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Particle characteristics and DNA damage induced by exhaust particulate matter collected from a heavy duty diesel engine using biofuels

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Background – Diesel exhaust particles

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- Polydisperse aerosol.
- Heterogeneous mix of elemental carbon, organic compounds, sulphates and metals.
- Often a significant contributor to particulate matter in an urban environment.
- Combustion of diesel fuel was responsible for 25% of US transport related CO_2 output in 2009.
- World oil reserves are finite and are likely to run out at some point during this century.
- Known to have a number of health effects in humans.

Research motivation

- Biofuels are becoming increasingly frequently used.
- The use of plant based renewable fuels in place of petroleum derived diesel is gaining increasing interest due to the potential for reduction in overall traffic related CO_2 emissions.
- Particles produced by engines running on biofuels differ from those produced by conventional diesel engines in terms of chemical composition and size.
- Current understanding of the potential health effects of biofuel combustion is very limited.

Biodiesel production in Europe 2002 - 2009

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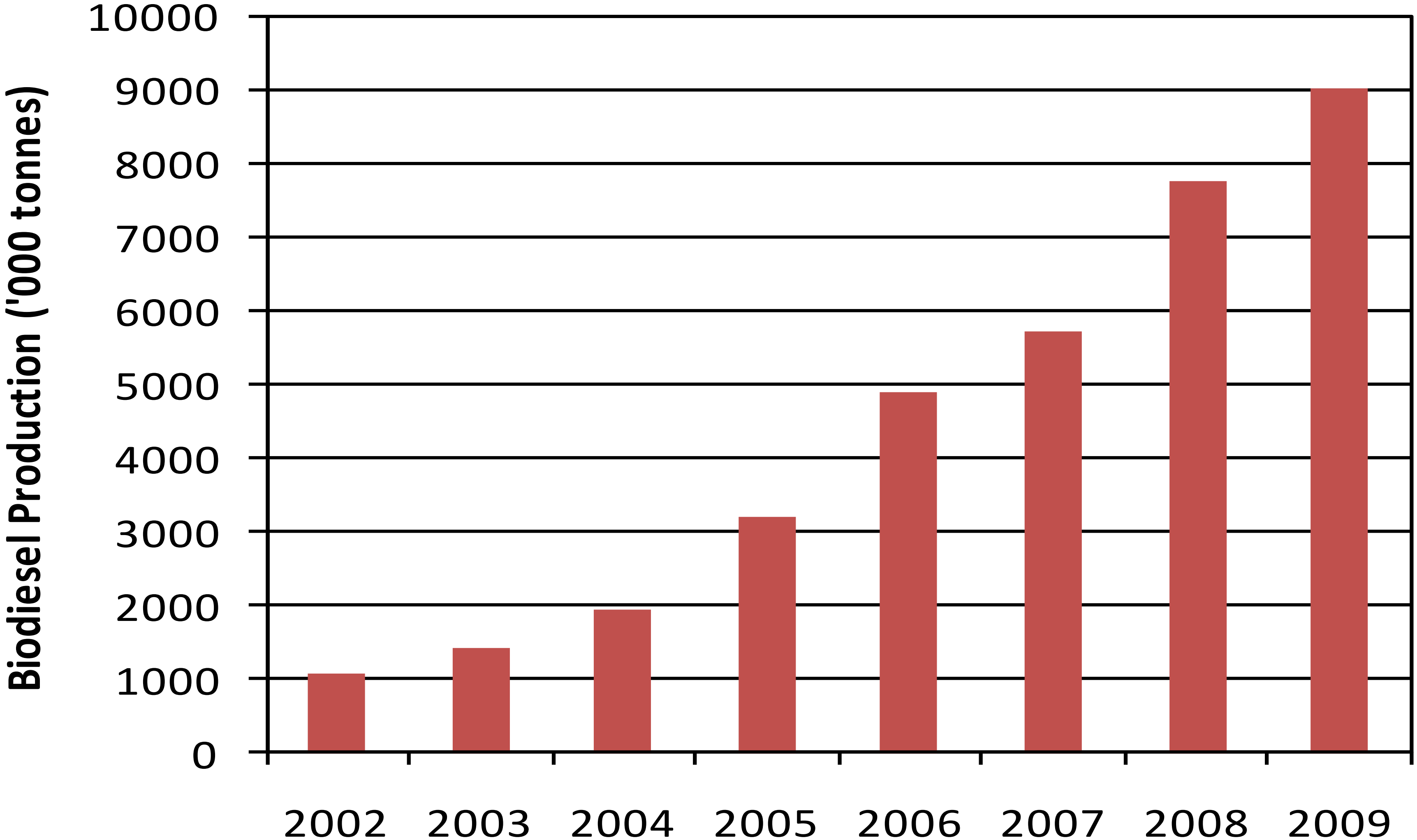

Minimally processed biofuels

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- The use of unprocessed plant oils results in impaired combustion characteristics when compared to conventional diesel fuel.
- Such oils commonly undergo transesterification in order to improve their combustion.
- However this process requires a significant energy input, lowering the net amount of useful energy.
- It has been proposed that minimally processed rapeseed oil (RSO) may serve as a diesel substitute.

Rapeseed oil as an alternative fuel

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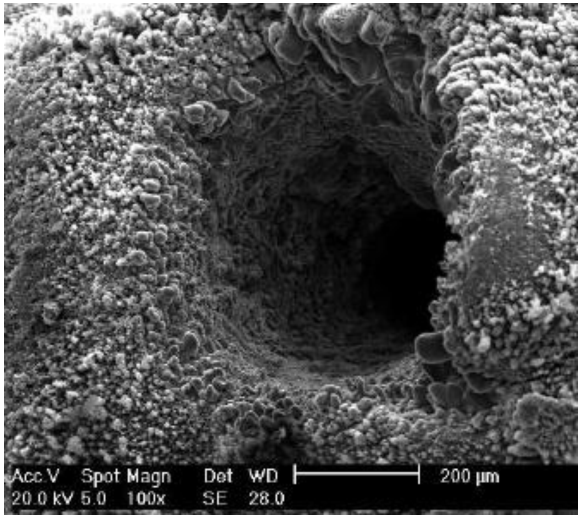
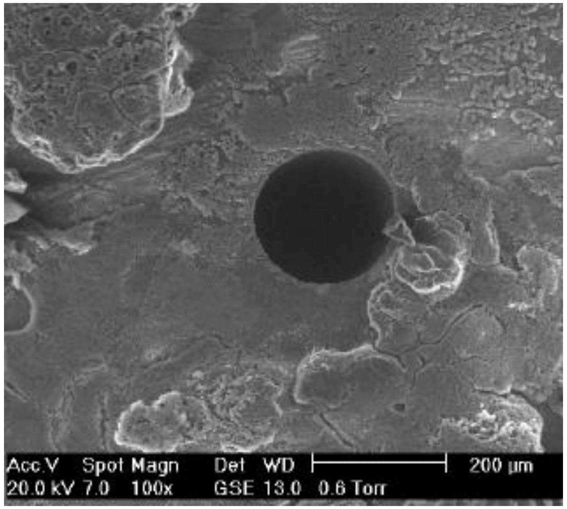
- Use of unprocessed rapeseed oil results in significant coking of fuel injectors.
- Carbonised injectors produce an uneven fuel distribution and decreased engine performance.
- The addition of an additive to the fuel limits injector carbonisation.





Particle collection from engine for diesel / biofuel analysis

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- In order to examine the potential health effects of biofuels engine exhaust particles (EEP) were collected directly from the exhaust of a heavy diesel engine.
- Three fuel types were used:
  - Conventional diesel.
  - Unprocessed rapeseed oil.
  - Rapeseed oil with 800ppm additive (RSOAd).
- Size segregated particles were collected using Anderson impactors and an electrical low pressure impactor (ELPI).
- Total suspended particle (TSP) samples were collected using a smoke meter.

Engine conditions

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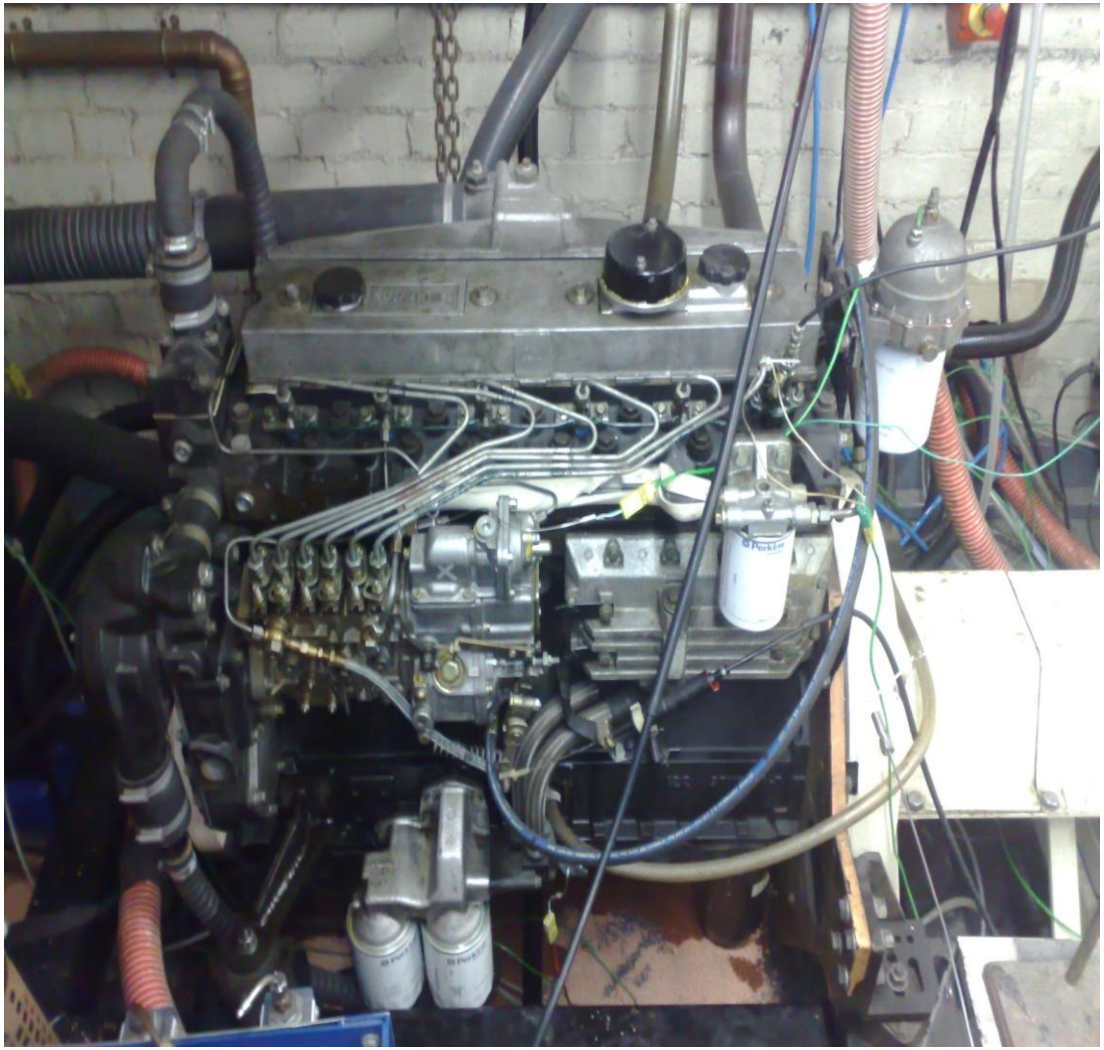
- Particulate samples were collected down stream of the catalytic converter from a 6 litre turbocharged Perkins “Phaser” diesel engine.
- Engine was run at a 47kW, 1500 RPM steady state condition (50% of maximum power output at this RPM).



Particulate matter specific emissions

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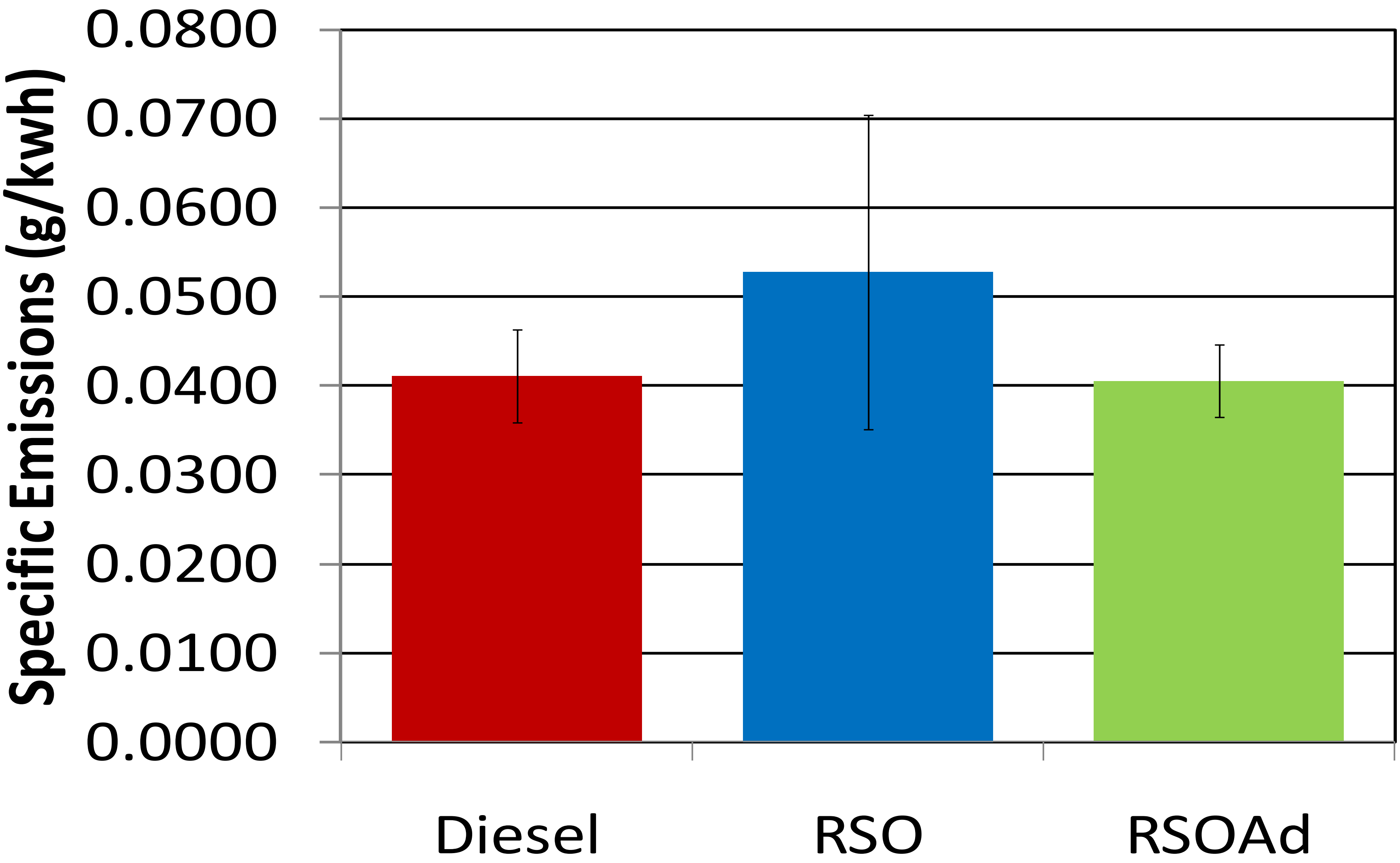

Collection of size segregated particulate material

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- Anderson impactors and ELPI sampling simultaneously.
- Anderson impactors collect particles with an aerodynamic diameter of between 0.43 and 10µm.
- The ELPI collects particles between 0.03 and 10µm.
- EEP for toxicological analysis were collected onto aluminium foil.
- EEP for chemical analysis were collected onto quartz microfibre disks.

Anderson and ELPI inertial impactors

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Anderson	
Stage	D50% (µm)
Stage 0	9
Stage 1	5.8
Stage 2	4.7
Stage 3	3.3
Stage 4	2.1
Stage 5	1.1
Stage 6	0.65
Stage 7	0.43
Backup Filter	<0.43

ELPI	
Stage	D50% (µm)
Stage 13	10
Stage 12	6.8
Stage 11	4.4
Stage 10	2.5
Stage 9	1.6
Stage 8	1
Stage 7	0.65
Stage 6	0.4
Stage 5	0.26
Stage 4	0.17
Stage 3	0.108
Stage 2	0.06
Stage 1	0.03

Particle size and mass distribution of EEP

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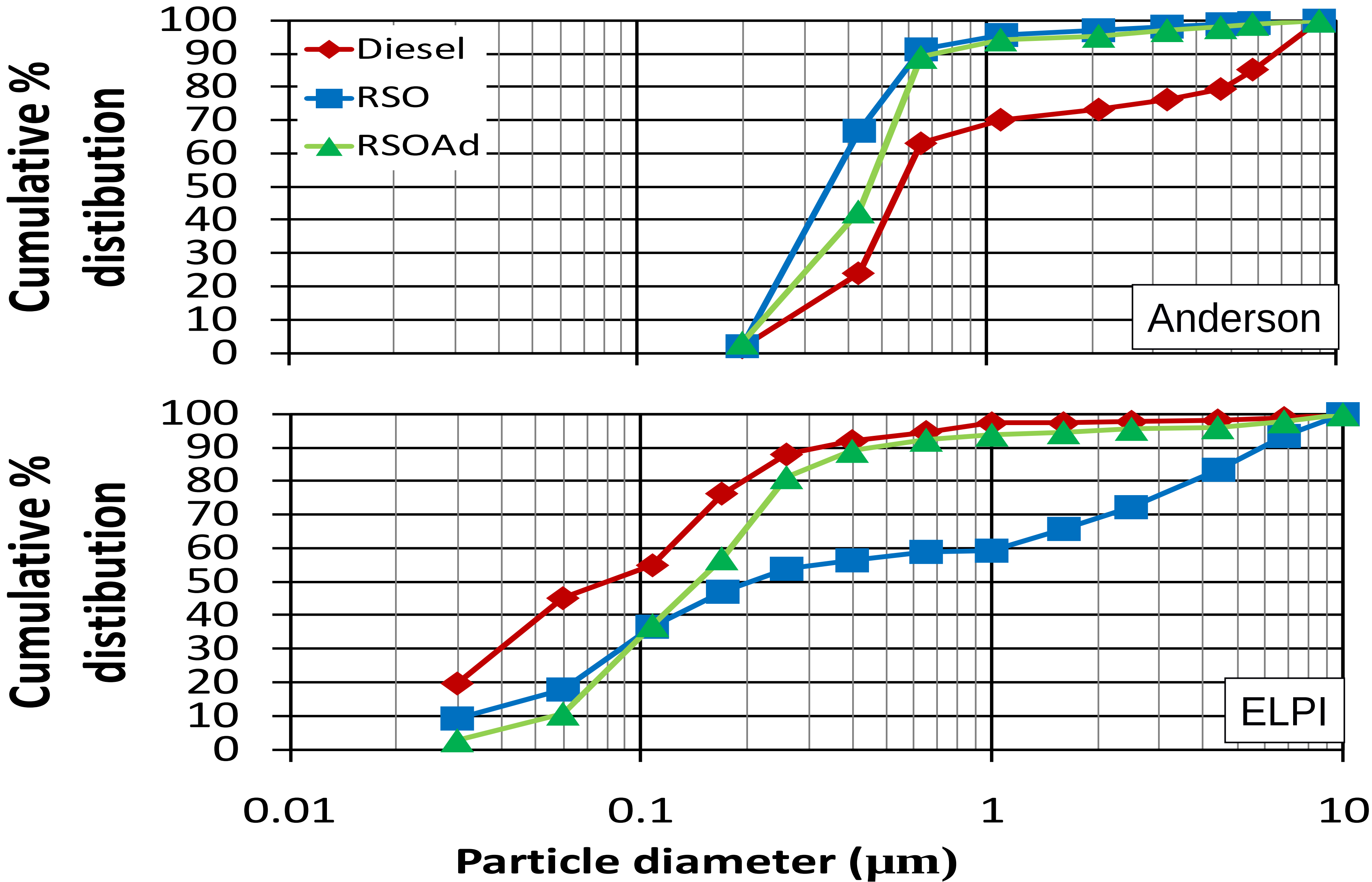

Particulate collection conclusions

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- Combustion of RSO produces a larger mass of particulate material than diesel.
- RSO PM production can be corrected through the use of an additive.
- Irrespective of fuel type the majority of particulate mass was found in the smallest size fractions.

Diesel engine exhaust and cancer

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- Epidemiological evidence has linked diesel exhaust particle (DEP) exposure with cancers of the lung, lymphatic system and bladder.
- In vitro bioassay based studies have demonstrated DEP to damage DNA and cause mutation.
- Diesel EEP are listed as a class 2A carcinogen by IARC (Probably carcinogenic to humans).
- A number of mechanisms for human health effects have been suggested:
  - Size.
  - Organic chemistry.
  - Metals.
  - Free radical production.

DNA damage

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- Damage to the DNA requires repair.
- Improperly completed repair can lead to a mutation.
- Mutations, particularly in genes regulating cell proliferation, are a precursor to cancer.
- May be caused by:
  - A bulky molecule binding with the DNA forming an adduct.
  - Oxidation of the DNA causing an oxidised base.
  - Physical breakage of a DNA strand.
- DNA damage is required for cancer development, but not all damage to DNA results in mutation.

Comet assay and plasmid strand break assay

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Comet assay	Plasmid Strand Break Assay
Measures DNA damage	Measures Free Radical Activity
Measures damage in cells	Measures damage in an acellular environment
Metabolism of chemicals on particles occurs, damage may be a results of free radical activity, metabolites, or cellular processes	No particle compounds are metabolised, damage is caused by free radicals only
DNA can be repaired by cellular processes	No possibility of DNA repair

Toxicological analysis - Comet assay

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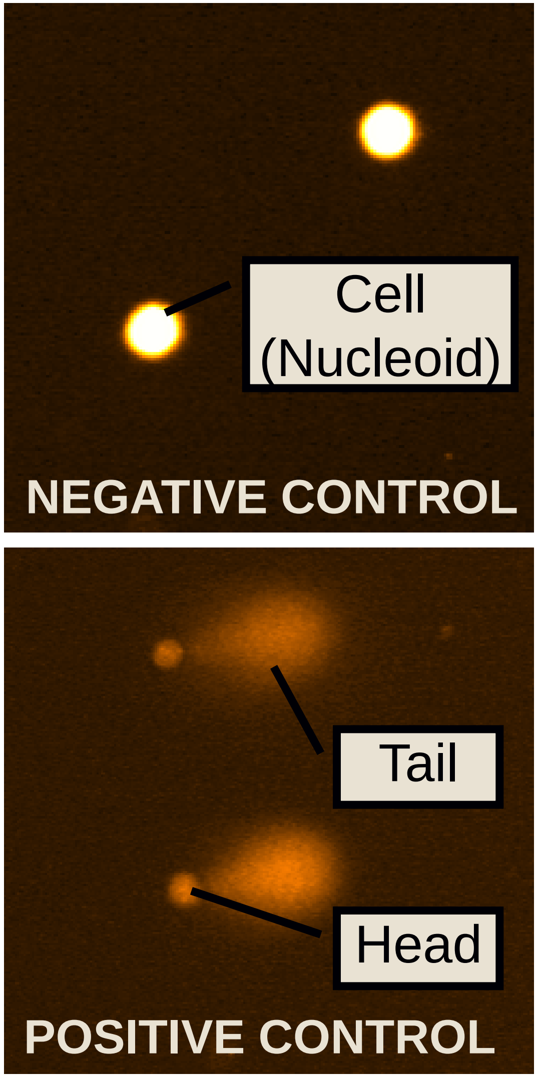
- A549 lung epithelium cells were exposed to particulate matter (50 µg/ml) for 24 hours.
- Treated cells are embedded in agarose, lysed and incubated in alkali solution.
- Many types of DNA damage are converted to DNA strand breaks by the high pH
- Fragmented DNA moves in an electrophoretic current
- More damage = more DNA in tail of “comet”, quantified by image analysis



Comet assay analysis of total suspended particle samples

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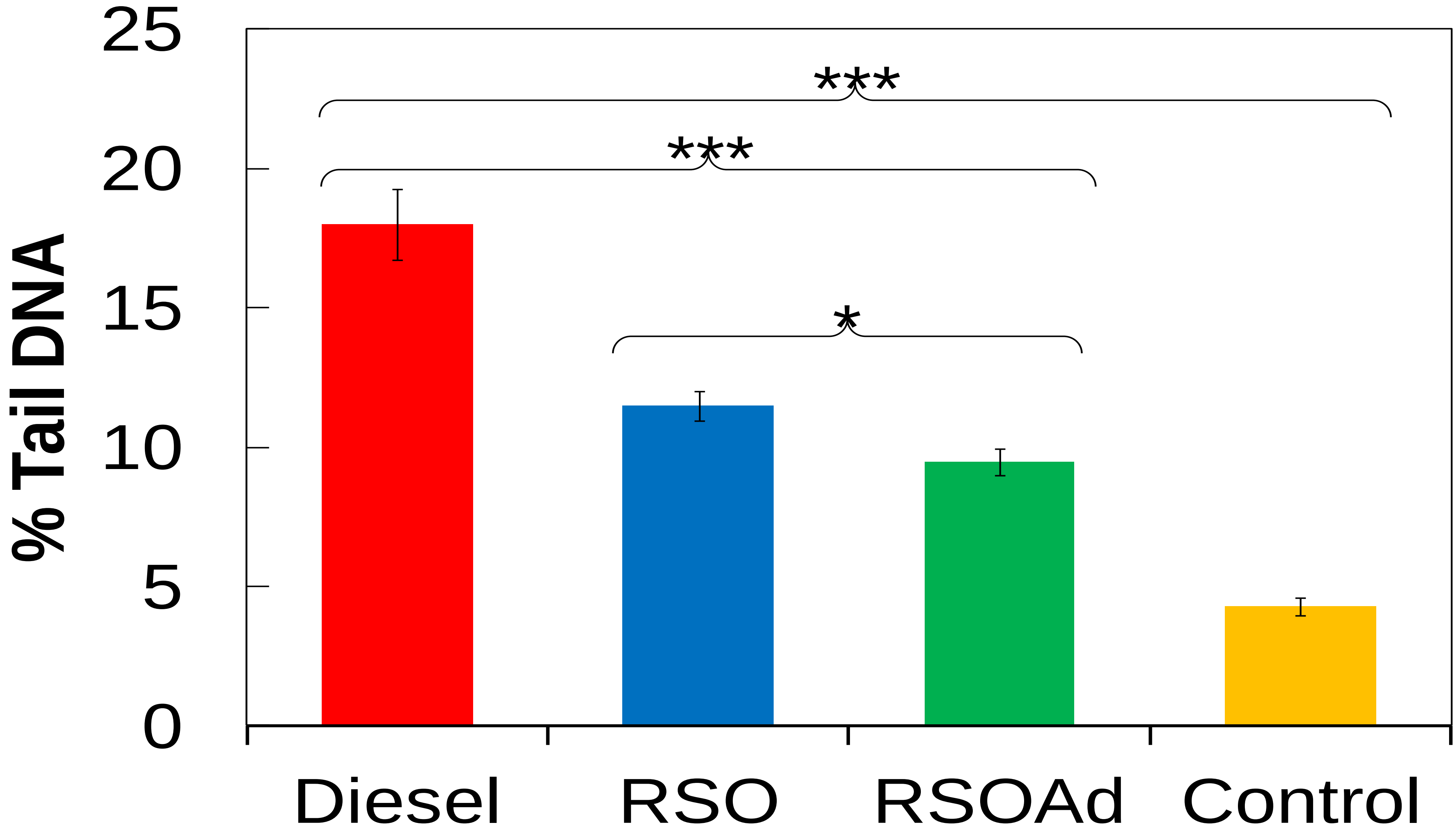

N = 9. Error bars = SEM. *P<0.05 **P<0.01 ***P<0.005

Grouping of size segregated particulate material – toxicological analysis

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Group	Min particle D50% (µm)	Max particle D50% (µm)
One	1	10
Two	0.65	0.65
Three	0.4	0.43
Four	0.17	0.26
Five	0.03	0.108

Comet assay analysis of total size fractionated EEP

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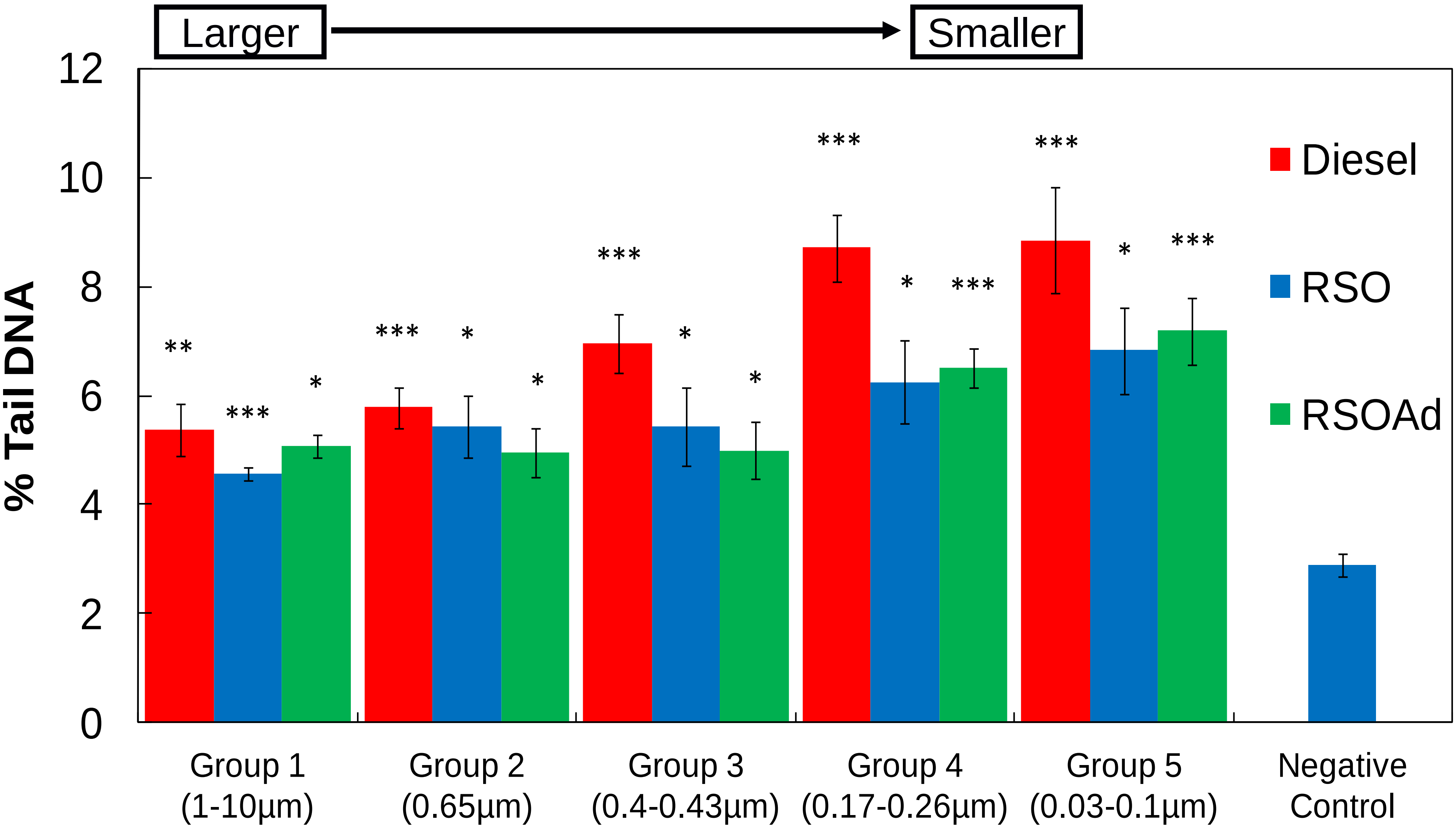

N = 6. error bars = SEM. *P<0.05 **P<0.01 ***P<0.005 VS negative control

Toxicological analysis - Plasmids

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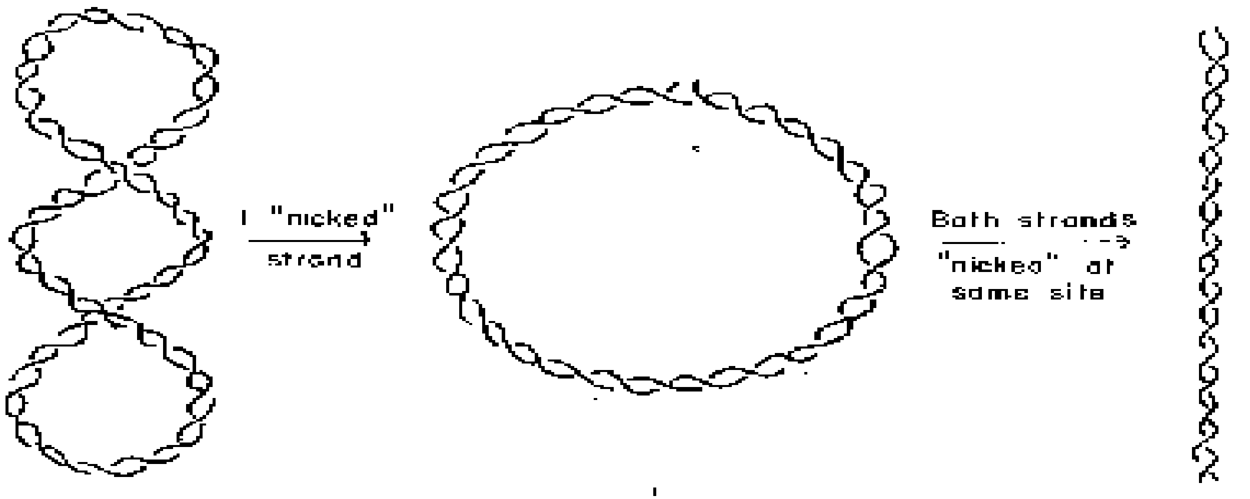

Supercoiled Relaxed Linear

- Plasmids are circular DNA passed between bacteria.
- Is normally supercoiled by cells.
- Free radicals damage supercoiled plasmid and cause relaxation by introducing a break (nick) in one strand.
- The relaxed form has same molecular weight but moves more slowly through an agarose gel during gel electrophoresis.

Plasmid strand break assay example image

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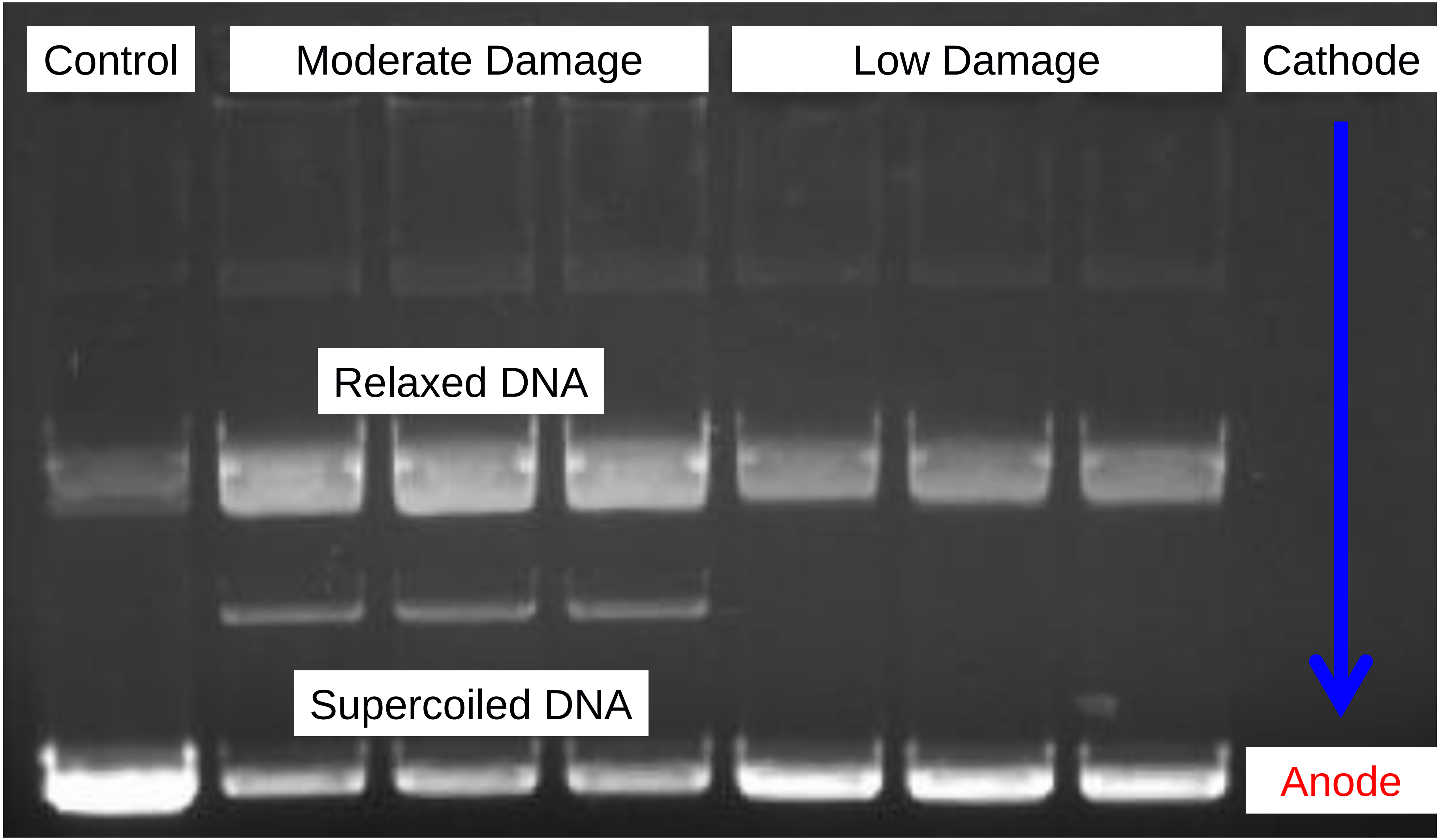

Toxicological analysis – Plasmid strand break assay

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- Quantitative measurement of free radical activity in an acellular environment.
- Supercoiled plasmid was exposed to particulate matter (50µg/ml) for 72 hours.
- Plasmid damaged by free radical activity (relaxed) will migrate more slowly than undamaged plasmid (supercoiled).
- Proportion of relaxed plasmid relative to negative control is reported.

Free radical induction by size fractionated EEP

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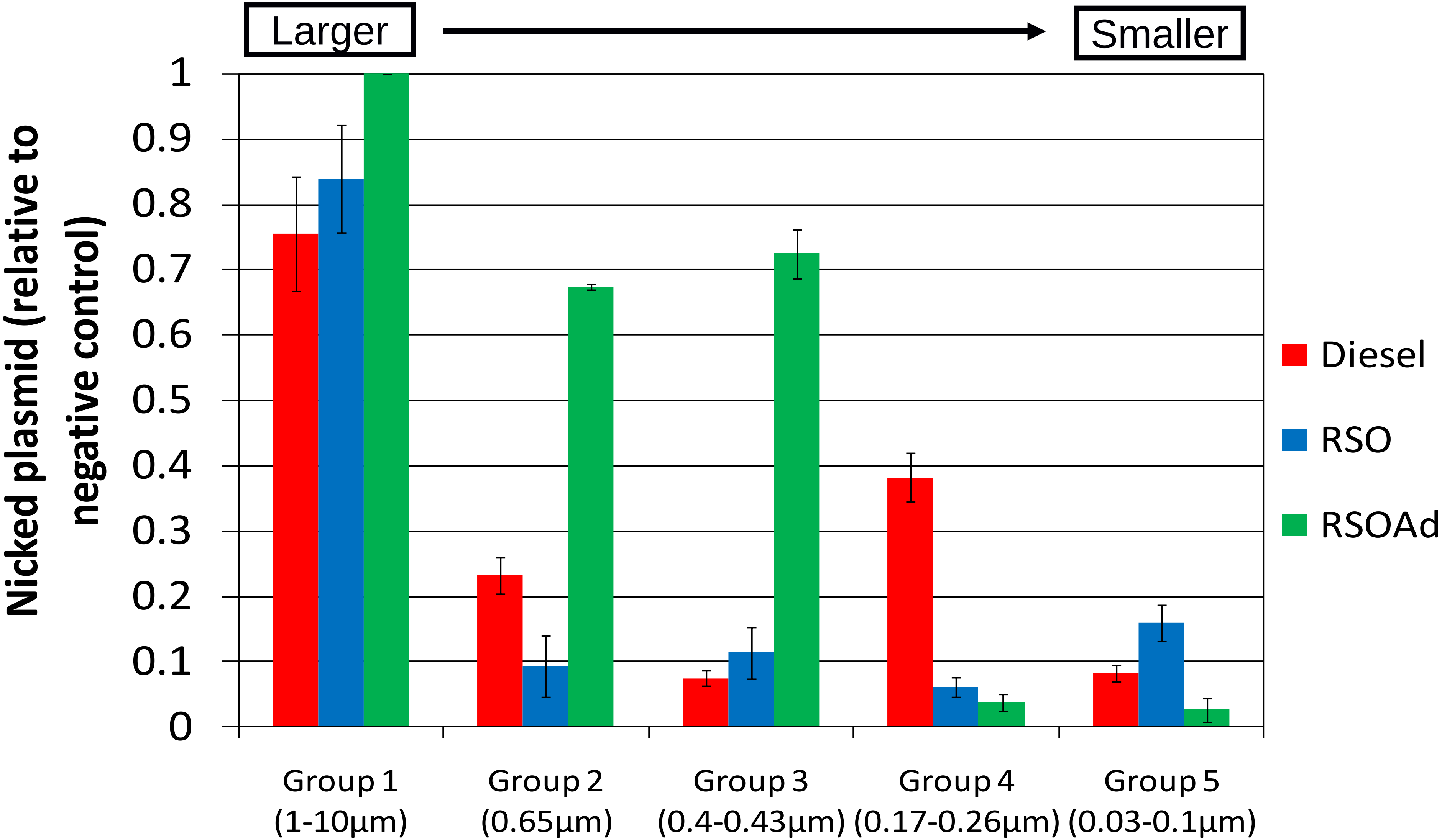

N = 6, error bars = SEM

Toxicity analysis conclusions

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- Whilst all TSP samples induced damage significantly above negative controls , diesel EEP produced significantly more DNA damage than RSO or RSOAd.
- In size fractionated samples diesel EEP genotoxicity was greater than RSO and RSOAd EEP.
- Diesel EEP genotoxicity was shown to be strongly dependant on particle size, with finer PM being the most toxic.
- For RSO and RSOAd exhaust particles the importance of size is less clear but smaller particles appear to be associated with greater levels of DNA damage.
- Coarse EEP induced higher levels of free radicals than fine PM, suggesting that free radicals are not the cause of the observed genotoxicity.

Polycyclic aromatic hydrocarbons (PAH)

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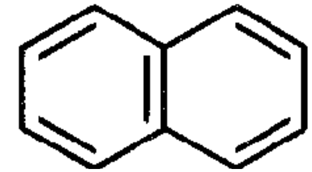
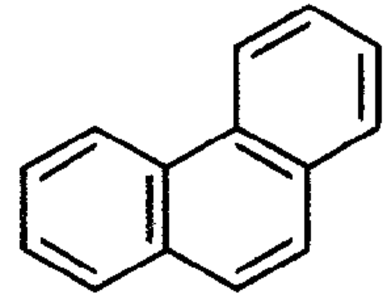
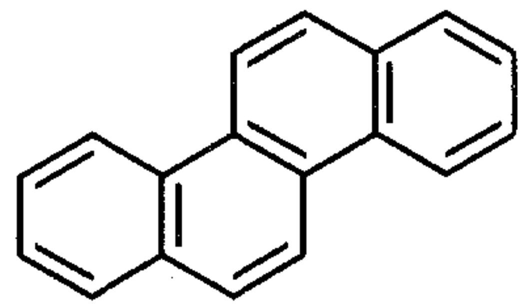
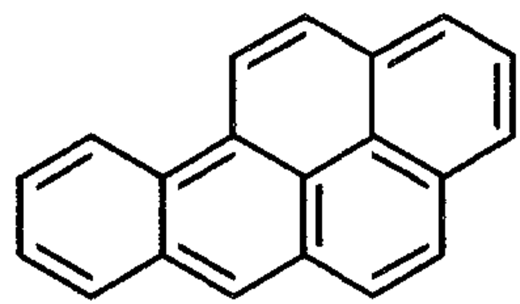
- Suggested as causative agents of diesel EEP mutagenicity and carcinogenicity.
- Toxic, and cellular processes will attempt to detoxify.
- Metabolic intermediaries can be mutagenic and carcinogenic.
- Metabolic processing generates free radicals.









Chemical analysis of EEP

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- Filters were extracted with an accelerated solvent extractor (ASE).
  - First extraction with hexane:acetone (1:1).
  - Second extraction with toluene.
- Extracts were concentrated with a centrifugal evaporator to 1mL.
- Chemical analysis of EEP extracts was carried out with a gas chromatogram mass spectrometer (GC/MS):
  - EPA16 priority PAH species.
  - Selective ion monitoring.
  - Internal standard quantification system.

Grouping of size segregated particulate material – Chemical Analysis

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Group	Min particle D50% (μm)	Max particle D50% (μm)
One	1.1	10
Two	0.65	0.65
Three	0.43	0.43
Four	~0.2	0.43

Particle phase PAH

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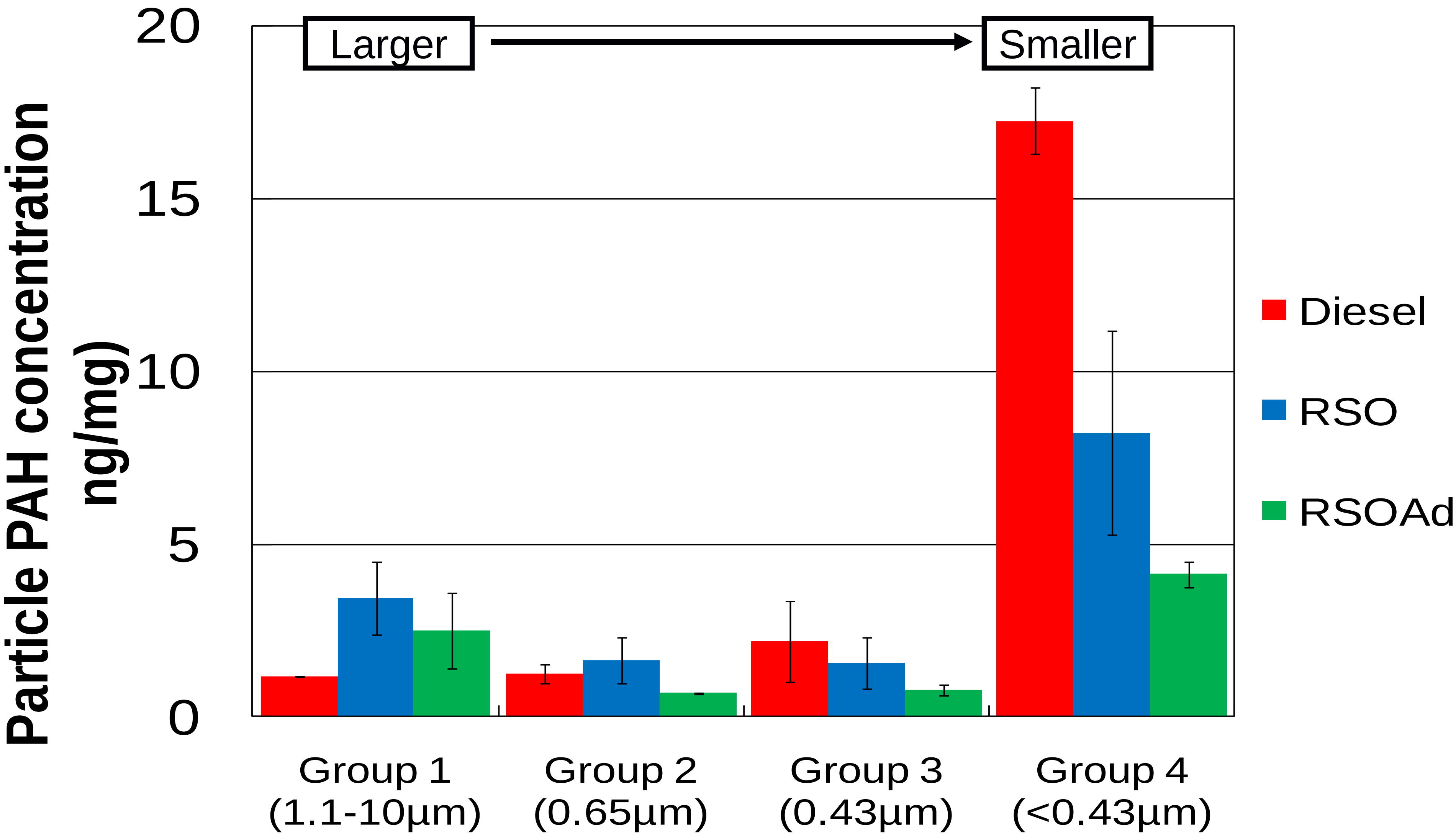

N = 2, error bars = SEM

Relationship between PAH and DNA damage

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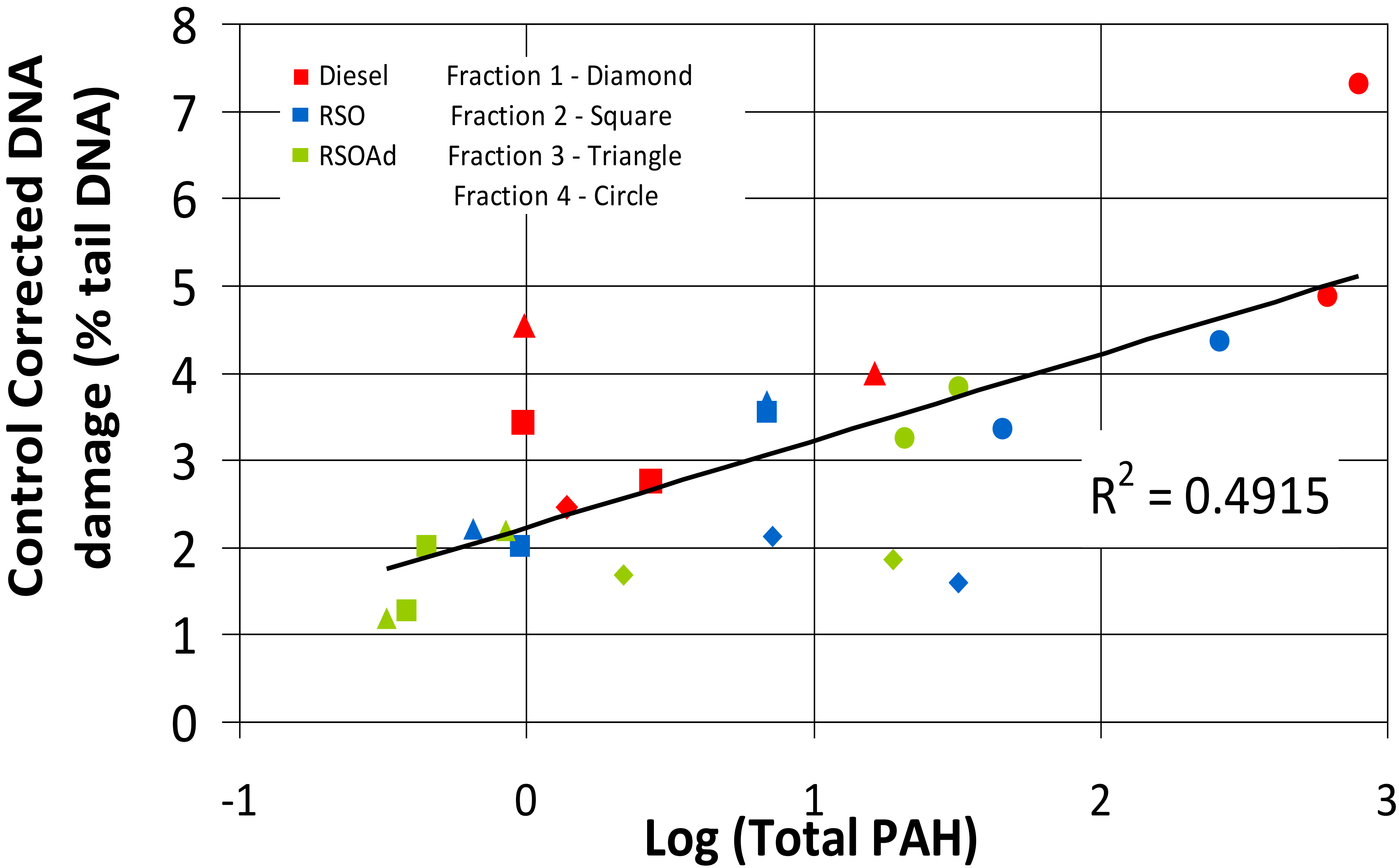

Chemical analysis - Conclusions

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- PAH are present at higher concentrations in diesel EEP than in RSO or RSOAd EEP.
- Finer EEP contain higher concentrations of PAH than coarse material.
- Diesel EEP PAH distribution is similar to what may be expected based on projected particle surface area.
- Particle PAH concentrations correlate well with observed DNA damage in the comet assay.

Conclusions - Summary

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- Combustion of RSO produces a greater mass of particulate material than diesel or RSOAd.
- Irrespective of fuel type the majority of particulate mass is found in the finest fractions.
- Diesel EEP are more genotoxic than RSO or RSOAd.
- Finer particles are more genotoxic than coarser material.
- Coarse EEP induce greater levels of free radicals in an acellular environment.
- Diesel EEP have greater levels of PAH.
- PAH tend to be more prevalent in finer particles.
- Comet assay DNA damage and PAH correlate well, suggesting that PAH may be responsible for genotoxicity.

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Thank you for listening

Thanks to

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