Unravelling the Health Impacts of Air Pollution (part II): a Focus on Toxicity Mechanisms

1 Introduction

Air pollution is the contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere. Air pollution causes respiratory and other diseases and is a major source of morbidity and mortality (Cohen *et al.*, 2017). In the UK, air pollution is the largest environmental risk to public health, leading to between 28,000 to 38,000 premature deaths every year. The current impact of air pollution, its sources and mitigation strategies were recently reviewed by the UK Chief Medical Officer (Chief Medical Officer's annual report 2022). Household combustion devices, motor vehicles, industrial facilities and forest fires are the common sources of air pollution.

Damaging air pollutants, for which national emission reduction commitments have been defined, are fine particulate matter (PM2.5; PM10), ammonia (NH_3), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and non-methane volatile organic compounds (NMVOCs). Other ambient (outdoor) air pollutants of major public health concern include ozone and carbon monoxide (CO). For detailed description of some of these pollutants, please read previous BTS statement on air pollution (BTS, 2019). Scientific advances continue to provide information on what are the toxicity mechanisms and exposure levels driving the health effects of air pollutants. As a consequence, in 2022 the World Health Organization (WHO) reviewed the recommended limits of annual average exposure for $PM_{2.5}$ to 5 µg/m^3 and PM_{10} to 15 µg/m^3. Despite considerable efforts to bring down levels of air pollution by governments and agencies, WHO data show that almost all of the global population (99%) breathe air that exceeds the existing guideline limits for pollution.

Defining a Causal Relationship. To justify public health interventions aimed at reducing problematic emissions, there is a requirement to first define whether the components of air pollution result in the observed health effects. This is known as demonstrating a causal relationship when one event is the result of the occurrence of the other.

Establishing a causal relationship involves describing the biochemical and molecular changes, known as toxic mechanisms, which connect chemical exposure to the development of adverse outcomes. In addition, the dose thresholds required to trigger disease progression by air pollutants must be defined. To improve our understanding of causal relationships, the cellular and tissue effects leading to disease are typically conceptualised and integrated into adverse outcome pathways (AOPs) (Figure 1; for an extended definition of AOPs, please see the BTS statement: A Decade of Toxicological Trends).

As explained above, air pollution arises from a variety of sources, and therefore, from a complex mixture of constituents. Although each constituent inflicts a certain type of damage on cells (primary toxicity), in real life exposure conditions there is a combination of constituents which often leads to additive effects (primary and secondary effects) (UKHSA, 2022). In this statement, we exemplify some of the primary mechanisms through which air pollutants can adversely affect our health (Mills *et al.*, 2009; Risom *et al.*, 2005).

2 Mechanisms by which air pollutants cause toxicity

2.1 Oxidative Stress

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of oxidants, leading to a molecular damage (Sies *et al.*, 2017). While organisms have a certain tolerance for oxidative stress, consistent or excessive direct pro-oxidant challenge from environmental oxidant toxicants can overwhelm cellular defences. Pro-oxidant toxicants can modify biomolecules, including DNA, proteins, and lipids. When oxidative stress leads to damage of biomolecules and if sustained for a prolonged period this can result in cell death.

Reactive oxygen species (ROS), derived mainly from reactions involving oxygen, are the most studied reactive species that can cause oxidative damage to cells. In addition to ROS, reactive nitrogen species and reactive sulphur species also have significant impacts on redox biology and oxidative stress (Zheng *et al.*, 2020). Certain components of air pollution can generate pro-oxidant reactive species inside the cells. The components found in air pollution include metals (e.g., Cu^{+}, Fe^{2+}) and polyaromatic hydrocarbons (and their derivatives) which originate from diesel exhaust particles. Other components of air pollution, such as quinones, can generate ROS through an indirect mechanism called one-electron reduction. These oxidants can lead to the formation of reactive species, including superoxide anion radicals, contributing to oxidative damage. There are other multiple processes that can generate ROS, such as the two-electron reduction of quinones (Penning *et al.*, 1999).

Pro-oxidant reactive species can react with proteins (protein oxidation), which results in the formation of various oxidation products in amino acid side chains, such as 3-nitro-tyrosine (Zheng *et al.*, 2020). Reactive pollutants can also react with lipids, generating damaged molecules such as hydroperoxides, lipid hydroxides, epoxides, malondialdehyde (MDA), and other aldehydes and ketones. Among the DNA bases, guanine is particularly susceptible to oxidative damage. The major DNA mutagenic lesion is 8-oxo-7,8-dihydroguanine, which can lead to transversion mutations during replication. Moreover, the accumulation of 8-oxoguanine can cause mitochondrial dysfunction and promote oncogenesis (Risom *et al.*, 2005). Circulating levels of 8-oG is used as a biomarker of oxidative damage in population studies.

2.2 Inflammation

Inflammation is a process that involves the activation of immune and non-immune cells to protect the host from pathogens and promote tissue repair (Furman *et al.*, 2019). There are direct (e.g., the interaction of particles with cell receptors) and indirect (e.g., interplay between oxidative stress, resulting damage and inflammation responses) ways by which chemicals present in air particulate can induce inflammation (Bauer *et al.*, 2012) and disrupt immunomodulatory pathways. These include the direct activation of Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–like receptor (NLR, or the inflammasome) (Bauer *et al.*, 2012; Yazdi *et al.*, 2010). TLRs are a group of cell surface proteins that recognise

pathogen-associated debris (e.g., from viruses and bacteria) and initiate the immune response with the intent to protect the host. Exposure of immune cells to different particular matter (i.e., different pollutants) can activate specific TLRs, which then induce an immune response such as cytokine secretion (inflammation messenger molecules). For example, $PM_{2.5}$ contains high levels of redox active metals and low levels of endotoxin, mainly inducing cytokine secretion in a TLR2-dependent mechanism. By comparison, exposure to PM_{10}, which has high levels of endotoxin, induces cytokine secretion via a different TLR receptor (TLR4-dependent) mechanism (Bauer *et al.*, 2012). Other air pollutants, such as asbestos particles, cigarette smoke and nanoparticles (e.g. titanium dioxide) can induce pulmonary inflammation and respiratory disease through alternative mechanisms which involve the inflammasome and other receptors (e.g. IL-1 receptor) (Yazdi *et al.*, 2010).

Pollutant-induced alteration of the immune system results in increased susceptibility to diseases such as viral infections and can lead to the pathogenesis of airway diseases such as asthma (Estrella *et al.*, 2019). Inflammatory responses elicited by $PM_{2.5}$ particles can also result in lung cancer initiation and promotion (Vineis & Husgafvel-Pursiainen, 2005).

2.3 DNA damage

Exposure to air pollutants can result in DNA damage, as touched upon above, which forms by-product signatures and/or specific marks. For this reason, DNA damage is usually considered as an endpoint of the effects of air pollutants, especially bulky DNA adducts (a chemical covalently bound to DNA bases) that can be used as signatures of exposure to genotoxic compounds (Vineis & Husgafvel-Pursiainen, 2005). Formation of adducts (e.g., chemical addition at the N7 position of guanine) can render the base-sugar bond unstable and lead to loss of the adducted base (depurination or depyrimidination) resulting in a change (mutation) of the DNA sequence. For example, benzo[a]pyrene derived from incomplete combustion of organic matter is ultimately metabolised to anti-BaP-7,8-diol-9,10-epoxide (diol-epoxide), which binds to DNA to form N2-deoxyguanosine (dGuo) adducts. These adducts can lead to transversion mutations, which are associated with the induction of tumours in target tissues (Risom *et al.*, 2005; Vineis & Husgafvel-Pursiainen, 2005). Some types of damaged DNA bases can be cleaved by repair enzymes and excreted to body fluids where they can be detected (e.g., urine, plasma). This is the case of 8-oxo-dGuo, which is the most abundant oxidative metabolite of guanine and adenine. Other examples of oxidative DNA damage modifications include 8-oxo-7,8-dihydroguanine (8-oxoGua) (Risom *et al.*, 2005; Phillips, 2005).

3 What has been achieved and future directions

Toxicologists have achieved a remarkable understanding of how chemicals can damage our cells. This has led to policies aimed at reducing the content of harmful components in products (e.g., metals in brake pads), banning of harmful substances (e.g., poly chlorinated bisphenols) or reducing population exposure to contaminants (such as ultra-low emission zones). However, there remain significant knowledge gaps on air pollution and toxicity mechanisms, which warrant further research. For example, understanding toxicity mechanisms in real life exposure conditions is technically challenging. Toxicologists are now shifting from the study of single chemicals to addressing complex mixtures in the environment (UKHSA, 2022). Such an approach is important to understand potential synergistic effects occurring in chemical mixtures and because novel technical and societal changes (e.g., electrification of transport, new materials) continue to re-shape the composition and biochemical properties of the air we breathe. Another avenue for further research will involve

describing how chemicals can trigger other types of stress mechanisms contributing to cell toxicity, such as endoplasmic reticulum stress or mitochondrial damage (Daiber *et al.*, 2020). If we are to advance on these topics, there is a need to develop better laboratory models and biomarkers that can inform on the complex physiological changes and early toxic mechanisms of air pollution (Inesta-Vaquera *et al.*, 2023). Advanced mechanistic biomarkers may be the key to explain outstanding questions, such as how toxic particles exacerbate pathologies originated in tissues distant to lung deposition (Raftis & Miller, 2019). Together, this information is required for the correct risk assessment of air toxicity and policy development to safeguard population health.

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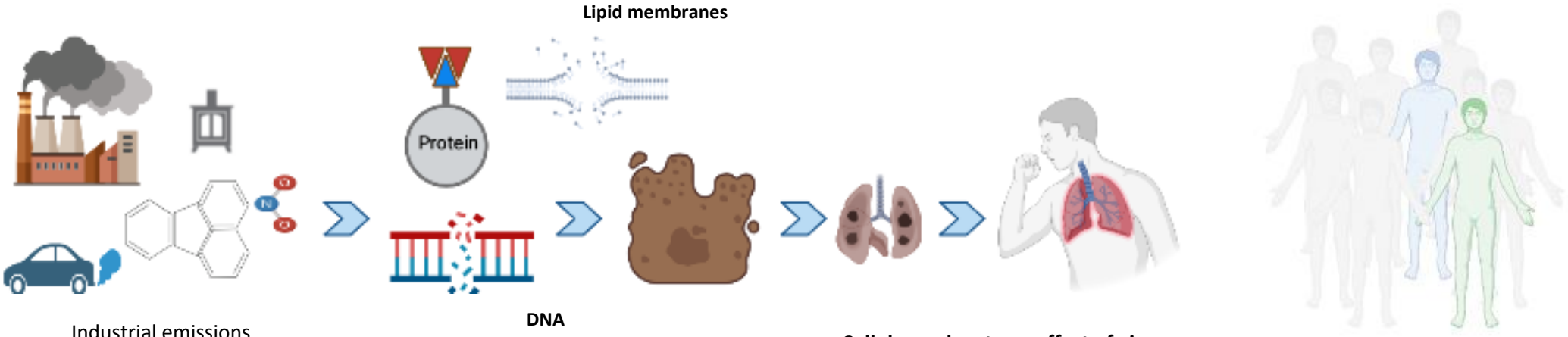
Complex mixtures composition:
Gases: SO_2, NOx.
Inorganics: metals, other elements.
Organics: e.g., Polyaromatic hydrocarbons.
Secondary organic components.

Emission sources:

Mechanisms of toxicity:

Negative impacts:



Industrial emissions
Vehicular emissions
Crustal elements
Biomass burning
Household emission
Desert dust incursions

DNA
Oxidative effects of toxic components:
Proteins → 3-nitro-tyrosine.
Lipids → malondialdehyde
DNA oxidation → 8-oxo-7,8-dihydroguanine.
Immune effects of particles:
PM2.5 → TLR2 activation
PM10 → TLR4 activation
Indirect immune regulation

Cellular and systems effect of air particles:
Endothelial dysfunction and alterations in vascular tone.
Inflamed environment that encourages proliferation of cells.
Others.

Population effects:
Increased cardiorespiratory diseases.
Poor cognitive health.
Deleterious effects on neonates.

Figure 1. AOPs for PM toxicity: exemplification of sources, components and mechanisms of toxicity leading causal association between air pollution and observed health effects in exposed populations.