Supplement to "Inflammation, oxidative stress and genotoxicity responses to biodiesel emissions in cultured mammalian cells and animals"

Supplementary table S1. Genotoxicity, oxidative stress and inflammation in cultures of mammalian cells

Diesel type and content of PAHs and transition metals	Model (cell and exposure)	Genotoxicity[a]	Oxidative stress[a]	Inflammation[a]	Reference
RME100, HVO100 and petrodiesel with or without catalyst (DOC/POC). *PAHs:* Heavy PAHs (>228 g/mol) not detect in the samples. *Transition metals:* HVO100/D100 = 0.75 RME100/D100 = 1.37	Mouse macrophage RAW264.7 (24 h) DEP (15, 50, 150 and 300 µg/ml; only 50-300 µg/ml for genotoxicity)	+SB (comet). Highest concentrations omitted in the review due to high level of cytotoxicity and apoptosis	+ROS (DCFH-DA). Biodiesel (RME100 and HVO100) without catalyst most potent. Addition of catalyst to petrodiesel increased the ROS production, but had little effect on biodiesel samples	+TNF (ELISA) +MIP-2 (ELISA) RME100 less potent than HVO100 and D100 (HVO100 and D100 have same potency)	(Jalava *et al.* 2010)
RME30, RME100, HVO30, HVO100 or D100 with or without catalyst (DOC/POC). *PAH content:* HVO100/D100 = 0.99 HVO30/D100 = 0.75 RME100/D100 = 0.87 RME30/D100 = 0.60 *Transition metals:* HVO100/D100 = 1.13 HVO30/D100 = 0.65 RME100/D100 = 2.54 RME30/D100 = 0.58	RAW 264.7 (24 h) DEP (15, 50, 150 and 300 µg/ml)	+SB (comet, HVO100). Highest concentrations omitted in the review due to high level of cytotoxicity and apoptosis. No effect of RME30 and RME100 at 50 µg/ml	+ROS (DCFH-DA, at highest concentration) for D100, HVO30 and RME. Chemical catalyst decreased the ROS production	+TNF (ELISA), all samples have similar response +MIP-2 (ELISA), RME lower than D100 and HVO	(Jalava *et al.* 2012)
RME20, AFME20 and petrodiesel in Euro 2 or Euro 4 engines (SRM2975	A549 (3 h, comet assay and ROS production) and THP-1	+SB (comet) +Fpg (comet)	+ROS (DCFH-DA). Same ROS production for biodiesel and	-CCL2 (mRNA) -IL8 (mRNA)	(Hemmingsen *et al.* 2011)

as benchmark). Hydrodynamic particle size (Nanosight Tracking Analysis) for Euro 4 engine was 148 nm (D100), 149 (AFME20) and 134 nm (RME20) *PAH content:* AFME20/D100 = 0.65 (only reported for Euro 2 engine as the combustion in the Euro 4 did not generate PAHs)	(cytokines). DEP (0.25, 25 and 100 µg/ml)		petrodiesel		
Biodiesel (10% butanol and 20% or 40% waste-cooking oil biodiesel) or petrodiesel (containing 2% biodiesel) *Organic carbon:* B20/D100 = 0.79 B40/D100 = 0.59	CHO-K1 (5, 10 or 20 µg/ml for 3 h, MN assay) or A549 cells (10 µg/ml for 24 h, comet assay)	+SB (comet) +MN (40% biodiesel blend generated less DNA damage than D100)			(Yang *et al.* 2017)
Commercial neat biodiesel (RME100) and D100 diesel fuel from a local fuelling station	A549 exposed to EOM (DCM, corresponding to 0.001 m^3 per mL of undiluted exhaust gas) for 4 or 24 h	+SB (comet, not conclusive)[b] +Fpg/EndoIII (comet, not conclusive)[b]			(Novotna *et al.* 2019)
FAME7, FAME20 or synthetic hydrocarbon biofuel (7% FAME + 13% HVO)	BEAS-2B and A549 cells (24 or 48 h). DEP (1, 10, 25 or 50 µg/ml)	+SB (comet, greatest effect on SB formation by FAME7 exposure) -Fpg (comet) -γH2AX +MN			(Kowalska *et al.* 2017)
RME30, RME100 and petrodiesel *PAH content:*	BEAS-2B (28 or 48 h) exposed to EOM (DCM: 1, 10 and 25	+MN (highest effect at lowest concentration and 48 h)			(Cervena *et al.* 2017)

FAME30/D100 = 1.32 FAME100/D100 = 3.38 Methylated, oxygenated, nitrated and dinitrated PAHs have also been measured	μg/ml)				
RME7, RME30 and petrodiesel[c]	A549 (3 h) in air-liquid interphase system (10% diluted)	+γH2AX (no difference between fuels)	-ROS production (ESR) -CAT and SOD (activity) -total GSH		(Barraud *et al.* 2017)
RME (7% or 30%) and petrodiesel	A549 or rat lung slices (3 or 24 h) to air flow, particles or EOM (DCM)	-PAH-DNA adducts (^{32}P-post-labelling)			(Andre *et al.* 2015)
Soy (20%) or petrodiesel *<u>PAH content:</u>* B20/D100 = 0.43	BEAS-2B and THP-1a (1, 2, or 24 h) DEP (10 or 20 μg/ml)		+ROS (DCFH-DA, THP-1a, 1 and 2 h). Biodiesel lower effect than petrodiesel fuel -ROS (DCFH-DA, BEAS-2B, results not shown)	+IL8 (BEAS-2B, THP-1a) +G-CSF (THP-1a, null in BEAS-2B) +TNF (THP-1a) +MCP1 (BEAS-2B, null in THP-1a) -IL6 (BEAS-2B, THP-1a) -MIP1α (BEAS-2B) Cytokines/chemokines have been measured using a multiplex system	(Fukagawa *et al.* 2013)
Biodiesel (20%, not specified) or petrodiesel. Samples collected from the inside of the cabin of a heavy duty front loader (particles extracted in water) *<u>Transition metals:</u>* B20/D100 = 0.49	BEAS-2B exposed to 15, 30 or 60 μg/ml. ROS production potential assessed after 24 h exposure		+ROS (60 μg/ml, DCFH-DA). Same response for biodiesel and petrodiesel		(Martin *et al.* 2019)
FAME7, FAME20 or	BEAS-2B (2 h (ROS) or		+ROS (DCFH-DA, post-	+IL8 (A549, mRNA; not	(Lankoff *et al.*

synthetic hydrocarbon biofuel (7% FAME + 13% HVO)	6 h (gene expression). DEPs (25, 50 and 100 µg/ml). A549 cells for cytokine expression(6 h exposure)		exposure) +HMOX1 (mRNA)	BEAS-2B) +CCL2 (A549 cells, mRNA; not BEAS-2B) Decrease IL1β (BEAS-2B)	2017)
RME30, RME100 or petrodiesel. Mean particle number distribution similar between D100 and RME30 (approximately 50 nm). RME100 had a similar size distribution peak around 50 nm and a smaller size distribution (less than 10 nm). *PAH content:* RME30/D100 = 2.1 RME100/D100 = 2.8	BEAS-2B (4 or 24 h). EOM (50 µg extract/ml, DCM)		-ROS (DCFH-DA, 4 h) -GSH (4 h) +HMOX1 (mRNA, 4 or 24 h) +TXNRD1 (mRNA, 4 or 24 h)		(Libalova *et al.* 2016)
FAME7, FAME100 or synthetic hydrocarbon biofuel (7% FAME + 13% HVO)	BEAS-2B (4 or 20 h). DEP (10, 50 and 100 µg/ml; 10 µg/ml corresponds to 1.5 µg/cm^2)		+HMOX1 (mRNA)	+IL6 (mRNA) +IL8 at 20 h (mRNA; unaltered at 4 h)	(Skuland *et al.* 2017)
Biodiesel (source not specified) or petrodiesel. Count mean diameter in exhaust was 32 nm (biodiesel) and 64 nm (D100) *Organic carbon:* Biodiesel/D100 = 4.53	Human bronchial epithelial cells (HNBE) exposed for 1 h air exhaust with (≤4 ng/cm^2) or without filter (petrodiesel: 230-340 ng/cm^2, biodiesel: 45-50 ng/cm^2) in air-liquid interphase system		+HMOX1 (mRNA). No difference between fuels		(Hawley *et al.* 2014)
RME20, RME100 or	3D culture consisting		Increased GSH level[d]	+TNFα (modest effect on	(Steiner *et al.*

petrodiesel. Count mean diameter in exhaust showed similar distribution between RME20 and D100 (approximately 50 nm). RME had a bimodal particle size distribution with peaks at approximately 20 and 50 nm	of epithelial cells (16HBE14o-), monocytes and dendritic cells. Exposed for 2 or 6 h to exhaust in air-liquid interphase system (one exposure level)		Increased HMOX1 (mRNA) Difference in oxidative stress response between diesel and biodiesel is equivocal as it is not consistent across biomarkers (i.e. GSH and HMOX1) and time point	secretion level for B100) -IL8 (gene expression and protein) Results overall regarded as equivocal	2013)
Biodiesel (type not specified) or petrodiesel (Euro 3 truck engine). Extracted to ethanol and DCM (1:1 ratio) *<u>Total PAHs:</u>* B5/D100 = 1.15 B10/D100 = 1.20 B20/D100 = 1.20 B100/D100 = 0.20 Oxy-PAH and nitro-PAHs have similar distribution as total PAHs	Mouse macrophages (RAW264.7). Exposure conditions not specified		Unaltered HMOX1 (mRNA, results not shown)		(Kooter *et al.* 2011)
Biodiesel (20% 50% or 100%) from coconut oil or petrodiesel. Particle size distribution for B20 and B50 unimodal with a peak around 100 nm, B100 and D100 were bimodal with peaks around 100 and 10 nm.	Primary human bronchial epithelial cells exposed for 30 min via air-liquid interphase system and subsequent 5-hours exposure		-HMOX1 (equivocal oxidative stress response)[e]	+IL8 (100% biodiesel, not 20% or 50%, or 100% diesel) -IL6	(Vaughan *et al.* 2019b)
Biodiesel (5% 15% or 20%) from coconut oil) or	Human bronchial epithelial cells		-HMOX1 (equivocal oxidative stress	+IL8 (effect depends on the percentage of coconut oil:	(Vaughan *et al.* 2019a)

petrodiesel	(16HBE) exposed for 30 min via air-liquid interphase system and subsequent 3-hours exposure		response)[f]	100% diesel and 20% biodiesel showed effect; no effect by 15% or 5% biodiesel) +IL6 (effect depends on the percentage of coconut oil: 100% diesel and 20% biodiesel show effect; no effect by 15% or 5% biodiesel)	
Soy methyl ester or soy ethyl ester (100%), petrodiesel or SRM1975 _PAH content_: Below detection limit	BEAS-2B (24 h) EOMs (10, 25, 40 µg PM eq/ml)			+IL6 (ELISA, greatest effect after biodiesel exposure) +IL8 (ELISA, greatest effect after biodiesel exposure)	(Swanson *et al.* 2009)
Waste cooking oil (B50 or B100) or D100 _Transition metals:_ B100/D100 = 1.3 (metals only reported for B100)	A549 cells exposed for 48 h to filters with particles (effect attributed to soluble metals as filters were immersed in the cell culture medium)		+GSH/GSSG ratio (decreased). Strongest antioxidant depletion by B100 samples		(Betha *et al.* 2012)
Soybean (100%) or reference diesel (with 5% biodiesel). Extracted in hexane or acetone _PAH content:_ B100/D100 = 3.3 _Transition metals:_ B100/D100 = 3.24 (Fe was less than the detection limit, which is therefore set to the lowest detected iron level in the samples)	BEAS-2B cells exposed for 24 h to acetone or hexane extracted materials (75 µg/ml)			No effect on IL6 and IL8 secretion (protein). Positive control (LPS) showed increased expression of IL6 and IL8	(Gioda *et al.* 2016)

FAME20 or petrodiesel collected particulate matter less than 10 nm by bubbling the exhaust through water (Euro 4 engine)	A549 cells exposed for 24 h to 1.2 or 4 ppm material (article also contain parallel experiments in HaCaT cells)			Generally lower cytokine production in exposed A549 cells.[g] Pro-inflammatory response in HaCaT cells (e.g. IL6 and IL8, multiplex platform). Petrodiesel seems to be more potent than biodiesel	(Malorni *et al.* 2017)
FAME20 or FAME100, from canola, a variety of rapeseed) and petrodiesel. No difference in geometric mean particle diameter (bimodal distribution, approximately 16 and 60 nm)	NuLi and 10KT airway epithelial cells in an exposure chamber (exposed for 1 h with 6, 12 or 24 h post-exposure). No information about suspension (e.g. hydrodynamic particle size)			+IL6 (protein) +IL8 (protein) +RANTES (Strongest effect at 24 h post-exposure). Overall similar results between petrodiesel, B20 and B100	(Mullins *et al.* 2016)
RME50 and petrodiesel in Euro4 engines operated under "rural" or "urban" running conditions	BEAS-2B (24 h, 6.25-200 µg/ml, protein; 4 h, 5.25-50 µg/ml; 10 µg/ml corresponds to 1.6 µg/cm^2 is all suspended particles were deposited)			+IL6 (protein, mRNA) -IL8 (protein, mRNA) Biodiesel more potent than petrodiesel. Rural condition more potent than urban running condition	(Gerlofs-Nijland *et al.* 2013)
Waste yellow grease (used cooking oil) as 20% biofuel or petrodiesel combusted in non-road heavy-duty engine. Particle size in tail pipe exhaust was 11 nm for both B20 and D100. Particle size by transmission electron	THP-1a or BEAS-2B cells (10 or 20 µg/ml) for 24 h			THP-1a: +TNFα (biodiesel) +IL8 (both) +MIP1α (all) -IL1β BEAS-2B: +IL6 (all) +IL8 (petrodiesel)	(Traviss *et al.* 2014)

microscopy was 99 and 109 nm for B20 and D100, respectively Organic carbon: B20/D100 = 1.63					
Soy-, animal-based or "renewable hydrotreated) biodiesel (20%, 50% or 100%), petrodiesel or SRM1650	Activated U937 cells (macrophage-like) exposed for 24 h to PM (extracted with DCM and suspended in DMSO), corresponding to 5-50 µg/ml of the total mass of particles			+IL8 (mRNA) +COX2 (mRNA) (soy-based diesel higher than normal diesel)	(Vogel *et al.* 2019)
B20 (source not reported) and D100 *Organic carbon:* B20/D100 = 0.45 *Transition metals:* B20/D100 = 45.2	Primary rat alveolar macrophages exposed for 24 h (1, 10 and 100 µg/ml)			+MIP2 (D100, mRNA) +COX2 (B20, D100, mRNA) PGE_2 (B20, protein) not conclusive	(Bhavaraju *et al.* 2014)

Abbreviations: AFME: animal fat methyl ester, CAT: catalase, COX2: cyclooxygenase 2 (also known as prostaglandin-endoperoxide synthase 2), DCFH-DA: 2',7'-dihydrofluorescin diacetate, DCM: dichloromethane, DEP: diesel exhaust particles, DMSO: dimethyl sulfoxide, DOC: diesel oxidation catalyst, EOM: extractable organic matter, EndoIII: endonuclease III, ESR: electron spin resonance, FAME: fatty acid methyl ester, G-CSF: granulocyte-colony stimulating factor, GSH: reduced glutathione, GSSG: oxidized glutathione, Fpg: formamidopyrimidine DNA glycosylase: HMOX1: heme oxygenase 1, HVO: hydrogenated vegetable oil, IL: interleukin, LPS: lipopolysaccharide, MCP1: monocyte attractant protein 1 (also known as CCL2), MIP1-alpha: macrophage inflammatory protein 1 (also known as chemokine (C-C motif) ligand 3 (CCL3)), MIP2: macrophage inflammatory protein 2 (also known as CXCL2), MN: micronuclei (or micronucleus assay), , PAH: polycyclic aromatic hydrocarbons, PM: particulate matter, PGE2: prostaglandin E2, POC: particle oxidation catalyst, RANTES: Regulated on Activation, Normal T Cell Expressed and Secreted (also known as chemokine (C-C motif) ligand 5 (CCR5)), RME: rapeseed methyl ester, ROS: reactive oxygen species, SB: strand breaks, SOD: superoxide dismutase, THP-1a cells: activated THP-1 cells (i.e. cells that have been treated with a phorbol ester to develop a macrophage-like phenotype), TNF: tumor necrosis factor, TXNRD1: thioredoxin reductase.

[a]The response is reported as positive (+) or null effect (-), based on the statistical analysis in the original publication. [b]The article indicate that only one experiment was conducted (i.e. lack of independent replication) and therefore it is not conclusive evidence of genotoxicity. The measurement of oxidatively damaged DNA was assessed by incubation with Fpg and EndoIII. [c]The study included measurements of 8-oxodG in cells and extracellular medium. These endpoints are not considered relevant as the antibody-based detection of 8-oxodG is considered to be unspecific and 8-oxodG in extracellular medium is not a DNA lesion. [d]The increased level of GSH goes against the notion that oxidative stress is caused by depletion of antioxidants. Thus, we have considered the results as equivocal evidence of oxidative stress. [e]Gene expression of HMOX1 (mRNA levels) measured as a marker of “antioxidant protection”; however it demonstrates both increases and decreases in a seemingly random way that is difficult to interpret. [f]Gene expression of SOD1, SOD2 and HMOX1 (mRNA levels) measures as markers of “oxidative stress”; SOD1 and SOD2 have opposite results and HMOX1 was unaltered. Given the fact that the results seems derived from mRNA analyses and they are opposite in direction, the overall result is inconclusive with regard to oxidative stress. [g]The study has not assessed the difference between biodiesel and petrodiesel (only multiple Student’s t-tests).

Supplementary table S2. Genotoxicity, oxidative stress and inflammation in the lungs of animals (lung tissue unless specified otherwise)

Diesel type	Model (animal)	Genotoxicity[a]	Oxidative stress[a]	Inflammation[a]	Reference
RME20 in a Euro5 engine (including particle-filtered air as control)	Adult (age not specified) male F344 rats (2.0 mg/m^3) by whole-body inhalation exposure for 7 (6 h/day) or 28 days (6 h/day, 5 days/week). <u>*Dose*</u>: 84 and 60 mg*h/m^3 per week, respectively	-SB (comet) -Fpg (comet, results not shown, no assay description)	Minor effects (*SOD*, *GPX*, *THPO, HMOX1*). We have regarded the gene expression results to show minor and inconsistent effects	+(modest effect on histology, gene expression (*TNF*, *IL1β*, but not *IL6*) and neutrophils in BALF (2.5% neutrophils at day 28)	(Magnusson *et al.* 2017)
FAME7 or synthetic hydrocarbon biofuel (7% FAME and 13% HVO) in Euro5 engine	Adult (age not specified) male F344 rats (2.0 mg/m^3 of PM) by whole-body inhalation exposure for 7 (6 h/day) or 28 days (6 h/day, 5 days/week). <u>*Dose*</u>: 84 and 60 mg*h/m^3 per week, respectively	-SB (comet) -Fpg (comet)	Minor effects (gene expression of *Cat* and *HMOX1*)	Minor effects (no influx of neutrophils or macrophages, no difference in BALF cytokines)	(Magnusson *et al.* 2019)
RME30 in Euro4 engine (before or after particle filter)	Male Wistar rats (age not reported) exposed to 24 mg/m^3 of PM by inhalation 3 h per day for 5 days/week during 3 weeks[b]. <u>*Dose*</u>: 360 mg*h/m^3 per week	-γH2AX -PAH-DNA adducts -8-oxodG[c] -Telomere length (qPCR; also unaltered telomerase activity and gene expression level)	-GSH/GSSG -ROS production -Catalase (activity) -SOD (activity) -GPX (activity) -Protein carbonyls		(Douki *et al.* 2018)
Soybean oil (100%)	Male and female rats, 5-7 weeks old, exposed by inhalation (6 h/day, 5 days/week) for 13 weeks by inhalation (0.04, 0.2 or 0.5 mg/m^3).			+(presence of macrophages, assessed by histology)	(Finch *et al.* 2002)

	Dose = 1.2, 6 and 15 mg*h/m^3 per week, respectively. NO_x was 5, 25 and 50 ppm, respectively				
Soy biodiesel (100%) or petrodiesel. Mass median aerodynamic diameter of biodiesel and D100 were 113 and 168 nm, respectively. *PAH content:* B100/D100 = 0.38	Female Balb/cJ mice (10-12 weeks old) exposed to 50, 150 or 500 µg/m^3 (4 h/day, 5 days/week) for 4 weeks. *Dose:* 1, 3 and 10 mg*h/m^3		+lipid peroxidation (4-HNE) +protein carbonyls +GSH depletion Strongest response for biodiesel	+IL6, MCP1, TNF α, INFγ, IL12p70 and MPO activity Strongest response for biodiesel	(Shvedova *et al.* 2013)
Soy biodiesel (20% or 100%) or D100 *Organic carbon:* B20/D100 = 1.44 B100/D100 = 2.44 The content of organic compounds, including in PAHs in EOM was assessed by Mutlu *et al.* 2015a	Female Balb/cJ mice (6-8 weeks old) exposed to 50, 150 or 500 µg/m^3 (4 h/day, 5 days exposure, or 4 weeks exposure (5 days/week). *Dose:* 1, 3 and 10 mg*h/m^3			+Neutrophils (BALF, 500 µg/m^3) and MIP2 (protein, 150 and 500 µg/m^3) at 2 h after a single 4-hour exposure to biodiesel. No effect 24 h after a single exposure, 2 or 24 h after a 5-day exposure or 4 weeks exposure. No effect on IL6 and TNFα levels. Biodiesel generated less pulmonary inflammation than D100	(Gavett *et al.* 2015)
Soy (20% or 100%) or D100. *Organic carbon:* B20/D100 = 1.44 B100/D100 = 2.44 The content of organic compounds,	Male Wistar-Kyoto or spontaneous hypertensive rats (6-8 weeks old) exposed to 50, 150 or 500 µg/m^3 for 2 days (4 h/day) or 4 weeks (4 h/day and 5 days/week). *Dose*: 0.4, 1.2 and 4.0 mg*h/m^3 per week (short-			Slightly increased influx of neutrophils in D100 (1-day, less than 2-fold increase). Unaltered number of macrophages in BALF and toxicity (protein, albumin, LDH)	(Bass *et al.* 2015)

including in PAHs in EOM was assessed by Mutlu *et al.* 2015a	term exposure) and 1, 3, and 10 mg*h/m^3 per week (long-term exposure)				
Soy (20% or 100%) or D100. *__Organic carbon:__* B20/D100 = 1.44 B100/D100 = 2.44 The content of organic compounds, including in PAHs in EOM was assessed by Mutlu *et al.* 2015a	Male spontaneous hypertensive rats (12 weeks old) exposed to 50, 150 or 500 µg/m^3 for 2 days (4 h/day). *__Dose__*: 0.4, 1.2 and 4.0 mg*h/m^3 per week			Unaltered influx of cells in BALF (results are not reported in the article)	(Farraj *et al.* 2015)
Soybean (50% or 100%) or D100. *__PAH content:__* B50/D100 = 0.08 B100/D100 = 0.01 Based on median between minimum and maximum values. *__Transition metals:__* B50/D100 = 1.06 B100/D100 = 1.17 *__Volatile compounds:__* less aromatics, alkanes, alkenes and alkadiesel in biodiesel than D100	Male Balb/c mice (6-8 weeks old) exposed for 1 h to 550 µg/m^3 and sacrificed 24 h post-exposure. *__Dose__*: 0.55 mg*h/m^3 per week			Modest increase (reported as a modest increase of neutrophils in BALF after exposure to B50, but the corresponding figure in the articles does not indicate an effect on neutrophils). Likewise, there are not statistically significant effects on neutrophils in lung parenchyma. There appears to be modest evidence of increased number of total cells, which is mainly driven by macrophages. Inflammation reported to be higher in biodiesel exposed mice as compared to D100	(Brito *et al.* 2010)

Sewage methyl esters or D100	Male Balb/c mice (6-8 weeks old) exposed to 600 or 1200 µg/m^3 by inhalation for 1 h and sacrificed at 24 h post-exposure. _Dose_: 0.6 and 1.2 mg*h/m^3 per week			+influx of neutrophils and macrophages (BALF, histology). Same response in biodiesel and petrodiesel	(de Brito *et al.* 2018)
Soy (20%) or D100. Mean particle number diameter in aerosol of biodiesel (32 nm) and D100 (51 nm) _PAH content:_ B20/D100 = 0.43	C57BL/6 mice (age not reported) exposed to 84 µg by oropharyngeal aspiration once a day for 3 days. _Dose_: 1.3 mg/g_{lung} tissue		+Protein carbonyls +GCLC	+influx in BALF (macrophages, lymphocytes, neutrophils) +G-CSF, IP-10, IL6 in BALF and lung tissue (higher response with biodiesel as compared to D100)	(Fukagawa *et al.* 2013)
Soybean (100%, Euro2 engine) and petrodiesel (Euro1 or Euro4 engine). _PAH content:_ B100/D100 = 2.53 (D100/Euro 4) B100/D100 = 1.08 (D100/Euro 2). _Transition metal:_ B100/D100 = 0.88 (D100/Euro 4) B100/D100 = 1.64 (D100/Euro 2)	Female Balb/CJ mice (12-14 weeks old) exposed for 24 h after an i.t instillation of 50 or 100 µl extracted particles (reported to contain 0.2 µg/µl, corresponding to instilled doses of 10 and 20 µg). _Dose_: 0.05 or 0.1 mg/g_{lung}			-(number of neutrophils in BALF). Increased BALF protein at highest concentration (direct comparison between diesel types not possible because of the use of different engines)	(Tzamkiozis *et al.* 2010)
Neat corn-based FAME100 or D100. Hydrodynamic diameter by dynamic light scattering was 216	C57BL/6 female mice (8-10 weeks old) exposed to 9 or 18 µg of total carbon by pharyngeal aspiration and sacrifice at 24 h, 7 days or 28 days.		+Protein carbonyls +4-HNE (biodiesel≥D100)	+BALF cells +MPO +cytokines (BALF) Generally greater response in mice after exposure to biodiesel than D100	(Yanamala *et al.* 2013)

(FAME) and 312 nm (D100). *<u>Organic carbon:</u>* B100/D100 = 1.88	*<u>Dose</u>*: 0.05 and 0.09 mg/g_{lung} (assuming that total carbon represents 80% of the PM as reported in the article and a weight of 20 g per mouse)				
PM from the exhaust pipe of a public bus running on diesel fuel with 7% biodiesel. Hydrodynamic particle size (dynamic light scattering) was 390 nm	Female C57BL/6 mice (8-10 weeks old) exposed by intra-nasal instillation to 250 or 1000 µg/day over 5 consecutive days[d]		+ROS (cells in BALF) +CAT, SOD, GPX, GCLC and GCLM (decreased in lung tissue) measured by immune blot	+Macrophages in lung parenchyma +TNFα (BALF)	(Cattani-Cavalieri *et al.* 2019)

Abbreviations : BALF: bronchoalveolar lavage fluid, CAT: catalase, D100: petrodiesel, FAME: fatty acid methyl ester, GCLC: Glutamate-cysteine ligase catalytic subunit, GCLM: Glutamate-cysteine ligase regulatory subunit, GPX: glutathione peroxidase, G-CSF: granulocyte-colony stimulating factor, GSH: reduced glutathione, GSSG: oxidized glutathione, Fpg: formamidopyrimidine DNA glycosylase: HMOX1: heme oxygenase 1, HVO: hydrogenated vegetable oil, IL: interleukin, INFγ: interferon gamma, IP-10: interferon gamma induced protein 10 (also known as chemokine C-X-C motif 10 (CXCL10)), LDH: lactate dehydrogenase, MCP1: monocyte attractant protein 1 (also known as CCL2), MIP2: macrophage inflammatory protein 2 (also known as chemokine C-X-C motif ligand 2 (CXCL2), MPO: myeloperoxidase, PAH: polycyclic aromatic hydrocarbons, PM: particulate matter, RME: rapeseed methyl ester, SB: strand breaks, SOD: superoxide dismutase, THPO: Thrombopoietin, TNF: tumor necrosis factor, 4-HNE: 4-Hydroxynonenal, 8-oxodG: 8-oxo-7,8-dihydroguanosine-2'-deoxyguanosine.

[a]The response is reported as positive (+) or null effect (-), based on the statistical analysis in the original publication. The age of the animals is reported as stated in the publications, which is typically the age at arrival. [b]The exposure concentration is excessively high and it does not appear to be in line with the author's statement that it is 75% of the concentration inside a vehicle in urban traffic. The levels of NOx (83 ppm) and NO (34 ppm) suggest relatively low level of diesel exhaust. [c]Null effect finding on 8-oxodG should be viewed in the light that the baseline level of DNA damage (≈33 to 44 lesions per 10^6 dG) was much higher than the commonly accepted level of less than 5 lesions/10^6 dG. [d]The deposited dose in the peripheral lung would be relatively high (6.3 and 25 mg per gram lung tissue) assuming 100% transport of particulate matter into the lung, which is uncertain.

Supplementary table S3. Common and official names and abbreviations of pro-inflammatory mediators

Common abbreviation	Common name	Official abbreviation	Official abbreviation
MIP1α (or MIP-1)	Microphage inflammatory protein 1	CCL3	C-C motif chemokine ligand 3
MIP2α (or MIP-2)	Microphage inflammatory protein 2	CXCL2	C-X-C motif chemokine ligand 2
MCP1	Macrophage chemoattractant protein 1	CCL2	C-C motif chemokine ligand 2
IL1β	Interleukin 1β	IL1B	Interleukin 1 beta
IL8	Interleukin 8	CXCL8	C-X-C motif chemokine ligand 8
IL6	Interleukin 6	IL6	Interleukin 6
IL12p70	Interleukin 12 (active heterodimer of IL12A and IL12B)	IL12A and Il12B	Interleukin 12A and Interleukin 12B
INF gamma	Interferon gamma	IFNG	Interferon gamma
IP-10	Interferon gamma induced protein 10	IP-10	C-X-C motif chemokine ligand 10
RANTES	Regulated on activation normal T expressed and secreted protein	CCR5	C-C motif chemokine receptor 5 (gene/pseudogene)
TNF	Tumor necrosis factor	TNF	Tumor necrosis factor
G-CSF	Granulocyte-colony stimulating factor	CSF3	Colony stimulating factor 3

Supplementary table S4. Particle size of biodiesel and petrodiesel in studies included in the present review

Particle size of biodiesel (nm)	Particle size of petrodiesel (nm)	Matrix or vehicle	Method	Reference
90 nm (RME20, Euro 4) 130 nm (AFME20, Euro 2) 95 nm (AFME20, Euro 4)	139 nm (D100, Euro 2) 129 nm (D100, Euro 4) 132 nm (SRM2975)	Water	NanoSight Tracking Analysis	(Hemmingsen *et al.* 2011)
134 nm (RME20, Euro 4) 158 nm (AFME20, Euro 2) 149 nm (AFME20, Euro 4)	189 nm (D100, Euro 2) 148 nm (D100, Euro 4) 154 nm (SRM2975)	Cell culture medium (with serum)	NanoSight Tracking Analysis	(Hemmingsen *et al.* 2011)
32 nm	51 nm	Aerosol	Scanning mobility particle sizer	(Fukagawa *et al.* 2013)
113 nm (mass median aerodynamic diameter)	168 nm (mass median aerodynamic diameter)	Aerosol	Scanning mobility particle sizer	(Shvedova *et al.* 2013)
50 nm (B20) 15 nm and 50 nm (B100)	50 nm	Aerosol	TSI scanning mobility particle sizer	(Steiner *et al.* 2013)
216 nm	312 nm	Suspension	Dynamic light scattering	(Yanamala *et al.* 2013)
32 nm (count median diameter of exhaust)	64 nm (count median of exhaust)	Aerosol	Not described	(Hawley *et al.* 2014)
16 and ≈60 nm (FAME20 and FAME100)	16 and ≈60 nm	Aerosol	TSI scanning mobility particle sizer	(Mullins *et al.* 2016)
11 nm (B20, primary size) 99 nm (B20, agglomerate)	11 nm (primary size) 109 nm (agglomerate)	Dry condition	Transmission electron microscopy	(Traviss *et al.* 2014)
≈50 nm (B30) ≈10 nm and ≈50 nm (B100)	≈50 nm	Aerosol	TSI fast mobility particle sizer	(Libalova *et al.* 2016)
≈100 nm (B20) ≈12 nm and ≈100 nm (B50) ≈80 nm (B100)	Bimodal (≈20 nm and ≈100 nm)	Aerosol	DMS500	(Vaughan *et al.* 2019b)

Supplementary Table S5. Segregation of studies on toxicity relative to the content of organic carbon or PAHs. The “outcome” refers to genotoxicity, oxidative stress or inflammation, which is segregated into higher (i.e. D100 > Biodiesel), the same (i.e. D100 = Biodiesel), or lower (i.e. D100 < Biodiesel) toxicity than biodiesel exhaust. The content of organic carbon or PAH level has been categorized as highest content in petrodiesel exhaust (i.e. Ratio < 1), same content in biodiesel and petrodiesel exhausts (i.e. Ratio = 1), or highest content in biodiesel exhaust (i.e. Ratio > 1). The content of organic carbon (or PAHs) has been regarded as the same in biodiesel and petrodiesel exhausts if the difference was less than 10%. Supplementary tables S1 and S2 show the ratio between organic carbon (or PAH) content in biodiesel versus petrodiesel exhausts.

Organic carbon or PAH level (biodiesel/petrodiesel ratio)	**Outcome (D100 > Biodiesel)**	**Outcome (D100 = Biodiesel)**	**Outcome (D100 < Biodiesel)**
Ratio < 1 (highest content in D100)	**Genotoxicity** HVO30 (Jalava *et al.* 2012) RME100 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012) **Oxidative stress** S20 (Fukagawa *et al.* 2013) **Inflammation** No studies	**Genotoxicity** RME20 (Hemmingsen *et al.* 2011) AFME20 (Hemmingsen *et al.* 2011) **Oxidative stress** RME20 (Hemmingsen *et al.* 2011) AFME20 (Hemmingsen *et al.* 2011) HVO30 (Jalava *et al.* 2012) RME100 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012) **Inflammation** RME20 (Hemmingsen *et al.* 2011) AFME20 (Hemmingsen *et al.* 2011) B20 (Bavaruju *et al.* 2014) HVO30 (Jalava *et al.* 2012) RME100 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012)	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** S20 (Fukagawa *et al.* 2013) S50 (Brito *et al.* 2010) S100 (Brito *et al.* 2010) B100 (Shvedova *et al.* 2013)
Ratio = 1 (same content)	**Genotoxicity** No studies **Oxidative stress** HVO100 (Jalava *et al.* 2012)	**Genotoxicity** RME100 (Jalava *et al.* 2010) HVO100 (Jalava *et al.* 2010) HVO100 (Jalava *et al.* 2012) **Oxidative stress**	**Genotoxicity** No studies **Oxidative stress** No studies

	Inflammation RME100 (Jalava *et al.* 2010)	RME100 (Jalava *et al.* 2010) HVO100 (Jalava *et al.* 2010) **Inflammation** HVO100 (Jalava *et al.* 2010) HVO100 (Jalava *et al.* 2012)	**Inflammation** S100 (Swanson *et al.* 2009)
Ratio > 1 (highest content in biodiesel)	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** S20 (Gavett *et al.* 2015) S100 (Gavett *et al.* 2015) S20 (Bass *et al.* 2015) S100 (Bass *et al.* 2015)	**Genotoxicity** B30 (Cervina *et al.* 2017) B100 (Cervina *et al.* 2017) **Oxidative stress** RME30 (Libalova *et al.* 2016) RME100 (Libalova *et al.* 2016) **Inflammation** BD (Hawley *et al.* 2014) B20 (Traviss *et al.* 2014) B100 (Gioda *et al.* 2016) BD/Euro 4 (Tzamkiozis *et al.* 2010) BD/Euro 1 (Tzamkiozis *et al.* 2010) S20 (Farraj *et al.* 2015) S100 (Farraj *et al.* 2015)	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** B100 (Yanamala *et al.* 2013)

Abbreviations: BD = biodiesel (typically unspecified), S = soy.

Supplementary S6. Segregation of studies on toxicity relative to the content of transition metals. The "outcome" refers to genotoxicity, oxidative stress or inflammation, which is segregated into higher (i.e. D100 > Biodiesel), the same (i.e. D100 = Biodiesel), or lower (i.e. D100 < Biodiesel) toxicity than biodiesel exhaust. The content of transition metals has been categorized as highest content in petrodiesel exhaust (i.e. Ratio < 1), same content in biodiesel and petrodiesel exhausts (i.e. Ratio = 1), or highest content in biodiesel exhaust (i.e. Ratio > 1). The content of transition metals has been regarded as the same in biodiesel and petrodiesel exhausts if the difference was less than 10%. Supplementary tables S1 and S2 show the ratio between organic carbon (or PAH) content in biodiesel versus petrodiesel exhausts. For the calculation of transition metals we have used geometric mean of vanadium, chromium, manganese, iron, cobalt, nickel and copper.

Transition metal level (biodiesel/petrodiesel ratio)	**Outcome (D100 > Biodiesel)**	**Outcome (D100 = Biodiesel)**	**Outcome (D100 < Biodiesel)**
Ratio < 1 (highest content in D100)	**Genotoxicity** HVO100 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012) B20 (Yang *et al.* 2017) B40 (Yang *et al.* 2017) **Oxidative stress** HVO100 (Jalava *et al.* 2012) **Inflammation** No studies	**Genotoxicity** HVO100 (Jalava *et al.* 2010) **Oxidative stress** HVO100 (Jalava *et al.* 2010) RME100 (Jalava *et al.* 2012) BD (Martin *et al.* 2019) B100 (Kooter *et al.* 2011) **Inflammation** HVO100 (Jalava *et al.* 2010) HVO100 (Jalava *et al.* 2012) RME100 (Jalava *et al.* 2012) BD/Euro 4 (Tzamkiozis *et al.* 2010)	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** No studies
Ratio = 1 (same content)	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** No studies	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** No studies	**Genotoxicity** No studies **Oxidative stress** No studies **Inflammation** S50 (Brito *et al.* 2010)
Ratio > 1 (highest content in biodiesel)	**Genotoxicity** HVO30 (Jalava *et al.* 2012)	**Genotoxicity** RME100 (Jalava *et al.* 2010)	**Genotoxicity** No studies

	RME100 (Jalava *et al.* 2012) **Oxidative stress** No studies **Inflammation** RME100 (Jalava *et al.* 2010)	 **Oxidative stress** RME100 (Jalava *et al.* 2010) HVO30 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012) B5 (Kooter *et al.* 2011) B10 (Kooter *et al.* 2011) B20 (Kooter *et al.* 2011) **Inflammation** HVO30 (Jalava *et al.* 2012) RME30 (Jalava *et al.* 2012) B20 (Bhavaruju *et al.* 2014) B100 (Gioda *et al.* 2016) BD/Euro 1 (Tzamkiozis *et al.* 2010)	 **Oxidative stress** B50 (Betha *et al.* 2012) B100 (Betha *et al.* 2012) **Inflammation** S100 (Brito *et al.* 2010)

Abbreviations: BD = biodiesel (typically unspecified), S = soy.

Supplementary S7. Segregation of studies into the percentage of biodiesel in the fuel and country where the study has been conducted

Blend (%)	American studies	European studies	Studies from other countries
100	Soy (Finch *et al.* 2002) Soy (Vogel *et al.* 2019) Soy (Swanson *et al.* 2009) Soy (Shvedova *et al.* 2013) Animal (Vogel *et al.* 2019) Hydro-treated (Vogel *et al.* 2019) FAME (Yanamala *et al.* 2013)	RME (Jalava *et al.* 2010, 2012) RME (Cervena *et al.* 2017; Libalova *et al.* 2016; Novotna *et al.* 2019) RME (Steiner *et al.* 2013) HVO (Jalava *et al.* 2010, 2012) NR (Kooter *et al.* 2011)	<u>Australia</u> Coconut (Vaughan *et al.* 2019b) FAME (Mullins *et al.* 2014) <u>Brazil</u> Soy (Brito *et al.* 2010) Soy (Gioda *et al.* 2016) Sewage waste (de Brito *et al.* 2018) <u>Singapore</u> Cooking oil (Betha *et al.* 2012)
50	Soy (Vogel *et al.* 2019) Animal (Vogel *et al.* 2019) Hydro-treated (Vogel *et al.* 2019)	RME (Gerlofs-Nijland *et al.* 2013)	<u>Australia</u> Coconut (Vaughan *et al.* 2019b) <u>Brazil</u> Soy (Brito *et al.* 2010) <u>Singapore</u> Cooking oil (Betha *et al.* 2012) <u>Taiwan</u> Cooking oil/butanol (Yang *et al.* 2017)
30		RME (Jalava *et al.* 2010, 2012) RME (Cervena *et al.* 2017) RME (Andre *et al.* 2015; Barraud *et al.* 2017) RME (Douki *et al.* 2018) HVO (Jalava *et al.* 2010, 2012)	<u>Taiwan</u> Cooking oil/butanol (Yang *et al.* 2017)
20	Soy (Fukagawa *et al.* 2013) Soy (Vogel *et al.* 2019) Soy (Bass *et al.* 2015; Farraj *et al.* 2015; Gavett *et al.* 2015) Animal (Vogel *et al.* 2019) Hydro-treated (Vogel *et al.* 2019) NR (Bhavaraju *et al.* 2014) Cooking oil (Traviss et al. 2014) NR (Martin *et al.* 2019)	RME (Hemmingsen *et al.* 2011) RME (Steiner *et al.* 2013) AFME (Hemmingsen *et al.* 2011) FAME (Kowalska *et al.* 2017; Lankoff *et al.* 2017; Magnusson *et al.* 2017, 2019; Skuland *et al.* 2017) FAME (Malorni *et al.* 2017) FAME/HVO (Kowalska *et al.* 2017; ; Lankoff *et al.* 2017; Magnusson *et al.* 2017, 2019; Skuland *et al.* 2017)	<u>Australia</u> Coconut (Vaughan *et al.* 2019a; Vaughan *et al.* 2019b) FAME (Mullins *et al.* 2014)

15			<u>Australia</u> Coconut (Vaughan *et al.* 2019a)
7		RME (Andre *et al.* 2015; Barraud *et al.* 2017) FAME (Kowalska *et al.* 2017; ; Lankoff *et al.* 2017; Magnusson *et al.* 2017, 2019; Skuland *et al.* 2017)	<u>Brazil</u> NR (Cattani-Caveieri *et al.* 2019)
5			<u>Australia</u> Coconut (Vaughan *et al.* 2019a)
Not reported	NR (Hawley *et al.* 2014)		

NR: Not reported. Tzamkiozis et al. (2010) has not been included in the table as it used soybean (i.e. putative "American" type of fuel biodiesel) and Euro 1-4 type of engines (i.e. European regulation of exhaust emissions).

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