

Supplemental Material

Comparison of the toxicity of diesel exhaust produced by bio- and fossil diesel combustion in human lung cells *in vitro*

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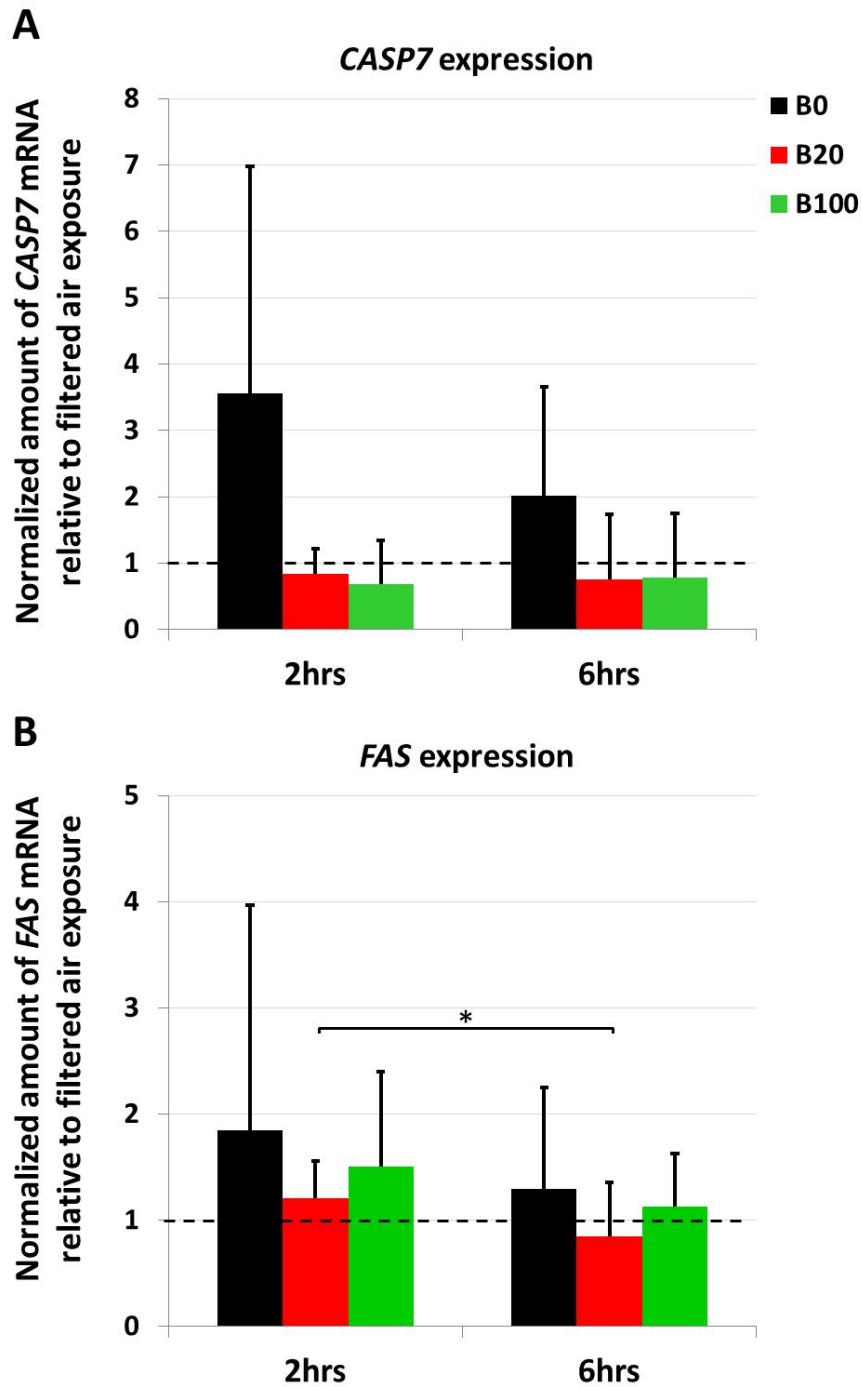
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Supplemental Material Figure S1: Induction of pro-apoptotic responses. A) transcriptional activity of *CASP7*, B) transcriptional activity of *FAS*. Results are reported normalized to the filtered air exposure (dashed line). Error bars indicate standard deviations, asterisks indicate statistical significance ($P < 0.05$)

Supplemental Material Table S1: GenBank accession numbers of the analysed genes and sequences of the primers used for real-time PCR

Gene	GenBank accession	Direction	Primer sequence
<i>GAPDH</i>	NC_000012	forward	AAC AGC CTC AAG ATC ATC AGC
		reverse	GGA TGA TGT TCT GGA GAG CC
<i>HMOX1</i>	CP002685	forward	TTC TCC GAT GGG TCC TTA CAC T
		reverse	GGC ATA AAG CCC TAC AGC AAC T
<i>TNF</i>	NM_000594	forward	CCC AGG GAC CTC TCT CTA ATC A
		reverse	GCT ACA GGC TTG TCA CTC GG
<i>IL-8</i>	NM_000584	forward	CTG GCC GTG GCT CTC TTG
		reverse	CCT TGG CAA AAC TGC ACC TT
<i>CASP7</i>	NM_001227	forward	CGG TCC TCG TTT GTA CCG TC
		reverse	GGT GGT CTT GAT GGA TCG CA
<i>FAS</i>	NM_000043	forward	AGC TTG GTC TAG AGT GAA AA
		reverse	GAG GCA GAA TCA TGA GAT AT

Supplemental Material Table S2: A list of all obtained results before normalization to the filtered air exposure. Extracellular LDH activity and gene expression data are expressed as fold change relative to the untreated control, total reduced GSH is expressed relative to the amount of protein in the cell cultures. Units can therefore not be assigned.

Biological endpoint	treatment		B0	B20	B100
			avg \pm SD	avg \pm SD	avg \pm SD
Extracellular LDH activity (relative to untreated control)	Untreated control	2hrs	1 \pm 0	1 \pm 0	1 \pm 0
	Filtered air exposure		1.1 \pm 0.2	1.0 \pm 0.0	1.0 \pm 0.1
	Exhaust exposure		1.0 \pm 0.1	1.2 \pm 0.2	1.0 \pm 0.1
	Untreated control	6hrs	1.0 \pm 0.0	1.0 \pm 0.0	1.0 \pm 0.0
	Filtered air exposure		1.2 \pm 0.4	1.1 \pm 0.2	1.4 \pm 0.5
	Exhaust exposure		1.2 \pm 0.1	1.1 \pm 0.1	1.8 \pm 0.9
Total reduced GSH / Total protein	Untreated control	2hrs	0.0016 \pm 0.0011	0.0063 \pm 0.0030	0.0044 \pm 0.0032
	Filtered air exposure		0.0015 \pm 0.0012	0.0050 \pm 0.0022	0.0057 \pm 0.0050
	Exhaust exposure		0.0002 \pm 0.0003	0.0007 \pm 0.0008	0.0006 \pm 0.0002
	Untreated control	6hrs	0.0015 \pm 0.0009	0.0074 \pm 0.0028	0.0050 \pm 0.0045
	Filtered air exposure		0.0014 \pm 0.0010	0.0071 \pm 0.0044	0.0036 \pm 0.0027
	Exhaust exposure		0.0002 \pm 0.0003	0.0007 \pm 0.0008	-0.0001 \pm 0.0006

Supplemental Material Table S2 continued

Biological endpoint	treatment		B0			B20			B100		
			avg	±	SD	avg	±	SD	avg	±	SD
Normalized amount of <i>HMOX1</i> mRNA relative to the untreated control	Untreated control	2hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		1.1	±	0.6	1.0	±	0.1	0.7	±	0.4
	Exhaust exposure		24	±	16	14	±	15	37	±	32
	Untreated control	6hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		3.3	±	4.1	1.5	±	0.7	1.7	±	0.3
	Exhaust exposure		84	±	71	32	±	16	52	±	33
Normalized amount of <i>TNF</i> mRNA relative to the untreated control	Untreated control	2hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		0.8	±	0.4	1.5	±	0.5	4.6	±	5.4
	Exhaust exposure		1.0	±	0.3	1.5	±	0.5	3.5	±	3.3
	Untreated control	6hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		1.0	±	0.6	1.2	±	0.6	0.7	±	0.5
	Exhaust exposure		1.7	±	1.0	1.4	±	0.9	1.1	±	0.5

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Biological endpoint	treatment		B0			B20			B100		
			avg	±	SD	avg	±	SD	avg	±	SD
Normalized amount of <i>IL-8</i> mRNA relative to the untreated control	Untreated control	2hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		1.9	±	1.3	2.8	±	1.1	2.1	±	1.7
	Exhaust exposure		7.5	±	12.0	4.9	±	3.5	3.7	±	2.4
	Untreated control	6hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		2.4	±	1.4	2.7	±	1.2	2.0	±	2.2
	Exhaust exposure		8.7	±	7.1	6.0	±	3.6	3.7	±	2.4
Normalized amount of <i>CASP7</i> mRNA relative to the untreated control	Untreated control	2hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		0.9	±	1.0	1.0	±	0.4	3.4	±	3.9
	Exhaust exposure		0.9	±	0.4	3.0	±	4.0	1.0	±	0.4
	Untreated control	6hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		0.8	±	0.6	1.0	±	0.5	0.6	±	0.6
	Exhaust exposure		1.4	±	1.5	0.7	±	0.4	0.3	±	0.2

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Biological endpoint	treatment		B0			B20			B100		
			avg	±	SD	avg	±	SD	avg	±	SD
Normalized amount of <i>FAS</i> mRNA relative to the untreated control	Untreated control	2hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		1.1	±	0.9	1.3	±	0.5	0.6	±	0.6
	Exhaust exposure		1.3	±	0.9	1.5	±	0.5	0.6	±	0.3
	Untreated control	6hrs	1	±	0	1	±	0	1	±	0
	Filtered air exposure		0.6	±	0.3	0.7	±	0.1	0.5	±	0.3
	Exhaust exposure		0.5	±	0.2	0.6	±	0.2	0.5	±	0.6

Supplemental Material Table S3. Properties of the used fuels. Analyses have been performed according to the standard test methods required by the council directive 70/220/EEC for reference fuel analysis.

Property	Unit	Result	
		Fossil fuel	RME
Density (15°C)	kg/L	0.83	0.88
Viscosity (40°C)	mm ² /s	2.5	4.76
Cetane number		53	52.3
Flash point	°C	> 62	174
Water	mg/kg	-	520
Sulfur	mg/kg	< 10	5.2
Aromatic hydrocarbons	Volume %	< 2	n/a
Methanol	Mass %	n/a	0.08
Ester content	Mass %	n/a	94.4
Linoleic acid methylesters	Mass %	n/a	12
Polyunsaturated methylesters	Mass %	n/a	< 1
Monoglyceride	Mass %	n/a	0.43
Diglyceride	Mass %	n/a	0.74
Triglyceride	Mass %	n/a	2.56
Free glycerol	Mass %	n/a	0.02
Total glycerol	Mass %	n/a	0.5