

Understanding how blending different ratios of renewable fuels with diesel impacts the toxicity of exhaust particulates to human health

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Abstract

The urgent need to decarbonise transport has increased the utilisation of renewable fuels blended with current hydrocarbons. Heavy duty vehicle electrification solutions are yet to be realised and therefore the reliance on diesel engines may still be present for decades to come. Currently, the diesel supplied to fuel stations across the UK is a 7% blended biodiesel, whilst in South Korea a 5% blend is utilised. Biodiesel is produced from renewable sources, for example, crops, waste residue, oils and biomass.

Particulates from diesel combustion are known to be toxic due to the presence of polyaromatic hydrocarbons (PAHs), however there is very limited understanding of blending oxygenated fuels on the toxicity of the particulates produced. PAHs are aromatic structures that can be metabolised into chemicals which can disrupt DNA replication and potentially influence cancer mechanisms if inhaled in high quantities.

Soyabean methyl-ester (SME) was blended at lower ratios, e.g., 5%, 10%, 15% and combusted in a light duty direct injection diesel engine to investigate and collect particulate emissions. Gas-Chromatography Mass-Spectroscopy (GC-MS) was used to characterise and identify PAH content of the collected particulate samples. Results showed that the lower blend fuels produced a greater amount of large ring PAHs, which correlated with a higher toxic effect in experiments undertaken using *in-vitro* cell models with collected soot samples. This toxic effect was of greater significance than that observed from combustion of either 100% fossil diesel (FD) or 100% SME biodiesel. The toxic effects found highlight the need to better understand impacts of renewable fuel utilisation on human health.

Introduction

The World Health Organisation (WHO) estimates that through implementation of regulations to lower concentrations of air pollutants, an equivalent of 300,000 deaths would be saved per year. [1] It is clear, therefore, that emissions of both gaseous and particulate pollutants must be minimised to reduce impacts on air quality and public health globally. Part of this effort to reduce gaseous and particulate pollutants is synergistic with the decarbonisation through electrification of the road transport system, a large contributor of atmospheric pollution worldwide. However, liquid fuels for combustion will still be required in the transport sector for decades to come, due to the ever-increasing demand and limited availability and function of alternatives. [2] Especially in

relation to the future of heavy-duty vehicles which will require utilisation of liquid fuels for longer than light-duty vehicles, whilst battery technology is still developing. [3]

Biofuels are a necessary step in the decarbonisation of transport, this is due to ease of integration with current vehicle stock, with no modifications or engine retrofitting required. Blend ratios with fossil fuel range across the world with different regulations. Currently B7 (Biodiesel 7%) is available in the UK and up to B5 (Biodiesel 5%) in South Korea. [4][5] Biofuels that are currently used to blend with fossil fuels range from waste products such as used cooking oils, factory effluent and slurry, as well as virgin crops such as palm, soy and sugars. [2] The blending of fossil fuels with renewable alternatives creates a change in composition of the fuels, which introduces species such as oxygenated compounds, which would not otherwise be present. The toxicological impact of pollutants produced from burning these new renewable fuels has yet to be fully investigated. However, there is increased attention due to wider awareness of the health effects of air pollution and the greater commercial availability of renewable fuels.

A crucial factor in investigating the potential health impacts arising from the combustion of renewable fuel blends is understanding the toxic compounds present in the particulate emissions; these are more persistent than gaseous counterparts such as NO_x, CO₂ and CO and can penetrate deep into the human lung system. [7] These particles deposit on surface lung tissue and potentially pass into the bloodstream and to organs around the body. The post-combustion emission profile of particulates will contain polycyclic aromatic hydrocarbons (PAHs), formed from the pyrolysis of the fuel and synthesis of intermediate compounds to form soot in which aromatic species are known intermediates. Therefore, the use of a hydrocarbon liquid fuel, whether fossil fuel, or from a renewable source will produce compounds which have been identified by the Environmental Protection Agency (EPA) as hazardous, carcinogenic and environmentally damaging, all to varying degrees of impact. [6]

Previous work by the authors highlighted the combustion emissions of 100% renewable fuels and the particulate bound PAH profiles of select blend ratios. [7] This prior study investigated the particulates and PAH produced during the combustion in a diesel engine of 20, 50 and 100% blends of a fatty acid methyl ester (FAME) biodiesel with fossil diesel (B0, B20, B50 and B100) A toxic impact from high blend levels of the investigated biodiesel was observed, but lower blend ratios are more representative of those currently utilised worldwide, e.g. B5 to B10, were not considered. A 10% blend (B10) was investigated in a later project which indicated that there was

potentially also an effect of the presence of the biodiesel on particulate toxicity at this low level. [8][9]

The use of fossil fuels for transport needs to be heavily reduced, however it must be reduced and replaced by alternatives that are less toxic. For small personal vehicles, it makes immediate sense to improve battery infrastructure and electrify the majority of small to medium cars and journeys. For larger freight infrastructure there needs to be more work done around using fuels which can decarbonise the transport system and mitigate the presence of harmful PAHs.

This paper will explain the methodology used to test the toxicology of the emissions of the biofuel combustion within a light duty diesel engine and preliminary characterisation of PAHs using GC-MS. Iterations of lower volumetric percentage blends were used, such as B5, B10, B15, to further investigate the effects of lower blend ratios, representative of practical fuel blends around the world. Lower blends of SME appear to exhibit an interesting toxicology, which could be attributed to the PAH profile identified using GC-MS, rather than the engine combustion conditions which were kept consistent.

Experimental Methodology

Engine Method

A Ford Duratorq 2.0 CD132 96kW engine serves as the basis of the naturally aspirated single cylinder diesel research engine facility, i.e. a donor Duratorq engine provided the cylinder head, intake manifold, a fuel injector and a piston, with variable engine braking performed by a David McClure dynamometer configured to stabilise speeds up to 1200rpm. Additionally, piezoelectric and piezoresistive pressure transducers monitored the in-cylinder and intake pressures. For the in-cylinder pressure and charge amplification, instruments were sourced from Kistler (parts 6056A and 5018 respectively), while the inlet manifold pressure and exhaust pressure instruments were sourced from Druck (PTX 717-3275). The once-per-cycle reference pressure for the in-cylinder piezoelectric pressure transducer was determined at bottom dead centre (at which time the inlet valves remained open) using the Druck piezoresistive transducer located 160mm upstream of the inlet valves, whereas the exhaust pressure and temperature were measured 150mm downstream of the exhaust port.

To improve consistency between different test fuels the engine oil was circulated at 80°C around the crankcase and cylinder head at 4bar. As well as this, coolant was distributed around the cylinder head at 70 °C.

Table 1. Naturally aspirated single cylinder engine specifications

Engine Head Model	Ford Duratorq
Engine Crankcase Model	Ricardo Hydra
Cylinder Bore (mm)	86
Crankshaft Stroke (mm)	86
Swept Volume Per Cylinder (cm ³)	499.56

Compression Ratio (Geometric)	18.3:1
Maximum In-Cylinder Pressure (bar)	150
Piston Design	Central ω-bowl in piston
Fuel Pump	Delphi (33100-4X400) single-cam radial-piston pump
Common Rail	Delphi (B47KA) solenoid controlled
Fuel Injector	Delphi DFI 1.3 6-solenoid valve injector
Crank Shaft Encoder	1800 ppr, 0.2 CAD resolution
Engine Oil	10W40 A3/B4

The combustion engine was supplied pressurised fuel for direct injection via one of two systems; the first employed a high-pressure fuel pump fed by a two-litre tank to supply the fuel common rail and was connected directly to the engine fuel injector. The second system used reference diesel pressurised by the common rail as a hydraulic fluid to pressurise and deliver a test fuel contained in a bespoke pressure vessel, details of which can be found in prior publications. [8][10] The engine was warmed up with a ‘conditioning run’ each testing day to help prevent inconsistencies from cold starts. This conditioning run involved combusting fossil diesel to minimise contamination in the test fuel lines, this procedure was also carried out between samples as a means of purging test fuels from the engine and low volume fuel system.

Test fuel blend ratios were selected to replicate the current regulated fuel market, as well as selecting both extremes; 100% Soya Methyl Ester (SME100) and 100% Fossil Diesel (FD). The fuel blends were prepared at volumetric ratios v/v%, with the blend type indicated by the renewable fuel content, i.e., SME05 is 5% SME blended with 95% fossil diesel. Tests with fuel blends FD, SME05, SME10, SME15, SME20 and SME100 were repeated 4 times to detect and reduce errors and increase the quantity of particulates collected. The sample housing setup drew untreated exhaust particulate emissions through an exhaust pipe tapping, with a negative pressure gradient obtained using a high-pressure vacuum pump. The solid particulates were filtered through a 70mm-diameter microfibre glass filter paper (25µm pore size) placed within a heated (180°C) sample chamber, which ensured no condensation in the sample or housing. Sampling occurred over 20-minute periods for consistency between runs, enough time for most fuel blends to saturate the filter paper with particulates.

To ensure consistency, engine speed, injection pressure and sampling duration were all kept constant during test runs as shown in Table 2. An indicated mean effective pressure (IMEP) of 7bar was maintained during testing and achieved by the adjusting the injection duration for each test fuel. An injection pressure of 550bar was chosen to intentionally exacerbate incomplete combustion thus enhancing soot production.

Table 2. Operating conditions of the single cylinder engine system

Engine speed (rpm)	1200
IMEP (bar)	~7.0
Injection Pressure (bar)	550
Combustion timing (CAD)	360
Injection Timing (CAD)	2.8-7.3 BTDC
Injection Duration (μs)	715-900
Sampling Duration (min)	20
Vacuum Pressure (bar)	-0.8

The filtered particulate samples were extracted to a liquid phase using a Dionex ASE 150 to remove the organic polar compounds from the particles. A high pressure of 100bar and extraction temperature of 125°C was used to extract a 60mL final volume of organic compounds in DCM (HPLC grade, Thermo Scientific Chemicals). This 60mL volume was reduced to 1mL under constant nitrogen flow. Samples maintained in DCM were processed through GC-MS and when required for cell culture, solvent swapped into DMSO. DCM was not viable to use in the cell system due to its innate toxicity which would cause the cells to rupture; DMSO was a compromise solvent chosen due to its low levels of toxicity and this was also used as a control to take into consideration any impact the control had on untreated cells.

PAH content was quantified by use of an Agilent 7890B Gas Chromatography and Agilent 5977A single quadrupole Mass Spectrometer (GC-MS), set up with a 30m Restek Rxi-17Sil MS, fused silica column of internal diameter 0.25 mm and a 5m Restek Rxi guard column. Selective Ion Monitoring (SIM) was used to analyse known PAHs with higher resolution and precision than Scan mode. Table 3 shows the operating condition of the GCMS used to resolve species from the calibration curve and was developed to optimise elution of individual compounds. Chromatograms were generated for each test fuel with reference standards of known deuterated samples to enable quantification.

Table 3. Operating conditions of the GCMS.

Running conditions	Temperatures
Oven Ramping	65°C for 2 mins
	15°C min ⁻¹ (65-155°C)
	5°C min ⁻¹ (155-280°C)
	10°C min ⁻¹ (280-340°C)
	340°C for 10 mins
MSD Transfer Line	340°C
MSD Source	230°C

For accurate quantification of PAH present on exhaust particulate samples collected during the combustion of the biofuel blends, the Quebec PAH standard was built upon to incorporate the 24 compounds identified by the EPA. [11] The standard included

quinones which are known to be metabolised into toxic metabolites in the body and have been identified previously in combustion studies. [13] The range of PAHs to investigate was therefore extended to 35 compounds. Additional toxic standards were produced with the help of the Bloomsbury Isotope Facility at UCL, where a microbalance increased resolution and accuracy of the mass standards to be added into a calibration stock.

Calibration curves of a variety of concentrations of the external standard (4- 0.01 ug/mL) were produced on the day of GC-MS testing and were vortexed before use in the instrument. Each level of calibration was prepared independently to remove carry over error and to increase accuracy of individual calibration standard points. A known internal standard was also added at a concentration of 0.6%, or 1.548μg/mL, which contained Naphthalene-d8, Acenaphthene-d10, Phenanthrene-d10, Chrysene-d12, Perylene-d12. Ions that shared similar elution timings to the standard compounds were calculated in reference to respective internal standard.

Mass spectrometry data was confirmed from the calibration curve list of potential PAHs as well as integration of the NIST MS search library, which helped to define the probability of a match to the reference library. Individual undetermined peaks were selected within the Agilent MassHunter Qualitative analysis and compared against the library.

Chromatographs were produced using peak fitting functions within MATLAB and using gaussian fitting functions to distinguish peaks from background noise. Peak areas were converted to concentrations using a scaling factor relevant to the internal standard concentration, as this was known. The m/z values were used to confirm presence of peaks and qualifying ions, which justified the presence of the compounds and allowed for accurate quantitative analysis in the MassHunter Quantitative analysis suite.

Cellular Exposure Method

Cell work was undertaken at the UK Health Security Agency (UKHSA) in Harwell Science Campus, where facilities were available to grow both human primary small airway epithelial cells (hSAEC) and primary immune cells for toxicology testing. Human donor small airway cells and CD34+ derived macrophages (immune cells) were grown independently and differentiated into organotypic cell types prior to particulate exposure. hSAEC cells were expanded for two weeks and then differentiated for 21 days at the Air-Liquid Interface (ALI), until the cells had developed organotypic features such as multiciliation. CD34+ derived immune cells were grown over three weeks before treatment with a soot suspension. The protocol used for the immune cell methodology is found in “Mononuclear phagocyte sub-types in vitro display diverse transcriptional responses to dust mite exposure”. [13] The ALI growth is explained in greater detail within “Biodiesel Exhaust Particle Airway Toxicity and the Role of Polycyclic Aromatic Hydrocarbons”. [7]

Exposure concentrations were related to dispersion papers which discussed a dosing equivalence range from 150μg/mL to 1500μg/mL. [9] To ensure that maximum dosing was assessed, a dosing of 1250μg/mL was used in DMSO solution within the hSAEC. The immune cells were dosed with particulates alone, whereas the hSAEC were dosed with extracted organic material from the soot. As soot particulates are greatly hydrophobic, to blend the particulates into a single cell suspension, vortexing and sonication was required to blend the material fully into water with 0.1% Tween 80. The Tween 80 acted as a detergent to create emulsification of the soot with the water

and was used as an in plate experimental control to ensure the results did not alter the endpoints assessed.

The methodology for the hSAEC exposure to particulates has yet to be refined and therefore organic extracts were tested, post extraction through the ASE Dionex. Extracts were solvent swapped from highly toxic DCM to the biocompatible solvent dimethyl sulfoxide (DMSO) concentrated down to high concentrations in DMSO. As DMSO is slightly toxic to human cells, the percentage DMSO must be below 1% in a solution, therefore a 0.1% solution was chosen to decrease effects from DMSO alone. [14] DMSO and a filter paper (DMSO +FB (Filter Blank)) were used as a control measure to ensure no changes were related to these components. Exposure occurred over a 24-hour period to invoke a response in specific genes related to carcinogenicity such as CYP1A1 and CYP1B1. These genes are sensitive to the presence of PAHs, especially in relation to carcinogenesis. [15] After this exposure time, cells were frozen immediately to preserve the effects.

Gene expression was analysed using real time QuantStudio™ 6 flex qPCR (quantitative polymerase chain reaction) from ThermoFisher. Specific genes sensitive to PAHs were chosen to be analysed against, such as CYP1A1 and CYP1B1. As mentioned above, these specific genes were selected as they have been related to carcinogenicity. [16]

Results and Discussion

The results from the emissions of blended ratios of fuels indicated that there were specific effects in all the samples, the PCR data was displayed in percentage fold (i.e. multiples) over the value of the control. Controls for these data points were the untreated cell wells and effects were normalised to these untreated cell levels. The toxicology data was processed for specific genes. CYP1A1, a gene that responds to the presence of aryl hydrogen groups, was used for the PCR of the hSAEC and CD34+ cells, which were exposed to the particulates. Aryl hydrogen groups are very abundant in PAHs. HSAEC processed data from the results for exposure to SME10 and SME20 particulates and extracts were more like the reference fossil diesel (FD) results. SME05 with the same dosing had a significant increase in fold over control relative to the other blends, this can be seen in Figure 1.

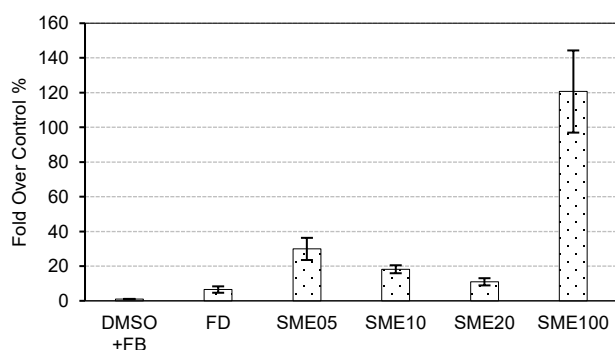


Figure 1. CYP1A1 response to particulate extracts from the various biodiesel blends and reference diesel in the hSAEC after a 24hr exposure.

SME100 exhibited the strongest CYP1A1 response in the ALI cell model (Figure 2), however as it is less likely to be adopted at a 100% fuel than a lower fuel blend, the 100% level was not investigated further in the current study. DMSO+FB control had no effect on the

ALI cells. Here the “fold over control” represents the percentage effect increase over the untreated cells.

Alike to the results in the hSAEC model, the immune cells showed that the lower blend ratios, at comparable exposure, reacted in a very similar way in the two different cell models. The SME05 has the greatest response again and then a downwards trend through SME10, SME20 and then back up to a heightened response of SME100. The results for the CYP1B1 gene indicated that the fossil diesel samples created similar responses as observed for the SME10 and SME20, whereas the SME05 treatment created a significantly higher response, as can be seen in Figure 2.

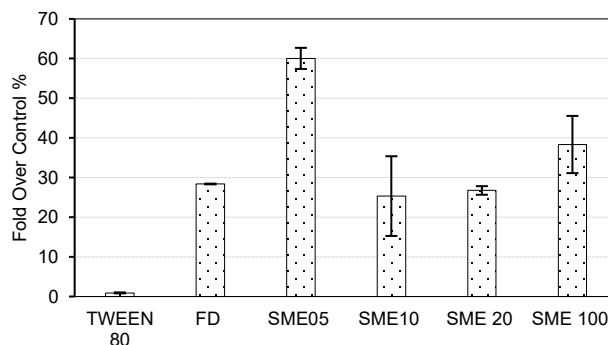


Figure 2. CYP1B1 response to particulates from the various biodiesel blends and reference diesel in the CD34+ derived macrophages after a 24hr exposure.

The immune cell model had a dampened reaction to the SME100, when compared to the ALI model, however the overall trends of the other samples remained the same. It can be also seen that the control had no effect on the cells (Figures 1 and 2).

Regarding the combustion characteristics of the fuels, the biodiesel blends of SME05 exhibited lower peak heat release rate than the FD, which can be seen in Figure 3. The consideration of heat release rate in the context could be tied to the way the fuel combusted in the engine. It is likely that the toxicological differences were tied to the presence of specific compounds that are formed due to the addition of SME. It is thought, currently, that the toxicity effects of different fuel blends are caused by the variation in the profile of the particulate borne PAH and, in turn, this variation in PAH arise from differences in combustion of the blends. Beyond the lower heat release seen in Figure 3, extensive further research is required to link the PAH profiles for different blends to their combustion characteristics.

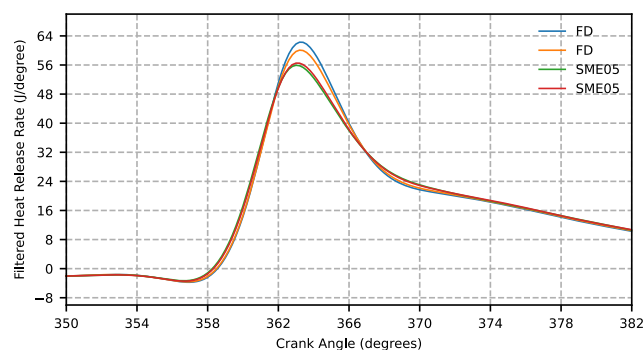


Figure 3. Apparent net heat release rate during combustion of FD and SME05 at constant engine operating conditions.

Table 4 shows the average IMEP, and standard deviation of the values recorded during combustion of the FD and SME05 shown in Figure 3. Constant IMEP of ± 0.025 bar was maintained for all tests and that despite displaying lower peak heat release rate (Figure 3) marginally higher IMEP was recorded for the 5% biodiesel blends. As expected with the addition of a renewable fuel, the heat release rate is slightly lower than the FD graph, indicating a different combustion that is likely tied to viscosity. There was no evidence of a large difference in the ignition timing and overall, the runs were very similar. The consistency in the engine conditions in Table 4 stands to suggest that there is a very slight difference in the combustion properties of both fuels when compared to each other. There is a slightly later ignition in the FD than the SME05, with a lag of 0.2 CAD. The faster ignition of the SME05 suggests a shorter air-fuel mixing period than diesel. The presence of ester functional groups in the SME05 blends could also be affecting temperature and nucleation of soot, which produces a slightly different combustion to the FD.

Earlier research around combustion of SME05 showed a reduction in gaseous emissions, which suggests the blended fuel has greater combustion efficiency than fossil diesel alone. [16]

Table 4. Indicated mean effective pressure (IMEP) during combustion of FD and SME05 shown in Figure 3 and standard deviation of the average values.

Test fuel	Average IMEP (bar)	Standard Deviation
SME05	6.95	0.0555
SME05	6.95	0.0794
FD	6.91	0.0442
FD	6.90	0.0894

To further investigate the effects of the 5% SME blend observed in the toxicity data, the particulate extract samples were analysed and quantified using GC-MS as described. As the retention times for the PAHs were predetermined using a developed calibration standard, the compounds present in the samples could be identified.

Figures 5 and 6 show the chromatogram peaks identified from the samples of FD and SME05 extracted exhaust particulates respectively. The visible scaling of the graphs shows that the fossil diesel exhibited approximately half the intensity counts (Figure 5) of that displayed by the SME05 extract (Figure 6) for the earliest peak-naphthalene. Visually the distribution of PAH identified produced were comparable, with most peaks appearing in both chromatograms.

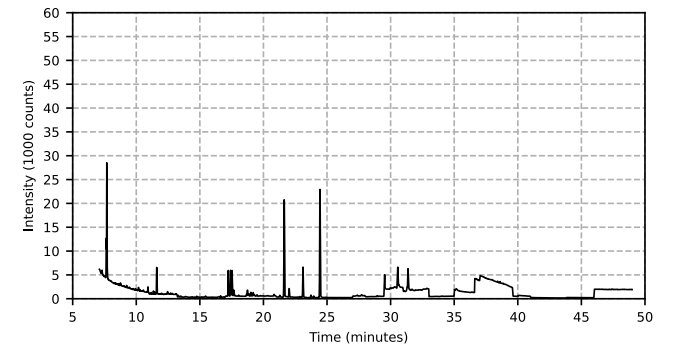


Figure 5a Peaks identified in the reference fossil diesel particulate extract during SIM GC-MS quantification. With y- axis scaled to the largest peak of the SME05 data. X-axis represent retention time. Y-Axis represents the TIC intensity at the detector.

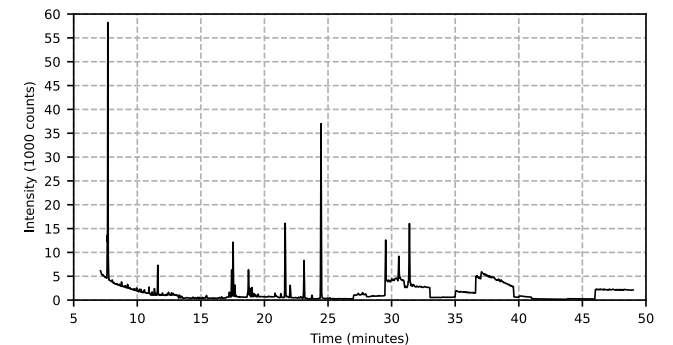


Figure 5b. Peaks identified in the 5% SME blend particulate extract during SIM GC-MS quantification.

Integrating the peaks using the Agilent Mass Hunter technology produced a list of peak areas and retention times for FD and SME05. Internal standards were spiked in both samples, in order to apply a scaling factor for the other peak areas to a known concentration ($0.12\mu\text{g/mL}$).

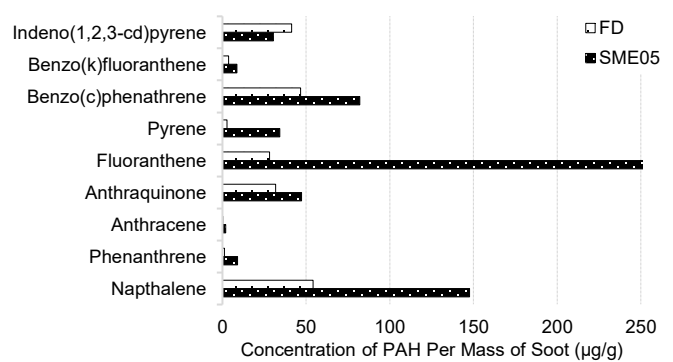


Figure 7. Distribution of specific PAHs found in both reference fossil diesel and SME05 blend particulate extracts.

The largest differences between the levels of PAH measured in both extracts are indicated in Figure 5a and 5b, with the intensity of quantified PAHs present in the SME05 at least double the amount present in the fossil diesel alone. The concentration of fluoranthene present in the SME05 greatly exceeds the concentration in the fossil diesel sample and is a large proportion of the total PAH concentration equivalence. In addition, the naphthalene content of the SME05 extract is appreciably higher than that of the FD extract. Naphthalene is present in fossil diesel fuel prior to combustion and has previously been found to survive into the exhaust emission. [17] It is likely that the SME05 combustion is changing the level of unburnt fuel from which the naphthalene can originate from, as the concentration result is significantly higher than in FD alone. Therefore the naphthalene is potentially surviving the combustion in greater amounts in the SME05 than in the FD.

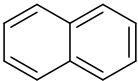
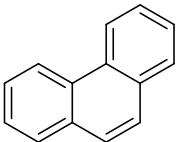
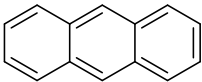
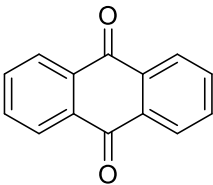
Other large ring number structured PAHs were identified in the SME blend and FD extracts are shown in Table 5 and Figure 4, alongside their respective chemical structures. At least three of the identified PAH peaks in higher concentrations from the SME05 were structures

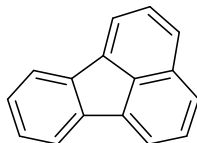
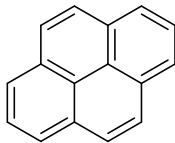
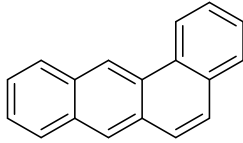
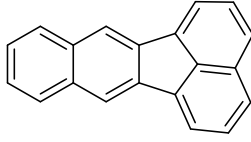
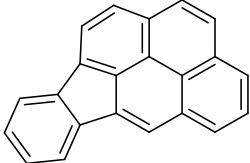
that contain a 5-membered joining ring, which are kinetically easier to form than 6 membered ring structures and can form at faster rates. [18] This could indicate that the SME fuel changed the reaction kinetics of PAH formation post combustion, due to the presence of methyl esters in the initial fuel composition.

The peak of benzo(k)fluoranthene shares a similar retention time, when part of the soot matrix, to the peak of benzo(a)pyrene, one of the most toxic carcinogens, with identical m/z ratio traces; the compounds share the same fragmentation pattern and qualifying ions of 252.1 and 126. [19] It was difficult, therefore, to distinguish between the two compounds and further work is set to explore deconvoluting these peaks to ensure validity. It was determined through the NIST library that the benzo(k)fluoranthene was more likely the compound at RT 35.30 mins. It is highly likely however that the benzo(a)pyrene is present within the sample but has yet to be identified fully.

All of the PAHs found in the soot are identified as being toxic to different levels as categorised by the EPA [6]. The degree of toxicity of individual PAHs is known in terms of the theoretical toxic effect, however this measure is a mass-by-mass basis compared to benzo(a)pyrene in an inhalation study [20]. As well as this it is likely that a lot of toxicological effects are produced from a compound effect of a multitude of different PAHs and may not be as relevant when taken out of the context. It may be difficult to mitigate for specific effects of only one PAH as the formation mechanisms are similar.

Table 5. Specific PAHs present in both the FD and SME05 samples, GC-MS retention times (RT) on the specific column and their chemical structures.

Sample ID	RT(mins)	Structure
Naphthalene	7.68	
Phenanthrene	17.54	
Anthracene	17.68	
Anthraquinone	22.03	

Fluoranthene	23.13	
Pyrene	24.45	
Benzo(c)phenanthrene	30.31	
Benzo(k)fluoranthene	35.30	
Indeno(1,2,3-cd)pyrene	40.58	

The profile of PAHs in the SME05 soot is suggested as a large contributor to the increase in response shown by the equivalent toxicological data relative to the control. However specific toxicity can be attributed to different PAHs and linked to a toxic equivalency factor which was developed as a result of multiple in vivo studies in the late 1900s. [20] The specific toxicity of the PAHs listed in Table 5 and found in the FD and SME05 particulate extracts are all included in the list, apart from Benzo(c)phenanthrene, demonstrating that these 9 compounds have been considered as hazardous to human health for many decades and have varying implications.

In earlier work looking at BaP alone there was significant toxic effect caused by the presence of a single PAH. [21] A greater toxicological impact found in lower blends of these emissions product could result in heightened levels of toxic exposure in the environment as there are a variety of PAHs present.

Summary/Conclusions

With the increased usage of renewable fuels in the road transport sector, it is important that public health is a priority for the changing future. There is evidence from the work that the lower blends of renewable fuels, currently in usage across the world, have the

potential to cause an increase in toxicity compared to fossil diesel alone. SME05 also has the greatest toxic effect compared to the other lower blends in both models of primary lung airway cells, as well as this it has the highest quantified concentration of PAHs.

There is current discord around SME fuel blends in literature, some may have reduction of PAHs but there is no systematic consensus due to the variety of different test conditions[9] Further work needs to be done to further quantify PAH particulate profiles, note differences in fuel stocks and identify changes to the fuel with aging, as well as changes to the emission profile when the operating engine is changed.

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Definitions/Abbreviations

PAH	Polyaromatic hydrocarbons
SME	Soya methyl ester
GC-MS	Gas chromatography- mass spectroscopy
B7,5	Biodiesel 7%, 5%
FD	Fossil diesel
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide

