsorb high concentrations of toxic air pollutants leading to further harmful effects (Lu et al., 2015).

Due to the considerable rise of the nanotechnologies, engineered nanoparticles are increasingly used and incidentally or intentionally released in the environment. We are thus potentially increasingly exposed to these particles. Compared to ultrafine particles, less information is available regarding manufactured nanoparticles in terms of the toxicity induced after co-exposure with other pollutants. However, as ultrafine particle and nanoparticle physicochemical features may overlap, it has been suggested that their behaviors and toxicities may also overlap

(Stone et al., 2017). In addition, beside inhalation, nanoparticles can also enter the body through oral exposure and co-ingestion of nanoparticles and other pollutants such as pesticides for instance may also induce adverse effects on human health, especially on the gastro-intestinal tract (Cao et al., 2021, 2019; Dussert et al., 2020; Shi et al., 2010; Zheng et al., 2012). Moreover, nanoparticles incorporated in consumer products can be freed during the life cycle of the products, from their manufacture to their end-of-life. Especially, when this latter occurs through incineration, it can be accompanied by the formation of by-products which can impact deeply the release particle toxicity (Chalbot et al., 2017; Chivas-Joly et al., 2019, 2016; Singh et al., 2017). Consequently, some concerns have raised about the combined toxicity of air particulates and other air pollutants on human health.

1.2. Poly-exposure issue

As previously mentioned, it has been well documented that ambient air is a complex mixture of particles and air pollutants. However, their combined effects on human health remain unclear (Lu et al., 2015; Miller et al., 2012). Although constantly exposed to multiple sources of pollutants, the interactions between the contaminants and their resulting combined toxicity are poorly described in the literature. Indeed, the biological effects triggered by nanoparticles are usually assessed focusing on individual nanoparticles (Carlsten, 2018; Rosário et al., 2020). However, the nanoparticles released into the environment can interact with other pollutants or adsorb them on their surface, helping them enter in the organism (Abbas et al., 2020). This can lead to a significantly altered toxicological profile. This is why, although these interactions can be challenging to characterize, they should be carefully taken in consideration. Conversely, isolating the impact of a specific component present in a mixture and drawing firm conclusions on the health effects it induces can be complex (Miller and Newby, 2020). It has even been suggested that even if the toxicities of individual compounds are well characterized, unexpected

adverse effects can be induced by the mixing of such compounds (Silins and Högberg, 2011; Zheng et al., 2012), arguing for a joint toxicity assessment (Yang et al., 2018). Even worse, compounds that do not trigger adverse effects individually can induce a significant toxicity when in mixture, resulting in an underestimation of the risk. This is perfectly exemplified by the study from Jiang *et al.* who assessed the combined toxicity of carboxylated black carbon and lead acetate on human bronchial epithelial cells at the no-observed-adverse-effect level (NOAEL). They demonstrated that co-exposure to these compounds could induce some unexpected toxicity, even beyond their known individual toxicities (Jiang et al., 2020).

The toxicological profile of a compound can be dramatically altered depending on a single or co-exposure with other agents. Interactions between the compounds may result in additive or synergistic effects. Indeed, for combined exposures the health effects may increase more than what would be expected from simply adding the effects of the individual components (Cedergreen, 2014). Antagonistic effects can also be observed (Asweto et al., 2017; Carlsten, 2018; Liu et al., 2018; Silins and Högberg, 2011).

Taken together, it highlights the need to consider pollutant combination, leading to “mixture toxicology” (Deville et al., 2016), not considering anymore pollutants individually but shifting to an integrated multipollutant approach (Yun et al., 2015). Only a better knowledge in this field will allow a better and reliable risk assessment/ management. A recent attempt to reach this objective relies on the study of the “exposome”. This concept firstly introduced in 2005 by C.P. Wild (Wild, 2005), can be defined as the cumulative measure of environmental (*i.e.* non genetic) influences and the associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes (Miller and Jones, 2014; Niedzwiecki et al., 2019; Vermeulen et al., 2020). Research on the human exposome is thus paramount to potentially understand the causal pathways leading to common diseases. Although the concept is very appealing, in practice, its development is extremely challenging.

Unlike the genome, the exposome is a highly variable and dynamic entity that evolves throughout the lifetime of the individual. Advances will require increasing collaboration between epidemiologists, biostatisticians, experts in bioinformatics, and laboratory and environmental scientists (Miller and Jones, 2014; Wild, 2005).

2. A literature review on the joint toxicity of nanoparticles and co-contaminants

Studies on the combined toxicity of nanoparticles and other air pollutants are still scarce (Deng et al., 2017; Liu et al., 2018; Lu et al., 2017, 2015; Wu et al., 2016). To illustrate the lack of data on the joint toxicity and bioaccumulation of nanoparticles and co-contaminants a bibliographic review was carried out. Using PubMed, as a major search engine for papers on life sciences and biomedical topics, we queried the database with the following search terms: “(nanoparticles OR nanomaterials OR ultrafine particles) AND (mixture or co-exposure) AND toxicity AND human health”. It turned out that 433 papers have been published on this topic by March 2021. By comparison, the same research without the terms (mixture OR co-exposure) resulted in 10 112 references. As shown by Figure 1, very few papers were published until 2004 (less than 10 per year), but a rise in the number of publications was observed roughly in the last decade reaching almost 50 articles published each year during the last three years.

Figure 1 – Distribution of the 433 papers published per year.

Figure 2 schematically summarizes the selection process of the papers. We thoroughly reviewed the 433 articles focusing our attention on studies dealing with the toxicity induced by mixture of nanoparticles and co-pollutant by comparison with the effects induced by the same substance separately. We focused our attention on human health, we therefore excluded from our analysis studies dealing with plants and non-mammalian animal models, we also excluded reviews. We also focused on the issue of pollution and thus we excluded studies assessing nanoparticles and other components in the context of nanomedicine or environmental remediation (depollution with the use of nanoparticles to make the co-contaminant less available). We also excluded studies that did not include experimental data (*i.e.* studies based on digital, computing models for toxicity prediction).

Figure 2 – Selection process of the papers.

Based on these criteria, we retained 39 publications. The list of these papers and their comprehensive analysis are reported in Table 1. The main results are summarized in Table 2.

Table 1 - Analysis of the 39 publications selected from the literature review on the joint toxicity of nanoparticles and co-contaminants.

Authors	Title	Type of mixture	Biological model	Conclusions of the study	Conclusions on the observed effects	Reference
<u>Studies targeting the respiratory system</u>						
Lee et al.	Combination effect of nanoparticles on the acute pulmonary inflammogenic potential: additive effect and antagonistic effect	Binary mixtures of carbon black (CB), nickel oxide (NiO), and copper oxide (CuO) NPs	Rats	Additive effect of CuO and NiO NPs on the percentage of neutrophils and ROS generation potential. Antagonistic effect of a combined treatment with CuO or NiO NPs on the percentage of neutrophils.	Both	(Lee et al., 2021)
Guo et al.	Environmentally relevant iron oxide nanoparticles produce limited acute pulmonary effects in rats at realistic exposure levels	Nano-sized iron oxide particles (Fe ₃ O ₄ , α -Fe ₂ O ₃ and γ -Fe ₂ O ₃)	Rats and human bronchial epithelial cells (BEAS-2B)	No adverse effects observed.	None	(Guo et al., 2021)
He et al.	Autophagy changes in lung tissues of mice at 30 days after carbon black-metal ion co-exposure	Carbon black (CB) and Ni	Mice	No adverse effects observed.	None	(He et al., 2020)
Köerich et al.	Toxicity of binary mixtures of Al(2)O(3) and ZnO nanoparticles toward fibroblast and bronchial epithelium cells	Zinc oxide (ZnO) and aluminum oxide (Al ₂ O ₃) NPs	Mouse fibroblast cells (L929) and human bronchial epithelial cells (BEAS-2B)	Cytotoxicity of binary mixtures of Al ₂ O ₃ and ZnO NPs produced greater effects compared to toxicity of either individual compound.	Additive/synergistic	(Köerich et al., 2020)
Rosario et al.	Unravelling the potential cytotoxic effects of metal oxide nanoparticles and metal(loid) mixtures on A549 human cell line	Binary mixtures of cerium oxide (CeO ₂) NPs, titanium dioxide (TiO ₂) NPs, mercury (Hg) and arsenic (As)	Lung epithelial cell line (A549)	Combined exposure of TiO ₂ NP or CeO ₂ NP mitigated As adverse effects. Only CeO ₂ NP co-exposure reduced Hg toxicity.	Antagonistic	(Rosário et al., 2020)
Wang et al.	Complex to simple: in vitro exposure of particulate matter simulated at the air-liquid interface discloses the health impacts of major air pollutants	Silica NPs and bisulfate	Human bronchial epithelial cells (BEAS-2B)	Sulfate exacerbates the toxic effects of inhalable PMs.	Additive/synergistic	(Wang et al., 2019)

Zeng et al.	Cerium dioxide (CeO ₂) nanoparticles decrease arsenite (As(III)) cytotoxicity to 16HBE14o- human bronchial epithelial cells	Cerium dioxide (CeO ₂) NPs and arsenite (As(III))	Human bronchial epithelial cells (16HBE14o-)	Co-exposure of CeO ₂ NPs and As(III) resulted in reduced toxicity.	Antagonistic	(Zeng et al., 2018)
Lu et al.	Combined exposure to nano-silica and lead induced potentiation of oxidative stress and DNA damage in human lung epithelial cells	Silica NPs (nano-SiO ₂) and lead (Pb)	Lung adenocarcinoma cells (A549)	Exposure to the combination of nano-SiO ₂ and Pb potentiated oxidative stress and DNA damage in A549 cells.	Additive/synergistic	(Lu et al., 2015)
Lehto et al.	Co-exposure with fullerene may strengthen health effects of organic industrial chemicals	Fullerene and chemicals (acetophenone, benzaldehyde, benzyl alcohol, m-cresol, and toluene)	Human THP-1-derived macrophages	Co-exposure to organic chemicals with fullerene may strengthen the health effects of the chemicals.	Additive/synergistic	(Lehto et al., 2014)
Wu et al.	The chronic effect of amorphous silica nanoparticles and benzo[a]pyrene co-exposure at low dose in human bronchial epithelial BEAS-2B cells	Silica NPs (SiNPs) and benzo[a]pyrene (B[a]P)	Human bronchial epithelial cells (BEAS-2B)	Severe synergistic and additive toxic effects induced by SiNPs and B[a]P co-exposure for long-term in BEAS-2B cells even at low dose.	Additive/synergistic	(Wu et al., 2019)
Ahamed et al.	Preventive effect of TiO ₂ nanoparticles on heavy metal Pb-induced toxicity in human lung epithelial (A549) cells	TiO ₂ NPs (nTiO ₂) and lead (Pb)	Human lung epithelial cells (A549)	In the co-exposure group (nTiO ₂ +Pb), nTiO ₂ effectively attenuated the cytotoxicity, oxidative stress and apoptotic responses of Pb in A549 cells.	Antagonistic	(Ahamed et al., 2019a)
Wu et al.	Co-exposure to amorphous silica nanoparticles and benzo[a]pyrene at low level in human bronchial epithelial BEAS-2B cells	Silica NPs (SiNPs) and benzo[a]pyrene (B[a]P)	Bronchial epithelial cells (BEAS-2B)	Co-exposure to SiNPs and B[a]P exerts synergistic and additive cytotoxic and genotoxic effects.	Additive/synergistic	(Wu et al., 2016)
Yu et al.	Combined toxicity of amorphous silica nanoparticles and methylmercury to human lung epithelial cells	Silica NPs (nano-SiO ₂) and methylmercury (MeHg)	Lung adenocarcinoma cells (A549)	Additive and synergistic interactions were responsible for the combined toxicity of nano-SiO ₂ and MeHg.	Additive/synergistic	(Yu et al., 2015)
Ballesteros et al.	Nanoceria, alone or in combination with cigarette-smoke condensate, induce transforming and epigenetic cancer-like features in vitro	Nanoceria and cigarette smoke condensate	Human bronchial epithelial cells (BEAS-2B)	Nanoceria exposure shows a positive interaction with the cell-transforming effects of cigarette smoke condensate.	Additive/synergistic	(Ballesteros et al., 2021)

Ahamed et al.	Single-walled carbon nanotubes attenuate cytotoxic and oxidative stress response of Pb in human lung epithelial (A549) cells	Single-walled carbon nanotubes (SWCNTs) and lead (Pb)	Human lung epithelial cells (A549)	SWCNTs attenuated the cytotoxic and oxidative stress response generated by Pb exposure in A549 cells.	Antagonistic	(Ahamed et al., 2020b)
Jiang et al.	Low-dose combined exposure of carboxylated black carbon and heavy metal lead induced potentiation of oxidative stress, DNA damage, inflammation, and apoptosis in BEAS-2B cells	Carboxylated black carbon (c-BC) and lead acetate (Pb)	Human bronchial epithelial cells (BEAS-2B)	Combination of c-BC and Pb could significantly increase cytotoxicity.	Additive/synergistic	(Jiang et al., 2020)
Son et al.	Effects of antioxidants on oxidative stress and inflammatory responses of human bronchial epithelial cells exposed to particulate matter and cigarette smoke extract	PM10 and cigarette smoke extract (CSE)	Human bronchial epithelial cells (BEAS-2B)	Exposure of epithelial cells to PM or CSE induced cytotoxicity, inflammation, and oxidative stress, all of which were dramatically increased when cells were exposed to the combination of CSE and PM.	Additive/synergistic	(Son et al., 2020)
Azari et al.	Additive toxicity of co-exposure to pristine multi-walled carbon nanotubes and benzo α pyrene in lung cells	Pristine multi-walled carbon nanotubes (P-MWCNTs) and benzo α pyrene (BaP)	Human epithelial lung cells (A549)	The co-exposure to P-MWCNTs and BaP enhanced the cytotoxicity compared to exposure to P-MWCNTs and BaP individually, but not statistically significant.	Additive/synergistic	(Azari et al., 2020)
Azari et al.	Antagonistic effect of co-exposure to short-multiwalled carbon nanotubes and benzo[a]pyrene in human lung cells (A549)	Short-multiwalled carbon nanotubes (S-MWCNTs) and benzo a pyrene (BaP)	Human epithelial lung cells (A549)	Co-exposure to S-MWCNTs and BaP demonstrated a significant reduction in ROS generation and apoptosis compared with the sum of their separate toxic effects at the same concentrations.	Antagonistic	(Azari et al., 2019)
Azari et al.	Individual and combined toxicity of carboxylic acid functionalized multi-walled carbon nanotubes and benzo a pyrene in lung adenocarcinoma cells	Acid functionalized multi-walled carbon nanotubes (F-MWCNTs) and benzo a pyrene (BaP)	Human epithelial lung cells (A549)	Combined exposure to F-MWCNTs and BaP decreases cytotoxicity compared to individual exposure.	Antagonistic	(Rezazadeh Azari et al., 2019)
Yun et al.	Synergistic effects of particulate matter (PM10) and SO ₂ on human non-small cell lung cancer A549 via ROS-mediated NF- κ B activation	Particulate matter 10 (PM10) and sulfur dioxide (SO ₂)	Human lung adenocarcinoma cells (A549)	The concomitant treatment of PM10 and SO ₂ led to synergistic injury.	Additive/synergistic	(Yun et al., 2015)

Studies targeting the digestive system

Zheng et al.	Effects of the interaction of TiO ₂ nanoparticles with bisphenol A on their physicochemical properties and in vitro toxicity	TiO ₂ NPs (nano-TiO ₂) and bisphenol A (BPA)	Human embryo hepatocytes (L-02)	The mixture of nano-TiO ₂ and BPA increased toxicity.	Additive/synergistic	(Zheng et al., 2012)
Shi Y et al.	Synergistic genotoxicity caused by low concentration of titanium dioxide nanoparticles and p,p'-DDT in human hepatocytes	Titanium dioxide NPs (nano-TiO ₂) and dichlorodiphenyltrichloroethane (p,p'-DDT)	Human embryo hepatocytes (L-02)	Dose-dependent synergistic genotoxicity induced by combined exposure of trace p,p'-DDT and nano-TiO ₂ .	Additive/synergistic	(Shi et al., 2010)
Ahamed et al.	Co-exposure to SiO ₂ nanoparticles and arsenic Induced augmentation of oxidative stress and mitochondria-dependent apoptosis in human cells	Silica NPs (nSiO ₂) and arsenic (As)	Human liver (HepG2) and human fibroblast (HT1080) cells	Co-exposure of a non-cytotoxic concentration of nSiO ₂ significantly augmented the As induced toxicity in both cell types.	Additive/synergistic	(Ahamed et al., 2019b)
Dussert et al.	Toxicity to RAW264.7 macrophages of silica nanoparticles and the E551 food additive, in combination with genotoxic agents	3 types of silica NPs (LS30, E551, fumed silica) and benzo[a]pyrene (B[a]P) and methane methylsulfonate (MMS)	Mouse macrophages (RAW 264.7) and colorectal adenocarcinoma cells (Caco-2/ HT29-MTX coculture)	Co-exposure to SiO ₂ and MMS causes DNA damage in a synergistic way.	Additive/synergistic	(Dussert et al., 2020)
Ahamed et al.	TiO ₂ nanoparticles potentiated the cytotoxicity, oxidative stress and apoptosis response of cadmium in two different human cells	Titanium dioxide NPs (nTiO ₂) and cadmium (Cd)	Human liver (HepG2) and breast cancer (MCF-7) cells	Non-cytotoxic concentration of nTiO ₂ enhanced the toxicological potential of Cd in human cells.	Additive/synergistic	(Ahamed et al., 2020a)

Studies targeting the cardiovascular system

Yang et al.	Co-exposure of silica nanoparticles and methylmercury induced cardiac toxicity in vitro and in vivo	Silica nanoparticles (SiNPs) and methylmercury (MeHg)	Rats and human cardiomyocytes (AC16)	Additive and synergistic interactions were responsible for the cardiac toxicity induced by co-exposure of SiNPs and MeHg <i>in vitro</i> and <i>in vivo</i> .	Additive/synergistic	(Yang et al., 2018)
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Feng et al.	Co-exposure subacute toxicity of silica nanoparticles and lead acetate on cardiovascular system	Silica NPs (SiNPs) and lead acetate (Pb)	Rats	Co-exposure to SiNPs and Pb could aggravate the cardiovascular toxicity and cardiac injury in vivo.	Additive/synergistic	(Feng et al., 2018)
Feng et al.	Low-dose combined exposure of nanoparticles and heavy metal compared with PM(2.5) in human myocardial AC16 cells	Silica NPs (SiNPs) and lead acetate Pb(AC)2; carbon black nanoparticles (CBNPs) and Pb(AC)2; SiNPs and CBNPs	Human myocardial cells (AC16)	The combination of SiNPs and Pb(AC)2/CBNPs as well as the combination of CBNPs and Pb(AC)2 resulted in increased toxicity via additive and synergistic interactions.	Additive/synergistic	(Feng et al., 2017)
Asweto et al.	Combined effect of silica nanoparticles and benzo[a]pyrene on cell cycle arrest induction and apoptosis in human umbilical vein endothelial cells	Silica NPs (SiNPs) and Benzo[a]pyrene (B[a]P)	Human umbilical vascular endothelial cells (HUVEC)	Interactions between SiNPs and B[a]P synergistically potentiated toxicological effects on HUVECs.	Additive/synergistic	(Asweto et al., 2017)

Studies targeting other systems

Roje et al.	Synergistic effects of parabens and plastic nanoparticles on proliferation of human breast cancer cells	Plastic NPs and parabens	Breast cancer cell lines (MDA-MB 231 and MCF-7)	Parabens increased proliferation of oestrogen-sensitive breast cancer cells, and this effect became synergistic in the presence of plastic nanoparticles.	Additive/synergistic	(Roje et al., 2019)
Lai et al.	Coexposed nanoparticulate Ag alleviates the acute toxicity induced by ionic Ag(+)in vivo	Nanoparticulate and ionic silver: AgNPs and Ag ⁺	Rats	AgNPs reduced the Ag ⁺ induced toxicity.	Antagonistic	(Lai et al., 2020)
Schirinzi et al.	Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells	Fullerenes and malathion, sodium dodecylbenzenesulfonate and diethyl phthalate	Human cerebral (T98G) and epithelial (HeLa) cells	Fullerene presented a predominantly antagonistic effect in binary mixtures with other organic contaminants.	Antagonistic	(Schirinzi et al., 2017)
Xia B et al.	Cellular oxidative damage of HEK293T cells induced by combination of CdCl ₂ and nano-TiO ₂	Titanium dioxide NPs (nano-TiO ₂) and cadmium chloride (CdCl ₂)	Human embryo kidney 293T cells (HEK293T)	CdCl ₂ and nano-TiO ₂ exert synergistic effects on the cellular oxidative damage of HEK293T cells.	Additive/synergistic	(Xia et al., 2011)

Teng et al.	Oral co-exposures to zinc oxide nanoparticles and CdCl ₂ induced maternal-fetal pollutant transfer and embryotoxicity by damaging placental barriers	Zinc oxide NPs (ZnO NPs) and cadmium chloride (CdCl ₂)	Pregnant mice	Synergistic embryotoxicity by nanoparticle/pollutant combination.	Additive/synergistic	(Teng et al., 2020)
Cotena et al.	In vitro co-exposure to CeO ₂ nanomaterials from diesel engine exhaust and benzo(a)pyrene induces additive DNA damage in sperm and cumulus cells but not in oocytes	Cerium dioxide nanomaterials (CeO ₂ NMs) and Benzo(a)pyrene (BaP)	Human (sperm) and rat gametes: sperm and cumulus (oocytes) cells	Co-exposure to the CeO ₂ NMs-BaP mixture induced additive DNA damage in sperm and cumulus cells, whereas no additive effect was observed in rat oocytes.	Both	(Cotena et al., 2021)
Ku et al.	Synergistic effects of particulate matter (PM _{2.5}) and sulfur dioxide (SO ₂) on neurodegeneration via the microRNA-mediated regulation of tau phosphorylation	Particulate matter (PM _{2.5}) and sulfur dioxide (SO ₂)	Mice and primary cortical neurons	Particulate matter (PM _{2.5}) and sulfur dioxide (SO ₂) co-exposure synergistically leads to neurodegeneration.	Additive/synergistic	(Ku et al., 2017)
Deville et al.	Interaction of gold nanoparticles and nickel(II) sulfate affects dendritic cell maturation	Gold NPs (GNPs) and nickel (II) sulfate (NiSO ₄)	Dendritic cells	When cells were exposed to a mixture of GNPs and NiSO ₄ , the observed cell activation pattern indicated a competitive rather than an additive effect of both inducers.	Antagonistic	(Deville et al., 2016)
Courter et al.	Urban dust particulate matter alters PAH-induced carcinogenesis by inhibition of CYP1A1 and CYP1B1	Benzo[a]pyrene (B[a]P), dibenzo[a,l]pyrene (DB[a,l]P) and urban dust particulate matter (UDPM)	Mice	UDPM significantly delayed the onset of B[a]P-induced tumor initiation. When co-treated with UDPM, DB[a,l]P-treated animals displayed no significant difference in tumor-initiating activity, compared with DB[a,l]P alone. Co-treatment of UDPM plus B[a]P or DB[a,l]P resulted in increased DNA strand breaks, compared with PAH treatment alone.	Both	(Courter et al., 2007)

Table 2 – Main parameters considered in our literature review. The proportion of the selected studies related to each parameter is reported. For the biological endpoints, the percentage of studies assessing the indicated variables is indicated. NP: nanoparticle.

Nature of the mixture studied	Biological models used	Target organs investigated	Biological endpoints mainly assessed	Nature of the effects observed
NP/NP 10% NP/other 85% Both 5%	<i>In vitro</i> 77% <i>In vivo</i> 15% Both 8%	Respiratory 54% Digestive 13% Cardiovascular 10% Other/various 23%	Cell viability/cytotoxicity 67% Oxidative stress 59% Apoptosis 46% Genotoxicity 33% Cell cycle 18% Cell/organ injury 15% Cell proliferation 10% Inflammation 10% Immunotoxicity 5% Carcinogenesis 5%	Additive/synergistic 64% Antagonistic 23% Both (depending on the model or parameter assessed) 8% No effect 5%

We first analyzed the type of mixture investigated, indeed, nanoparticles can be co-exposed with a wide variety of compounds including organic contaminants, metal/metalloid ions, inorganic ligands, or other nanoparticles (Deng et al., 2017).

It clearly appeared that the toxicity from multiple nanoparticles exposure was poorly investigated. Indeed, only 10% of the studies investigated the joint toxicity upon exposure to two types of nanoparticles (Feng et al., 2017; Guo et al., 2021; He et al., 2020; Köerich et al., 2020; Lee et al., 2021; Rosário et al., 2020).

This overlooked type of mixture can however provide critical information. For instance, while investigating the combined effects of zinc oxide nanoparticles (ZnONPs) and copper nanoparticles (CuNPs) in a human hepatoma cell line Li *et al.* observed that the cytotoxic effect of CuNPs was significantly enhanced by the ZnONPs. However, the cytotoxicity triggered by

CuNPs was not affected by co-incubation with medium containing only zinc ions, indicating that the increase in toxicity might be attributed to the particle form of ZnONPs (Li et al., 2015). Therefore, the study of binary mixtures of nanoparticles seems highly relevant to better understand the combined toxicity of pollutants.

Only two papers reported both binary mixture of nanoparticles and nanoparticle/other compound (Feng et al., 2017; Rosário et al., 2020).

Much more data is available on the toxicity induced by a co-exposure of a nanoparticle and another compound. Indeed, nanoparticles have been associated with organic contaminants, metal/metalloid ions, inorganic ligands, thus studies reported the joint effects of nanoparticles and common environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), metal, chemicals, insecticide, pesticide, surfactant, plasticizer, plastics, paraben, preservatives, *etc.* (For further details, please refer to Supporting Information).

Interestingly, no combination nanoparticle/gas was reported, maybe because this association is technically challenging to reproduce. Wang *et al.* generated aerosols constituted by silica nanoparticles and bisulfate to simulate a model of particle-associated high-sulfur air pollution. They used bisulfate-coated silica nanoparticles ($\text{SiO}_2\text{-NH}_2\text{@HSO}_3$) and compared their effects to those produced by nano-silica or bisulfite aerosol individually (Wang et al., 2019).

We then analyzed the biological models used and target organs investigated in these studies of combined toxicity. Regarding the biological systems, we clearly observed that the vast majority of the studies were performed using *in vitro* models, only 15% used *in vivo* models and even fewer used a combined approach (8%). Studies mainly targeted the respiratory system, which seems quite logical considering that they were carried out in a context of air pollution. Indeed, as mentioned before, this topic is a major public health issue worldwide as it has been reported that air pollution is a complex, multi-source mixture of gases and particulate matter, containing

organic and inorganic compounds. It is recognized as a significant cause of adverse effects on human health, leading to illnesses and being responsible for millions of premature deaths (Guo et al., 2021; Son et al., 2020; WHO, 2014). As the respiratory tract is directly exposed to the atmospheric environment it represents a target of choice for studies aiming to assess the toxicity of air pollutants. The small size of nanoparticles facilitates their entry into the lungs and they can even reach its deepest structures, the alveoli, where they can accumulate if not cleared. As the toxic compounds can adsorb at the surface of the nanoparticles they can benefit from the carrier effect and reach the alveoli, causing further damages (the underlying mechanism will be further described in the next section) (Lu et al., 2015).

Still because of their small size, nanoparticles can translocate and reach the systemic circulation to be potentially distributed to other organs. This explains why some studies investigated the damages to the cardio-vascular system (Asweto et al., 2017; Feng et al., 2018, 2017; Yang et al., 2018) and to other organs (Cotena et al., 2021; Courter et al., 2007; Deville et al., 2016; Ku et al., 2017; Lai et al., 2020; Roje et al., 2019; Schirinzi et al., 2017; Teng et al., 2020; Xia et al., 2011).

Among the different organs, the digestive system is quite well studied (Ahamed et al., 2020a, 2019b; Cao et al., 2021, 2019; Dussert et al., 2020; Shi et al., 2010; Zheng et al., 2012). Indeed, inhaled nanoparticles can reach this system after translocation or due to swallowing after mucocilliary clearance of particles from the airways. They can also be ingested directly as nanoparticles are widely used as additives in cosmetics, toothpaste, food industry, pharmaceuticals, or water treatment. This is especially the case for silica (E551) and titanium dioxide (E171) nanoparticles. Nanoparticles can also be included in products in contact with food (food containers or packaging) and be found in presence of other environmental contaminants such as traces of pesticides (Shi et al., 2010), arsenic (Ahamed et al., 2019b), bisphenol A (Zheng et al., 2012), cadmium (Ahamed et al., 2020a), or benzopyrene (Dussert et

al., 2020). Cao *et al.* clearly highlighted that co-exposure to E551 or E171 and the pesticide boscalid increased the cytotoxicity and bioavailability of the pesticide in a model of small intestine epithelium. They further demonstrated an increased translocation of the intestinal barrier correlated to altered cell junctions (Cao et al., 2021, 2019).

In Table 2, the category other/ various includes studies on the nervous system (Ku et al., 2017; Schirinzi et al., 2017), immune system (Deville et al., 2016), kidney cells (Xia et al., 2011), breast cells (Roje et al., 2019), on carcinogenesis (Courter et al., 2007), reproductive organs (Cotena et al., 2021), fetus (Teng et al., 2020) or deal with global effects, affecting several organs (Courter et al., 2007; Lai et al., 2020; Teng et al., 2020).

We then considered the biological endpoints mainly used in these studies. Cell viability/cytotoxicity was the most commonly assessed biological endpoint (evaluated in 67% of the studies), followed by oxidative stress, a mechanism usually associated with cell damages caused by nanoparticles. This latter includes the assessment of different parameters such as the pro-oxidants generation (reactive oxygen species and lipid peroxidation which is a cellular effect induced by these radicals) and antioxidants depletion (glutathione level, and glutathione reductase, superoxide dismutase and catalase activities). Apoptosis (as reported by the upregulation of caspases and pro-apoptotic members of the Bcl-2 family and the downregulation of anti-apoptotic members of the Bcl-2 family) and genotoxicity (as evaluated by DNA damages) are also frequently investigated. Finally, in less than 20% of the cases were studied parameters such as cell cycle, cell/organ injuries, cell proliferation, inflammation (secretion of pro-inflammatory cytokines), immunotoxicity (effects on cells from the immune system) or impacts on carcinogenesis.

Finally, we evaluated the nature of the biological effects observed following a co-exposure to nanoparticles and a co-contaminant. Most of the selected studies reported that co-exposure of nanoparticles and co-contaminant potentiated the biological effects compared to the effects of each compound assessed individually. Additive and/or synergistic effects were indeed observed in 64% of the cases. On the contrary, protective, antagonistic effects were observed in 23% of the studies. It should also be mentioned that sometimes no effect was observed (Guo et al., 2021; He et al., 2020) or opposite effects could be shown depending on the nature of the mixture, the biological model or the biological endpoint considered (Cotena et al., 2021; Courter et al., 2007; Lee et al., 2019).

3. Underlying mechanisms of combined nanoparticle/other contaminant toxicity

Considering the simultaneous presence of nanoparticles and other pollutants in the environment, various kinds of interactions may occur. In particular, because nanoparticles are characterized by a huge surface/volume ratio, they can adsorb compounds on their surface, either through hydrophobic, electrostatic, π - π stacking, covalent and/or hydrogen bonding interactions and/or surface complexation (Deng et al., 2017; Liu et al., 2018). The complexes formed between nanoparticles and contaminants will have a deep impact on their biodistribution, their fate within the organism and eventually on the biological outcome.

Figure 3 schematically summarizes the different mechanisms that can be responsible for the joint toxicity of co-exposed contaminants as discussed below. First, nanoparticle can provide a vehicle for co-contaminants and play the role of carrier.

Figure 3 – Summarizing scheme of the potential mechanisms underlying the combined toxicity of nanoparticle and co-pollutant. NP: nanoparticle, [pollutant]_i: intracellular concentration of pollutant, [pollutant]_e: extracellular concentration of pollutant, [NP]_i: intracellular concentration of nanoparticle, ROS: reactive oxygen species, $\downarrow$: decrease, $\uparrow$: increase.

3.1. A carrier effect leading to increased toxicity

The capacity of nanoparticles to adsorb a co-pollutant can play a critical role in the toxicity of either the nanoparticle or the pollutant, especially by facilitating the entry of a contaminant in the organism through the uptake of nanoparticle-contaminant complexes. Once inside, the complexed contaminants can be released in the organism increasing their concentration and thus their bioavailability and toxicity. This mechanism is referred to as the “Trojan horse” effect (Deng et al., 2017; Liu et al., 2018). This concept explaining the facilitated entry of toxic molecules adsorbed on nanoparticles and resulting in an enhanced intracellular concentration of pollutants was illustrated by numerous studies (Ahamed et al., 2019b; Dussert et al., 2020; Guo et al., 2013; Roje et al., 2019; Yu et al., 2015; Zheng et al., 2012). For instance, Yu *et al.* exposed human lung cells to either silica nanoparticles, methylmercury or a combination of both. They observed that the intracellular content of silicon and mercury in the combination group were much higher than those in single exposure groups. This locally increased concentration of methylmercury was correlated with enhanced biological damaging effects (Yu et al., 2015). When assessing the combined toxicity of TiO₂ nanoparticles and bisphenol A, Zheng *et al.* observed that because these two compounds formed complexes, they were efficiently taken up by cells, leading to increased intracellular concentrations of both TiO₂ nanoparticles and bisphenol A, and ultimately resulting in synergistic toxicity (Zheng et al., 2012). Ahamed *et al.* also showed a higher intracellular level of arsenic when cells were co-exposed with silica nanoparticles compared to cells exposed only to arsenic. In addition, most of the arsenic present in culture media was adsorbed on the surface of the nanoparticles in the co-exposure group, suggesting that nanoparticles can act as a cargo, facilitating arsenic cellular uptake and accumulation in cells (Ahamed et al., 2019b).

Although many studies have reported this carrier effect as responsible for an enhanced cytotoxicity, other have reported the exact opposite, *i.e.* a protective effect following a nanoparticle/ other contaminant co-exposure.

3.2. A carrier effect leading to decreased toxicity

Indeed, the bioavailability, *i.e.* the accessibility, of a contaminant to the cells will strongly influence the induction of adverse effects. This is why sorption of contaminants by nanoparticles may decrease the bioavailable concentration in the exposure medium, which would subsequently lower the toxicity caused by these contaminants, assuming that the nanoparticle-contaminant complexes do not enter the organism. But even if the complexes are successfully internalized, toxicity may be mitigated if desorption of contaminants from the nanoparticles-contaminant complexes is limited or incomplete (Deng et al., 2017). As an example, Ahamed *et al.* reported that adverse effects were attenuated in human lung cells co-exposed to TiO₂ nanoparticles and lead (Pb) compared to cells exposed only to Pb. Cellular uptake experiments showed that nanoparticles facilitated the entry of Pb into cells but due to strong adsorption free Pb ions were not available even inside the cells. From these results it was concluded that TiO₂ nanoparticles significantly prevented the bioavailability and toxicity of Pb (Ahamed et al., 2019a). Similarly, Zeng *et al.* showed in a model of human bronchial cells that CeO₂ nanoparticles were able to reduce available arsenite through adsorption, resulting in reduced toxicity (Zeng et al., 2018). Azari *et al.* also concluded from two studies that benzo[a]pyrene (B[a]P) adsorbed on multi-walled carbon nanotubes, resulting in a decrease of the bioavailability of B[a]P and consequently an attenuation of its adverse effects (Azari et al., 2019; Rezazadeh Azari et al., 2019). Similar results were observed with single-walled carbon nanotubes that can adsorb Pb at their surface leading to a decreased bioavailability and toxicity of Pb (Ahamed et al., 2020b). Furthermore, we should consider carefully the kinetic aspect in

these studies as the bioavailability of the co-pollutant depends on its desorption rate and effects may not be observed if too early time points are considered (effects may just be delayed). This underlines the importance of investigating the effects of pollutants co-exposure over a sufficient period.

Therefore, a same mechanism, the adsorption of a contaminant on nanoparticles, can lead to opposite effects, either 1) facilitating the transport into cells or organisms, increasing the cellular dose and amplifying the toxic effect of the contaminant or 2) decreasing its availability, resulting in a reduction of contaminant uptake by organisms (Liu et al., 2018; Rosário et al., 2020; Schirinzi et al., 2017). It adds further complexity to combined toxicology and draws attention on the fact that nanoparticles/ co-contaminants interactions should be considered carefully.

3.3. Opposite effects depending on the biological models or endpoints considered

Furthermore, variable effects can be observed depending on the biological models, the biological endpoints or the experimental conditions. For instance, Köerich *et al.* observed that while additive effects of ZnO and Al₂O₃ nanoparticles were observed on the cytotoxicity on human bronchial cells, effects were less uniform on murine fibroblasts where additive effects were reported only for the lowest concentrations. However, at the highest concentrations antagonistic effects were observed as the cytotoxicity of ZnO nanoparticles was attenuated in case of co-exposure with Al₂O₃ nanoparticles. It suggests concentration and cell line-dependent effects (Köerich et al., 2020). Similarly, the combined effects of cadmium chloride and TiO₂ nanoparticles on cellular oxidative damage varied from additive to synergistic depending on the proportion of the compounds in the mixture (Xia et al., 2011).

Such variable effects were also observed *in vivo*. For example, Courter *et al.* investigated the effects of a co-exposure to benzo[a]pyrene (B[a]P) and urban dust particulate matter (UDPM)

on the bioactivation and tumor initiation in an initiation-promotion tumorigenesis model. While UDPM significantly delayed the onset of B[a]P-induced tumor initiation, it increased DNA damages compared to those induced by B[a]P alone (Courter et al., 2007). Besides, Teng *et al.* observed effects dependent on the timing of exposure. Indeed, they assessed the combined toxicity of zinc oxide nanoparticles and cadmium chloride using two pregnant mice models established by oral administration during peri-implantation or organogenesis period. They observed that exposures to combined pollutants only at organogenesis stage induced higher fetal deformity rate compared to co-exposures at peri-implantation stage (Teng et al., 2020). Cotena *et al.* showed that co-exposure to CeO₂ nanoparticles and benzo[a]pyrene induced additive DNA damage in sperm and cumulus cells compared to those generated by the individual pollutants, whereas no additive toxic effect was observed in rat oocytes. They attributed this result to the structural protection of the oocyte by cumulus cells and to the oocyte's efficient system to repair DNA damage compared to that of cumulus and sperm cells (Cotena et al., 2021).

Also at a population level, co-exposure to pollutants can impact differently individuals, especially in susceptible populations. Jia *et al.* investigated the joint toxicity of zinc oxide nanoparticles and lead in healthy overweight and healthy normal weight mice. Co-exposure resulted in an increased deposition of lead in all major organs in the overweight mice compared to that in the normal mice and was consequently associated with an enhanced toxicity in overweight mice (Jia et al., 2017).

3.4. Effects of the co-pollutant on the nanoparticles

3.4.1. Direct effects

So far, we discussed the influence of nanoparticles on the accumulation and toxicity of co-exposed contaminants, but the reverse relationship should also be considered. Indeed,

contaminants can alter the physicochemical features of nanoparticles, especially in relation with surface properties (chemistry, charge, hydrophobicity, agglomeration state, *etc.*) and consequently result in a modified toxicological profile. As an example, Deville *et al.* examined the impact of a combined exposure of nickel (II) sulfate (NiSO₄) and gold nanoparticles (GNPs) on the maturation response of dendritic cells. Antagonistic effects were observed although GNPs and NiSO₄ were shown not to directly interact with each other. A further physiochemical characterization allowed to conclude that the presence of NiSO₄ induced changes in GNPs' charge and their associated protein corona (content and composition). This latter, as the new nano/bio interface is acknowledged to play a key role in cell/ nanoparticle interactions (Forest, 2019; Forest et al., 2015; Forest and Pourchez, 2017). This alteration could contribute to a decreased cellular uptake of GNPs, sustaining the nickel-induced dendritic cells maturation (Deville et al., 2016). Similarly, Teng *et al.* observed that the surface charge of ZnO nanoparticles was modified in case of co-exposure with cadmium chloride (because of Cd²⁺ adsorption at the nanoparticle surface), promoting enhanced maternal-fetal transfer of both pollutants and aggravated embryotoxicity (Teng et al., 2020). Finally, functional groups present at the surface of the nanoparticles can be modified by co-contaminant, resulting in an altered toxicity (Deng et al., 2017).

3.4.2. Indirect effects

Co-exposed pollutant can impact the cell membrane (in terms of hydrophobicity, fluidity, permeability or physical integrity) leading to an enhanced nanoparticle internalization and toxicity (Deng et al., 2017; Liu et al., 2018). Similarly, exocytosis can be impaired by a pollutant, leading to intracellular accumulation of nanoparticles, enhancing their bioavailability and thus their potential adverse effects (Deng et al., 2017). Some pollutants can also directly affect the physiological activities of organisms and change receptor tolerance to the

nanoparticles. As an example, Dhasmana *et al.* showed that benzo[a]pyrene (BaP) entered lung carcinoma cell nucleus via aromatic hydrocarbon receptors, but TiO₂ nanoparticles having a higher binding affinity for these receptors than BaP, they reduced nuclear internalization and cytotoxicity of BaP upon co-exposure (Dhasmana et al., 2014). Other contaminants can mitigate nanoparticles toxicity by scavenging the reactive oxygen species (ROS) the latter produce. On the contrary, contaminants can potentiate nanoparticles adverse effects by generating additional ROS production (Deng et al., 2017).

3.5. Sensitizing effect

Another mechanism that can contribute to an enhanced toxicity in case of combined exposure to different pollutants is that one of them can make the cells more sensitive to the second one. This is what observed Jiang *et al.* suggesting that the intracellular perturbations caused by black carbon made the cells susceptible to lead toxicity (Jiang et al., 2020). Li *et al.* described that by accumulating in cells, zinc oxide nanoparticles altered cell membranes and enhanced the toxicity of co-exposed copper nanoparticles (Li et al., 2015).

3.6. Oxidative stress, inflammation, DNA damage and cell death

Oxidative stress and inflammation have been shown to be two major mechanisms involved in the toxicity of nanoparticles. In addition, they seem to act synergistically to amplify each other's effects (Leni et al., 2020; Miller et al., 2012; Miller and Newby, 2020).

Oxidative stress corresponds to excessive reactive oxygen species (ROS), overwhelming the organism's antioxidant defense system. ROS can lead to cell damages, including DNA damages, and impaired cell functions (Miller et al., 2012). ROS can activate inflammatory cells and oxidize circulating lipids, promoting their accumulation (Miller et al., 2012).

Inflammatory cells within the lung represent the first line of defense against inhaled pollutants. In particular, alveolar macrophages are responsible for the uptake and clearance of foreign particles. When the phagocytic capacities of the macrophages are exceeded (because of an accumulation of particles or because of a too high particle concentration), cells from the immune system are stimulated continuously, leading to a chronic inflammation (Miller and Newby, 2020).

In addition, the smallest particles are more reactive and more prone to penetrate deeply in the lungs, up to the alveoli, where they can cross the lung epithelial barrier and enter the blood circulation (particle translocation), to be distributed to other organs, explaining extra-pulmonary effects (Miller and Newby, 2020).

Oxidative stress has been shown to be a major contributor to the toxicity induced by a combined exposure to carbon black and maghemite iron oxide (Fe_2O_3) nanoparticles on human lung epithelial cells. Indeed, whereas exposure to either nanomaterial alone induced no significant oxidative stress, the simultaneous exposure to both materials induced a synergistic oxidative stress which was largely attributed to the surface reductive capacity of the carbon black nanoparticles. This latter enabled redox cycling of the iron ions and hence caused significant oxidative stress in cells (Berg et al., 2010).

Oxidative stress can also lead to DNA damages. When these latter are too severe to be repaired by the cellular systems, cell death will occur (Silins and Högberg, 2011; Yu et al., 2015).

4. Conclusion

The current literature on the joint toxicity of nanoparticles and co-contaminants is still limited. Moreover, contradictory findings have been reported making it difficult to draw firm conclusions. Co-exposure to different compounds may induce unexpected toxic effects, even if their individual toxicities are well known. Consequently, the estimation of the combined

toxicity of two compounds based only on their toxicity characterized separately can potentially lead to underestimation. Although the issue is complex (there are countless potential mixtures in our environment) and can be technically challenging to study, it appears of paramount importance to take into account the mixture effects to be more representative of real-life conditions. However, this layer of complexity is often overlooked, using simplified models leading to a reductionist view of the problem. It argues for an urgent need to get data on that topic for a better and reliable sanitary risk assessment and management. It could be starting by binary mixtures and moving forward to mix of increasing complexity to be more representative of the interactions existing between various environmental pollutants (*i.e.* multilayered combination of exposures, beyond combinations of two factors alone to mimic more realistic exposure scenarios). Introducing incrementally such layers of complexity can unveil effects hidden when using simplified models. Exposomics could also greatly contribute to provide relevant information for the accurate environmental risk assessment of multi-pollutant exposure.

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