Annex B to TOX/2020/15

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

First draft statement on the potential risks from exposure to microplastics

Potential risks from exposure to microplastics: Background on tyre wear

Introduction

1. It was previously mentioned in the initial discussion paper (TOX/2019/62), that there is some debate as to whether rubber tyre particles are considered microplastics. Tyres were initially made of natural rubber from; the Brazilian rubber tree (Hevea brasiliensis*)*, however, currently tyres are synthesised from a mixture of natural and synthetic materials. Synthetic rubbers are made from petroleum and are functionalised with the addition of; sulphur (1-4%), zinc oxide (1%), carbon black/silica (22-40%) and oil (Kole et al., 2017).

2. Wagner et al., (2018) provides the ranges for major compounds present in tyres presented in Table. 1 (listed in order of w/w%; high to low).

Table. 1 – ranges for major compounds present in tyres (reproduced from Wagner et al., 2018).

Compounds	Content (%)	Ingredients
Rubber/elastomer	40-60	Poly-butadiene, styrene-butadiene, neoprene isoprene, polysulphide
Reinforcing agents (fillers)	20-35	Carbon black, silica, silanes
Process oils	12-15	Mineral oils
Additives	5-10	Zinc oxide, sulphur, selenium, tellurium
Textile and metal net	5-10	-
Vulcanisation agents	1-2	Preservatives (halogenated cyanoalkanes), anti-oxidants (amines, phenols), desiccants (calcium oxides), processing aids (mineral oils, peptisers).

3. Car tyres release wear particles through mechanical abrasion, resulting from the contact between the road surface and the tyre. The amount and particle size are dependent on several factors such as; climate (temperature), composition and

structure of the tyre, tyre age, road surface, driving speed and style and nature of the contact. Experimental set-up is also an important factor to consider. In general, most tyre wear and tear are conglomerates with road wear (Kole *et al*., 2017).

4. The morphologies of tyre materials vary under various sampling conditions. Those collected from road runoff and shredded tyres have elongated shapes, whilst samples generated from road simulator systems in laboratories range from jagged, droplets, granules, warped, porous, irregular, and near spherical (Wagner et al., 2018).

5. The European Tyre and Rim Technical Organisation ranked these key influencing factors (Fig.1), which suggests that driving styles and road and vehicle characteristics can together have a bigger influence on the rate at which tyre and road wear particles (TRWP) are formed than tyre design alone (ETWRP, 2019).

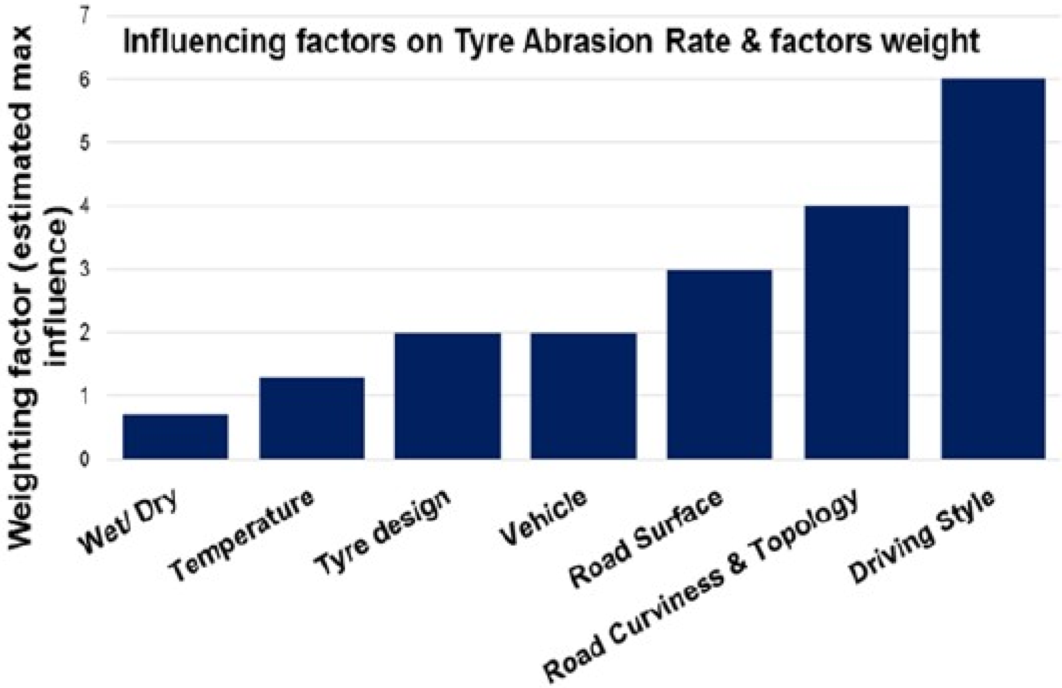

Figure. 1 – A bar chart to illustrate the influencing factors on tyre abrasion rate and their associated weight (reproduced from ETRWP, 2019).

6. Post-consumer tyre uses such as those seen in civil engineering applications (*e.g.* landfill, lightweight fill and soil reinforcement, drainage applications, erosion control, artificial reefs, noise barriers, thermal insulation, hydrocarbon retardation[2], planters *etc.*), also lead to tyre wear particles present in the environment (Hylands & Shulman, 2003).

7. Highways England has funded trails into a new asphalt mix developed by Tarmac in August. 2019, which recycles tyres (up to 750 waste tyres/kilometre of road surface) by adding granulated rubber to the mix. The trial is being run on a

[2] Hydrocarbon retardation (in-ground barriers); a process where shredded tyres have been utilised to mop-up petroleum-based hydrocarbons to prevent entry to groundwater and surface water resources.

section of road between junctions 23 and 22 on the southbound carriageway of the M1, it will test the effective durability of the road surface on a highly trafficked network[3].

8. A tyre loses approximately 10-20% of its weight in a lifetime, the Environmental Agency (EA) estimates that 140g of tyre-derived particles are eroded per metre of road per year (EA,1999).

9. The Department for Transport (DfT) commissioned a study to investigate the relationship of tyre ageing and its effects on material properties and structural integrity, this was carried out by TRL Limited. The report was published in May. 2019. Gathered data suggests that the physical properties of the tyre are affected by the length of time that the tyre is in service, it was noted that observable changes have occurred within the first 3-4 years of the tyre's service life. Furthermore, scattered data was gathered from tyres of similar age, which suggests that the variability is likely to be influenced by the particular service use and the employed maintenance regime during the tyres use. The following limitations were identified by the authors; limited number of samples which comprised of only a single tyre brand, and the history of the tyre (*i.e.* in-service conditions including; under-inflation (to any degree), overloading, and the heat history) (TRL, 2019).

10. Following this the DfT also carried out a consultation on banning tyres aged 10 years and older from heavy goods vehicles, heavy trailers, buses, coaches and minibuses in June. 2019. Evidence was also sought on whether to introduce a similar maximum age for tyres fitted to taxis and private hire vehicles. The consultation closed in September. 2019[4]. Currently, the Dft recommends that tyres over 10 years old should not be fitted to front steered axles of public service vehicles (DfT, 2013). Further updates were not yet made available during the completion of this review, although, should the new law be supported it could be in force by early 2020.

11. A review by Kole et al., (2017) presented a size distribution range of tyre wear and tear particles from 6 - 350,000 nm based on a literature search. The authors concluded there is considerable variation in the size distribution and data interpretation presented complications due to the use of different metrics.

12. Amato et al., (2011) investigated the spatial and chemical properties of the strength of emission source (road dust particles <10 µm) in three European cities; Barcelona and Girona in Spain, and Zürich in Switzerland. Eight sites were sampled in each city, the loadings of road dust (<10 µm) varied; Zürich ranged from 0.2-1.3 mg/m^2, Girona had a range of 1.3-7.1 mg/m^2, whilst Barcelona had a range of 3.7-23.1 mg/m^2. The four main sources that were found to drive the variability were: mineral (road wear and urban dust generated mostly by construction emissions), motor exhaust, brake wear and tyre wear. This was determined by a standard multivariate receptor model[5]. The authors conclude (taking into account the

[3] Further information on the tarmac trial available at: https://www.gov.uk/government/news/tackling-tyre-graveyards-as-new-road-surface-using-rubber-is-trialled
[4] Further details on the DfT consultation on a proposed ban on tyres aged 10 years or older available at: https://www.gov.uk/government/consultations/banning-tyres-aged-10-years-and-older
[5] Standard multivariate receptor model:

uncertainty of the model) that the road wear/mineral is the dominating source in Spanish cities (~60%) but represents only 30% of road dust loadings in Zürich where contributions are more equally distributed among the four main sources of road dust.

13. Singh et al., (2008) analysed the contributions from road traffic emissions to fine particulate matter ($PM_{2.5}$) concentrations within London for 2008. Predicted and measured hourly time series of concentrations at 18 sites in London were modelled. According to predictions, urban increment on average was 18%, 33%, 39% and 43% of the total $PM_{2.5}$ in suburban environments, in the urban background, near roads and near busy roads, respectively. Although, the highest values of urban traffic increment can be up to ~50% of the total $PM_{2.5}$ concentrations near motorways and major roads. The total urban increment close to busy roads was ~7- 8 μg/m^3, in which the estimated traffic contribution is >2 μg/m^3. On average, the contribution is ~1 μg/m^3 of $PM_{2.5}$ to the urban background across London. According to modelling ~2/3 of the traffic increment originates from exhaust emission and the remaining was due to tyre and brake wear.

14. In addition to TRWPs, end-of-life tyre applications *e. g.* granule production (as part of end of life (ELT) management in the circular economy) may also pose as a source of microplastics in the environment. The EU treatment route for used tyres in 2015 involved five sectors; landfill/unknown (8%), energy recovery (28%), material recovery (46%), retreading (6%) and reuse and export (12%) (ETRMA, 2017).

15. The highest markets for ELT granules and powder in 2014 in Portugal, France, Italy and Spain was for synthetic turf (26%), undetermined (export, trader) at (21%), moulded objects (22%) sport and children playgrounds (20%). Other market use included, other uses (10%) and asphalt and road paving (1%) (ETMRA, 2014).

Sources of exposure

16. Councell et al., (2006) (abstract only) assessed the non-point sources of zinc (Zn) in the environment in the US for 1936-1999. Tyre tread material was stated to have a zinc content of ~1 wt%. From their analyses, in 1999 the quantity of Zn released by tyre wear in the US was ~10,000-11,000 metric tons. A case study on Zn sources and sinks in an urban-suburban watershed (Lake Anne) in Washington, DC, US in the late 1990's. The atmospheric flux of total Zn (wet deposition) to the watershed was 2 μg/cm²/year. The flux of Zn to the watershed estimated from tyre wear was 42 μg/cm²/year. The measured concentration accumulation rated of total Zn in age-dated sediment cores from Lake Anne was 27 μg/cm²/year. The author concluded that from the data tyre wear Zn inputs to urban-suburban watersheds can be significantly greater than atmospheric inputs, however, it was noted that watersheds have the capacity to retain appreciable quantities of vehicular Zn inputs.

17. Unice et al., (2019a) characterised the environmental fate of land based TRWP by *in silico* modelling. In brief, the parameters were based under 4 themes; the vehicle kilometres travelled, development of a geospatial estimate of TRWP emissions based on a consideration of emission factors and road type, a description of TRWP to runoff, road-side soil and the atmosphere, and finally the application of hydrological and mass balance models to assess the freshwater fate of TRWP

released to surface waters in post-treatment runoff. *Figure. 2* provides a visual representation of the data.

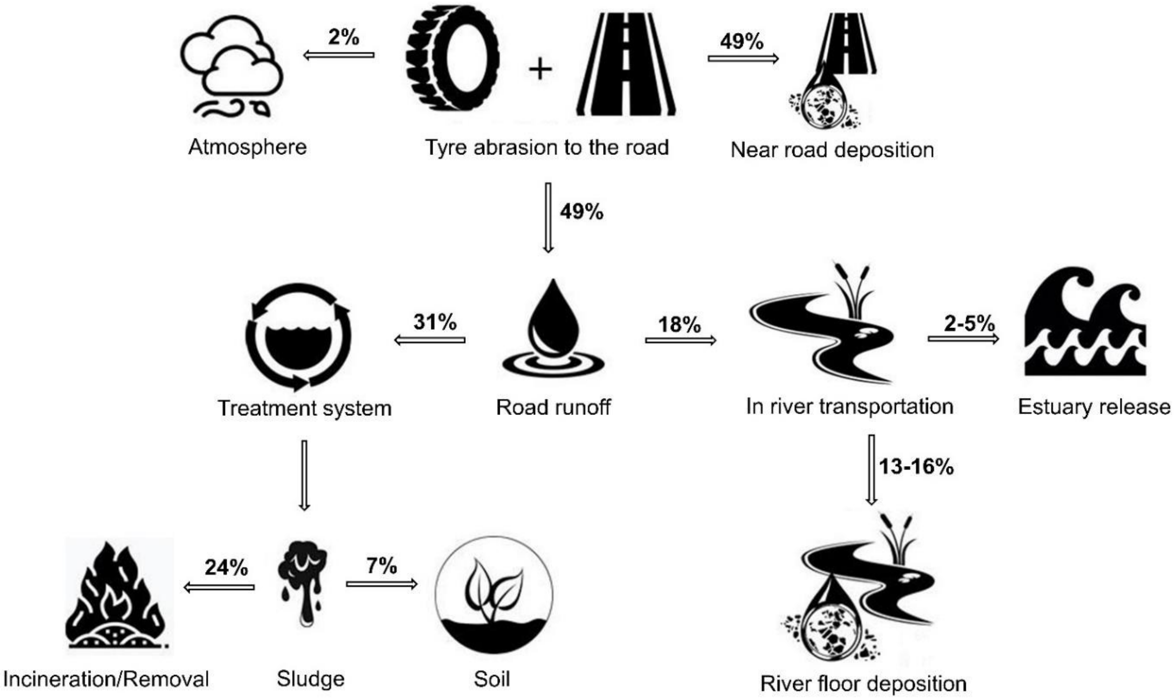

Figure. 2 hypothesised flow-diagram of the environmental fate of tyre and road wear particles based on an *in-silico* model by Unice *et al*., (2019a) (reproduced from European TRWP Platform, 2019).

18. The authors note that the above model is specific to that observed in the Seine watershed and that partition between these five pathways is highly variable and depends on location, rainfall events and drainage design (in both rural and urban settings).

Air

19. Harrison et al., (2012) estimated the contributions of brake dust and tyre wear resuspension to non-exhaust traffic particles derived from atmospheric measurements. Sampling was carried out in month long campaigns conducted in 2007, 2009, 2010 and 2011. Particulate samples (10 size fractions from <0.2 to >21 µm aerodynamic diameter) were collected from the air quality monitors of the Automatic Urban and Rural Network (AURN) at Marylebone Road, London. Traffic flow was estimated to be 80,00 vehicles/day on a six-lane highway.

20. Simultaneous background samples of particulate matter were collected within an urban park at Regent's college; ~0.4km north of Marylebone Road in 2007. For the following years background samples were collected at the AURN background site at North Kensington, London; ~4.0km west of Marylebone Road.

21. Zinc has often been taken as a tracer of tyre rubber emissions, although it is recognised that it does have other sources related to traffic. The aerodynamic diameter range was 0.9-11.5 µm of brake and tyre dust. The mean contributions at the Marylebone Road sampling site are estimated as 55.3% and 10.7% for brake and tyre wear respectively. The authors noted that zinc contamination of road dust from other sources (*e.g.* combustion products, asphalt, brake wear and industrial sources) may also contribute to this estimation, and it is important to note that results are site specific and are therefore subject to uncertainties which were yet to be unquantified.

22. It should be noted that an International Organisation for Standardisation (ISO) method has been developed in 2017[6] (ISO/TS 20593:2017 Ambient air — Determination of the mass concentration of tire and road wear particles (TRWP) — Pyrolysis-GC-MS method). This specifies a method for the determination of the airborne concentration mass concentration (µg/g) and mass fraction (%) of tyre and road wear particles (TRWP) in ambient PM samples. It further establishes principles for air sample collection, the generation of pyrolysis fragments from the sample, and the quantification of the generated polymer fragments.

Soil

23. As seen in Fig. 2, near road deposition represents as a major fraction of TRWP (~49%). TRWP can also be present in the soil *via* aerial deposition and the application of sludge. Soil is therefore an important sink for TRWP. On road-side soils, they are eventually degraded or bound into the soil matrix as hydrophobic persistent organic pollutants. It has been observed that the concentration of TRWP decreases with the distance from the edge of the road. There is limited information available on the degradation of TRWP in soils, due to the lack of analytical methods (Jekel, 2019). A study by Cadle and Williams (1980) was identified. They investigated the degradation of styrene-butadiene rubber (SBR) in test soils, and the resulting half-life was ~490 days.

Water bodies

24. Road-run off also presents as a major fraction of TRWP (~49%). As previously mentioned, in the case of microplastics wastewater treatment plants in England are not designed to retain microplastics and the current percentage of microplastic particles captured in wastewater treatment sludge ranges from 65-100% (Hann *et al*., 2018).

25. It is then considered logical to extend this in the case of TRWP; however, one must consider the differences in particle density and size. Within the literature, some are in the view that max fluxes of TRWP in combined sewer overflows and run-off are likely to be more important than the treated wastewater effluents (Jekel, 2019).

26. Sediment deposition of TRWP in receiving freshwater bodies is expected, however, there are no studies that reviews this pathway due to the lack of sensitive analytical method for biota samples. Unice *et al*., (2019b) estimated a half-life of

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[6] The full ISO specifications is available at: https://www.iso.org/standard/68470.html.

~5,000 days in their model, it was noted that density and size are the most influential parameter of the settling rate. Particles with a diameter of ≤20 μm and with a density closer to water were not prone to settling. The average contents of TRWP in sediments (by models for erosion and TRWP settling) were 0.24 % (2.400 ppm) and 0.32 % (3.200 ppm) for the Seine and Scheldt sediments, respectively.

27. As for rivers and estuaries, the predicted TRWP outflow amounts to 2% of the mass generated on roads (Unice *et al*., 2019a).

28. In reference to both modelling studies, the authors acknowledged that there is a need for *in-situ* characterisation of TRWP size and densities at the interface to validate their model results.

Dermal contact

29. With reference to synthetic turf, there have been growing concerns about the safety of synthetic turf fields, particularly the infill materials. Synthetic fields share the same basic composition; polyethene synthetic grass fibres, infill and carpet backing. Crumb rubber is commonly utilised as the infill material, which is produced by fragmentation of end-of-life tyres. Some of the specific chemicals are measured in crumb rubber include; polyaromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and metals (*e.g.* zinc and lead).

30. Human exposure to crumb rubber-derived chemicals may occur through inhalation, ingestion and/or dermal contact. The route is dependent based on the compound's physicochemical properties. For example, SVOCs, such as PAHs, are more likely to be absorbed *via* inhalation. In contrast, metals may be more readily absorbed *via* non-intentional ingestion of crumb rubber particles.

31. The route of exposure may also be influenced by player characteristics and behaviour, *i.e.* age, type of sport, use of gloves and mouth guards, as well as their field position. Younger players may exhibit greater hand-to-mouth contact and abrasions through falls during competitive sports. Increased ventilation rates may also be considered as a factor that affects exposure, especially for volatile compounds. It is also important to note that, the age of the infill later can affect the concentration of chemicals found within the crumb layer, *i.e.* newer synthetic turf fields have higher levels of PAHs and benzothiazole in crumb rubber samples than those collected from older synthetic fields (Li *et al*., 2010).

32. To date, exposure measurement studies of crumb-derived chemicals are limited.

Literature search

33. The following search strategies were combined to identify literature relevant to the exposure and toxicity of tyre wear to humans. Pubmed, Science Direct and Google Scholar databases were searched using single words or combinations of terms as described in Annex A. Reports from authoritative bodies that have reviewed

the toxicity and human health effects of exposure to tyre wear particles (TWP) and or TRWP were appraised and relevant literature cited within these reports were identified.

Publications by other assessment groups

European Tyre and Road Wear Particles Platform - Tire Industry Project (TIP) subgroup

34. The European TRWP platform, is a multi-stakeholder platform launched by the European Tyre and Rubber Manufacturers' Association (ETMRA) and facilitated by the European Business Network for Corporate Social Responsibility in 2018.The platform brings together governments, academia, non-governmental organisations and industries. The Platform aims to share scientific knowledge, achieve a common understanding of the possible effects of particles generated during normal tyre use and wear, and co-design mitigation options to reduce TRWP.

35. Jekel (2019) prepared a scientific report on TRWP in the aquatic environment for the ETRMA. The report is briefly summarised in the following paragraphs.

36. The author echoed that the definition of tyre wear particles under the generic term "microplastic" is not yet fully clarified, however, the elastomers in tyres are considered to be a type of different polymers which are the main constituents of other plastics and microplastics. Throughout the report the author refers to TWP, as the mass loss of tyres in driving and the mass and modelling of tyre mass into the environment (*i.e.* it does not include roadway particles). TRWP were defined as generated particles from tyres that have interacted with a road surface.

37. In terms of identification and mass quantification of TWP and TRWP, SBR and Zn are useful as markers, however, it must be noted that other sources of Zn is present in the environment. For example, industrial activities, brake wear, automobile exhaust, lubricants, and metallic road barriers.

38. In support to the data presented by Kole *et al.*, (2017), whereby TRWP are generated as a result of abrasion; TRWP particles can also be formed as evaporative emissions due to the heating of the tyre followed by condensation and coagulation. *Table. 2* presents an in-depth description regarding the factors of influence on TRWP generation, as supplement information to paragraph. 9.

39. Some tyre wear mass balances were presented. A range of ~0.28 – 4.7 kg/capita and year (the upper bound value relating to the United States of America), and a global average of 0.81 kg/capita and year. It was noted that the present knowledge of tyre wear rates seems to be well established for passenger cars, but there remains an uncertainty with heavy-duty vehicles (utilised for freight transport) and increasing trends towards the utilisation of electric and/or hybrid cars and their contribution to TRWP (heavier cars due to battery weight and their high instantaneous torque).

<u>Table. 2</u> – influencing factors on TRWP generation, associated with its impact level and stakeholder category (reproduced from Jekel, 2019).

Influencing factors	**Impact on the tyre generated by**	**Impact level on tyre wear generation**	**Stakeholder category**
Tyre characteristics	Tyre type, tyre tread, and rubber compounds	Very high	Tyre manufacturers
	Tyre size	Medium	
	Mileage and age	Medium	
Vehicle characteristics	Vehicle weight	High	Vehicle manufacturers
	Suspension type: toe angle (single angle)	High	
	Suspension type: camber angle	Low	
	Vehicle control	Medium to high	
Road surface characteristics	Road surface	Very high	
	Road and tyre interaction	High	Highway agencies (road makers)
	Humidity and seasonal effects	Medium	
Driving behaviour characteristics	Speed	Medium	Drivers
	Acceleration, braking, cornering	Very high	
Driving behaviour characteristics	Inflation pressure	High	
	Tyre maintenance (e.g. storage)	-	

40. The author was in the view that current methodology for sampling, treatment and analysis of TRWP is insufficient and is neither harmonised nor standardised. Furthermore, the methods utilised for microplastics (*e.g.* infrared or Raman spectroscopy) are only partially suitable for TWP/TRWP since their composition is complex; homo- and hetero-coagulation with other particulates on roads can occur which may increase the effective diameter of aggregates. Additionally, the stability of TRWP in view of their content of tyre and road material is another unknown factor. It was suggested that gross parameters of the particles (*e.g.* size, shape, density and TWP-content) may be more reasonable to sample, as these are more influential on the fate of TRWP in aquatic systems.

41. The report then proceeded to summarise the studies carried out by Unice *et*

al., (2019a), as previously detailed in paragraphs. 17-18. The author concluded that based on this model; road-side soils, drainage treatment systems and the river sediments are the dominating sinks for TRWP, whilst the influential factors are limited to gross properties such as the particle diameter, density and the possible role of biofilms (and its effects in changing size and density). The model, however, requires validation by monitoring soils, sediments and freshwater flows to estuaries by sensitive methods.

42. The report also describes the reported mass fluxes of TRWP in comparison with other microplastic within the literature. *Table. 3* presents the reviewed studies.

Table. 3 – Reviewed mass fluxes of TRWP studies in comparison with other microplastics by Jekel, 2019.

Share of tyre wear on the total emissions of microplastics into the aquatic environment (%)	Reference
60	Lassen et al., (2015)
28-46	Boucher & Friot (2017)
20	Hann et al., (2018)
42	Siegfried et al., (2017)
30-42	Bertling et al., (2018)

43. The author summarised that the cited studies in *Table.3* shows a trend that tyre wear has a significant share in general microplastic emissions, even though the percentages are not quite comparable due to difference in assumptions and target points in the environment.

44. The following data gaps were identified by the author. That there is a need for a reliable and representative tyre abrasion test, as well as analytical tools. Further research on the effects of influencing factors (*Table. 2*) and the generated TRWP. Field studies involving the environmental pathway of TRWP to support model validation. Additional, degradation studies in soils were further recommended since they are important sinks for TRWP.

Joint Research Centre (JRC)

JRC non-exhaust traffic related emissions; brake and tyre wear particulate matter report

45. The JRC published a literature review on non-exhaust emissions (NEE) traffic related emissions specifically from brake and tyre wear particulate matter (Grigoratos & Martini, 2014).

46. The report provided an absolute concentration of TRWP in ambient air based on the highest concentrations of tyre markers reported in the literature. This value was 0.2-11 μg/m^3. Tyre wear contribution to PM_{10} has been reported to be significantly higher when studded tyres are employed and/or nor well-maintained porous pavements are used.

47. It must be noted that not all generated tyre wear particles become airborne, a large proportion of abraded rubber is >20 μm which are deposited on the road or

nearby. It is estimated that only 0.1-10% of generated TWP become airborne. Tyre wear is generally regarded to be a significant source of NEE (5-30% by mass), particularly at curbside sites, however, this contribution may also include road wear.

48. In terms of mass distribution, the reviewed literature was difficult to compare due to differences in the parameters (type of tyre, vehicle load and speed, type of pavement, experimental assembly *etc.*,), however, the majority of studies reported bimodal mass size distribution; one peak among the fine mode ($PM_{2.5}$) and coarse mode ($PM_{2.5-10}$). It was highlighted that tyre rubber has the tendency to develop electrostatic charge leading to a fraction of the particles being adhered to vehicle surfaces, and thus affecting the study of particle size distributions (Thorpe & Harrison, 2008).

49. Trace elements of inorganic compounds utilised in tyre manufacturing processes have been detected in airborne wear particles generated in the tyre-pavement interface. These include silicon, aluminium, calcium, titanium, sulphur, potassium, copper, iron, lead, magnesium, tellerium, selenium, and cadmium. It has been proposed that only a relatively small mass contribution comes from tyres, and that the majority are attributed to pavement wear. Tyres contain ~1% zinc (present as either inorganic ZnO and ZnS) or in the form of organic compounds.

50. The total PAH content in tread wear particles was attributed to 5% of the total PAH content of the road wear particles, with other sources such as asphalt, automobile exhaust, fuel combustion products and natural ambient sources being the dominant PAH contributor.

51. Available studies suggest that toxicity of tyre particle leachates in laboratory animals and *in vitro* tests have been mainly attributed to the presence of Zn, as well as the extractable organic compounds present in TWPs. Studies whom investigated chelated particle leachates exhibited lower toxic effects on water fleas (*Daphnia magna* and *Ceriodaphnia dubia*), and microalga (*Pseudokirchneriella subcapitata*). compared to unchelated leachates. In terms of the human related effects of Zn, it has been associated with acute respiratory responses.

52. Organic constituents of tyre wear debris have also been attributed to have the ability to pose adverse health impacts to humans; this relates mainly to the presence of PAHs. The Kreider *et al*., (2010) study found that the total PAHs content of the tread wear particles represent only 5% of the total PAHs content of the road wear particles (<150 µm). In addition to this, the EU have discontinued the use of high aromatic oil containing PAHS in the manufacture of tyres since 2010. Non-polar organic compounds were also attributed to toxic effects on invertebrate models (water fleas; *Daphnia magna* and *Ceriodaphnia dubia*, and microalga; *Pseudokirchneriella subcapitata*) based on tyre particle leachate studies carried out by Wik & Dave, (2009).

53. Exposure of human alveolar lung cells (A549) to organic extracts of tyre particles (10-80 µm) caused dose-dependent increase in cell mortality and DNA damage, as well as modifications in cell morphology (observed at higher doses). Organic particle extracts were further shown to cause the production of reactive oxygen species (ROS) in human alveolar lung cells, and at higher ROS levels;

inhibition of protein synthesis was hypothesised to occur, which culminates in cell toxicity (Gualtieri *et al*., 2005; 2008).

54. In relation to the presence of natural rubber latex (NRL) proteins present in tyre particles, some researchers have reported that it can contribute to the likelihood of increasing the development of latex allergy and asthma (Dorsey *et al*., 2006) (abstract only), however, some debated that the levels of bioavailable natural rubber latex proteins from tyre particles in the air are low (~0.004 ng bioavailable NRL antigen/m^3) and therefore represents a non-significant source contributor to these health problems (Finley *et al*., 2003) (abstract only).

55. To conclude, the JRC literature review found that exhaust and non-exhaust sources approximately contribute equally to total traffic related PM_{10} emissions. The relative contributions of NEE for brake wear is 16-55%, tyre wear is 5-30% and road dust suspension are 28-59%. In general, tyre wear contribution is much higher in areas where studded tyres are used. It is foreseen that the relative contribution of NEE to traffic related emissions (for both PM_{10} and $PM_{2.5}$) will increase due to stricter controls in exhaust emissions.

56. 0.1-10% of tyre wear is estimated to be airborne PM_{10} and larger size fractions may be deposited on the roadside/curbside or be attracted by the vehicle. Due to the various factors that affect both physicochemical characteristics and generation rates of tyre wear particles, the study of these particles is complex. The varied methodologies utilised to sample and analyse these particles lead to non-comparable and often contradictory results.

57. Mass distribution studies suggest that tyre wear PM_{10} has both unimodal and others bimodal size distributions, based on the available data; tyre wear PM_{10} lies within the fine size fraction (2.5 µm). Particle number distributions of tyre wear PM_{10} suggest a unimodal distribution, however, there is no consensus as to where the peak of the distribution is found.

58. Based on the available data, the JRC deemed the following chemicals important constituents of PM_{10} tyre wear particles Zn, organic Zn, Cu, S, Si, organic carbon, and elemental carbon in fine ($PM_{2.5}$) and Zn organic Zn, Cu, Si and Mn in coarse particle fraction ($PM_{2.5-10}$), respectively.

59. Zinc, benzothilazoles and SBR are utilised as markers/tracers for tyre wear particles. The relative PM_{10} emission factor of tyre wear 4-13 mg/km/vehicle for light-duty vehicles. The calculated average value for PM_{10} emission factors of tyre wear based on the reviewed literature was 6.3 mg/km/vehicle.

60. In terms of adverse human health effects, the JRC were in the opinion that tyre wear contains particles from all fractions involved in respiratory function. Furthermore, it was acknowledged that some constituents of TWPs have been recognised as dangerous (*e.g.* PAHs) or potentially dangerous for humans (*e.g.* presence of zinc and NRL), however, there were no comprehensive studies linking TWPs with adverse effects on human health, and available *in vitro* studies were contradictory.

JRC migration of polycyclic aromatic hydrocarbons (PAHs) from plastic and rubber articles report

61. The JRC further published a technical report on the migration of PAHs from plastic and rubber articles in 2018 (Barrero-Moreno *et al*., 2018). The report addressed the development of a migration measurement method.

62. Regulation (EU) No. 1272/2013 establishes content limits for eight PAHs[7] of 0.5 mg/kg for plastic and rubber components, there were considered the target PAHs to be detected in representative test materials including; low-density polyethylene, polystyrene (PS) and polyvinyl chloride (PVC) as plastic matrices and ethylene-propylene diene monomer, natural-butadiene rubber, and silicone as rubber matrices. Recycled granules (coated and uncoated) originating from end of life tyres (manufactured before and after 2010), as well as rubber tiles made of the recycled coated granules, were also tested in this study.

63. The maximum concentration of PAHs in the test materials, expressed as the sum of the eight target PAHs was ~20 mg/kg in rubber, 15 mg/kg in plastic materials and 5 mg/kg in recycled rubber granules. The release of target set of PAHs in migration media of various stimulants (skin surface film liquid including sweat plus sebum; SSFL), artificial sweat and saliva simulants and 20% ethanol (described in the literature as a good model for human absorption simulation). Migration tests were carried out by total immersion[8] (0.2 dm^2 surface area of test sample in 20 mL) at 40 °C for 24 hours.

64. Under these conditions very low amounts of PAHs were released and were often below the quantification limit of method (range of 0.2-0.6 ng/mL depending on the specific substances detected). None of the plastic polymeric materials led to detectable levels of the target PAHs.

65. Migration of PAHs from rubbers revealed that release is related to the type of extender oil is utilised in their manufacturing process since no release was observed from matrices containing treated distilled aromatic extract. Qualitative analysis suggest that PAHs contained in extender oils migrate more readily than those in carbon black component of the rubbers.

66. In the migration experiments with 20% ethanol, chrysene and benzo[e]pyrene were the substances that were released in higher amounts. The concentration levels of PAHs released, expressed as a function of the surface contact area, ranged from 0-21.17 ng dm^{-2}, or 0-0.09% relative to the total content in the material.

67. PAHs release from coated recycled rubber granules was two to three times lower, than uncoated granules, which suggests that coating acts as a barrier to the chemical migration of target substances. Although the PAHs content was lower compared to other polymeric matrices, the relative migration expressed in mass percentage was higher, however, in terms of surface contact area migration; this was

[7] The eight PAHs are: benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene and dibenzo[a,h]anthracene.
[8] Total immersion studies are considered worst-case exposure scenarios.

not calculated due to the difficulties associated with measuring the surface area of the weighted granules. Furthermore, their exact composition was unknown.

68. Results from migration tests with SSFL, revealed that PAH migration to SSFL increased with migration time and with the sebum content.

The Department for Environment, Food and Rural Affairs (DEFRA)

69. DEFRA has called for evidence on brake, tyre and road surface wear in July 2018 with the consultation period ending in September 2018[9]. Fifty-four response were received, and they suggested that a range of technologies and approaches are available to reduce PM emissions (*e.g.* driving style, alternative materials for brakes, tyres and roads, or regenerative braking), however, no data was provided in order to quantify their effectiveness or combined effects. Responses further highlighted that there is a lack of internationally agreed measurement methods for non-exhaust emission particles, and that the emission factors currently used to estimate national emission levels from these sources may have become dated as technologies have evolved[10].

70. The Air Quality Expert Group (AQEG) identified that particles from brake wear, tyre wear and road surface wear currently constitute 60% and 73% (by mass), respectively, of primary $PM_{2.5}$ and PM_{10} emissions from road transport. These figures are predicted to become more dominant in the future. Currently, they contribute 7.4% and 8.5% of all UK primary $PM_{2.5}$ and PM_{10} emissions, however, it is predicted to account for 10% of national emissions of $PM_{2.5}$ by 2030. Tyre-wear emissions are estimated to be at higher concentrations on high-traffic trunk roads and motorways, in both urban and rural settings.

71. The Group commented that emissions vary widely according to brake, tyre and road-surface material, and with driving style. NEE emission factors have a widespan of uncertainty, factors greater than two is considered typical. An additional source of uncertainty is that emission factors are largely based on historic data from the 1990s and are not necessarily representative of the change seen in vehicle design and fleet composition.

72. NEE particles were found to be an important source of metals to the atmosphere; the National Atmospheric Emissions Inventory estimates contributions of 47% and 21% for copper and zinc, associated with brake wear and tyre wear respectively (AQEG, 2019).

73. DEFRA has stated that the data submitted for the Call of Evidence, and the AQEG 2019 report (as discussed), will be considered in the conclusions and recommendations in deciding further action(s) to take in order to address emissions from brake, tyre and road surface wear.

[9] DEFRA overview on call for evidence on brake, tyre and road surface wear – Available at: https://consult.defra.gov.uk/airquality/brake-tyre-and-road-surface-wear/
[10] Further details on the responses received are available at: https://www.gov.uk/government/consultations/air-quality-brake-tyre-and-road-surface-wear-call-for-evidence/outcome/brake-tyre-and-road-surface-wear-call-for-evidence-summary-of-responses#conclusion

Health and Safety Executive (HSE)

74. Historically an increased risk of bladder cancer in tire industry was evident, this was associated with exposure to beta-naphthylamine[11] present in Nonox S (banned in the UK since the 1950's).

75. The Rubber Industry Advisory Committee (RUBIAC) has produced a statement on occupational cancers in 2001, which was updated 2007[12]. Based on recent epidemiological data, these increased risks are no longer present in the tire industry, however, observations of multiple myeloma cases were present in the general rubber goods sector only, which remains under investigation.

76. The current workplace exposure limits (WELs) are 6 mg/m^3 for rubber process dust and 0.6 mg/m^3 for rubber fume in an 8-hour time weighted average (*i.e.* representative of a typical working day). It is to be noted that the rubber dust refers to dust produced from rubber manufacture; where ingredients are handled weighed, added to or mixed with uncured natural or synthetic elastomers. It does not include dusts arising from the abrasion of cured rubber (HSE, 2011). The RUBIAC is of the opinion that it is possible to achieve exposure levels of at least 50% of the current WELs, and the key for achieving such levels is to ensure good control processes where rubber dust and fumes are emitted.

Committee on the Medical Effects of Air Pollutants (COMEAP)

77. The COMEAP focussed on assessing the strength of evidence that some components of particulate matter, or particulate matter from some sources are more hazardous to human health than others – in their 2015 statement (COMEAP, 2015). They considered whether the evidence supports suggestions that primary particles[13] are more detrimental to health than secondary particles[14].

78. They acknowledged that non-exhaust sources such as brakes and tyres, which have been found to have been found to have high oxidative potential can plausibly exert the health effects of particulate matter.

79. The COMEAP Committee highlighted that it is unlikely that all components of particulate matter have the same potency in causing adverse health effects. Therefore, they concluded that the reviewed evidence is mixed and remains insufficient to draw reliable conclusions regarding the most health-damaging components or sources of ambient particulate matter. Available epidemiological studies at the time of the review were insufficient to conclude which particulate matter constituents were the most important. Further to this, the COMEAP Committee were not able to recommend differential coefficients for quantification.

[11] Beta-naphthylamine was utilised as an antioxidant additive in the tyre industry.
[12] RUBIAC 2007 statement available at: https://www.hse.gov.uk/rubber/cancerstatement.htm
[13] Primary particles: Particles emitted directly to the air *e.g.* particles emitted during combustion, break and tyre wear, and crustal materials such as road dust resuspended by vehicles.
[14] Secondary particles: Particles formed from pre-cursors by atmospheric processes *e.g.* nitrates and sulphates, which are major components of the secondary inorganic aerosol.

80. The COMEAP Committee concluded that since there is evidence to suggest that both primary and secondary (particularly sulphate) particulate matter are detrimental to health. Therefore, the COMEAP Committee were in the view that reductions in both types of particulate matter are likely to be beneficial to health.

World Health Organisation (WHO)

81. The WHO published a review of evidence on health aspects of air pollution (REVIHAPP project) in 2013 (WHO, 2013). The project highlighted that there was a limited number of studies to suggest that traffic-generated dust, including road, brake and tyre wear, also contribute to the adverse effects of health. As such, available evidence does not allow discernment of the pollutants or pollutant combinations that are related to different health outcomes, although association with tailpipe primary particulate matter was identified increasingly.

The Dutch National Institute for Public Health and the Environment (RIVM)

82. RIVM published a report on the emission of microplastics and potential mitigation measures specifically on abrasive cleaning agents, paints and tyre wear (Verschoor *et al*., 2016).

83. It was concluded that all three materials can release microplastic particles, which are subsequently distributed in soil, water and air. Out of the three, tyre wear was the largest contributor with an approximate total emission in the Netherlands of 17, 300 tons/year[15].

84. Road transport vehicle tyres were estimated to contribute 1,800 tons of particles from tyre tread wear per year into surface water through run-off from pavements, effluents and overflows of sewage systems. An estimated 6,200 tons of particles/year were attributed to soil, whilst 900 tons of particles/year of fine particulate matter from tyres were estimated to be released to the air. Additionally, it was estimated that 7,400 tons of particles/year were captured in pen asphalt concrete roads. These estimated values were calculated based on the wear of tyres on 9 different vehicle types including; trucks, passenger cars, buses and motorcycles.

85. The contribution of road-traffic-related NEE wear to total PM_{10} emissions in the Netherlands is ~10%, an estimated 35% of which is caused by tyre wear, 20% by brake wear and the remaining 45% by road wear.

86. Fine particulate matter has been associated with adverse health effects as a result of inhalation exposure. It was highlighted that European emission standards do not specifically address “non-exhaust” emissions from tyre wear. The presence of tyre wear particles in the soil and water result in the leaching of metals, PAHs and other potentially toxic additives, which have been shown to cause adverse effects on

[15] Note that for the estimation of microplastic emissions, the dataset for 2012 and the method of the Dutch Pollutant release and Transfer Register was used (http://www.emissieregistratie.nl/erpubliek/bumper.en.aspx).

aquatic ecosystems, however, the concentrations utilised in these studies are not representative *i.e.* are higher than what is currently present in the environment.

87. The report details that the total amount of tyre tread material lost per kilometre varies widely and depends on several parameters including; tyre characteristics, vehicle characteristics, road surface characteristics and vehicle operation.

88. The characterisation of microplastics from road vehicle tyres is due to the fact that synthetic rubber is manufactured from styrene and butadiene monomers. The styrene/butadiene ratio influences the properties of the polymer; a greater styrene content results in an end product that is harder and less rubbery.

89. It was reported that the general composition of the rubber blends commonly used in passenger vehicles is; natural rubber (40%), styrene-butadiene rubber (30%), butadiene rubber (20%) and other rubber (10%). Fillers are also added, commonly carbon black, although this has been partially substituted by silicone/silica materials.

90. Particles generated during the use of a tyre results in a mixture of the road pavement and the tyre, thus resulting in TRWP. Tyre tread wear by road vehicles were considered to be microplastics, since the particulates partly consist of tyre particulates, a cut-off value for a minimum polymer content has not yet been decided on in both academia and government settings.

91. The density of TRWP was estimated to be 1.2-1.3 g/cm^3. Density is an important characteristic that determines the distributions of the particles in the environment. Since particles are heavier than water, they tend to deposit in the sediment, however, in situations with high flow velocities and turbulence, the particles are present in the water column.

92. In general terms, Verschoor *et al*., (2016) is in the opinion that particles that are emitted into the soil, surface water and sewer will have an approximated size range of 10-400 µm. The size of particles largely depends on characteristics during wearing.

93. The distribution of car tyre wear amongst environmental compartments were; road residue (43%), soil (36%), surface water *via* sewerage (8%), surface water direct (3%), air (5%), sludge (6%).

94. In order to limit uncertainties and to improve emission estimates, the following recommendations were made: additional data are needed to improve the emissions factors of tyre wear and the influence of a particular road surface on the wear factor remains largely unknown, the durability of porous asphalt (with 40% retention of tyre wear particles) needs to be further investigated, the contribution of car tyre wear relative to other traffic-related emissions of polymeric particles (*e.g.* brake system, road surface, road paints, and thermoplastic road markers) requires further investigation, further research on fate and spatial distribution of TRWP (especially the distribution pathways to sewerage treatment plants), additional data are needed on the effect of vehicle (*e.g.* wheel alignment, tyre pressure) characteristics and driver behaviour, generation of monitoring data to assess actual exposure and

effects of the particles on aquatic organisms, and lastly, the contribution of different routes of human exposure to microplastics from these sources needs further quantification, with respect to TRWP, past research have focused on human exposure through inhalation of traffic dust in the air (5% of TRWP in the air when compared to 36% in soil), however, it was acknowledged that there was a lack of data to support concerns resulting from oral exposure.

Synthetic turf

95. Li *et al*., (2010) characterised substances released from crumb rubber material (CRM) used on artificial turf fields (n=18; 0.84-2.0 mm diameter, made from either recycle tyre rubber or other alternative rubber material). A qualitative method based on solid phase micro-extraction (SPME) coupled with gas chromatography-mass spectroscopy (GC-MS) was developed to identify the major volatile and SVOCs out-gassing from CRM samples. GC-MS was applied for the quantitative analysis.

96. Ten selected organic compounds which were known to the added to tyre manufacturing were identified in the vapour phase by the SPME method. These were naphthalene, benzothiazole, 2-methylnapthalene, 1-methylnaphthalene, butylated hydroxyanisole, butylated hydroxytoluene, 4-tert-octylphenol, phenanthrene, fluoranthene, and pyrene. Volatile benzothiazole was detected at the highest level in all commercial samples, ranging from 8.2-69 ng/g CRM.

97. Outdoor experiments under natural weathering conditions (rainwater) showed a significant reduction of out-gassing organic compounds from the CRM in the first 14 days, however, values remained consistent up to 70 days. Zinc was the most abundant element in the acidified leachate (ranging from 220-13,000 µg/g), whilst leachable benzothiazole was detected at relatively small amounts.

European Synthetic Turf Organisation (ESTO)

98. The ESTO published a guidance document on minimising the risk of synthetic turf surfacing being a sour of microplastic pollution in 2018 (ESTO, 2018).

99. Synthetic turf surfaces were described as hard-wearing and low maintenance, in comparison to natural turf alternatives. These is a wide range of synthetic turf surfaces, some with or without infill material. The infill may comprise of natural materials (*e.g.* sand and cork) or can be made from rubber or other forms of polymer.

100. Most synthetic turf football and rugby surfaces (also described as third-generation synthetic turf sports surfaces) contain granular rubber infill within the pile of synthetic turf carpet. Some infill can have localised movement within the field and potentially unto the surrounding grounds.

101. It was stated that good infra-structure design and regular maintenance can minimise the effects of infill movements. The following examples of good practice were listed: use of raised perimeter edge details, the use of entrance mats and metal foot-grills to capture infill being walked off a field, use of slit traps or special filer

areas in draining devices around the boundaries of fields and changing rooms, use of better quality synthetic turf systems that either have a lower potential for infill movement and or the use of synthetic turf systems that require less infill, and use of infills that are less prone to movement and migration.

European Chemicals Agency (ECHA)

102. ECHA evaluated the possible health risks of recycled rubber granules used as infill in synthetic turf sports field in 2017 (ECHA, 2017). Note that some information regarding rubber granules as a source for microplastics in the environment were also provided, however, the Commission did not request ECHA to evaluate the risk of rubber granules to the environment.

103. By 2020, it is estimated that 21,000 full size pitches and ~72,000 mini pitches will exist in the European Union (EU). In the EU, rubber granules are utilised as infill material which are mainly produced from EU manufactured ELT. It is believed that the quantity tyres and recycled rubber granules imported into the EU is small, however, this has not been verified by ECHA. In the UK, there were >5,000 artificial grass pitches and ~2,750 third generation pitches in 2016.

104. A literature review was performed, and the studies covered ~50 samples from new recycled granules and several hundreds of samples taken from more than 100 fields. The samples were collected from ~10 member states and in addition ECHA received studies from industry.

105. Recycled rubber granules were regarded as mixtures. ECHA screened the reported substances by considering their Classification, Labelling and Packaging (CLP) status. Of the screened substances, 20 had were harmonised as carcinogenic, reprotoxic and mutagenic in the CLP legislation. In addition, 17 skin sensitisers (*e.g.* formaldehyde and 2-mercaptobenzothiazole) and of these one was also a respiratory sensitiser; cobalt was also found.

106. Substances that were identified to be commonly present in recycled rubber granules from the reviewed literature by EHCA included; PAHs, metals, phthalates, VOCs and SVOCs. Substances selected for a more detailed investigation in the ECHA report are listed in Table. 4.

107. ECHA investigated the risks to children playing football (and other sports) on synthetic sports fields (including goalkeepers), adults playing professional sports and workers installing/maintaining the fields. The considered exposure routes to rubber granules were skin contact, ingestion and inhalation (of substances evaporating from the granules, as well as the dust generated by the granules themselves).

108. Exposure assessment was performed by measuring airborne impurities in the breathing zone of the workers or at waist height of the players. The latter was considered worst-case scenarios and also the exposure of children. Based on the reviewed literature, the highest measured value for inhalable dust has been 3.1 mg/m^3, whilst for respirable dust it was 1.4 mg/m^3. The maximum concentration for PM_{10} was 40 mg/m^3.

109. In terms of dermal contact, the migration factors of PAHs to artificial sweat have been detected from 0.007-0.02%, absorption of 100% was used in the risk characterisation, which takes into account the effects of any abrasion of the skin.

110. As for oral exposure, the accidental swallow of rubber granulate for children and adults were 0.05 and 0.01 g, in one event respectively.

111. The exposure to EU-8 PAHs was evaluated for cancer risk. The lowest benchmark dose level that corresponds to 10% extra risk ($BMDL_{10}$) was 0.49 mg/kg bw/day for the mixture of 8 PAHs derived by EFSA from a 2-year dietary carcinogenicity study in female mice with coal tar mixtures by Culp *et al*, (1998). It is worth noting that the EFSA 8 PAHs replaced BeP and BjFa with benzo[ghi]perylene and indeno(123-cd)pyrene, however, ECHA assumed that to toxicological potency of the 8 PAHs doesn't cause a significant change.

112. The $BMDL_{10}$ was converted to a human $BMDL_{10}$ by applying an allometric scaling factor of 7 for mice. It was assumed that oral absorption in humans is equal to oral absorption in mice, and no other assessment factors were applied.

113. The dermal $BMDL_{10}$ value was derived from the oral value by taking into account oral (rat) and dermal (human) absorption fractions. An oral absorption fraction of 0.5 was assumed and for dermal 0.2 was used.

114. The dermal $BMDL_{10}$ value was derived from the oral value by taking into account oral (rat) and dermal (human) absorption fractions. An oral absorption fraction of 0.5 was assumed and for dermal 0.2 was used.

115. The excess lifetime cancer risk, for EU-8 PAHs was calculated and was below one in a million for players, goalkeepers and workers. The full set of calculations are available in Annex VII[16] of the ECHA 2017 report.

116. ECHA concluded that there was a very low level of concern from exposure to PAHs from recycled rubber granules utilised as infill for synthetic turfs. Firstly, it was stated that the concentrations of PAHs in recycled rubber granules have normally been well below the limit values set in the REACH restriction relevant for such mixtures and therefore the concern for lifetime cancer risk for players and workers was considered very low (Table. 4).

117. Secondly, the limited available data regarding migration of metals, showed negligible concern to players and workers, since they were below the limits allowed in accordance to the EU toys legislation (Directive 2009/48/EC[17]), when compared with limit values for dry powder like or pliable toy materials, as example.

[16] Annex VII available at: https://echa.europa.eu/documents/10162/13563/annexes_to_axv_report_rubber+granules_en.pdf/f3cc9f58-8ab3-8e4a-0258-51466817f0fd (pp.82).
[17] Please refer to Annex II Chapter III Article 13. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009L0048-20171124&from=EN.

<u>Table. 4</u> – lists the substances selected for further investigation and review in the ECHA report and their average concentration in new rubber granules and field samples (mg/kg) (ECHA, 2017).

		Average concentration in rubber granules (mg/kg)			
Substance	**Name**	**New granules**	**Field samples**	**recycled samples**	**limit value under REACH XVII* (w/w%)**
Polycyclic aromatic hydrocarbons (PAHs) – collectively called EU- 8 carcinogenic PAHs	Benzo(a)pyrene (BaP) Benzo[e]pyrene (BeP) Benzo(a)anthracene Chrysene Benzo(b)fluoranthene	Total PAH; 9.12-58.21. Total EU-8 carcinogenic PAHs; 2.12-22.78. BaP; detection limit (<0.08)- 1.19	Total PAH; 1.90-72.94. Total EU-8 carcinogenic PAHs; 0.98-42.88. BaP; detection limit (<0.01)-2.38.	Total EU-8 PAHs: 20.	0.0001 0.0001 0.0001 0.0001

	Benzo(j)fluoranthene (BiFa) Benzo(k)fluoranthene Dibenz(a,h)anthracene				0.0001 0.0001 0.0001 0.0001
Phthalates	Di-2-ethylhexylphthalate Di-isobutylphthalate Dibutyphthalate Benzylbutyl phthalate				0.1 Data unavailable 0.1 0.1
Other	Formaldehyde Benzothiazole Benzothiazole-2-thiol Methyl iso butylketone Benzene				Data unavailable Data unavailable Data unavailable Data unavailable 0.1

	Cadmium†			0.11-2.38	0.1
	Cobalt†			3.5-268	Data unavailable
	Lead†			Detection limit (<0.5)-308)	0.05

* REACH Annex XVII: Is a list of restricted substances in the EU; either on its own or in a mixture, or to an article containing the substance.

† In relation to the metals found in rubber granules, only those whose elemental metal is itself classified were selected by ECHA for preliminary evaluation, since it was not possible to determine in which form the metals were in the recycled rubber granules. This is an uncertainty identified in the report, as it is possible that some of the compounds of the metals that are classified as CMR were not considered.

NOTE: That empty cells does not necessarily reflect the absence of data, however, due to the variety of substance types and methodologies from literature, it was difficult to extract an average concentration in rubber granules in the unit of mg/kg.

118. Thirdly, no concerns were found to players and workers from the concentrations of phthalates, benzothiazole, and methyl isobutyl ketone in rubber granules, since these were below the concentrations that would lead to adverse health effects.

119. Lastly, ECHA acknowledged that reports that VOCs emitted from rubber granules in indoor halls may cause irritation to the respiratory track, eyes and skin.

120. The following uncertainties identified by ECHA are hereby summarised. ECHA's conclusions were based on available studies from ~10 Member states, although no bias could be identified, it is uncertain as to what extent they are representative for recycled rubber granules utilised in sports fields across the EU. There were also knowledge gaps in characterising substances present and their concentrations in rubber granules typically utilised for the purpose of infill in synthetic turf field applications. Although the number of imported tyres and granules entering the EU was considered small, these materials are of unknown composition and therefore may have different concentrations of substances when compared to those in the reviewed studies.

121. The combined effects of all the substances present in rubber granules were not known and was difficult to assess, however, ECHA stated that this uncertainty is not considered to affect the main conclusions of their evaluation.

122. There were also assumptions related to the exposure scenarios. For example, the oral route was not considered a relevant exposure for workers since it was expected that workers do not accidentally swallow the granules (due to good hygiene practices). Infrequent exposures were also considered into account (*e.g.* spectators), however, it was assumed that these exposures were covered under the worst-case scenarios.

123. ECHA highlighted that some of the input values used in the risk assessment were assumptions, and that these assumed values were conservative, and so this approach reduced the uncertainty of the evaluation.

124. Based on their evaluation, ECHA recommended the following; firstly, the REACH regulation may be changed to ensure that rubber granules are only supplied with very low concentrations of PAHs and other relevant hazardous substances.

125. Secondly, the owners and operators of existing (outdoor and indoor) fields should measure the concentrations of PAHs and other substances in the rubber granules used in their fields and make this information readily available to interested parties. In conjunction to this, producers of rubber granules and their related associations should develop guidance on how to test their material to aid manufacturers and importers of (recycled) rubber infill. The European sports and football associations and clubs also have a play to role in ensuring effective and transparent communication of information related to the safety of rubber granules in synthetic turf to players and the general public. It was recommended that owners and operators of existing indoor fields with rubber granule infills should ensure adequate ventilation.

126. Lastly, ECHA recommended that players should also take basic hygiene measures after playing on artificial turf containing recycled rubber granules (*e.g.* washing of hands, attending to cuts and scrapes quickly, removal of cleats and soiled uniforms outside the house (in order to prevent entry of rubber crumb material), and in the case of accidental entry of crumb into the mouth – it must not be swallowed.

127. In September 2019, new evidence arose which lead to the Committee for Socio-economic Analysis adopting its final opinion on the proposal for the restriction of the EU-8 PAHs found in granules and mulches used in synthetic turf pitches and playgrounds[18]. The restriction proposal lowers the total concentration limit of 8 PAHs to 20 mg/kg, equivalent to 0.002 % by weight (as supposed to having individual restrictions; *Table. 4*). Whilst the levels of PAHs are currently low, the aim of the restriction is to ensure that the cancer risk remains at a low level from those coming into contact with the granules and mulches (ECHA, 2019).

Toxicity

Human data

In vivo studies

Occupational exposure

128. Savary & Vincent (2011) assessed occupational exposure in four French facilities where used tyres were turned into rubber granulates. Particulate exposure levels were measured using filter samples and gravimetric analysis (n=30 and n=21 ambient concentration measurements). VOCs screening was also carried out in parallel, followed by analysis using GC-MS (n=6). Details of the number of workers at each facility and the processing capability are presented in *Table*. *5*.

[18] Summary article available at: https://echa.europa.eu/-/echa-s-scientific-committees-support-restricting-pahs-in-granules-and-mulches

Table. 5 – The number of workers for each facility and the number of processed tyres annually (Savary & Vincent, 2011).

Facility	Number of workers	Working pattern	Equipment installation	Processed tyres (metric tons/years)
A	2	N/A	One outside shredder	~30,000
B	6	N/A	One inside line of four shredders series	~12,000
C	24	Three 8-hour shifts	Three inside lines of three shredders in series	~38,000
D	18	Three 8-hour shifts	Two inside lines of two shredders arranged in series	~40,000

129. The exposure estimates were taken by fitting workers with a pump and a sampling head mounted in the workers breathing zone for 4-8 hours. Static samples were also set up to measure particles in the workshops, these were taken by placing a sampling system; pump and support, close to the installations, at airway level. Tared glass fibre membranes were places on a cellulose plug in a 37 mm filter holder with a 4 mm opening. Sampling was carried out at a controlled flow rate of 2 L/min. The measured results were calculated as an 8-hour time-weighted average.

130. Dust samples taken from the shredding installations mainly comprised carbon and sulphur particles, with silica and iron oxide at their surface. This was also observed to have mixed with textile fibres that had an average length of 830 µm and a median diameter of 15 µm.

131. Exposure level medians were between 0.58 and 3.95 mg/m^3, however, the highest measured concentration can reach up to 41 mg/m^3 in instances where clogging of the textile fibres separation systems occurred. The lowest exposure level was 0.31 mg/m^3. The ambient concentrations were in the range of 0.17 – 6.23 mg/m^3. VOC levels of >1 ppm was not detected.

132. The number of shredders arranged in series and the daily output appeared to have no influence on the values measured.

133. The authors concluded that there was insufficient background information for risk evaluations and that the particle measurements could not be compared to the 8-hour occupational exposure limit value for inhalable fraction of airborne particles of 10 mg/m^3 since microscopic analyses indicated the presence of carbon black, sulphur, silicon, and iron oxide.

134. Chien *et al*., (2003) assessed the occupational health hazards in two scrap-tyre shredding facilities in Taiwan. The average process loadings for plants A and B were 26,500 and 55,000 kg/day, respectively. Both plants utilised a two shift/day working schedule, with each shift lasting for 10 and 8 hours, and have 9 and 19 workers in plants B and A, respectively.

135. Total particulate/dust was collected using PVC filters (5 µm pore size), fitting in a 37 mm cassette and personal sampling pumps, running at 1.7 L/min. In order for total suspended particulates to be sampled, high-volume samplers were also used at a sampling rate of ~1.38 L/min. VOCs were sampled through multi-bed sorbent tubes.

136. The mass collected for the latter samples was mainly used for subsequent mutagenicity test (Ames *Salmonella typhimurium* test; strains TA98 and TA100 with or without bio-activation). The sampled filters were extracted with acetone, under ultra-sonication. Dimethyl sulfoxide was added to each residue to create a concentration of 20 mg/mL.

137. Levels of volatile organics were not significant, but carcinogenic/mutagenic chemicals were identified, such as styrene, benzothiazole, phthalate ester and naphthalene. The total particulate levels ranged from 0.43 – 6.54 mg/m^3, whilst respirable particulates ranged from 0.23 – 1.25 mg/m^3.

138. Ames testing revealed indirect mutagenicity on strain TA98, indicating possible effects on frame-shift type mutagens, however, the authors concluded that the mutagenic/carcinogenic property of tyre shredding/crumb material requires further confirmation.

Epidemiological studies

139. Epidemiological studies related specifically to non-exhaust sources are relatively few, due to limitations and difficulties in obtaining specific tracers related to chronic exposure and the lack of personal exposure data for risk assessment studies.

140. Stafoggia *et al*., (2013) estimated the association between daily concentrations of fine ($PM_{2.5}$), coarse particles (PM_{10}) and their difference ($PM_{2.5\text{-}10}$) with hospitalisations for cardiovascular and respiratory conditions in 10 Southern European cities (within the MED-PARTICLES Project; these included Milan, Turin, Bologna, Parma, Reggio Emilia, Modena and Rome, Italy; Marseille, France; and Madrid and Barcelona, Spain)[19] between 2001 and 2010.

141. Daily concentrations of PM_{10} and $PM_{2.5}$ were highest in Milan and Turin (46.9 and 48.1 PM_{10} µg/m^3; and 32.9 and 34.4 $PM_{2.5}$ µg/m^3, respectively). Madrid had the smallest daily $PM_{2.5}$ concentrations (17.2 µg/m^3) and the largest $PM_{2.5\text{-}10}$ concentrations (17.5 µg/m^3).

[19] Mediterranean particles (MED-PARTICLES) project: Full details available at: http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=3974. Note that in the reference study; Parma, Reggio Emilia and Modena were analysed together as a single conurbation called “Emilia Romagna”.

142. The total cardiovascular admissions for all cities was 727,579; daily mean of 51.4, whilst the total respiratory admissions were 459,261; daily mean of 32.5. Significant associations were found between all PM fractions and cardiovascular admissions. Increases of 10 µg/m^3 in $PM_{2.5}$, 6.3 µg/m^3 in $PM_{2.5-10}$, and 14.4 µg/m^3 in PM_{10} were associated with increases of 0.51%, 0.46%, and 0.53%, respectively. As for associations with respiratory hospitalisations, these were 1.15% for PM_{10} and 1.36% for $PM_{2.5}$.

143. The authors concluded that $PM_{2.5}$ and $PM_{2.5-10}$ were positively associated with cardiovascular and respiratory admissions in the Mediterranean cities included.

144. Meister et al., (2012) estimated the effects of short-term exposure of $PM_{2.5-10}$ on daily mortality in Stockholm, Sweden for the years 2000-2008. The analysis was stratified by period because the composition of particles varies seasonally. In Sweden, passenger cars, light-weight trucks and light-weight buses are required to have winter tyres (studded or non-studded) from the 1st of December-31st of March, however, studded winter tyres were allowed from the 1st of October-31st of April. During the study period 70-75% for vehicles in Stockholm utilised studded tyres. The months from November-May was referenced as the period of interest, since this represent the highest concentrations of $PM_{2.5-10}$, whilst June-October was regarded as the reference season.

145. The daily mean concentrations of $PM_{2.5-10}$ were highest during late winter and spring, which was associated to increase suspension of road dust particles during dry road conditions. The authors described that this is mainly because of the wear of stone materials in the asphalt. It was observed that coarse particles ($PM_{2.5-10}$) had a significant increase on daily mortality (1.7% per 10 µg/m^3) in Stockholm, Sweden.

146. Ostro et al., (2011) examined the effects of various PM sources on daily mortality for 2003 to 2007 in Barcelona, Spain. Vehicle exhaust, fuel oil combustion (ship emissions and industrial combustion), secondary nitrate/organics, minerals (urban and construction dust) and secondary sulphate/organics (power plants, ship emissions, long-range transport) and road dust (brake/tyre/road wear and re-entrained PM) were found to have had significant associations with all-cause and cardiovascular mortality.

147. Up to 4% increase all-causes mortality risk for an interquartile range increase of road dust contributions only (in $PM_{2.5}$), which was larger than the risk from vehicle exhaust emissions.

148. One epidemiological study by Perez *et al*., (2009) analysed the respiratory, cardiovascular and cerebrovascular mortality risk associated with non-combustion, traffic related processes at different PM size fractions in Barcelona (Spain) during March 2003-December 2005. These size fractions were coarse ($PM_{2.5-10}$), intermodal ($PM_{1-2.5}$) and very fine PM (PM_1). Daily mortality in Barcelona was obtained from the Catalan mortality registry for 2003-2005, specifically for respiratory, cardiovascular and cerebrovascular mortality.

149. It was observed that a significantly increased risk ratio $PM_{10-2.5}$ of 5.9% and 9.8% for cardiovascular and cerebrovascular causes, respectively. It is worth noting

that the chemical composition data did not detect the typical tyre marker (Zn), it is assumed that Si was utilised as a substitute marker. $PM_{2.5-10}$ contained the highest concentration of crustal elements (Ti, Li, Rb, Sr, La, Ce, P and Th), however other elements were also characterised (Sb, Cu, Mn, Cr, Co, Sn, Tl, Ba, and Si).

In vitro studies

150. Yanosky et al., (2012) measured the oxidative potential (OP) of PM_{10}, which was measured as the rate of depletion of the antioxidant reduced glutathione (GSH) in an acellular model of human lung respiratory tract lining fluid; cultures were exposed for 4 hours. Data on traffic density and emissions were obtained from the London Atmospheric Emissions Inventory for the year 2005. Traffic emissions data were grouped in three: heavy-goods vehicles, light-goods vehicles, and other vehicles (buses, cars, motorcycles, and taxis). Two statistical models were utilised to predict the weekly average at unmeasured locations anywhere within the study domain throughout 2002-2006; cross-validation (CV R^2) which focused on modelling spatial variation and a model that calculated the square root of the mean of the squared prediction errors (RMSPE), where prediction errors were defined as predicted minus the measured values.

151. The total weekly PM_{10} emissions for all three vehicles are provided in *Table. 6* It was observed that differences in predictive accuracy were more pronounced when comparing spatial rather than weekly CV R^2 values.

152. The results suggest that brake wear and tyre wear particles have higher oxidative potential than other traffic-related sources and their effect is very local (50-100 m from the source). The authors postulated that this effect might be due to the metal content in the brake material. It was also highlighted that GSH OP does not serve as a direct measure of particle toxicity, it only captures components that do not require cellular metabolism to induce oxidative stress, and as such, the total oxidative stress burden may be underestimated.

Table. 6 – Glutathione oxidative potential model performance statistics by emission type and vehicle group categories (reproduced from Yanosky et al., 2012).

		CV R^2	CV R^2	RMSPE	RMSPE
PM10 emissions within 50 m	Vehicle group	Weekly	Spatial	Weekly	Spatial
Tail pipe	Total	0.40	0.63	0.31	0.15
	Heavy-goods vehicle	0.35	0.51	0.32	0.17
	Light-goods vehicle	0.19	0.00	0.36	0.26
	Other	0.41	0.65	0.30	0.14
Brake and tyre wear	Total	0.44	0.73	0.29	0.13
	Heavy-good vehicle	0.28	0.15	0.34	0.22

	Light-goods vehicle	0.36	0.51	0.32	0.17
	Other	0.43	0.71	0.3	0.13

153. Karlsson et al., (2011) examined the toxicoproteomic effects on human monocyte derived macrophages after exposure to TRWP generated from the interface of studded tires and a granite containing pavement (bimodal size distribution; 4-5 and 7-8 µm) for 18 hours.

154. The macrophage proteome was separated using two-dimensional gel electrophoresis, detected proteins were quantified and identified. Seven were significantly decreased and three were increased as a result of exposure to TRWP when compared to control samples. Overall, proteins associated with inflammatory responses were increased and proteins involved with cellular functions (*e.g.* redox balance, anti-inflammatory response, and glycolysis) were decreased.

155. Gualtieri et al., (2008) investigated if ROS production and heat-shock protein 70 (Hsp70) expression are involved in the cased of toxic effects produced on the A549 (human alveolar lung cells) post-exposure to tyre debris organic extract (TDOE; obtained by extraction in soxhlet apparatus[20]). Cells were exposed with the following TDOE concentrations; 10, 50, 60, and 75 µg/mL.

156. ROS production was analysed after 2 hours of treatment, a dose-response relationship was observed. At 50 µg/mL, ~82% ROS production increase is evident, at 60 µg/mL the increase was measured to be ~99%, and at 75 µg/mL becomes ~127%.

157. An increased expression of Hsp70 was observed at lower doses (10 and 50 µg/mL) and at longer exposure times (72 hours). Densitometric analysis of immunoblots, showed that at 10, 50 and 75 µg/mL the percentage of increase of Hsp70 expression was ~42%, 31% and 12%, respectively.

158. The conclude the authors suggest that ROS production may be the first event caused by A549 exposure to TDOE and that the under expression of Hsp70 probably results from the inhibition of protein synthesis.

159. Gustafsson et al., (2008) characterised TRWP and evaluated their potential for inducing inflammation on human monocytes (release of tumour necrosis factor-α; TNF-α, interleukin (IL)-6 and IL-8). Cells were incubated with a final concentration of 10, 50, 100, 250 and 500 µg/mL for 18 hours.

160. Cell viability was also determined by Trypan Blue. TRWP were generated on road simulator using two different common road surface materials either dense asphalt concrete with granite (ABT) or stone mastic asphalt with quartzite (ABS). Two types of winter tyres were used; light duty vehicle studded tyres with light weight studs and light duty vehicle friction tyres. Fine and coarse fraction samples were collected on 47 mm diameter filters and the particle size distribution was determined by aerodynamic particle sizer.

161. PM_{10} samples were collected during early spring in Hornsgatan (a highly traffic intensive street) and a subway station (Mariatorget) in Stockholm, Sweden using a high-volume sampler with glass fibre filters (pore retention 1.2 µm).

[20] Soxhlet apparatus: it has a flask, an extraction chamber, and a condenser. It can be used for solid-liquid extractions.

162. TRWP particle size was at 4-5 µm (mode) and 7-8 µm (peak). Ultrafine particles (<0.1 µm) were also generated for ABT tyre interaction; 40 nm dominated the number distribution. The particle diameter was also found to be affected by speed. At 70 m/h, the peak appears at about 20 nm, and at 50 and 30 m/h, the peak shift below the lower size limit (16nm). This was attributed to the higher tyre temperatures observed at higher speeds, where loosely bound reinforcing filler material and evaporation of semi-volatile softening oils result in increased tyre emissions.

163. ABT generates much more particles than ABS, however there is not enough knowledge about which pavement properties are the most important for particle formation. Elemental analyses of PM_{10} particles show that most originates from the pavement, and only a relatively small mass contribution from tyres.

164. In terms of secretion of cytokines, only extracted material from subway filters induced a significant release of TNF-α at 6.4 pg/10^4 cells, in comparison to 1.7 pg/10^4 cells in control cells. Other cytokines did not differ from the controls. ABT particles induced an increase of TNF-α for all doses except between 100-250 µg/mL. Street particles showed an increase of TNF-α between 10 and 50 µg/mL and thereafter a decrease. ABT and street particle samples induced higher TNF-α secretion, when compared to ABS and subway particles. All of the particle types sampled induced a liberation of IL-8 at the lowest dose (10 µg/mL).

165. From the results, the authors concluded that studded tyres strongly contribute to the concentration of particles with a diameter <10 µm and also have the potential to strongly contribute to the fraction of particles <2.5 µm. The cell study indicate TRWP can induce inflammation in airways and that the type of stone material present in the pavement is important for the contribution of this effect. Lastly, TRWP was observed to have at least as high inflammatory potential as diesel engine exhaust particles.

166. Beretta et al., (2007) investigated the potential toxicity of TDOE on human alveolar epithelial cells (A549). Cells were exposed to increasing organic extract concentrations (10, 60 and 75 µg/mL) for 24, 48 and 72 hours.

167. Within 24 hours of exposure, damage was evidenced by the presence of Trypan Blue in ~30% of cells. After 48 hours, an increase in lactate dehydrogenase (LDH) activity was observed. Only minor changes in the overall plasma membrane composition were found in exposed cells, suggesting that LDH release occurs through localised plasma membrane breaks. The authors concluded that the results present evidence that tyre debris exposure (from TDOE) produces lipid raft redistribution and cytotoxicity through activation of the necrosis pathway.

168. Lindbom et al., (2006) investigated the inflammatory effect of PM_{10} generated from wear of studded tyres on two types of pavements (asphalt/granite and asphalt/quartzite). Additionally, as a comparison, they further investigated PM_{10} from a traffic intensive road, a subway station and diesel exhaust particulates (DEP).

169. Human monocyte-derived macrophages, nasal epithelial cells (RPMI 2650) and bronchial epithelial cells (BEAS-2B) were exposed to different types of particulates (10, 50 and 100 µg/mL for DEP and 10, 50, 100, 250 and 500 µg/mL for all other particulate types for 18 hours) and the secretion of IL-6, IL-8, IL-10 and TNF-α into the culture medium was measured. Granite and street particulates resulted in the strongest response of TNF-α secretion. As for IL-8, the ranking was granite > subway > street > quartzite. IL-6 secretion was observed at the 50 and 100 µg/mL dose when compared to street and quartzite. IL-10 secretion was significantly higher in granite particulates when compared to quartzite and subway.

170. Based on the observed results the authors suggested that the airway inflammatory potential of wear particles from tires and pavement might be of greater magnitude than that of DEP.

171. Karlsson et al., (2006) investigated and compared the genotoxicity and the ability to induce inflammatory mediators of 9 different particle types including; wood and pellets combustion, TRWPs collected from an urban street and subway station. The Comet assay was utilised to assess genotoxicity and inflammatory effects were measured as induction of IL-6, IL-8 and TNF-α. There were four types of TRWPs collected. Three were TRWPs collected from running a road simulator with studded tires on different pavements; asphalt concrete (10 µm) and stone mastic asphalt (2.5 and 10 µm). Additionally, TRWPs (10 µm) were collected from running a road simulator with friction tires and sanded stone mastic asphalt. Note that all pavement types commonly used in Swedish roads.

172. In terms of exposure, particle suspensions were mixed with fresh medium and cells were exposed for 4 hours to 70 µg/mL. All particles tested caused DNA damage. TRWP generated from friction tyres and sanded stone mastic asphalt caused significant higher levels of DNA damage when compared to TRWP collected from asphalt concrete at 12% and 7% DNA damage tail, respectively. There was no significant difference between the different size fractions of TRWP collected from stone mastic asphalt. The subway particles were the most genotoxic (17% DNA damage tail), likely due to redox-active iron.

173. The most potent particles to induce all three cytokines were those collected from an urban street. TRWP collected when running studded tyres with stone mastic asphalt caused significant increase of all three cytokines, $TRWP_{10}$ was observed to be more potent than the $TRWP_{2.5}$.

174. Gualtieri et al., (2005) exposed A549 (human alveolar lung cells) for 24, 48 and 72 hours to 10, 50, 60 and 75 µg/mL of TDOE and characterised as isoprene with FTIR). MTT and Trypan Blue assays were used to evaluate cytotoxicity and Comet assay to evaluate DNA damage.

175. TDOE were found to induce a dose-dependent increase in cell mortality and DNA damage, significant toxicity was observed in the 60 µg/mL for 72 hours. At 24 hours, cell mortality varied from 12.9-27.8%, while at 48 hours it varied from 18-39.8%. It must be noted that the range of cell viability represents the lowest and the highest doses. The authors concluded that the dataset contributes to the knowledge

of the possible damage produced by tyre debris, which constitutes 5-7% of PM_{10} and 2% of $PM_{2.5}$.

In silico studies

176. Perkins *et al*., (2019) evaluated the potential carcinogenicity of organic chemicals in synthetic turf crumb rubber (commonly fabricated from recycled tyres) using ADMET Predictor®[21] and chemical classifications from the United States Environmental Protection Agency (US EPA) and ECHA. In Europe, there are currently ~13,000 synthetic turf fields, this is expected to rise to ~21,000 by 2020.

177. A literature review was carried out and 306 chemical constituents were identified to be present in crumb rubber infill from 20 publications (out of 43). These compounds spanned several chemical classes including PAHs, nitrosamines, furans, organochlorides, antioxidants and plasticisers.

178. The ADMET Predictor® output predicted 197/306 chemicals to have met the authors *a priori* carcinogenicity criteria. Of these 52, chemicals were also known, presumed or suspected carcinogens by the US EPA and ECHA. 6/109 remaining chemicals were missed by the computational programme since these were classified as known, presumed or suspected carcinogens by the US EPA and ECHA.

179. It was highlighted that; the majority of the crumb rubber constituents were not listed in the US EPA (n=207) and ECHA (n=262) databases. It was suggested that this was likely due to an absence of evaluation or there was insufficient information for a reliable carcinogenicity classification.

180. The authors concluded that the above findings related that computational toxicology assessment in conjunction with government classifications can be used to identify and prioritise hazardous chemicals to be examined in future exposure studies for users of synthetic turf fields.

181. Atkinson *et al*., (2016) reviewed the short-term exposure to traffic-related air pollution and daily mortality in London, UK. They assembled a database based on >100 daily, measured and modelled pollutant concentrations characterising air pollution in London during 2011-2012. Zn was utilised as a marker for tyre wear, as it is the only element in tyres with concentrations above those found in crustal material. Other pollutants were nitrogen monoxide (NO), carbon monoxide, elemental and black carbon, copper (brake wear), and aluminium (mineral dust).

182. Results were presented as percent change in mortality for an interquartile (IQR) increase in pollutant concentration to facilitate comparison of relative risks between pollutants. Zn had an IQR of 0.009 μg/m^3 based on 668 days at the 50th percentile (10th% 0.004 - 0.025 90th%); the associations of Zn and mortality were generally below 1% per IQR with confidence intervals that spanned 0% (*Table. 7*).

[21]ADMET Predictor®: Absorption, distribution, metabolism, excretion and toxicity (ADMET) is a property prediction and QSAR model-building application.

Table. 7 - results from two-pollutant models for percentage change in mortality (and 95% confidence intervals; CI) associated with an IQR increase in traffic-related cardiovascular and respiratory mortality in London, UK (reproduced from Atkinson et al., 2016).

Pollutant (IQR, µg/m^3)	Adjustment	Total	Mortality (95% CI) Cardiovascular	Respiratory
Zn	None PM10 PM10 + CO PM10 + O3 PM10 +SO2	-0.36 -0.12 0.08 0.02 0.02	-1.39 -1.58 -1.25 -1.34 -1.38	-0.44 -0.34 -0.74 -0.21 -0.31

183. Based on the results there were no evidence for associations between markers of tyre wear and mortality. The authors hypothesised that the lack of an association either suggests that population exposure away from the roadside are insufficient to overwhelm endogenous airway defences or that their toxic actions requires longer-term accumulation within the body, and so are therefore unlikely to be apparent and or significant when interrogating short-term health effects.

Mammalian data

In vivo studies

184. Gerlofs-Nijland *et al*., (2019) observed the inhalation toxicity of $PM_{2.5}$ released from studded winter tyres and asphalt concrete pavement in female Balb/cOlaHsd mice (n=4/5 per dose group). PM samples were collected as suspensions in water, the concentrations were up to 7 (6.7 – low,6.9 – medium,6.8 – high) mg/m^3. The exposure was administered *via* nose-only inhalation and lasted for 1.5, 3 or 6 hours.

185. No cytotoxicity was observed after exposure to re-aerosolised $PM_{2.5}$. The numbers of lymphocytes were not affected. Bronchial lavage fluid analysis revealed that LDH and GSH levels in the bronchoalveolar lavage fluid (BALF) were not affected. There were no signs of oxidative stress after exposure to re-aerosolised $PM_{2.5}$.

186. The authors also investigated the effects of other PM sources including; brake pads (low-metallic, semi-metallic, non-asbestos organic (NAO) and European market-NAO hybrid), tires and road pavement, poultry farm dust, as well as the combustion of diesel fuel and wood (modern and old-fashioned stove technologies). The potency based on pulmonary inflammation as assessed by the influx of polymorphonuclear neutrophils (PMN) in BALF was determined based on benchmark dose analyses. PM from tire/road wear and brake wear (low-metallic and semi-metallic) appeared to be less potent compared to PM from an old-fashioned stove, diesel combustion, modern stove, and brake wear (NAO and European market-NAO hybrid), which in turn are less potent compared to PM2.5 of the poultry farm.

187. Kreider et al., (2012) evaluated the effects of subacute inhalation of TRWP in Sprague-Dawley rats (n=40; 5 males and 5 females per group (n=4)). Four treatment

groups were investigated; target air concentrations were 0 (control), 10, 40 and 100 µg/m3. TRWP were collected at a road simulator laboratory located within the German Federal Highway Research Institute. Three tyre types including a silica-based summer and winter tyre from Michelin and Pirelli, respectively, as well as a carbon-based summer tyre from Bridgestone were used to generate the TRWP. Particles from each tyre were combined to create a composite sample (2:1:1 Bridgestone:Michelin:Pirelli).

188. Animals were exposed to filtered air or TRWP using the nose-only inhalation exposure system for 6 hours/day, 7 days/week, for 4 weeks. Animals were sacrificed a day after completion of the exposure. The average mean mass median aerodynamic diameter and percent of mass <3 µm suggests that TRWPs were inhalable for rats.

189. No signs of overt toxicity were observed in any treatment group. No deaths were recorded. No changes were also observed for the following endpoints; body and organ weights, food consumption behaviours, haematological and clinical chemistry parameters. Lavage fluid samples were analysed for markers of cytotoxicity (interleukin-6, growth-related oncogene-keratinocyte chemoattractant and tumour necrosis-α) and inflammation (total protein, lactate dehydrogenase and alkaline phosphatase activity). Expression levels remained unchanged for both markers. Lung tissues were analysed for markers of oxidative stress (homo-oxygenase 1 and thiobarbituric acid reactive substances), neither markers were elevated in the lung tissue in control and exposed groups.

190. Histopathological results are summarised here forth. Macroscopic examination of the lungs showed no abnormal lesions. Microscopic examination of the lungs of the 40 (n=1/10 rats; affected sex not reported) and 100 µg/m3 (n=3/10 rats; affected sex not reported) exposure groups showed rare to few widely scattered minimal focal areas of subacute inflammatory cell infiltration of mononuclear cells in the alveolar wall and alveolar spaces. The effects were therefore described to have a slight dose-response.

191. The authors then described the potential correlation of zinc in the ambient air with incidence of adverse effects seen in humans. As described in paragraph. 52, Zn contributes ~1% by mass to tire tread and ~0.3% by mass to TRWP. The potency of Zn is correlated with its solubility in water; thus, increasing its bioavailability. From unpublished data, the authors state the expected percentage of Zn in TRWP to be soluble in lung fluid is ~50%. The authors postulated that despite the TRWP being of high Zn content, only a fraction of Zn is bioavailable via inhalation, which could explain the low toxicity observed.

192. The authors concluded that TRWP is of low toxicity from inhalation and based on the results, a no adverse effect level of 112 µg/m3 was identified for future use in risk assessment. The authors further note that the highest concentration utilised in the study is above those detected in the environment (value not provided).

193. It should be noted that the study carried out was funded by the Tyre Industry Project.

194. Mantecca *et al*., (2010) further investigated the early acute (3 hours) effects of tyre particles at different sizes: 2.5 µm ($TP_{2.5}$) and 10 µm (TP_{10}) (TPs were obtained from a commercial tire recycling plant and size-fractionated in a lab) and $PM_{2.5}$ and PM_{10} collected in Milan in 2007 on male BALB/c mice (n=6/dose/size-fraction) post-instillation.

195. A single intratracheal instillation of 100 µg/µL solution containing either $PM_{2.5}$, PM_{10} or $TP_{2.5}$ and TP_{10} was performed, and after 3 hours mice were euthanized and the BALF was collected and analysed for cellular and biochemical inflammatory markers (as performed in the previous study discussed).

196. Both TP fractions induced a non-significant decrease in macrophage percentage. Only an increase in PMN percentage at the $TP_{2.5}$ fraction. No notable variations in lymphocytes were noted after both TP and PM exposure.

197. In terms of the levels of cytotoxic markers, there were no significant differences in total protein, LDH and alkaline phosphatase (AP) when compared to the control. Significant increases in macrophage-inflammatory protein 2 (MIP-2) and TNF-α secretion appeared to be largely induced by $TP_{2.5}$ and $TP_{10,}$ respectively was evident.

198. Results from histopathological analyses showed that TPs were abundantly present in airway and alveolar spaces lining the epithelial tissues, whilst $PM_{2.5}$ and PM_{10} particles were not easily distinguishable in the lung tissues. Slight tissue lesions were observed in PM-exposed lungs, with localised epithelial cell lyses of the bronchiolar epithelia post PM_{10} exposure and alveolar walls affected by $PM_{2.5}$ particles. The lungs of the TP_{10} exposed group presented large particle deposits throughout the terminal bronchioles and the proximal alveolar spaces. $TP_{2.5}$ deeply penetrated the alveolar sacs, engulfing macrophages and adhering to alveolar epithelial cells. Cellular debris and tissue exudate were commonly seen inside the alveolar lumen.

199. Protein analyses on lung tissues was also carried out. The transcription of TNF-α was not statistically significant in all treatment groups despite the increased level of TNF-α in the BALF. Caspase-8 transcription was increased in the lung tissues of PM-exposed mice and in TP2.5 treated mice. Haeme-oxygenase 1 transcription was also increased after TP2.5 instillation, however, PM_{10} induced the strongest production.

200. The authors concluded that the results provide evidence of variable inflammatory mechanisms in mouse lungs in response to both urban PM and tyre particles.

201. Manteca *et al*., (2009) evaluated the pro-inflammatory and toxic effects of size-fractionated ($TP_{2.5}$; <2.5 µMTP and TP_{10}; <10µm) in male BALB/c mice (n=12/dose/size-fraction) post-instillation (TPs were obtained from a commercial tire recycling plant and size-fractionated in a lab).

202. A single intratracheal instillation of 10, 100 or 200 µg/100µl was performed, and after 24 hours mice were euthanized and the BALF was examined for total protein, LDH, AP and β-glucuronidase as markers of cytotoxicity. Whilst, TNF-α,

macrophage inflammatory protein-2, and inflammatory cells were used as markers of inflammation.

203. The results are hereby summarised. Cellular inflammatory response to TP showed that both size fractions induced significant granulocyte infiltration (increase in PMN at all doses for $TP_{2.5}$ and at 100 and 200 µg/100µl of TP_{10}. As for cytotoxicity, no differences were observed between TP_{10} and control groups, whilst significant increases in total proteins, LDH and AP were observed resulting from $TP_{2.5}$ exposure, however, there was no evidence of a dose-response relationship. Oxidative potential was only observed at the highest dose of $TP_{2.5}$; increase in GSH and SOD was decreased at the highest dose of for TP_{10}. Levels of pro-inflammatory cytokine release were not commonly detected post 24 hours; the exception was an increase in MIP-2 at the 100 µg dose level for TP_{10}.

204. Histopathological results showed that TP_{10} exposed lungs had intact respiratory parenchyma with particle aggregates lining the alveolar walls, phagocytised TPs were localised in the alveolar walls, demonstrating efficient particle clearance, however, depositions interacting with epithelial layers could be found. At 100 and 200 µg exposure groups, inflammatory tissue infiltrations were localised at terminal bronchioles and alveolar ducts. Additionally, at the highest dose heavy inflammatory and degenerative processes with abundant granulocytes and tissue exudate were found to fil the alveolar lumina.

205. As for $TP_{2.5}$, particles were finely dispersed with fewer particle aggregates than TP_{10}. Macrophage mediated clearance, as well as inflammatory tissue infiltration, were not evident. Congested respiratory tissue with haemorrhagic phenomena were commonly observed, particularly in 100 and 200µg dose groups. The marked tissue damage was confirmed by lysis of alveolar walls coupled with extensive necrotic phenomena.

206. The authors concluded that lung toxicity induced by TP_{10} was primarily due to macrophage-mediated inflammatory events, while toxicity induced by $TP_{2.5}$ appeared to be related more closely to cytotoxicity.

207. Gottipolu *et al*., (2008) investigated the respiratory effects of a single dose of ground tyres of recycled SBR or dust from scrap tyres at 5 mg/kg in male Kyoto rats (n=6; per group). Pulmonary toxicity and cardiac mitochondrial enzymes were analysed 1 day, 1 week or 4 weeks post-administration of tyre particles and 4 or 2 hours later for metals (mainly Zn and Cu which were detected at high levels in water-soluble fractions).

208. A significant increase in lavage fluid markers of inflammation and injury were observed at day 1 (the effect of dust from scrap tyres was higher than ground tyres from recycled SBR), however, these observed effects were reversible after week 1. No effects on cardiac enzymes were noted resulting from either tyre particulate types.

209. Adverse effects were observed post exposure to soluble Zn and Cu, these included marked pulmonary inflammation and injury. Temporal differences were observed, in that the effects of Cu peaked earlier at 4 hours, whilst the effects of Zn

were observed after 24 hours). The instillation of Zn, Cu, and Zn with Cu decreased the activity of cardiac aconitase, isocitrate dehydrogenase, succinate dehydrogenase, cytochrome-c-oxidase and superoxide dismutase, which the authors suggested is an indication of mitochondrial oxidative stress.

210. The authors therefore concluded that the observed acute pulmonary toxicity of tyre particles could be due to the presence of water-soluble Zn and Cu, which at high concentrations may induce cardiac oxidative stress.

In vitro

211. Poma *et al*., (2019) investigated the effects of tyre particles (≤10 µm) on mouse RAW 264.7. Tyre particles were obtained from passenger and truck tyres (PT and TT; respectively), and cells were exposed to 10, 25, 50 or 100 µg/mL for 4, 24 and 48 hours. MTT assay[22] was utilised to assess cell viability. The cytokinesis-block micronucleus (CBMN) assay[23] was used to asses cell proliferation and genotoxicity, and the TNF-α ELISA mouse test was utilised to detect the levels of TNF-α.

212. The MTT assay showed a decrease in metabolic activity of ~30% at the concentration of 10 µg/mL of PT after 24 hours, whilst TT caused reductions of 20% and 10% at concentrations of 10 and 50 µg/mL, respectively. A significant increase in the number of micronuclei in cells incubated with PT at all concentrations was observed determined by the CBMN assay, whilst cells treated with TT particles showed an increase only at the 10 µg/mL concentration. After 4 hours of incubation, cells treated with PT and TT at 100 µg/mL only showed a slight increase of TNF-α, however, after 24 hours of incubation with both tyre samples, a significant increase of TNF-α was observed in cells treated with 50 and 100 µg/mL.

213. The authors concluded that from the data obtained, PT had higher cytotoxic, clastogenic/genotoxic and inflammatory potential when compared to TT.

214. Lindbom *et al*., (2007) also investigated the potential inflammatory effects of TRWP on murine macrophage like cell line; RAW 264.7. Much like the other study previously discussed, PM_{10} generated from wear of studded tyres on two types of pavements (asphalt/granite and asphalt/quartzite) and, as a comparison, PM_{10} from a traffic intensive road, a subway station and DEP were exposed to the cell line.

215. Cells were exposed to 1, 10 or 100 µg/mL of particles for 18 hours. Results show that all particles tested induced IL-6, TNF-α and NO, and those from the traffic intensive road were the most potent ones. In contrast, particles collected from a subway station were the most potent to induce lipid peroxidation, amino acid release, and formation of ROS. Particles from studded-tire pavement wear (generated using a road simulator), were able to induce inflammatory cytokines, NO, lipid peroxidation and ROS formation. Particles generated from pavement containing granite were more potent than those containing quartzite as the main stone material. Able to

[22] 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay: is a colorimetric method for sensitive quantification of viable cells in cell proliferation assay.
[23] Cytokinesis-block micronucleus (CMBN) assay: is a genotoxicity assay that provides information on a variety of chromosomal damage including chromosomal breakage, chromosome rearrangements, and gene amplification.

induce inflammatory cytokines (TNF-α, IL-6), NO, lipid peroxidation and ROS formation. Cell viability was not influenced the presence of the particle at any concentration; ≥90%.

Data on ecotoxicity

216. Wagner *et al*., (2018) reviewed the effects of TWP on biota in the aquatic environment. The effects on biota can be caused by physical interactions between particle and organism or as a consequence of toxic leachates. They noted that the majority of studies examined the effects of TWP leachates independent from the environmental compartments. The toxicity of TWP leachates was related to the presence of Zn, and other toxic metals and compounds such as lead and cadmium, benzothiazoles, phthalates, phenolic derivatives *etc.*

217. The bioavailability of PAHs from TWP to organisms in the environment is expected to be low, since PAH bound to tyres are not easily extracted even under harsh extraction conditions.

218. Due to varying test methodologies, the acute effect concentrations of TWP in aquatic media including marine environments were found to cover a range of 25-100,000 mg TWP/L, while chronic effect concentrations vary from 10 - 3600mg TWP/L. The sub-lethal concentrations for teratogenic, mutagenic and oestrogenic effects ranged from 500 – 500,000 mg TWP/L. It was highlighted that these TWP concentrations were not representative of those found in the environment. Reported environmental concentrations in river water do not exceed 10 mg TWP/L. Only in surface runoff have levels of >100 mg TWP/L have been reported. It was further mentioned that TWP obtained from road simulators are not appropriate to study ecotoxicological effects since they behave differently from those obtained *in situ*.

219. In terms of TWP toxicity in soils and sediments, the risk of leachate toxicity was considered low, since observed effects were low or absent at concentrations of ≥10,000 mg TWP/L.

220. To conclude, the authors recommended that in order to assess the risk of TWP in the aquatic environment, the environmental exposure conditions have to be known and that test conditions must realistic and representative.

Other

Mechanistic studies

221. Tyre rubber contains a wide variety of chemicals (Table. 1) that can produce biological and toxicological effects through many distinct mechanisms. The toxicological studies summarised below provide evidence on the possible modes of action that TWP exhibit to cause adverse effects.

222. He *et al*., (2011) investigated whether leachates from rubber tyre material induces aryl hydrocarbon receptor (AhR) signalling pathways. Analysis of extracts for their ability to stimulate AhR-dependent gene expression was carried out using

recombinant mouse hepatoma cell based CALUX[24] and CAFLUX[25] clonal cell lines. Cells were exposed to tyre extracts equivalent to 0.5 µg to 0.5 mg of the original tyre sample (1 g) for 4 hours.

223. The results demonstrated that tyre extract induced luciferase in a concentration dependent manner and that this stimulation is due to the ability of chemicals present in the extract to bind to and stimulate AhR transformation and DNA binding. In order to identify the specific chemical(s) responsible for activating the AhR and AhR signalling pathway, a toxicant identification evaluation was carried out *via* silica gel column chromatography; 22 fractions were eluted. 19/22 fractions exhibited AhR agonist activity, instrumental analysis by GC-MS reveal that the most active fraction contained several benzothiazole derivatives and several PAHs were also tentatively identified in one fraction. Whilst PAHs are known AhR agonists, the benzothiazole derivatives; mercaptobenzothiazole and 2-hydroxyenzothiazole were found to only weakly induce AhR-dependent gene expression and that benzothiazole and mercaptobenzothiazole were inactive (when compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin).

224. The authors concluded that further studies are needed to assess the contribution of other tyre additives to the overall AhR agonist activity of tyre extracts and to determine its potential for producing AhR-dependent toxic and biological effects.

225. Although strictly not a mechanistic study, Zhang *et al*., (2002) analysed the oestrogenic, anti-oestrogenic, progesteronic and anti-progesteronic activity of organic tyre extracts in two yeast constructs carrying hormone binding domain of human oestrogen receptor (strain KY320) or progesterone receptor (strain YPH500).

226. Twelve tyres with different types (not further defined) were collected and aliquots of 5 g were quantitively extracted in a Soxhlet apparatus. The final concentrations were 0.01, 0.1, 1, 10, 100, 1,000 and 10,000 µg/mL. Yeast cell cultures were exposed for 2 hours.

227. No detectable oestrogenic or progesteronic activity was observed for doses below the highest dose (10,000 µg/mL). The effective concentration causing 50% of anti-oestrogenic activity ranged from 0.07-9.90 mg/mL and 0.07-0.38 mg/mL for anti-progestronic activity.

228. The authors acknowledged that a yeast-based assay does not determine which entity present in the tyre extract causes the anti-activities, and therefore further *in vivo* assays are needed to determine this. Additionally, the concentrations required to elicit an adverse effect is not generally observed outside laboratory settings, although, occupational exposures may be of concern, however, these should be accounted for in occupational work exposure limits.

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[24]CALUX (Chemical-Activated luciferase gene expression); the clonal cell line contains an integrated AhR-/dioxin-response element driven firefly luciferase plasmid.
[25] CAFLUX (Chemical-Activated fluorescent gene expression); the clonal cell line contains an integrated Ahr-/dioxin-response element driven enhanced green fluorescent protein reporter gene plasmid.

Summary and conclusions

229. Tyres contain a wide range of chemicals, the bulk of tyre tread is composed of a variety of rubbers, including natural rubber co-polymers, poly-butadiene rubber, styrene-butadiene rubber, nitride rubber, neoprene rubber, isoprene rubber, and polysulphide rubber. The interaction of tyres and pavement alters both the chemical composition and characteristics of particles generated compared to the original tyre tread due to heat and friction, as well as incorporation of materials such as environmental "*dust*", brakes, fuels and the atmosphere, as well as roadway particles.

230. Human exposure to chemicals leached from tyres, shredded tyres, and tyre wear material can occur by dermal exposure from environmental sources and ingestion of contaminated materials, as well as inhalation of airborne particulate matter derived from tyre wear material.

231. Challenges associated with evaluating risk from exposure becomes complex when considering other factors such as the effects of weathering and ageing of tyre materials, the effects of temperature, pavement types and driving style. All in all, this will result in the generation of various chemicals with significantly different biological and toxicological effects and potencies.

Abbreviations

ABS	Stone mastic asphalt with quartzite
ABT	Asphalt concrete with granite
AhR	Aryl hydrocarbon receptor
AQEG	Air Quality Expert Group
AURN	Automatic Urban and Rural Network
BALF	bronchoalveolar lavage fluid
BMDL10	Benchmark dose level that corresponds to 10% extra risk
CI	Confidence interval
CLP	Classification, Labelling and Packaging
COMEAP	Committee on Medical Effects of Air Pollution
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CRM	Crumb rubber material
Cu	Copper
CV R2	Cross-validation
DEFRA	Department for Environment Food and Rural Affairs
DEP	Diesel exhaust particle
Dft	Department for Transport
DNA	Deoxyribonucleic acid
EA	Environmental Agency
ECHA	European Chemicals Agency
ELT	End of life
ESTO	European Synthetic Turf Organisation
ETMRA	European Tyre and Rubber Manufacturers' Association
ETWRP	European Tyre and Road Wear Platform
EU	European Union
GC-MS	Gas chromatography-mass spectroscopy
GSH	Glutathione
HSE	Health and Safety Executive
Hsp70	Heat-shock protein 70
IL	Interleukin
IQR	Interquartile
ISO	International Organisation for Standardisation
JRC	Joint Research Centre
LDH	Lactate dehydrogenase
MED-PARTICLES	Mediterranean particles
MIP-2	Macrophage-inflammatory protein 2
NAO	Non-asbestos organic
NEE	Non-exhaust emissions
NO	Nitrogen monoxide
NRL	Natural rubber latex

OP	Oxidative potential
PAHs	Polyaromatic hydrocarbons
PM	Particulate matter
PMN	Polymorphonuclear neutrophils
PT	Passenger tyre
PVC	Polyvinyl chloride
REVIHAPP	Review of evidence on health aspects of air pollution
RIVM	The Dutch National Institute for Public Health and the Environment
RMSPE	Square root of the mean of the squared prediction errors
ROS	Reactive oxygen species
RUBIAC	Rubber Industry Advisory Committee
SBR	Styrene-butadiene rubber
SPME	solid phase micro-extraction
SSFL	Skin surface film liquid including sweat plus sebum
SVOCs	semi-volatile organic compounds
TDOE	Tyre debris organic extract
TIP	Tire Industry Project
TNF-α	Tumour necrosis factor-α
TP	Tyre particle
TRWP	Tyre and road wear particles
TT	Truck tyre
TWP	Tyre wear particles
TWP/L	Tyre wear particles per litre
UK	United Kingdom
US EPA	United States Environmental Protection Agency
VOCs	Volatile organic compounds
WELs	Workplace exposure limits
WHO	World Health Organisation
Zn	Zinc

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

First draft statement on the potential risks from exposure to microplastics

Potential risks from exposure to microplastics: Background on tyre wear

Relevant literature was obtained from reviews published by authoritative bodies, as described in paragraph 33 of Annex E. In addition, searches for further literature relating to the toxicity of tyre wear and or tyre and road wear particles were carried out utilising the search terms below. The literature searches were performed by the Secretariat at the FSA, with no limit on the publication date.

Search terms

“Tyre wear particles OR Tyre and road wear particles &”

Toxicity
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
Acute Toxicity - oral
Sub(chronic)tox/ carcinogenicity
Human exposure
Human health effects
Risk assessment
Genotoxicity
Reprotoxicity
Reproductive toxicity
Developmental toxicity
Immunotoxicity
Respiratory effects
Inhalation
Food
Soil
Water - water treatment plants
Bioaccumulation/retention
Europe
United Kingdom - UK
Leachates
Ageing
Degradation