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The health effects of traffic-related air pollution: A review focused the health effects of going green

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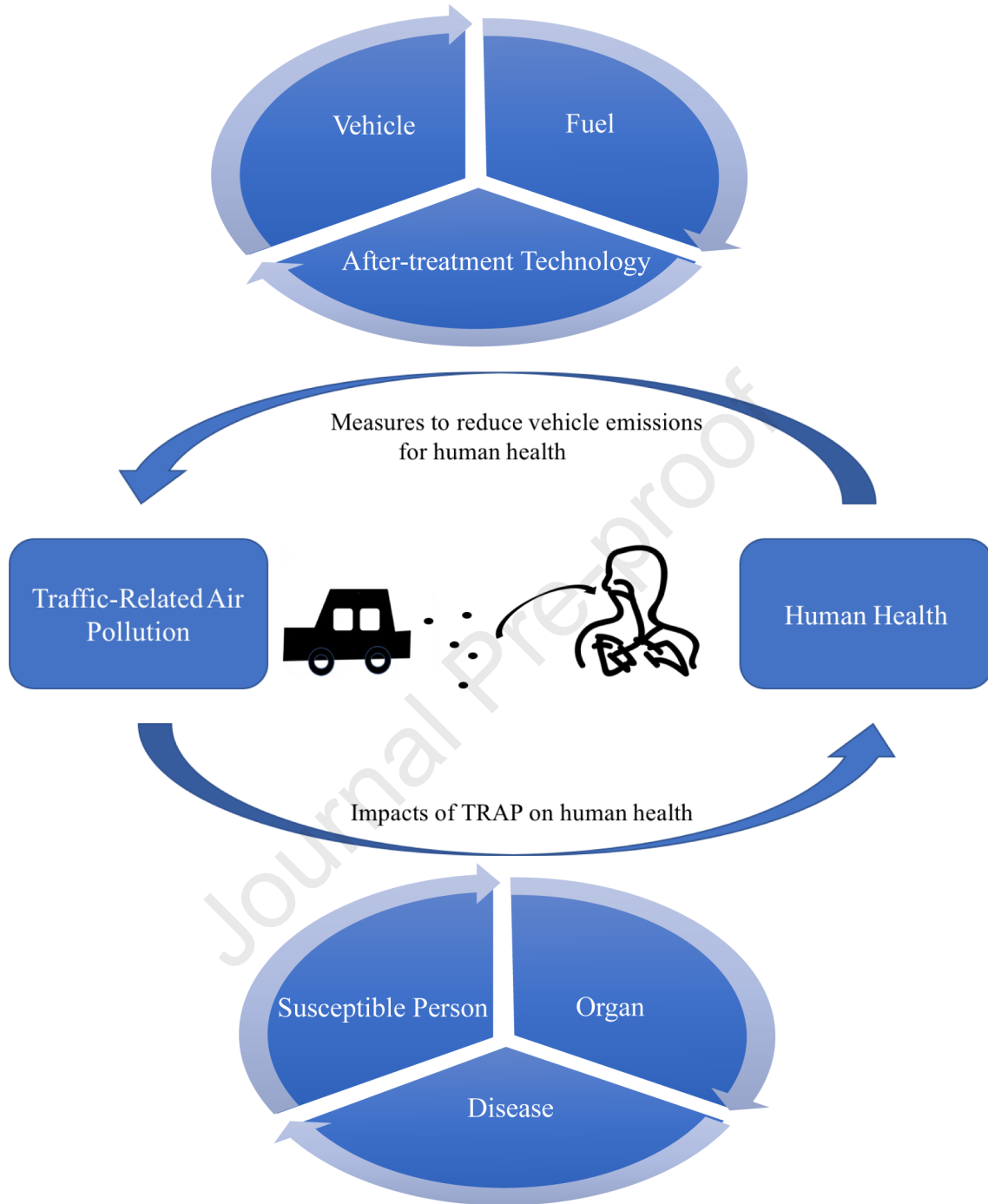
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1 **The health effects of traffic-related air pollution: a review focused the health effects of going**
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13
14 **Abstract**

15 Traffic-related air pollution (TRAP) is global concern due to both the ecological damage of TRAP
16 and the adverse health effects in Humans. Several strategies to reduce TRAP have been implemented,
17 including the use of sustainable fuels, after-treatment technologies, and new energy vehicles. Such
18 approaches can reduce the exhaust of particulate matter, adsorbed chemicals and a range of gases, but
19 from a health perspective these approaches are not always successful. This review aims to discuss the
20 approaches taken, and to then describe the likely health effects of these changes.

21 **Keywords:** Traffic-related air pollution, biodiesel, after-treatment technology, new energy vehicles,
22 particulate matter

23

24 1. Introduction

25 Air pollution has risen to the fourth largest cause of early death, according to the 'State of Global Air
26 2020 Report' (Institute 2020). This effect is likely to be observed for the foreseeable future as the
27 majority of Asia, Africa, and the Middle East's middle- and low-income countries continue to have
28 rising levels of air pollution, including increased particulate matter (PM), noxious gases, and various
29 hazardous chemical compounds. (Bunger et al. 2012, Sinharay et al. 2018, Institute 2020).

30
31 Air pollution is characterised as either outdoor air pollution or indoor air pollution depending upon
32 the pollutant source (Mannucci and Franchini 2017). Outdoor air pollution is made up of a variety of
33 pollutants generated primarily by vehicle traffic, industrial sources, power plants, agriculture, and
34 wildfires. Urbanisation and increased population density have both increased exposure to traffic-
35 related air pollution (TRAP), resulting in increased health concerns in both developing and developed
36 countries (Miller and Newby 2020). Outdoor air pollution causes considerable mortality, even in
37 areas thought to have good air quality. For example in 2018 outdoor air pollution caused 84,300
38 deaths in Italy, and 78,400 in Germany, 47,300 in France and 41,900 in the U.K. (Carvalho 2019).
39 TRAP is a major pollutant in cities, and can affect a large proportion of the population. In 2015 it
40 was estimated that 66% of the population in Beijing, 41% of New Delhi, 67% of Paris, and 96% of
41 Barcelona were exposed to high levels of TRAP (Barthelemy et al. 2020). Even in Australia, a
42 country with one of the lowest exposure levels to PM (less than $8\mu\text{g}/\text{m}^3$ on average), 16.9% of the
43 PM_{2.5} in Sydney is attributed to on-road vehicles. It is also worth noting that the peak concentration
44 of PM in Sydney can reach up to $280\text{ mg}/\text{m}^3$ (Broome et al. 2020, Forehead et al. 2020)

45

46 With the rising health concerns associated with TRAP, numerous studies have analysed the chemical
47 components of TRAP in an attempt to find causation. However, when considering the health effects
48 of constituents of TRAP it is worthwhile considering what it is feasible to measure in epidemiological
49 studies, the likely biological causality of the individual pollutant, and where the collection occurred
50 (the distance from the source). A simplistic overview of these questions leads to the following answers.

51

52 Commercially available equipment for assessing air pollution either assesses particulate matter (PM),
53 gasses, or a mixture of the two. It is worth noting that the assessment and collection of PM smaller
54 than 0.1 μM is technically difficult, and generally PM collection consists of PM₁ (PM $\leq 1\mu\text{M}$),
55 PM_{2.5} or PM₁₀. PM can be analysed for chemical content, but with different analysis technologies
56 and methodologies the range and sensitivity of detected constituents can be variable across studies.
57 Typically assessed constituents of TRAP consist of elemental carbon, organic carbon, inorganic
58 components (eg. metals, sulphate, and nitrate), and PM-bound organic components (eg. Polycyclic
59 Aromatic Hydrocarbons, PAH). Typical gaseous measurements include nitrogen oxides and ozone;
60 both are oxidants which can damage the lungs and other internal organs. Carbon monoxide, benzene,
61 and formaldehyde are also frequently assessed. All components of TRAP exhibit distance-decay
62 gradients, that is that their concentration is highest closest to the source. An elegant meta-analysis
63 summarised this, components which have a rapid decay (e.g. CO) within 150M from the road, those
64 which have a gradual decay over 500m from the road (e.g. nitrogen dioxide, and those which have
65 no appreciable decay over this distance (e.g. PM_{2.5}). It is also worth noting that PM, which has
66 been identified as the main bioactive component of TRAP, will vary according to vehicle type and
67 usage (Gupta and Elumalai 2019, Botero et al. 2020, Boongla et al. 2021). Compared to rural areas,
68 the constituents of PM were more complex in the urban regions attributable to population growth and

69 differences in vehicle usage (private vehicles, subway trains, light rails, and buses) (Ngoc et al. 2018).
70 Driving patterns, such as frequent stopping due to congestion, aggressive driving with high velocities
71 and acceleration, high-way traffic, and idling, can also have an impact on the amount and components
72 of PM (Botero et al. 2020).

73 2. PM and human health

74 TRAP contributes to daily PM exposure, exerting an important role in human health. More than 10 %
75 of the asthma cases were related to near road traffic-related pollution in 10 European cities (Perez et
76 al. 2013). Even TRAP is attributed to the development of hypertensive disorders of pregnancy (Sears
77 et al. 2018). Bus, car, and bicycle are the most common modes of commuting in urban areas. Various
78 studies have focused on the PM concentration in different travel modes, indicating highest PM
79 exposure associated with cycling, followed by bus, and the lowest exposure car (Peng et al. 2021,
80 Zheng et al. 2021). On average, the highest inhaled dose of PM_{2.5} was experienced while cycling
81 (55 µg), followed by bus (20.9 µg) and the lowest in car travels (17 µg) during a daily commuting
82 (Zheng et al. 2021). Apart from these common modes of transportation, heavy-duty trucks are also
83 the main source of PM in our environment. Diesel is the predominant fuel for heavy-duty vehicles
84 until now. A study have revealed that the level of 2-AFLU, major metabolites of diesel-specific nitrated
85 PAHs, was significantly associated with the buses and heavy-duty vehicles but not motorbikes, taxis,
86 or coaches (Yang et al. 2021). Thus, reducing the PM from different types of vehicle is of primary
87 importance to reduce environmental pollution and protect our health.

88
89 When considering the health effects of TRAP it is important to consider both the source of pollution
90 (for example engine type) and the amount of pollution produced. Trucks contribute significantly to

91 TRAP. For example, emissions of BC, NO_x, and PM₁₀ in TRAP were mostly attributed to heavy
92 duty trucks and buses. The greatest producers of PM_{2.5} are heavy duty trucks, followed by passenger
93 cars and bus, then light commercial vehicles. (Jiang et al. 2020). Furthermore, researchers have
94 created emission factors to normalize production of TRAP from different types of vehicles. When
95 considering PM_{2.5} the car's emission factor is 1.25 mg/km, while in trucks it is as high as 185 mg/km.
96 The age or condition of the diesel engine also effects emissions. Generally newer diesel vehicles
97 comply with emission standards such as EURO 5 or EURO 6 (Greim 2019) and have lower emissions,
98 but a lack of maintenance increases emissions as can also be seen for engines which have been used
99 more (Lin et al. 2019). Therefore, depending upon the composition of vehicles, the manufacturing
100 standards used and actual engine use and militance the relative exposure to TRAP from cars or trucks
101 will differ and therefore the relative contribution to TRAP associated health effects will also change.

102
103 Epidemiological studies have demonstrated that TRAP poses substantial risks to human health. (Hui-
104 Tsung et al. 2021). When considering PM from TRAP, fine particles (less than 10 µm) and ultrafine
105 particles (less than 0.10 µm) have large surface areas, which allows for more poisonous chemicals,
106 like metals and PAH, to be absorbed on the surface (Kim et al. 2015, Schraufnagel et al. 2019). Metals
107 and PM-bound organic components are the major constituents of TRAP PM, which can cause
108 inflammation, oxidative stress, genotoxicity, and cell death (Schraufnagel et al. 2019, Arias-Pérez et
109 al. 2020).

110
111 Epidemiological studies have demonstrated that TRAP poses substantial risks to human health, and
112 that some people have increased vulnerabilities to these effects. The most obvious effects are seen
113 when exposure occurs during pregnancy were exposure was related to low birth weight and preterm

114 birth, and an increased risk of developing lifelong chronic diseases (Lin et al. 2021). Furthermore,
115 people with preexisting diseases can have a disease flareup or exacerbation as a result of exposure to
116 TRAP. For example, patients with allergic asthma patients were more susceptible to air pollution-
117 induced exacerbations (Rosenquist et al. 2020), and TRAP levels are associated to coronary heart
118 disease and the incidence of fatal cardiac events (Rosenlund et al. 2008).

119

120 TRAP PM directly effects more than the lungs, for example PM expelled from the lungs in mucus is
121 often swallowed and therefore PM in respiratory mucous can potentially affect the gastrointestinal
122 system as well. Coarse PMs (2.5 - 10 μ m) deposit in the trachea and bronchi (Fig.1), whereas fine
123 particles, especially ultrafine particles, enter the lower airways and alveoli, where they reside for only
124 a short period on the luminal side of the airway epithelium (Schraufnagel et al. 2019). They are then
125 endocytosed and removed by alveolar macrophages or penetrate into the epithelial cells and have
126 even been found inside mitochondria, where cell injury, reactive oxygen species (ROS) generation,
127 and cell death can occur (Loxham et al. 2015, De Grove et al. 2018, Schraufnagel et al. 2019, Nääv
128 et al. 2020, Sharma et al. 2021). Apart from airway injury, PM, especially ultrafine and fine particles,
129 can penetrate through lung tissue and subsequently enter the bloodstream, reaching other organ
130 systems of the body, leading to cardiovascular, neurological, and reproductive disorders
131 (Schraufnagel et al. 2019, Chew et al. 2020). For example, air pollution contributed to the risk of
132 developing central nervous system diseases (Kim et al. 2020). A study has revealed that exposure to
133 PM was associated with the expression level of brain A β , suggesting a causative role in Alzheimer
134 disease (Calderon-Garciduenas et al. 2020, Alemany et al. 2021). In addition, PM can increase the
135 risk of liver toxicity, liver inflammation, and steatosis (E. G. Giannini, R. Testa et al. 2005). PM_{2.5}
136 exposure-induced metabolic damage promotes the development of non-alcoholic fatty liver disease

137 (Chen et al. 2021). Furthermore, exposure during pregnancy is associated with increased liver
138 enzymes levels, decreased birth weight and height in offspring (Bell et al. 2010). Even the occurrence
139 of kidney parenchyma cancer has been found to be associated with exposure to TRAP (Raaschou-
140 Nielsen et al. 2017).

141 **3. TRAP reduction approaches**

142 The health risks associated with exposure to air pollution are well known and the public is supportive
143 of technologies which claim to have reduced pollutants. In recent years on average, 25% of urban
144 ambient air pollution from PM is from traffic globally (Karagulian et al. 2015). At a regional level,
145 traffic can be the major contributor to PM_{2.5} and PM₁₀, for example 54% Africa. Similarly the
146 transportation sector accounts for a large proportion of primary emissions of NO_x and PM₁₀: 73%
147 (NO_x) and 42% (PM₁₀) in Paris; 50% (NO_x) and 50% (PM₁₀) in London (Font et al. 2019).
148 Fortunately, most countries now have measures in place to reduce industrial pollution, but
149 unfortunately, vehicle emissions are now the new source of air pollution in most cities. Attempts have
150 been made to decrease vehicular pollution. After-treatment technologies, renewable fuel, and the
151 development of cars powered by alternative energy sources are only a few of the primary strategies
152 for reducing TRAP PM (Fig.2). It has been suggested that future efforts to reduce TRAP should focus
153 on cleaner sources of fuel such as biodiesel, natural gas, hydrogen or electricity (Bartra et al. 2007).

154
155 China announced *The Strategic Plan of the Mid-and Long-Term Development of Renewable Energy*
156 in 2007, with the goal of increasing clean energy's share from 7.5 percent in 2005 to 15 percent by
157 2020 (Du and Liu 2012). Because of its increased oxygen content and fewer aromatics and sulphur
158 in its composition, biodiesel is viewed as a good choice for making the transition from diesel fuel to

159 renewable fuel while simultaneously minimising the health effects (Arias et al. 2021). For instance,
160 studies have shown that sustainable fuel can reduce PM-induced genotoxicity in lung cells and
161 cytotoxic effects on human bronchial epithelial cells (BEAS-2B) (Martin et al. 2017, Yang et al. 2017).
162 However, some studies reported that biodiesel has a greater potential to damage health compared to
163 diesel, by evaluating oxidative stress, cytotoxicity, and mutagenicity (Kisin et al. 2013, Agarwal et al.
164 2018). Another potential method to reduce PM emissions is using after-treatment technologies, such
165 as the diesel particulate filter (DPF) and the diesel oxidation catalyst (DOC), to filter most of the PM
166 and assist with the oxidation of CO, unburned hydrocarbons, and NO, thereby reducing the adverse
167 impacts on human health (Vaaraslahti et al. 2006, Lizarraga et al. 2011).

168
169 Diesel engines are the most common engines used by large vehicles, however, they produce more
170 pollutants in comparison to the petrol engine (Jhalani et al. 2019). Alternative-energy vehicles are
171 required to improve the current state of air pollution. Plug-in hybrid electric vehicles, battery electric
172 vehicles, hybrid-electric vehicles, compressed natural gas vehicles, and hydrogen vehicles have all
173 recently been launched to the market and are projected to dominate the market in the future,
174 particularly if local legislation supports them (Plotz et al. 2017). However, there is a lack of
175 information on the biological responses to the TRAP generated by these new energy vehicles,
176 especially if the energy source to produce electricity or hydrogen is factored in. Despite many other
177 factors that can influence the production of TRAP PM, such as operating conditions and weather, the
178 aim of this review is mainly to summarise the current understanding of the impact of the above three
179 factors, after-treatment equipment, renewable fuel, and new energy vehicles, on PM emissions and
180 the effect of these PM on human health, in order to identify knowledge gaps to guide much needed
181 future research in this field.

182

183

184 **3.1 Fuel type**

185 The global fuel crisis and the adverse health effects caused by vehicles powered by diesel or petrol
186 have driven research into clean and renewable fuel. The finite supply of conventional fuels and the
187 global interdependence on fuel supplies also contribute to innovation in the production of alternative
188 fuel sources. Thus, the requirement of clean combustion and replacing diesel or petrol with biodiesel
189 or another sustainable fuel have gained popularity in several countries, especially in the early 21st
190 century (Yang et al. 2017). Bioethanol is considered a renewable fuel that can be used in a modified
191 petrol engine, while biodiesel is the most popular alternative fuel for compression (diesel) engines
192 (Bunger et al. 2012). Nowadays, biodiesel is mainly generated from palm oil (36%, mainly in Spain,
193 Italy, and the Netherlands), rapeseed oil (16%, mainly in Europe), and waste cooking oils and soybean
194 oil (11% and 26%, respectively, in the US and Brazil) (Botero et al. 2020).

195

196 Studies have shown the correlation between the source of the fuel and PM concentration and chemical
197 constituent. Biodiesel mainly composed of unsaturated fatty acids is divided into the first generation
198 fuel from different plants (soy, rapeseed), second-generation fuel from waste cooking oil, and
199 hydrogenated vegetable oil, which results in a difference in the composition of metals, PAH, and other
200 pollutants found on PM between diesel and biodiesel (Martin et al. 2017, Moller et al. 2020). The
201 amount of metals (ie. S, Mg, K, Zn, Cu, Ca, and Fe) increases when waste grease is used as the fuel,
202 while S, Na, K, Ca, Fe, Zn, and Pb decrease when biodiesel made from soybean/tallow methyl esters
203 is used (Martin et al. 2017, Timmerman et al. 2019). In addition, blending biodiesel with diesel is

204 often considered to be a sustainable fuel (Emiroglu and Sen 2018). However, there are fewer studies
205 on PM derived from bioethanol and its blends with petrol, maybe due to the difficulty of collecting
206 such samples (Almeida et al. 2015, Akansu et al. 2017, Chansauria and Mandloi 2018). Similarly,
207 there is lack of knowledge on the health effects associated with different biodiesels and the resulting
208 PM, which requires future research to address. From a health perspective, the key purpose of using
209 renewable fuels is to provide cleaner sources of fuel, but the production of renewable fuels such as
210 biodiesel and bioethanol is often driven by local economic and geopolitical factors. Therefore,
211 investigating the health effects of different renewable fuels and corresponding emissions is urgently
212 needed.

213
214 Several studies have reported that biodiesel is a good option to prevent adverse health effects
215 compared to diesel (Larcombe et al. 2015, Emiroglu and Sen 2018). The reduction in ROS, and
216 cellular cytotoxicity are related to the lower exhaust of Cu, as well as water-soluble organic carbon
217 deposited in PM when waste grease was used as the fuel (Martin et al. 2019). Previous studies focused
218 on the mutagenicity by PM produced from biodiesel and diesel using bacterial assays, which yielded
219 inconsistent results (Kisin et al. 2013, Yang et al. 2017). Even in some animal studies, exposure to
220 PM from biodiesel and diesel did not cause significant genotoxicity, while such PM can cause
221 genotoxicity in cells *in vitro*, which may be due to the difference in dose, PM source, and
222 susceptibility (Moller et al. 2020). Therefore, there is a need to further investigate genotoxicity due
223 to PM exposure in animal models. It is worth highlighting that the difference in biological responses
224 can be attributed to the proportion of mineral diesel in biodiesel. Inflammation was reduced *in vitro*
225 when bronchial epithelial cells (BEAS-2B) were exposed to PM from 10% v/v biodiesel (coconut oil)
226 blended with mineral diesel and 15% v/v biodiesel blended with mineral diesel, while it was increased

227 when the fraction was high (20% v/v) (Cervena et al. 2017). Therefore, it is difficult to generalise the
228 likely health effects of using biodiesel compared to diesel. However, to the best of our knowledge,
229 there are no studies on the genotoxic effects of biodiesel emissions in the human population.
230 Some characteristics of biodiesel need to be improved, even if it is less harmful to human health.
231 These include the low volatility, high density, and high viscosity, which prevent pure biodiesel from
232 being directly used in most diesel engines. The addition of alcohol to biodiesel and diesel can modify
233 these characteristics, reducing the density and viscosity of biodiesel, so that overall fuel properties of
234 the fuel blend are improved (Emiroglu and Sen 2018). The mixture ratio of biodiesel to diesel can
235 change the property of biodiesel, resulting in the differences in the amount of emissions and biological
236 responses (Karavalakis et al. 2009). Compared with 20% v/v biodiesel blended with mineral diesel,
237 7% v/v biodiesel blended with mineral diesel generates more PAHs, leading to an increasing level of
238 single-strand DNA breaks in BEAS-2B and A549 cells (Kowalska et al. 2017). The lower amount of
239 PM generated from 20% v/v biodiesel blended with mineral diesel increased mutagenicity in TA98
240 bacteria, but reduced cytotoxicity and genotoxicity in A549 cells, compared to 10% v/v biodiesel
241 blended with mineral diesel (Botero et al. 2020). Therefore, choosing the correct blend ratio is crucial
242 to determine the biological response to PM from biodiesel.

243

244 Even though biodiesel is thought to be a more appealing alternative to gasoline, it has been reported
245 that biodiesel can have similar, if not more, toxic effects than diesel.(Topinka et al. 2012, Agarwal et
246 al. 2018). It has been demonstrated that the size of the PM from biodiesel can be smaller than that of
247 diesel, allowing a large number of toxic components, such as PAH, to be retained on its
248 surface.(Yanamala et al. 2013). A high concentration of PAH is suggested to be the major reason for
249 the lymphocytic infiltrate, impaired clearance of PM, and increased inflammatory cytokines and

250 chemokines, when corn-based fatty acid methyl ester is used as the fuel (Yanamala et al. 2013). These
251 findings were echoed by another study in which the emission exhaust from biodiesel increased the
252 cellular production of pro-inflammatory cytokines IL-8 and IL-6 in BEAS-2B cells (Swanson et al.
253 2016). In addition, PM from biodiesel exhibited higher genotoxic and mutagenic activity than diesel
254 due to a higher fraction of organic component and transition metals (Co, Cu, Ni, Zn) induced
255 oxidative stress and toxicity (Kisin et al. 2013, Agarwal et al. 2018). The conclusion was almost the
256 same when animals were exposure to PM from biodiesel, with more severe cardiovascular toxicity
257 and inflammatory respond than traditional diesel fuel (Brito et al. 2010). The outcomes may vary
258 depending on the source of the biodiesel, the difference in operating conditions, and the constituents
259 of PM, meaning that more in-depth studies are needed to be explored on the relationship between
260 biodiesel and human health in the future.

261 **Table 1 Biological responses in vitro and in vivo to exhausts from alternative fuels in comparison to diesel**

Biodiesel	Constituents of the Emission	Biological model (in vitro and in vivo)	Biological Response	Reference
Commercial-Grade B99	(↑) NO _x (↓) CO _x , Hydro-Carbon, PM, Elemental Carbon, Organic Carbon	NHBE cells (5, 20,60 min)	(-) Cytotoxicity (-) Oxidative stress: HO-1, and CYP1A1	(Hawley et al. 2014)
Soy-based Fatty Acid Methyl Ester	(↑) Co, Cu, Ni, Zn, organic compounds (↓) Elemental Carbon, PM	<i>Salmonella typhimurium</i> TA98 without S9 microsomal activation	(↑) Mutagenic activity	(Kisin et al. 2013)
Soy Ethyl Ester and Soy Methyl Ester	(↓) CO, Hydro-Carbon, PM (↑) NO _x	BEAS-2B Cells	(↑) Inflammation:IL-8 and IL-6 (-) Cytotoxicity	(Swanson et al. 2016)

Soy Biodiesel	<p>(↓) CO, PM</p> <p>(↑) NO_x, SO₂, Hydrogen Carbon, metals, PAH</p>	<p>HEK293T cells (50, 100, and 250 µg/mL of PM), for 48h;</p> <p><i>Salmonella Strains</i> TA98 and TA100 with and without S9 microsomal activation (500, 100, and 50 µg/mL)</p>	<p>(↑) Cytotoxicity</p> <p>(↑) Mutagenicity</p>	<p>(Agarwal et al. 2018)</p>
Corn-based Fatty Acid Methyl Ester	<p>(↑) Organic Carbon</p> <p>(↓) Elemental Carbon</p>	<p>C57BL/6 mice (8-10 weeks, 20.0 ± 1.9 g); Pharyngeal Aspiration--0, 9, and 18 µg/mouse of total carbon(60ul), 24 h, 7 days, and 28 days</p>	<p>(↑) Inflammatory response: immune cells in BALF, MPO activity in tissue homogenates, cytokines and chemokines in the BALF and lung homogenates</p> <p>(↑) Oxidative damage: protein</p>	<p>(Yanamala et al. 2013)</p>

			<p>carbonyls and 4-HNE in tissue homogenates</p> <p>(↑) Lung permeability: total protein in the BALF and in tissue homogenates</p> <p>(↑) Pulmonary damage: cytotoxicity in the BAL and tissue homogenates</p>	
Soybean Ethyl Esters	<p>(↑) Organic Carbon, S, Mg, K, Zn, Cu, Ca, and Fe, Black Carbon, Inorganics</p> <p>(↓) PM, VOCs</p>	<p>Adult male Balb/c mice (6–8 weeks, ~25g), 550ug/m³, for 1h;</p> <p>Heart rate, heart rate variability, and blood pressure were measured</p>	<p>(↑) Blood: Leucocytes and lymphocytes, platelets</p> <p>(↑) Bone marrow activation: Metamyelocytes</p> <p>(↓) Bone marrow activation: Monocytes</p>	(Brito et al. 2010)

		before exposure, 30 and 60 min after exposure; Bronchoalveolar lavage, blood, and bone marrow were collected to evaluate inflammation 24h after exposure;	(↑) BAL and tissue homogenates: total cell, neutrophils, macrophages (↑) Heart rate variability, blood pressure	
Palm Oil	(↓) PM, PAH	A549 cells for 24h; <i>Salmonella typhimurium</i> TA98 with S9 microsomal activation (10 uL sample)	(↑) Cytotoxicity (↑) Mutagenicity (↑) Genotoxicity	(Botero et al. 2020)
Soybean and Tallow Methyl Esters	(↑) NO, NO ₂ , and NO _x (↓) S, Na, K, Ca, Fe, Zn and Pb, PAH	Adult male Balb/C mice (6-8 weeks, ~25 g);	Biodiesel Group (↓) In blood: IgG1, IgG2a, IgE (↓) Inflammatory cells in BALF	(Timmerman et al. 2019)

		<p>House dust mite model from 1-9 day, daily exposure to house dust mite for 1 h; from day 9 to day 17 (Filtered air; 600 mg/m³ PM2.5 of diesel; 600 mg/m³ PM2.5 of biodiesel)</p>	<p>(↓) Cytokines: Th2 (IL-4, IL-5, and TSLP), Th1 (TNF-alpha and IL-2), and Th17 (IL17) in BALF</p> <p>Diesel Group</p> <p>(↑) In blood: IgG1</p> <p>(↓) Cytokines: Th2 --IL-4 in BALF</p> <p>Biodiesel+House Dust Mite Group</p> <p>(-) In blood: IgG1, IgG2a, IgE</p> <p>(-) Inflammatory cells in BALF</p> <p>(↓) Cytokines: Th2 (IL-4, IL-5, and TSLP), Th1 (TNF-alpha and IL-2), and Th17 (IL17) in BALF</p> <p>Diesel+House Dust Mite Group</p>	
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			(↑) Blood: IgG1, IgG2a (↑) Cytokines: IL-4 in BALF (↑) Inflammatory cells in BALF	
Waste Grease	(↑) Mn, Zn, Cu, and Mo, Total N-PAH, PM (↓) Pb, Total PAH, Elemental Carbon (-) PM2.5 mass concentrations	BEAS-2B cells (300, 150, and 50 µg/mL), for 24h.	(↓) Cytotoxicity	(Martin et al. 2017)
Waste Grease	(↓) Cu, Cr, and Zn, Water- soluble organic carbon and elemental metals	BEAS-2B cells (0,15, 30, and 60 µg PM/mL) for 24h	(↓) ROS levels (↓) Oxidative potential	(Martin et al. 2019)

Waste-Cooking-Oil and Butanol	(↓) NO _x , PM, Organic Carbon (↑) CO	A549 cells, for 24h CHO-K1 cells, for 3h	(↓) Mutagenicity (↓) Genotoxicity	(Yang et al. 2017)
Rapeseed Methyl Esters	(↓) PM	BEAS-2B cells (0–200 µg/mL), for 24 h	(↓) (-) Oxidative Potential (↑) Cytotoxicity (↑) Inflammation: IL-6	(Gerlofs- Nijland et al. 2013)
Coconut Oil	(↓) NO _x , NO ₂ (↑) CO, Hydro-Carbon	BEAS-2B cells, for 24h and 48h; 1, 10 and 25 ug/ml for organic matters; 25, 100 and 200 uM for B[a]P; 1, 5 and 10 uM for 3-NBA; and 1, 5 and 50 uM for 1-NP;	(-) DNA damage Low Fractional (↓) Inflammation (↑) Antioxidant High Fraction (↑) Inflammation (↓) Cytotoxicity	(Cervena et al. 2017)

<p>Coconut Oil/Triacetin and Coconut Oil</p>	<p>Coconut Oil-Diesel (↑) NO_x, CO₂ (-) Particulate mass and number Coconut Oil-Triacetin (↓) NO_x, Particulate mass (↑) Particulate number</p>	<p>pHBECs cells, for 30 min</p>	<p>Coconut Oil-Diesel (↑) Cytotoxicity (↑) Antioxidant: HO-1 mRNA (↑) Inflammation: IL-8 (↑) Xenobiotic metabolism: CYP1a1 mRNA Coconut Oil-Triacetin (↑) Cytotoxicity (↑) Antioxidant: HO-1 mRNA (↑) Inflammation: IL-8 (↑) Xenobiotic metabolism: CYP1a1 mRNA</p>	<p>(Vaughan et al. 2019)</p>
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Fatty Acid Methyl Esters/Hydro-treated Vegetable Oil	No Analyze	BEAS-2B and A549 cells (1, 10, 25, and 50 µg/mL), for 6, 24, or 48 h	(↓) Oxidative DNA damage and double-strand breaks. (↑) Single-strand breaks	(Kowalska et al. 2017)
Sewage Methyl Esters	(↑) (-) NO (↑) NO ₂ (↓) PM2.5	Adult male Balb/C mice (6–8 weeks, ~25 g), for 2 h; Filtered air (FA); DE-PM2.5: 600 and 1200 µg/m ³ ; BD-PM2.5: 600 and 1200 µg/m ³ ; HR, HRV, and BP were determined 30 min after exposure; Bronchoalveolar lavage,	(-) Heart rate (↑) In Bone marrow: Eosinophils (↑) In blood: Leukocytes, Lymphocytes, Reticulocytes (↑) In BALF: Total cells, neutrophils, and macrophages (↑) Cardiovascular effects: ET-Ar and ET-Br in the peribronchiolar vessels (↑) Pulmonary endothelial	(de Brito et al. 2018)

		blood, Bone marrow, and lung tissue were collected after 24 h	dysfunction: ET-Br and VCAM-1 in bronchial epithelium	
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262 ↑ for increase, ↓ for decrease, - for no change

Journal Pre-proof

263 **3.2 After-treatment technology**

264 Numerous studies have examined the exhaust from biodiesel to determine whether they meet the
265 requirements of clean combustion and low levels of pollutants, such as PM, PAH, HC, CO, NO, and
266 NO_x (Agarwal et al. 2018). Because of the global push for zero-emissions, sustainable fuel has grown
267 in popularity in recent years. However, emission technology is a critical component in accomplishing
268 this. As a result, post-combustion after-treatments such as DOC, lean NO_x trap (LNT), and selective
269 catalytic reduction (SCR) are typically used in conjunction with DPF to control emission
270 exhausts.(Ayodhya and Narayanappa 2018).

271
272 After-treatment technologies are the most effective way to reduce exhaust pollutants from
273 conventional engines (Fig.3). The DPF is one of the most widely used technologies for removing PM
274 from emissions, thereby significantly reducing the risk of developing cardiovascular and pulmonary
275 diseases, which increases by 1.3 percent and 1.1 percent, respectively, for every 10 ug/m³ increase in
276 PM_{2.5}. (Lucking et al. 2011, Ji and Zhao 2015, Bengalli et al. 2019). Furthermore, CO, NO, unburned
277 HC, and non-regulated emissions, such as aldehydes and PAH, are all toxic pollutants that need to be
278 removed. The DOC is a device used to oxidise these emissions by a chemical reaction, leading to a
279 high level of NO₂ and CO₂ (Castoldi 2020). Increased levels of NO₂ also has negative effects on
280 biological responses (Timmerman et al. 2019). To solve this problem, LNT and SCR were introduced
281 to turn NO₂ and NO into gasses found naturally in ambient air, N₂, H₂O, and CO₂, by adding
282 reductants or catalysing other chemical reactions (Moos 2010, Zhao et al. 2020). Hence, to meet
283 the upcoming stricter emission standards, the refinement of the after-treatment technologies is needed
284 in the future.

285 Improving engine technology has the potential to reduce PM emissions and, as a result, the negative
286 effects on health. Over the last few years, it has been demonstrated that emissions from traditional
287 diesel engines containing carbon particles with PAHs retained to them cause inflammation and
288 carcinogenicity in the lung (N. Yanamala, M. K. Hatfield et al. 2013); whereas emissions from
289 modern diesel engines with after-treatment are not as carcinogenic but still induce inflammatory
290 responses in the lung due to NO₂ (Timmerman et al. 2019). The new technology, DPF and DOC in
291 combination with ultra-low sulphur diesel fuel, made a major contribution to the reduction of
292 emissions by using the cooled exhausted gas recirculation system (Hesterberg et al. 2012, Akopian et
293 al. 2016). Furthermore, this technology was upgraded in 2016, named SCR, which was installed on
294 the engine directly. By increasing the temperature, this can be more efficient in reducing PM
295 emissions and increasing NOX conversion efficiency (Granger et al. 2019, Greim 2019). As a result,
296 the exhaust from engines installed with DOC and DPF showed less mutagenic and genotoxic in TA98
297 and TA102 than that from the conventional engine (Andre et al. 2015).

298
299 It has been reported that the efficiency of PM mass reduction can reach 90% by using DPF (Hawley
300 et al. 2014). However, the efficacy would be even better using multiple after-treatments rather than
301 single one alone (Akopian et al. 2016, Greim 2019). Studies have reported that even the exhaust from
302 the biodiesel or processed by the DPF was reduced, the markers of oxidative stress (HO-1) and
303 aromatic hydrocarbon response (CYP1A1) in human bronchial epithelial cells were still higher than
304 that from the engine without DPF due to NO₂ and organic carbon component in filtered exhaust
305 (Hawley et al. 2014). The DPF is thought to reduce the majority of the PM and HC in exhaust gases;
306 however, the small amount of gas-phase PAH compounds or NO₂ are potent enough to exert a similar
307 or even greater cellular response than unfiltered exhaust (Brito et al. 2010, Botero et al. 2020). A

308 second study also found that the level of NO₂ in the exhaust was increased when the engine was
309 installed with DPF, leading to a stronger inflammatory response (Karthikeyan et al. 2013). It is
310 suggested that increased levels of HO-1 or TNF- α were due to NO₂ using a rat model of exposure to
311 the exhaust generated from the SCR diesel engine (Tsukue et al. 2010). Nevertheless, the toxic
312 emissions can be reduced significantly when multiple after-treatments are used in combination. The
313 biological responses were compared between exhausts from different engines, either a combination
314 of a DOC and a DPF, or the DOC alone (Douki et al. 2018). Superoxide dismutase was only induced
315 when rats were exposed to the exhaust from the engine with DOC, rather than that equipped with
316 both DOC and DPF. Therefore, the combination of these after-treatments will be better to reduce the
317 adverse health impact of engine exhaust.

318

319

320

321 **3.3 Vehicle type**

322 Recently, government decision-makers and transportation authorities have emphasised the
323 importance of developing a green transportation system in order to focus on energy conservation and
324 emission reduction policies. New vehicle technologies have aided in the reduction of glasshouse gas
325 emissions. Hybrid-electric vehicles, plug-in hybrid electric vehicles, compressed natural gas vehicles,
326 and battery electric vehicles were introduced into the market with the goal of introducing sustainable
327 and clean energy. (Hassouna and Assad 2020).

328

329 Considering the cost, energy, and environmental protection regulations, the usage of the hybrid-

330 electric vehicles went up to 3% of all the on-road passenger vehicles since the large-scale promotion
331 in 2000 (Holmen and Sentoff 2015). It is estimated that the low emission vehicles, including hybrid-
332 electric vehicles, battery electric vehicles, plug-in hybrid electric vehicles, and other energy-saving
333 and emission reduction vehicles, will increase up to nearly 50% in 2040 (Holmen and Sentoff 2015,
334 Plotz et al. 2017). A study of real world usage 73,000 plug-in hybrid electric vehicles and 49,000
335 battery electric vehicles in the US and Germany, which also considered emissions generated during
336 manufacture found that over the lifespan of the vehicle both had reduced CO₂ emissions, but for
337 battery electric vehicles over a 4 year span their CO emissions were greater than conventional vehicles
338 (Plotz et al. 2017). In New York City, shared automated electric vehicles were introduced in 2017,
339 which are expected to dominate most of the market share of vehicles by 2050 (Bauer et al. 2018). The
340 economic and environmental impact of green house gases will be smaller than conventional internal
341 combustion engine vehicles, with a reduction in green house gas emissions by 73% and energy
342 consumption by 58% (Bauer et al. 2018). The use of electric vehicles is increasing globally, for
343 example in Malaysia, plug-in hybrid electric vehicle usage is improving year by year, but driving
344 range, vehicle ownership costs, and charging time are the major barriers to the widespread use of
345 plug-in hybrid electric vehicles by consumers. (Adnan et al. 2017).

346

347 Conventional vehicles are powered by the internal combustion engine, while hybrid-electric vehicles
348 are loaded with a larger battery and an electric motor. Plug-in hybrid electric vehicles are different
349 from conventional vehicles and hybrid-electric vehicles, with an additional plug-in charger in
350 addition to the standard engine (Sovacool 2010). Battery electric vehicles, a state-of-the-art
351 innovation in the transportation sector, are considered superior due to the minimal greenhouse gas
352 emission and the use of only electricity as energy. However, whilst there are no exhaust emissions,

353 there are still some other factors (eg. the source of electricity and the number of charging station)
354 influencing the implementation of these vehicles (Fig.4). To enable the wide use of battery electric
355 vehicles, technologies to improve combustion efficiency of coal or gas power stations and
356 improvements to the electric grid and charging stations should occur (Hassouna and Assad 2020).
357 Furthermore, the additional emissions from power generation impede the widespread use of the
358 battery electric vehicles from an air quality perspective. PM generated in coal-based power generators
359 can contribute more than 70% of the total PM emitted by battery electric vehicles (Huo et al. 2013).
360 Although battery electric vehicles are the best choice considering the energy saving compared to
361 compressed natural gas vehicles, they produced more PM10 and PM2.5 in most provinces in China
362 due to electricity generation derived air pollution (Huo et al. 2010) . In the future, if the proportion
363 of coal-based electricity can be reduced and/or the combustion efficiency of coal-fired power plants
364 can be increased, PM emissions related to battery electric vehicles energy supply can be reduced in
365 countries which rely on coal fired power stations for electricity generation (Plotz et al. 2017). When
366 it comes to other types of air pollution from electric vehicles, greenhouse gas emissions are mostly
367 neglected due to the difficulty to measure (Wu et al. 2019).

368

369 Given environmental regulations and the negative health effects of petrol and diesel, compressed
370 natural gas has been widely used in a variety of new types of vehicles (J. M. Luk, B. A. Saville et al.
371 2015). Compressed natural gas can also be used as a source of electricity generation for battery
372 electric vehicles. However, although compressed nature gas can reduce emissions, the demand for
373 natural gas will increase by 70% if electricity demand increases by only 5%. A 5% increase
374 corresponds to electric vehicles traveling 18000km/year. The likely flow on effect would be an
375 increase in the cost of natural gas power generation. As a result, lowering the cost of electricity

376 production is a top priority. Clean energy, such as wind farms and solar panels, may be preferable to
377 petrol fuels for producing electricity with low air pollutant emissions (B. K. Sovacool 2010).

378

379 Although the introduction of hybrid-electric vehicles, battery electric vehicles, and plug-in hybrid
380 electric vehicles will increase the amount of PM generated by power stations, the impact on human
381 health may be minimised because power stations are typically located outside or urban areas.
382 Furthermore, recharging is frequently done at night, avoiding peak times when the power plant will
383 not turn off to store spare capacity. As a result, it will not increase the load on the power plant,
384 implying that there will be few additional emissions as a result of these vehicles (Sovacool 2010).

385

386 The environmental impacts of the batteries in electric vehicles have attracted significant attention in
387 recent years. Lithium-ion batteries have been widely used in most electric vehicles, which can
388 produce 5.1kg CO₂ e/kg. LiMn₂O₄, Al, and Cu are the main materials that need to be recycled to
389 reduce the costs. However, during hydrometallurgical, intermediate physical, and direct physical
390 recycling, there are risks that the metals may leak out and contaminate the water or atmosphere,
391 posing a great threat to human health (Dunn et al. 2012). Although it is known that TRAP is harmful
392 to human health, leading to pulmonary diseases, few studies have focused on the potential health
393 impact of vehicles with new technologies. In addition, while tailpipe emissions from the engine may
394 be reduced (Sommer et al. 2018), a significant amount of PM is produced by the brakes and tyres,
395 which exist in all vehicles, including the electric vehicles (Sommer et al. 2018, Wahid 2018, Piscitello
396 et al. 2021)

397

398 4. **Conclusion**

399 Human health is affected by TRAP. While sustainable fuels, after-treatment technologies, and new
400 types of vehicles have been developed and implemented to reduce emissions, the chemicals contained
401 within can still cause inflammatory responses, oxidative stress, and genotoxicity. However, available
402 study limited, it is not fully discussed in this review that the other types of air pollution may have
403 additional or synergistic effects on human health with traps, which still needs further study. In addition,
404 there is still a significant gap in the relationship between air pollution caused by the use of renewable
405 fuels and various after-treatment modes and the corresponding health risks. Even though tailpipe
406 emissions have been decreasing as a result of various mitigation techniques, the percentage of non-
407 exhaust emissions is increasing, indicating that more attention should be paid to driving conditions,
408 traffic flow, and road and vehicle materials. As a result, given the situation to promote global
409 environmental quality, the evidence presented in this review emphasises the need for future studies
410 to focus not only on the implementation of various policies, but also on a deeper understanding of
411 these new technologies and mitigating strategies.

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416 **Declaration of competing interest**

417 The authors declare no conflict of interest.

418 **Figure legends**

419

420 **FIGURE 1.** Particulate matter deposit in different locations in the lungs dependent upon the size of
421 the particles, damaging the function and structure of the lung. PM can enter the bloodstream and
422 damage other organs (eg. brain, liver, kidney, and intestine), along with cell death, genotoxicity,
423 inflammation, and oxidative stress.

424

425 **FIGURE 2.** To alleviate the damage of TRAP PM to human health, sustainable fuel, after-treatment
426 technology, and vehicle using new types of energy are widely used to perform emission shifting and
427 fuel switching.

428

429 **FIGURE 3.** The combination of after-treatments (DFP: diesel particulate filter, DOC: diesel
430 oxidation catalyst, SCR: selective catalytic reduction, LNT: lean NOx trap) is more effective to reduce
431 the exhaust PM than either alone.

432 **FIGURE 4.** Exhaust emission and non-exhaust emission are the main sources of TRAP. Tire, road
433 wear particles (TRWPs), and sand are major components of non-exhaust traffic emissions. The
434 mitigation of emissions can be promoted with new types of electric vehicles. With the popularity of
435 electric vehicles, the additional potential hazards to human health are caused by the battery recycling
436 process and emissions from power plants.

437

438

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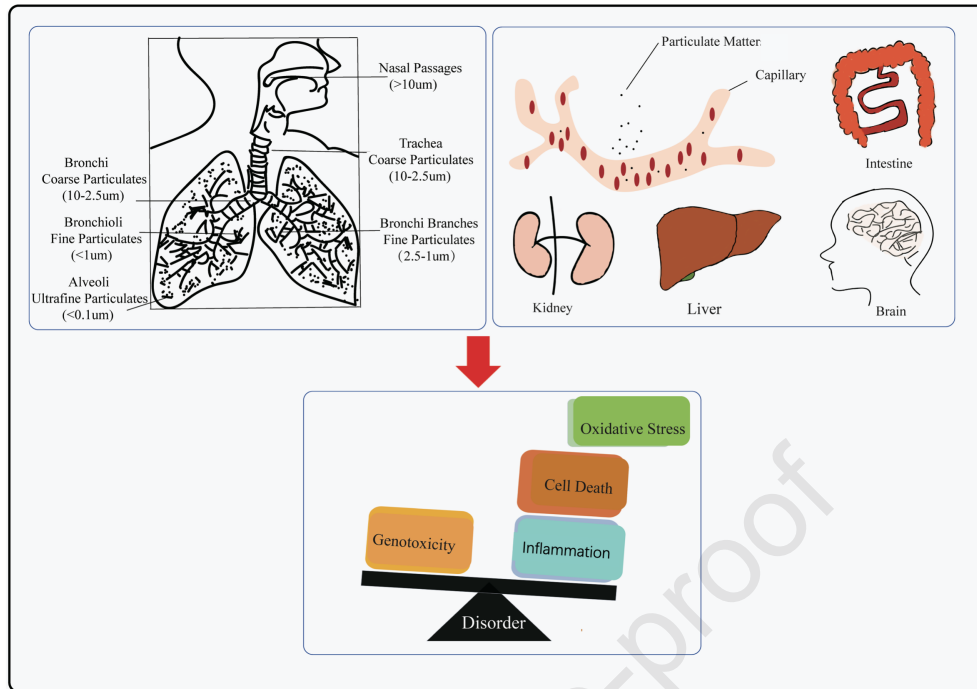
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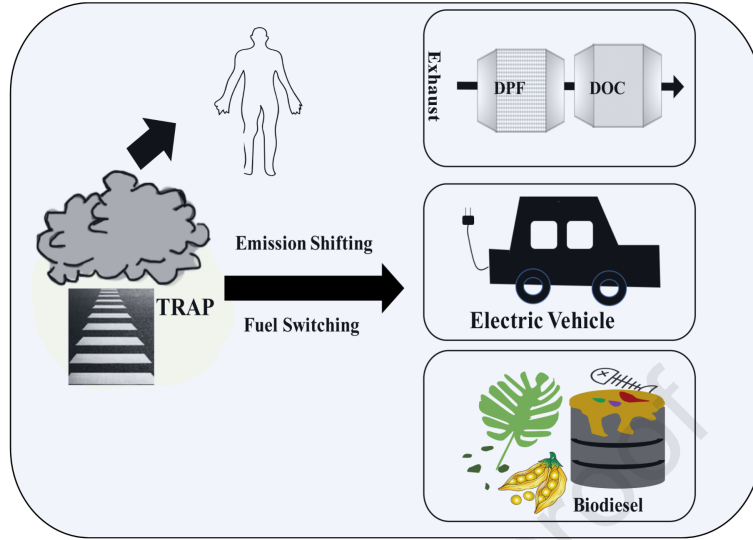
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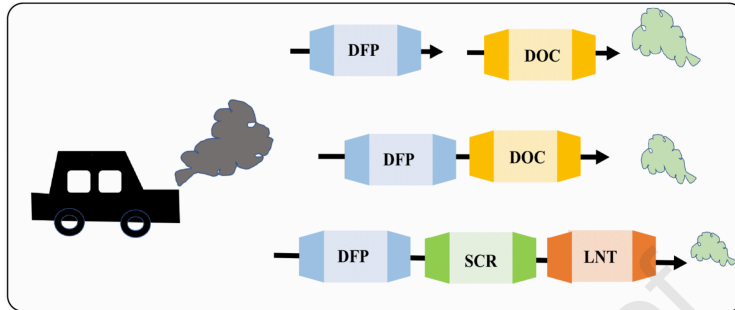
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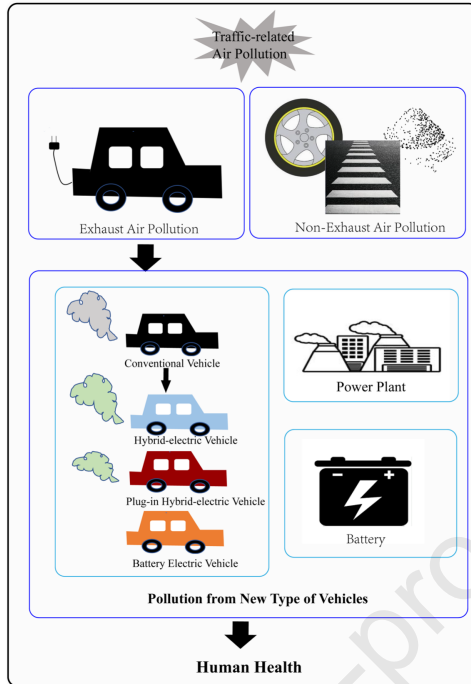
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Highlights

- TRAP has been targeted as important contributor to human health.
- Several strategies are emerging in the prevalent subject of reducing TRAP.
- The prevalence of technologies to reduce TRAP may increase the burden on other environmental pollution.
- The decrease of TRAP is supported to be accompanied by the low incidence of disease.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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