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Biological effects of Tire and Road Wear Particles (TRWP) assessed by *in vitro* and *in vivo* studies – a systematic review

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Abstract

Air quality is a critical issue because even small amounts of air pollutants can cause significant adverse health effects. Road traffic is a major contributor to air pollution both through aerosols from exhaust emissions (EE) and non-exhaust emissions (NEE). The latter result from mechanical abrasion of brakes and tires, erosion of road surfaces and resuspension of road dust into the atmosphere by passing traffic. EE have been extensively characterized and have declined over time due to mitigation measures. By contrast, NEE have been less studied, are not tightly regulated and there are limited data on their toxicity. Thus, NEE relative part has become prevalent, potentially making of these emissions a major human health concern. The aim of this systematic review was to provide an overview of the current state of knowledge on the biological effects of NEE. We paid particular attention to the toxicological effects of Tire and Road Wear Particles (TRWP) induced *in vitro* and *in vivo* in mammalian models. To this end, we performed a bibliographic search in two databases (PubMed and Web of Science). Of the 400 papers, 22 were found to be relevant and included in our analysis, confirming that the assessment of the TRWP toxicity in mammalian models is still limited. This review also reports that oxidative stress and inflammation are the main mechanisms underlying the toxicity of TRWP.

Key-words: Tire and Road Wear Particles (TRWP); Human health; Toxicity; Air quality; Non-exhaust emissions

1. Introduction

1.1. Air pollution

Air quality is a critical issue as reports indicate that even low levels of air pollutants can have significant adverse health effects, contributing in particular to respiratory and cardiovascular diseases (European Environment Agency, 2022; WHO, 2022). Particulate matter (PM) has been classified as a group I human carcinogen by the International Agency for Research on Cancer (IARC) based on data from human, animal and mechanistic studies (IARC, 2013; Loomis et al., 2013). PM has been shown to be correlated to the incidence and mortality of lung cancers (Cazzolla Gatti, 2021; Charloux et al., 1997; Hamra et al., 2014; Kurt et al., 2016; Mayoralas-Alises and Diaz-Lobato, n.d.; Nishida and Yatera, 2022; Raaschou-Nielsen et al., 2013; Tseng et al., 2019; Wong, 2014). However, other cancers are also suspected to be related to air pollution such as childhood leukemia (Amigou et al., 2011), gastrointestinal tract cancers (Pritchett et al., 2022), bladder and kidney cancers (Zare Sakhvidi et al., 2020) and breast cancer (Yu et al., 2021). Overall, it is estimated that air pollution is responsible for approximately 9 million premature deaths worldwide each year (Fuller et al., 2022).

Road traffic is a major contributor to air pollution (Amato et al., 2014; Harrison et al., 2021; Lewis et al., 2019; Piscitello et al., 2021). Emissions from road traffic include both gaseous pollutants and PM (Lewis et al., 2019), which can originate either from exhaust (tailpipe) or non-exhaust emissions (NEE). The former are the result of fuel oxidation and lubricant volatilization during the combustion process, while the latter arise from the mechanical abrasion of brakes and tires, erosion of road surfaces and resuspension of road dust into the atmosphere by passing traffic (Fussell et al., 2022; Harrison et al., 2021; Piscitello et al., 2021). NEE contribute mainly to PM₁₀ and, to a lesser extent to PM_{2.5} (Piscitello et al., 2021). In particular, the contact between the vehicle wheel and the road surface results in shear and frictional forces that lead to abrasion. Consequently, the emitted particles contain fragments of road surface material embedded in tire wear particles, which are referred to as Tire and Road Wear Particles (TRWP) (Harrison et al., 2021; Lewis et al., 2019).

Exhaust emissions (EE) have been extensively studied and progressively decreased thanks to successful mitigation measures. In contrast, NEE have not received such attention and there is currently no legislation to specifically reduce NEE particles. Consequently, there has been an increase in NEE, which is a significant source of PM₁₀ and PM_{2.5} in urban environments and now far exceeds EE in many countries (ADEME, 2022; Amato et al., 2014; Baensch-Baltruschat et al., 2020; Fussell et al., 2022; Harrison et al., 2021; Lewis et al., 2019; Piscitello et al., 2021). This relative contribution of NEE to air pollution may even increase in the future, making these emissions a major concern for human health. Indeed, vehicle traffic is expected to increase: the OECD estimates that NEE PM emissions from passenger cars will increase by 53.5% globally, from 0.85 million tons in 2017 to 1.3 million tons in 2030, based on the projected growth in the global vehicle stock (Harrison et al., 2021; OECD, 2020). In addition, the development of the electric vehicle fleet will lead to a further reduction in EE, but not NEE, which will actually increase in absolute terms because of the higher weight of electric vehicles, their increased power and torque capabilities, when compared with traditional gasoline vehicles (Amato et al., 2014; Baensch-Baltruschat et al., 2020; Lewis et al., 2019; Piscitello et al., 2021).

While NEE have been extensively described in terms of emission and physicochemical characterization (Amato et al., 2014; Gulia et al., 2019; Harrison et al., 2021; Lewis et al., 2019; Piscitello et al., 2021), very few data are available on their potential impact on human health.

We chose to pay particular attention to TRWP over brake wear because their mass emission is higher. It has been estimated that PM₁₀ emissions from a thermally driven vehicle NEE are composed of 25% brake emissions, 47% tire road contact emissions and 28% resuspended emissions. In addition, for electric cars, while brake emissions decrease to 3% due to regenerative braking, TRWP emissions increase to 61% (ADEME, 2022). Therefore, while we should continue to take measures to reduce brake emissions, further understanding of TRWP emissions is of utmost importance.

1.2. TRWP

Tire wear emissions result from the abrasion of tires on the road surface. Tire wear particles (TWP) are particles that are worn off tires and directly released into the air, whereas road wear particles are due to the fragmentation and degradation of road surfaces as a result of vehicle traffic (OECD, 2020). Consequently, it is difficult to measure tire wear particles separately from road wear particles. TRWP are therefore a complex mixture of tread rubber and encrusted mineral particles from the road surface. To better understand the composition of TRWP, it might be interesting to recall the composition of tires.

The main categories of materials in a tire by mass are as follows: rubber hydrocarbons (40–60%, natural and synthetic rubber (styrene butadiene rubber (SBR) or butadiene rubber (BR)), filler (20–35%, typically soot/carbon black, silica and chalk), softener (15%, hydrocarbon oils and resin), textile and metal reinforcement (5–10%), vulcanization agents (2–5%, sulphur and zinc oxide), other additives (5–10%, preservatives (halogenated cyanoalkanes), antioxidants (amines, phenols), desiccants (calcium oxides), and plasticizers (aromatic and aliphatic esters)) (Harrison et al., 2021).

Less than 1% by volume of the particles in tire wear emissions are smaller than 10 μm . Within PM₁₀, more than 60% of tire wear particles (by mass) are generally between 2.5 and 10 μm in size. However, a bimodal distribution is observed with peaks around 1 μm and between 5 and 8 μm . The use of studded tires increases TRWP emission by orders of magnitude, and produces nanometer-scale particles due to the interaction between the studs and the pavement (OECD, 2020).

Depending on whether or not road- and stormwater-runoff are treated, TRWP can also enter rivers, estuaries and coastal waters, or reach agricultural lands through the application of sewage sludge (Turner and Rice, 2010). Once in the oceans, TRWP can leach various substances including toxic metals such as zinc, lead and cadmium, and organic compounds such as hydrocarbons and benzothiazole derivatives (Page et al., 2022). For this reason, the adverse effects of TRWP on ecosystems have been studied primarily by assessing the toxicity of the leached fraction, known as 'leachate', which

corresponds to chemicals released from tire particles into water (Halle et al., 2021). For more details, see the references to publications on ecotoxicology in Supplementary Table 1.

The situation is quite different for humans, as the risk to human health is primarily associated with inhalation of TRWP released into the atmosphere (Baensch-Baltruschat et al., 2020). Because the lungs are directly and continuously exposed to air pollutants, considerable attention has been paid to the effects of air pollution in general and PM in particular on the respiratory system. *In vitro* studies have shown that TRWP can induce cytotoxicity at high doses, through several biological mechanisms, the most studied being inflammation and oxidative stress (Fussell et al., 2022).

The aim of this paper was to present the current state of knowledge on the biological effects of TRWP through a systematic review.

2. Systematic review of the literature

Figure 1 schematically illustrates the method, the results and the analysis we performed, which is explained in more detail below.

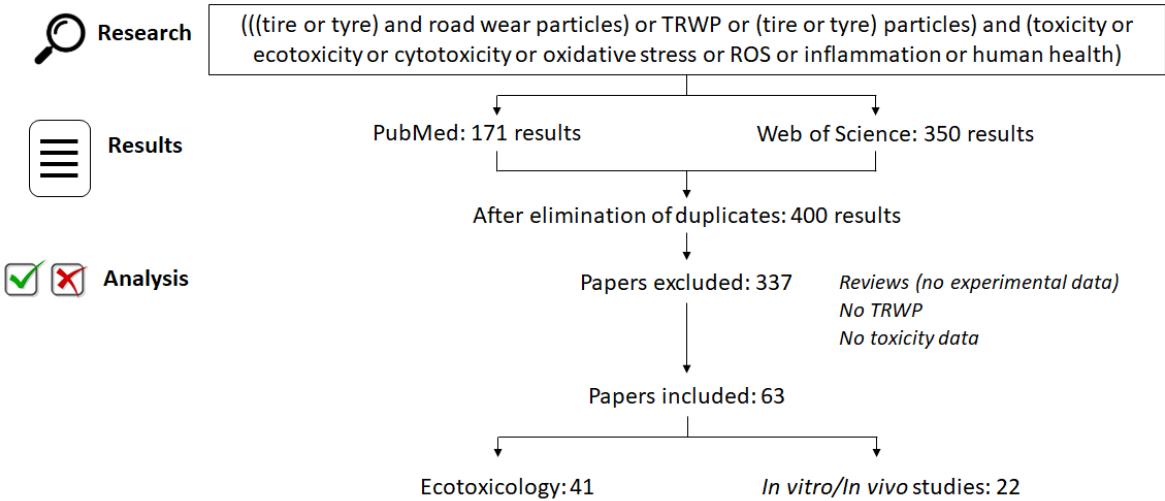


Figure 1 – Graphical summary of the research performed and the results obtained.

2.1. Method

Because we wanted to focus our research on the effects of TRWP on human health and the environment we selected the following keywords: (((tire OR tyre) AND road wear particles) OR TRWP OR (tire OR tyre) particles) AND (toxicity OR ecotoxicity OR cytotoxicity OR oxidative stress OR ROS OR inflammation OR human health).

We searched two databases: PubMed and Web of Science. We eliminated duplicates and analyzed the remaining papers. Papers were excluded from our selection based on 3 criteria: i) if they were reviews and thus did not contain original experimental data, ii) if the studies did not involve TRWP and iii) if no toxicity data were reported (*e.g.* if the studies focused only on the emission or the physicochemical characterization of TRWP).

2.2. Results

As shown in Figure 1, the search (performed on March 21, 2023) in PubMed yielded 171 papers, while the search in Web of Science yielded 350 references. After eliminating duplicates, 400 papers remained to be further analyzed. 337 papers were excluded and the remaining 63 papers were classified as addressing either the environmental (ecotoxicology) or human health (*in vitro/ in vivo* studies) effects of TRWP, corresponding to 41 and 22 references, respectively. Although our study focuses on the human health effects of TRWP we also considered papers addressing ecotoxicity to ensure that we did not overlook any data that could be, even indirectly, related to human health. However, these studies were considered separately (see below).

2.3. Analysis of the results

Supplementary Table 1 lists the papers that relate to the field of ecotoxicology. As mentioned earlier, we focused our analysis on studies conducted *in vitro* and *in vivo* in mammalian models to better assess the health-related impact of inhaled TRWP. Table 1 summarizes the findings.

Table 1 – Summary of the references assessing the *in vitro* and *in vivo* effects of TRWP.

Author	Year	TRWP source	Biological model	Biological endpoints	Main results / conclusions	Reference
<i>In vitro</i> studies						
Poma et al.	2023	Tire particles obtained using a road simulator device	Macrophage cell line (RAW264.7), exposed to 25 and 100 µg/mL of particles for 24, 48, and 72 h	Cell viability and morphology, genotoxicity (comet assay), expression of apoptosis-related genes and proteins (p53, p21, Bax, Bcl-2, cleaved caspases 3 and 9)	Initial induction of the apoptotic process, subsequently reversed by Bcl2. No genotoxic damage, but mild cellular abnormalities	(Poma et al., 2023)
Poma et al.	2019	Fragmented samples of passenger and truck tires	Murine alveolar macrophages (RAW264.7) exposed to 10, 25, 50 and 100 µg /mL of particles for 4, 24 and 48 h	Cell viability, metabolic activity, genotoxic potential, inflammatory response	Higher cytotoxic, clastogenic/genotoxic and inflammatory effects of passenger compared to the truck tire particles	(Poma et al., 2019)
Liu et al.	2022	Tire particles produced by milling, and photoaged by irradiation with a xenon lamp	Macrophage cell line (RAW264.7) exposed to 1 mg/mL of particles for 24 h	Cytotoxicity (cell viability), oxidative stress (ROS production), gene expression analysis of inflammatory factors (IL-6, iNOS, TNF-α)	Decrease of cell viability and the increase of oxidative stress response and inflammatory factor secretion after exposure to photoaged particles	(Liu et al., 2022)
Lindbom et al.	2007	Particles from studded tire-pavement wear, generated using a road simulator	Murine macrophage like cell line (RAW 264.7) exposed to 1, 10, or 100 µg/mL of particles for 18 h	Analysis of cell viability, inflammatory effects (induction of nitric oxide (NO), IL-6, TNF-α, arachidonic acid (AA), and lipid peroxidation) and ROS formation	Induction of inflammatory cytokines, NO, lipid peroxidation, and ROS formation	(Lindbom et al., 2007)
Karlsson et al.	2011	Wear particles from the tire/pavement interface generated using a road simulator	Human monocyte-derived macrophages exposed to 100 µg/mL of particles for 18 h	Cell viability, proteomic analysis	Increase of proteins associated with inflammatory response and decrease of proteins involved in cell functions (redox balance,	(Karlsson et al., 2011)

					anti-inflammatory response, and glycolysis)	
Gustafsson et al.	2008	Wear particles generated from studded and friction tyres using a road simulator	Human monocyte-derived macrophages exposed to 10, 50, 100, 250 and 500 µg/mL for 18 h	Cell viability (Trypan blue), pro-inflammatory response (IL-6, IL-8, TNF-α production)	Airways inflammation induced, dependent of the type of pavement	(Gustafsson et al., 2008)
Lehner et al.	2020	Tire rubber	3D intestine barrier model (human colon cell lines: Caco-2/HT29-MTX-E12 and human blood monocyte-derived macrophages and dendritic cells) exposed to 823.5–1380 µg/cm ² of particles for 6, 24 and 48 h	Cell viability (LDH release), pro-inflammatory response (IL-8, TNF-α and IL-1β release), barrier integrity (TEER measurements)	No significant cytotoxicity or release of pro-inflammatory cytokines and no change in the barrier integrity of the co-culture	(Lehner et al., 2020)
Lindbom et al.	2006	Wear particles from the tire/pavement interface generated using a road simulator	Human monocyte-derived macrophages, nasal epithelial cells (RPMI 2650), and bronchial epithelial cells (BEAS-2B) exposed to 10, 50, 100, 250, and 500 µg/mL of particles for 18 h	Pro-inflammatory response (secretion of IL-6, IL-8, IL-10, and TNF-α)	Significant release of cytokines from macrophages and BEAS-2B cells, no detectable cytokine release from RPMI 2650 cells	(Lindbom et al., 2006)
Karlsson et al.	2006	Particles from tire-road wear collected using a road simulator	Human lung epithelial cell line (A549) and human monocyte derived macrophages exposed to 100 µg/mL of particles for 18 h	Genotoxicity (Comet assay), pro-inflammatory response (secretion of IL-6, IL-8 and TNF-α)	Particles were genotoxic and able to induce cytokines	(Karlsson et al., 2006)
Karlsson et al.	2008	Wear particles originating from the tire-road interface were generated by a road simulator	Human lung epithelial cell line (A549) exposed to 70 µg/mL of particles for 8 h	Mitochondrial depolarization, intracellular ROS production	No significant change compared to control cells	(Karlsson et al., 2008)

Gualtieri et al.	2008	Organic fractions of tire debris	Human lung epithelial cells (A549) exposed to 10, 50, 60 and 75 µg/mL of tire debris organic extract for 24, 48 and 72 h	ROS production, Hsp70 expression	Tire debris organic extracts induce a dose-dependent ROS production and increase the expression of Hsp70	(Gualtieri et al., 2008)
Gualtieri et al.	2005	Tire debris organic extracts	Human alveolar lung cells (A549 cell line) exposed to 10, 50, 60 and 75 µg/mL of tire debris organic extract for 24, 48 and 72 h	Cytotoxicity (MTT, Trypan Blue), cell cycle analysis, genotoxicity (Comet assay)	TD extracts induced a dose-dependent increase in cell mortality and DNA damage	(Gualtieri et al., 2005b)
Beretta et al.	2007	Organic extracts of tire debris	Human alveolar epithelial cells (A549) exposed to 10, 60 and 75 µg/mL of tire debris organic extract for 24, 48 and 72 h	Cell viability (Trypan Blue, LDH release, MTT), analysis of modifications produced on the plasma membrane	No major modifications in plasma membrane composition, but time- and dose dependent toxic effect	(Beretta et al., 2007)
Jiang et al.	2022	Tire grinded with a carbon steel flat file	Human airway organoids generated from primary human bronchial epithelial cells (HBECs) exposed to 50, 100 and 200 µg/mL of particles for 48 h	Cell viability, apoptosis, oxidative stress (ROS production), gene expression analysis of inflammatory factors (TNF- α , IL-6, CCL2) and oxidative stress related genes (CAT, SOD2)	Tire wear particles had an inhibitory effect on the growth of human airway organoids. They induced significant cell apoptosis and oxidative stress. They also significantly upregulated the expression of genes involved in the inflammation response.	(Jiang et al., 2022)
Vicente et al.	2022	Wear particles from the tire/pavement interface generated using a road simulator. Selection of PM10	Two <i>Salmonella typhimurium</i> test strains (TA98 and TA100)	Mutagenicity assay (Ames test)	No mutagenic potential observed	(Vicente et al., 2022)
<i>In vivo studies</i>						
Li et al.	2022	Tire grinded with a carbon steel flat file	C57BL/6 mice exposed through aerosol inhalation to 0.125, 0.5 and 1 mg/kg of particles (single exposure). On day 28 after	Cell analysis in bronchoalveolar lavage fluids, histopathological analysis of lung tissues	Major pulmonary histopathological changes and pulmonary inflammatory response	(Li et al., 2022)

			particles inhalation, analyses were performed			
Kreider et al.	2012	TRWP collected in a road simulator	Male and female Sprague-Dawley rats exposed to aerosolized TRWP via nose-only inhalation at concentrations of 10, 40 or 100 µg/m ³ of particles for 6 h/day, 7 days/week, for 28 days. Analyses were performed one day after completion of the exposure	Bronchoalveolar lavage fluids analysis for markers of cytotoxicity and inflammation, oxidative stress, histopathology of the lungs	No effects on survival, clinical observations, body or organ weights, gross pathology, immune system endpoints, serum chemistry, or biochemical markers of inflammation or cytotoxicity	(Kreider et al., 2012)
Mantecca et al.	2010	Tire particles obtained after grinding and milling of tires. Size-fractionation to get TP2.5 and TP10 (tire particles < 2.5 and <10 µm respectively)	Male BALB/c mice, exposed to TP through intratracheal instillation of aerosolized particles (100 µg). Analyses were performed after 3 h	Bronchoalveolar lavage fluids analysis, markers of cell viability, pro-inflammatory response, histological analyses	The TP2.5 fraction reached the alveolar spaces and produced an acute inflammatory response	(Mantecca et al., 2010)
Mantecca et al.	2009	Tire particles obtained after grinding and milling of tires. Size-fractionation to get TP2.5 and TP10 (tire particles < 2.5 and <10 µm respectively)	BALB/C mice exposed to TP through intratracheal instillation (single exposure). Analyses were performed after 3 and 24 h	Markers of cytotoxicity, oxidative stress, inflammation, histological analyses of the lungs	Significant increase in pro-inflammatory cytokine production 3h post-instillation of both TP10 and TP2.5. Cytotoxic effects detected mainly for TP2.5. Cytokine secretion no longer detected after 24h, dose-dependent responses of inflammatory cells after TP10 exposure, strong cytotoxic effects of TP2.5	(Mantecca et al., 2009a)

Mantecca et al.	2009	Tire particles obtained after grinding and milling of tires. Size-fractionation to get TP2.5 and TP10 (tire particles < 2.5 and <10 µm respectively)	BALB/C mice exposed to TP through intratracheal instillation (10, 100 and 200 µg, single exposure). Analyses were performed after 24 h	Bronchoalveolar lavage fluids analysis for markers of cytotoxicity, oxidative stress, inflammation, and histological analyses of the lungs	Dose-dependent inflammation after TP10 exposure. Strong cytotoxic effects associated to TP2.5 exposure	(Mantecca et al., 2009b)
Gottipolu et al.	2008	2 types of tire particles (TP) obtained by mechanical milling	Male Wistar Kyoto rats exposed to TP via intratracheal instillation (5 mg/kg, single exposure). Analyses were performed after 1 day, 1 and 4 weeks	Analysis of pulmonary toxicity and cardiac mitochondrial enzymes	Increases in lavage fluid markers of inflammation and injury observed at day 1, but changes reversed by week 1. No effects on cardiac enzymes with either TP	(Gottipolu et al., 2008)

Both *in vitro* and *in vivo* studies

Gualtieri et al.	2005	Tire debris eluates and tire debris organic extracts	Human lung A549 cell line exposed to 10, 50, 60 or 75 µg/mL of tire debris extracts for 24, 48 and 72 h and <i>Xenopus laevis</i> embryos exposed to 50, 80, 100 or 120 µg/mL of tire debris extracts for 120 h.	Cell viability (Trypan Blue), genotoxicity (Comet assay), embryotoxicity	TD organic extract toxic to A549 cells affecting cell morphology, cell proliferation and DNA. TD also produced severe malformations in developing <i>X. laevis</i> embryos	(Gualtieri et al., 2005a)
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First, it should be noted that studies on the inhalation toxicity of TRWP in mammalian models remain quite sparse. While some works do not find any harm induced by TRWP (Karlsson et al., 2008; Kreider et al., 2012; Lehner et al., 2020; Vicente et al., 2022), others clearly indicate a significant toxicological effect (Beretta et al., 2007; Gottipolu et al., 2008; Gualtieri et al., 2008, 2005a, 2005b; Gustafsson et al., 2008; Karlsson et al., 2011, 2006; Li et al., 2022; Lindbom et al., 2007, 2006; Liu et al., 2022; Mantecca et al., 2010, 2009a, 2009b; Poma et al., 2019). From the analysis of these papers, we can conclude that the TRWP effects may be mediated mainly by a pro-inflammatory response and oxidative stress, as described below.

3. Mechanisms of toxicity

Airborne tire wear particles have been shown to induce *in vitro* inflammatory response, in various cell models such as human alveolar cells (Gualtieri et al., 2008, 2005b, 2005a), macrophages, nasal epithelial and bronchial epithelial cells (Karlsson et al., 2011, 2006; Lindbom et al., 2007, 2006). It was observed that the intensity of the inflammatory response varied depending on the nature of the road pavement or depending on the type of tire. Indeed, TRWP containing granite pavement had a significantly higher capacity to induce cytokine release than particles generated at the surface of quartzite pavement, both in murine and human macrophages (Lindbom et al., 2007, 2006). Similarly, higher inflammatory effects were induced in macrophages by particles from passenger car tires than by particles from truck tires, which may be related to the higher content of natural rubber in truck tires (Poma et al., 2019).

Furthermore, tire wear particles have been shown to induce oxidative stress through the formation of Reactive Oxygen Species (ROS) in human alveolar cells or macrophages (Gualtieri et al., 2008; Li et al., 2022; Lindbom et al., 2007). The formation of ROS may be related to the metallic components of the TRWP, which can catalyze the formation of free radicals through the Fenton and Haber-Weiss reactions

in respiratory lining fluids, thereby attacking antioxidants and metal-binding proteins that protect the epithelial surface of the lung (Fussell et al., 2022).

Genotoxicity is another mechanism of toxicity that has been reported as DNA damage was observed after exposure of human lung cells to TRWP (Gualtieri et al., 2005b; Karlsson et al., 2008, 2006; Lindbom et al., 2007, 2006). Similarly to inflammatory effects, higher clastogenic/genotoxic effects were induced in macrophages when exposed to particles from passenger car tires than those from truck tires (Poma et al., 2019).

The toxicity of TRWP has been associated mainly with the metallic constituents of non-exhaust PM but some studies have shown that these particles also contain polycyclic aromatic hydrocarbons (PAHs) (Vicente et al., 2022), many of which are potent mutagens and carcinogens. Therefore, metals (in particular Zn, Cd and Cu) and polycyclic aromatic hydrocarbons (PAHs) are key components driving the toxicity observed, which is consistent with the general literature on ambient PM. *In vivo* studies examining the effects of rodent exposure to TRWP have focused primarily on lung effects and similar to *in vitro* studies, report mainly oxidative stress and inflammation as major mechanisms for toxicity (Fussell et al., 2022; Harrison et al., 2021; Lewis et al., 2019).

It is also worth mentioning that PM can induce different biological effects depending on their size (Piscitello et al., 2021). This was illustrated by the studies of Mantecca *et al.* in which tire particles were obtained after grinding and milling tires and then size-fractionated into tire particles less than 2.5 μm in size (TP2.5) and tire particles less than 10 μm in size (TP10). After mice were exposed to 100 μg of these two size fractions by intratracheal instillation (single exposure), TP10 elicited an inflammatory response whereas TP2.5 rather triggered strong cytotoxic effects (Mantecca et al., 2009a, 2009b). The authors suggested a different mechanism of action between the two particle fractions, with macrophages being significantly involved in the responses to TP10 and cellular cytotoxicity being largely governed by TP2.5. They confirmed this finding in another study, which also showed that TP2.5 were able to reach the alveoli whereas TP10 were mainly distributed in the bronchi (Mantecca et al.,

2010). They also studied the effects of similarly sized urban PM collected in Milan, Italy and showed that the PM_{2.5} size fraction provoked an enhanced pulmonary response (inflammation, apoptosis and some signs of adverse histopathology), whereas PM₁₀ generally did not. Actually, lung immunohistochemistry showed that fine particles, especially TP, are able to penetrate deeply into the parenchyma and rapidly induce inflammatory responses, involving even endothelial cells, whereas PM₁₀ elicits a strong proinflammatory response mediated by the bronchiolar cells and resident macrophages of the proximal alveolar sacs, probably due to its larger size and endotoxin content. From these results, the authors concluded that different inflammatory mechanisms are at play in mouse lungs in response to urban PM and tire particles.

Gottipolu *et al.* also showed that after instillation of two types of respirable TWP into the lungs of rats (5 mg/kg, single exposure), both increased several markers of pulmonary inflammation, with TWP that contained more Cu and Fe having stronger effects (Gottipolu *et al.*, 2008). However, these effects were shown to be reversible, returning to baseline after one week. Li *et al.* also observed inflammatory responses after exposure of mice to TRWP (0.125 - 1 mg/kg of particles delivered through aerosol inhalation, single exposure) (Li *et al.*, 2022). Moreover, they reported a restricted ventilatory dysfunction and fibrotic pathological changes. Indeed, they observed an increase in tissue damping and elasticity in TRWP-treated mice compared with controls. Furthermore, lung inhalation capacity analysis showed that TRWP caused a decrease in expiratory peak flow, as well as a decreased lung ventilation in mice exposed to 1 mg/kg TRWP. These findings suggest that TRWP may play a role in the development of respiratory diseases (Li *et al.*, 2022).

4. Discussion

Although the above studies have provided clear evidences of the biological effects of TRWP and the underlying mechanisms, we should consider the limitations of these studies.

First, TRWP emission depends on many parameters, *e.g.* tire composition, type of road surface, driving style, climate, etc. (Fussell et al., 2022; Piscitello et al., 2021). In fact, the specific composition of tires varies greatly depending on the type of vehicle (*e.g.*, car, truck) and manufacturer (Fussell et al., 2022; Harrison et al., 2021). As an example of the different composition of tires, a truck tire contains 80% natural rubber, while passenger car tires contain only 15% (Fussell et al., 2022). It also varies by geographical area, for example, northern countries use studded tires which improve grip in icy conditions (Harrison et al., 2021). Similarly, the rate of tire wear is affected by the roughness of the road surface by a factor of two to three, and there may be increased emissions if the road surface is not paved (Harrison et al., 2021). We should also keep in mind that TRWP are generated by the contact between the tire and the road surface during driving, so their properties and, accordingly, their behavior in the environment depend not only on the components of the tire tread but also on the generation process. Heat and friction generated during the interaction between tire and road surface change the chemical composition of the generated particles compared to the original tire tread. Moreover, material from the road surface is incorporated into the tire wear particles and varies depending on the road surface material (asphalt, granite, etc.) (Baensch-Baltruschat et al., 2020; Fussell et al., 2022; Gustafsson et al., 2008; Lindbom et al., 2007, 2006; Piscitello et al., 2021). Due to these parameters, the physicochemical composition of TRWP can vary significantly, which can greatly affect the effects that TRWP can cause *in vitro* or *in vivo*. Table 1 clearly shows that different sources of TRWP have been used for toxicological studies. It should be taken into account that their composition differs depending on the way they are obtained. Depending on their origin, particles can be divided into tread particles, tire wear particles, and road collected particles. Tread particles include particles that have been manually abraded from tires that have never been used and therefore do not contain road wear. Tire wear particles, on the other hand, are generated by tire wear on a road or in a laboratory road simulator, and therefore contain road wear. On-road collected particles are a mixture of rubber tire particles, road dust and other car emissions; this form of particle is mostly sampled from the environment (Piscitello et al., 2021). Because of their different composition and generation mode,

particles collected on the road or obtained with a road simulator appear to better reflect real-world emissions, although the other types of particles still provide informative data.

The second limitation of the studies discussed is the heterogeneity of the toxicological assays performed, which make it difficult to draw firm conclusions about the toxicity of inhaled TRWP in mammalian models. For example, *in vitro* studies used a variety of methodological designs, dose ranges, and cell types, although lung epithelial cells are the most commonly studied (Fussell et al., 2022).

The third limitation of the studies discussed is that assessing the effects of TRWP is quite difficult because they are mixed with other emissions (other NEE as well as EE) and interference can occur (Forest, 2021; Fussell et al., 2022; Piscitello et al., 2021), potentially leading to additive, synergistic or antagonistic effects. It is therefore difficult to distinguish between “direct” wear emissions (tire, brake or road wear) and “resuspended” wear emissions, and consequently to determine their relative contribution to PM levels in the atmosphere (Piscitello et al., 2021).

Mitigation measures have been proposed to reduce NEE such as reductions in traffic volumes, vehicle speed/velocity, and aggressive driving styles to reduce brake, tire, and resuspension emissions (Fussell et al., 2022; Lewis et al., 2019; Piscitello et al., 2021). To reach this objective, a range of legislative, traffic management, and scientific engineering measures are needed (Fussell et al., 2022).

5. Conclusion

The publications analyzed in this systematic review have confirmed that the assessment of the toxicity of inhaled TRWP in mammalian models is still limited. *In vivo* and *in vitro* studies have nevertheless shown that oxidative stress and inflammation are the main mechanisms underlying the toxicity of TRWP. Further research, preferentially using standardized samples and assays is warranted to better characterize the toxicity of TRWP.

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Supplementary data

Table S1 – List of the studies related to the ecotoxicity of TRWP.

Author	Title	Journal	Year
Selonen et al.	Modulation of chlorpyrifos toxicity to soil arthropods by simultaneous exposure to polyester microfibers or tire particle microplastics	Applied Soil Ecology	2023
Kim et al.	Toxicity assessment of tire particles released from personal mobilities (bicycles, cars, and electric scooters) on soil organisms	Journal of hazardous materials	2022
Carrasco-Navarro et al.	Toxicity of Tire Rubber Microplastics to Freshwater Sediment Organisms	Archives of environmental contamination and toxicology	2022
LaPlaca et al.	Chronic toxicity of tire crumb rubber particles to mummichog (<i>Fundulus heteroclitus</i>) in episodic exposures	Science of the total environment	2022
Cunningham et al.	Toxicity of micro and nano tire particles and leachate for model freshwater organisms	Journal of hazardous materials	2022
Schell et al.	Effects of polyester fibers and car tire particles on freshwater invertebrates	Environmental toxicology and chemistry	2022
Magni et al.	Ecological impact of end-of-life-tire (ELT)-derived rubbers: acute and chronic effects at organism and population Levels	Toxics	2022
Shin et al.	Phenotypic toxicity, oxidative response, and transcriptomic deregulation of the rotifer <i>Brachionus plicatilis</i> exposed to a toxic cocktail of tire-wear particle leachate	Journal of hazardous materials	2022
Fort et al.	Environmental Consequences of Rubber Crumb Application: Soil and Water Pollution	Polymers	2022

Chibwe et al.	A deep dive into the complex chemical mixture and toxicity of tire wear particle leachate in fathead minnow	Environmental toxicology and chemistry	2022
Siddiqui et al.	Internalization, reduced growth, and behavioral effects following exposure to micro and nano tire particles in two estuarine indicator species	Chemosphere	2022
Taltec et al.	Chemical effects of different types of rubber-based products on early life stages of Pacific oyster, <i>Crassostrea gigas</i>	Journal of hazardous materials	2022
Page et al.	Toxicity of tyre wear particle leachates to marine phytoplankton	Aquatic toxicology	2022
Dolar et al.	Time-dependent immune response in <i>Porcellio scaber</i> following exposure to microplastics and natural particles	Science of the total environment	2022
Yang et al.	Acute toxicity of tire wear particles, leachates and toxicity identification evaluation of leachates to the marine copepod, <i>Tigriopus japonicus</i>	Chemosphere	2022
Ding et al.	Leachable additives of tire particles explain the shift in microbial community composition and function in coastal sediments	Environmental science & technology	2022
Garrard et al.	Tyre particle exposure affects the health of two key estuarine invertebrates	Environmental pollution	2022
Liu et al.	Toxicity of tire wear particles and the leachates to microorganisms in marine sediments	Environmental pollution	2022
Masset et al.	In vitro digestion of tire particles in a fish model (<i>Oncorhynchus mykiss</i>): solubilization kinetics of heavy metals and effects of food coingestion	Environmental science & technology	2021
Halle et al.	Tire wear particle and leachate exposures from a pristine and road-worn tire to <i>Hyaella azteca</i> : Comparison of chemical content and biological effects	Aquatic toxicology	2021
Carrasco-Navarro et al.	Altered gene expression in <i>Chironomus riparius</i> (insecta) in response to tire rubber and polystyrene microplastics	Environmental pollution	2021
Kim et al.	Time-dependent toxicity of tire particles on soil nematodes	Frontiers in environmental science	2021

Lehtiniemi et al.	Exposure to leachates from post-consumer plastic and recycled rubber causes stress responses and mortality in a copepod <i>Limnocalanus macrurus</i>	Marine pollution bulletin	2021
Selonen et al.	Exploring the impacts of microplastics and associated chemicals in the terrestrial environment - Exposure of soil invertebrates to tire particles	Environmental research	2021
Sheng et al.	Ecotoxicological effects of micronized car tire wear particles and their heavy metals on the earthworm (<i>Eisenia fetida</i>) in soil	Science of the total environment	2021
Sourkova et al.	The influence of microplastics from ground tyres on the acute, subchronical toxicity and microbial respiration of soil	Environments	2021
Ding et al.	Exposure to heavy metal and antibiotic enriches antibiotic resistant genes on the tire particles in soil	Science of the total environment	2021
Koski et al.	Effect of environmentally relevant concentrations of potentially toxic microplastic on coastal copepods	Aquatic toxicology	2021
LaPlaca et al.	Toxicological effects of micronized tire crumb rubber on mummichog (<i>Fundulus heteroclitus</i>) and fathead minnow (<i>Pimephales promelas</i>)	Ecotoxicology	2020
Ding et al.	Dysbiosis in the gut microbiota of soil fauna explains the toxicity of tire tread particles	Environmental science & technology	2020
Kolomijeca et al.	Increased temperature and turbulence alter the effects of leachates from tire particles on fathead minnow (<i>Pimephales promelas</i>)	Environmental science & technology	2020
Khan et al.	Acute and long-term toxicity of micronized car tire wear particles to <i>Hyalella azteca</i>	Aquatic toxicology	2019
Leads et al.	The effect of microplastic ingestion on survival of the grass shrimp <i>Palaemonetes pugio</i> (Holthuis, 1949) challenged with <i>Vibrio campbellii</i>	Environmental toxicology and chemistry	2019
Redondo-Hasselerharm et al.	Ingestion and chronic effects of car tire tread particles on freshwater benthic macroinvertebrates	Environmental science & technology	2018

Panko et al.	Chronic toxicity of tire and road wear particles to water- and sediment-dwelling organisms	Ecotoxicology	2013
Marwood et al.	Acute aquatic toxicity of tire and road wear particles to alga, daphnid, and fish	Ecotoxicology	2011
Turner et al.	Toxicity of tire wear particle leachate to the marine macroalga, <i>Ulva lactuca</i>	Environmental pollution	2010
Camponelli et al.	Impacts of weathered tire debris on the development of <i>Rana sylvatica</i> larvae	Chemosphere	2009
Mantecca et al.	Tire debris organic extract affects <i>Xenopus</i> development	Environment international	2007
Wik et al.	Acute toxicity of leachates of tire wear material to <i>Daphnia magna</i> - Variability and toxic components	Chemosphere	2006
Wik et al.	Environmental labeling of car tires - toxicity to <i>Daphnia magna</i> can be used as a screening method	Chemosphere	2005