Science Advances
AAAS

Supplementary Materials for

The efficiency of EURO 6d car particulate filters is compromised by atmospheric aging: In vitro toxicity of gasoline car exhaust

Mathilde N. Delaval *et al.*

Corresponding author: Mathilde N. Delaval, mathilde.delaval@helmholtz-munich.de;
Hendryk Czech, hendryk.czech@uni-rostock.de

Sci. Adv. **11**, eadq2348 (2025)
DOI: 10.1126/sciadv.adq2348

This PDF file includes:

Figs. S1 to S7
Tables S1 to S5

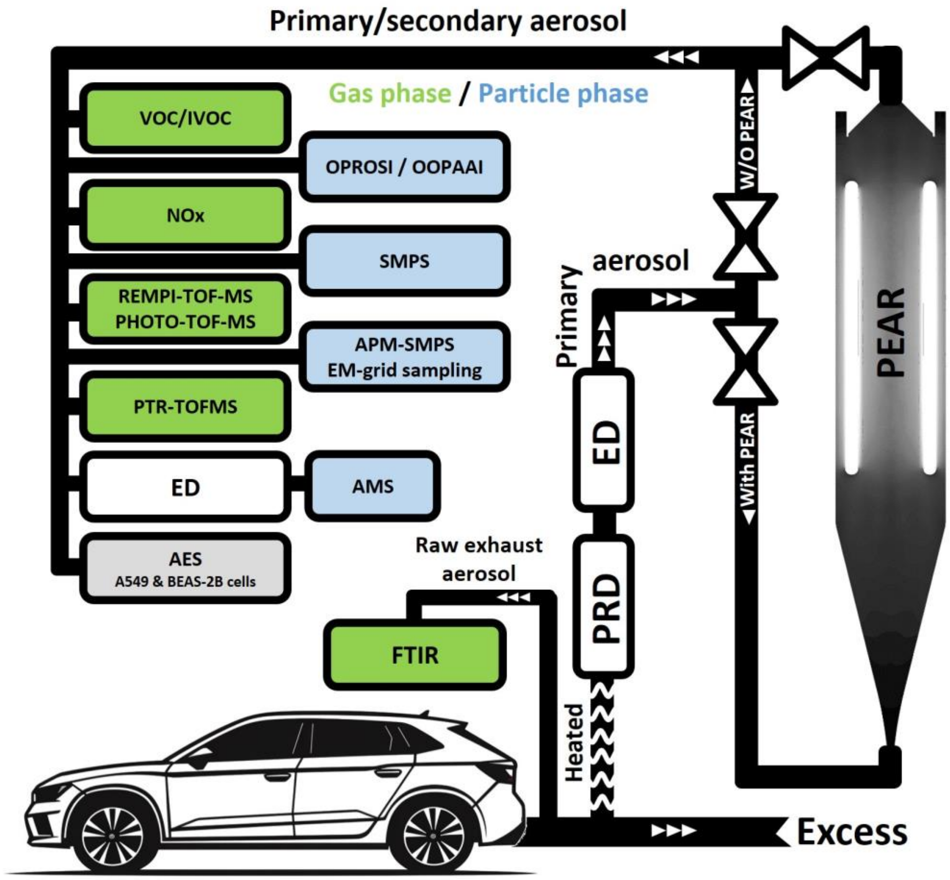

Fig. S1. Experimental set-up. *Emissions from a gasoline car were analyzed by various analytical techniques for gas phase (green) and particle phase species (blue) at different levels of dilution (white boxes) in parallel to cell exposure (grey).*

AES: automated exposure station; AMS: aerosol mass spectrometer, APM: Particle Mass Analyzer*; SMPS: scanning mobility particle sizer; ED: ejector dilutor; EM: Electron microscopy; FTIR: Fourier-transform infrared multicomponent gas analyzer; IVOC, OOPAAI: Online oxidative potential ascorbic acid instrument; OOPROSI: Online particle-bound ROS instrument; NOx: nitrogen oxides; PEAR: Photochemical Emission Aging flow tube Reactor, PHOTO-TOF-MS: time of flight mass spectrometry, PRD: porous tube diluter, PTR-TOF-MS: proton-transfer reaction time-of-flight mass spectrometer; REMPI-TOF-MS: resonance-enhanced multiphoton ionization time-of-flight mass spectrometer, VOC: volatile organic compounds.*

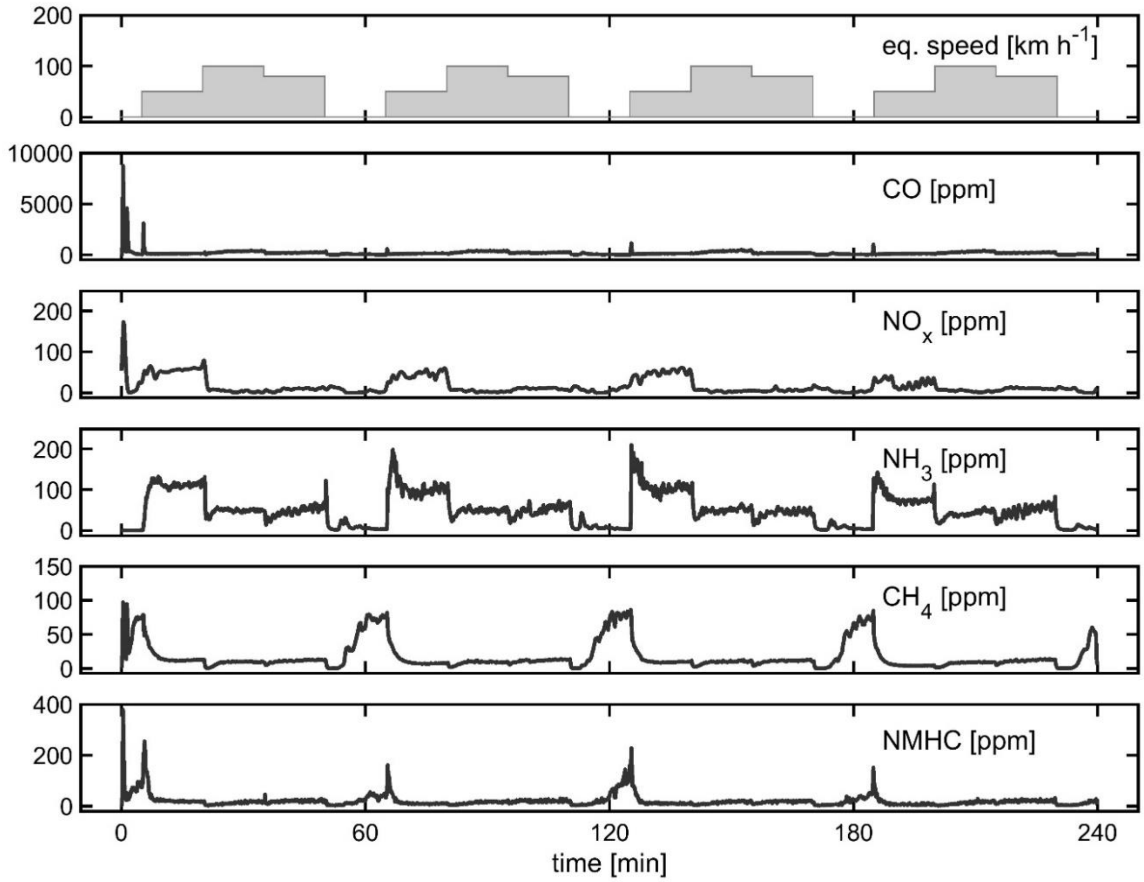

Fig. S2. Equivalent speed during a 4-hour experiment and targeted Fourier-transform infrared spectroscopy (FTIR) analysis. *Equivalent speed of four sequential 1h-driving cycles including idling, 50 km h-1, 120 km h-1, and 80 km h-1. Dynamic emission release were observed for the main exhaust gaseous CO, NOx (NO+NO2), NH3, CH4, and non-methane hydrocarbons (NMHC).*

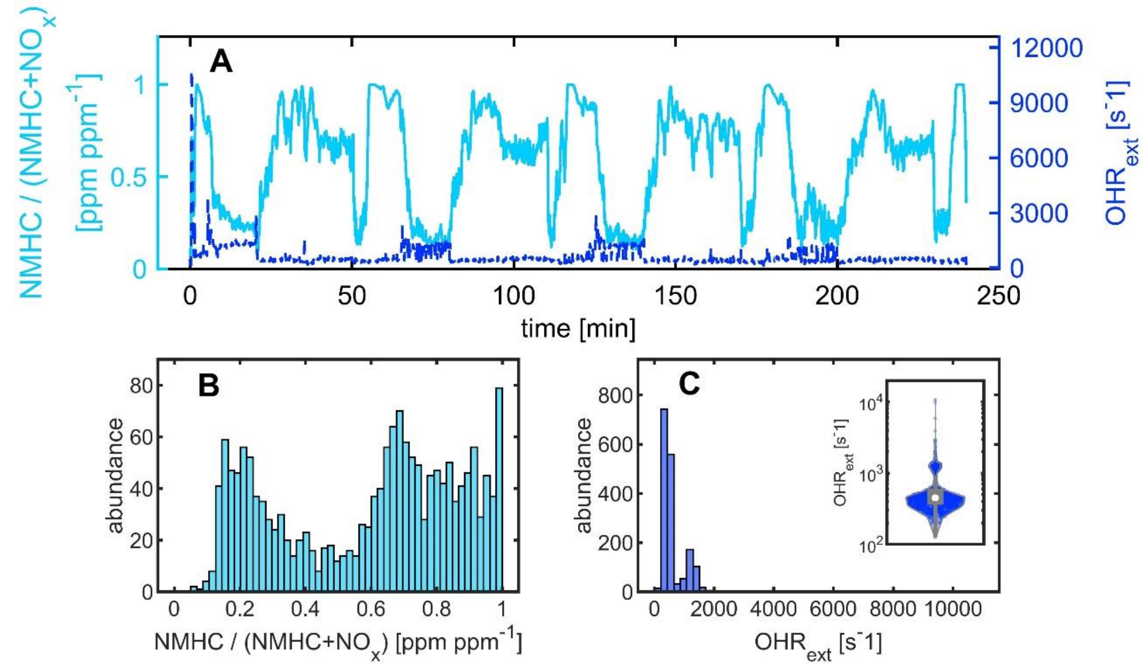

Fig. S3. Representative VOC-to-NO$_x$ and external OH reactivity (OHR$_{ext}$). *(**A**) Percentage of non-methane hydrocarbons (NMHC) to sum of NMHC and NO$_X$ (NO+NO$_2$) and external OH reactivity (OHR$_{ext}$) over four repeated driving cycles. (**B**) Distribution of NMHC / (NMHC+NO$_X$). 64% of the emissions contain more NMHC than NO$_x$. **(C)** Distribution of OHR$_{ext}$ with additional boxplot visualization. The median OHR$_{ext}$ (white dot) accounts for 449 s^{-1}, OHR$_{ext}$ larger than 2000 s^{-1} were only observed for time intervals of less than 30 s during engine start and first acceleration to 50 eq. km h^{-1}.*

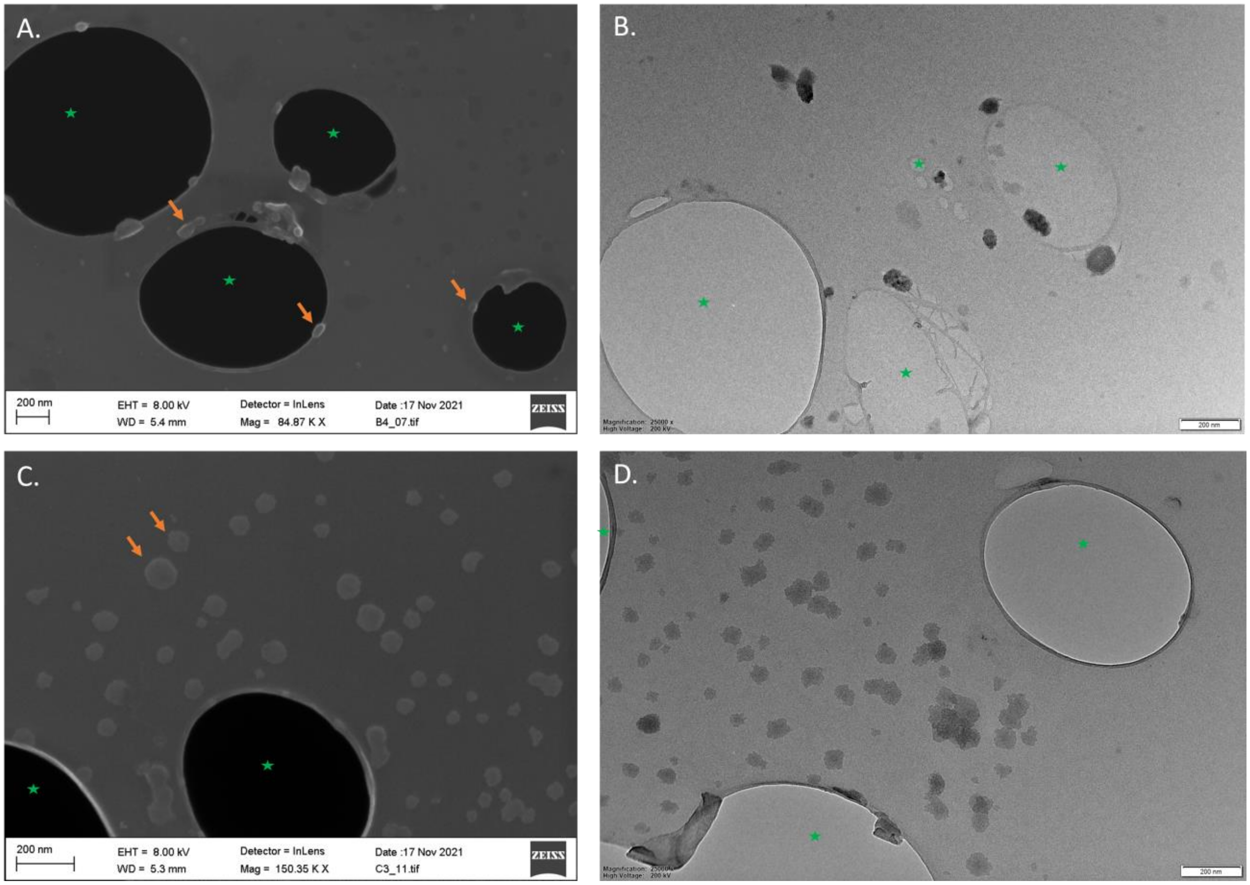

Fig. S4. Particles in the aged exhaust aerosol viewed by electron microscopy. *Particles were separately collected for all velocity phases in a driving cycle on holey carbon substrates with copper supported grids using an aspiration sampler. Particles collected during cold idle (A, B) and warm idle (C, D) were analyzed using a field emission SEM (A, C) and a field emission TEM (B, D). Orange arrows indicate some of the particles and green stars indicate the holes in the grids.*

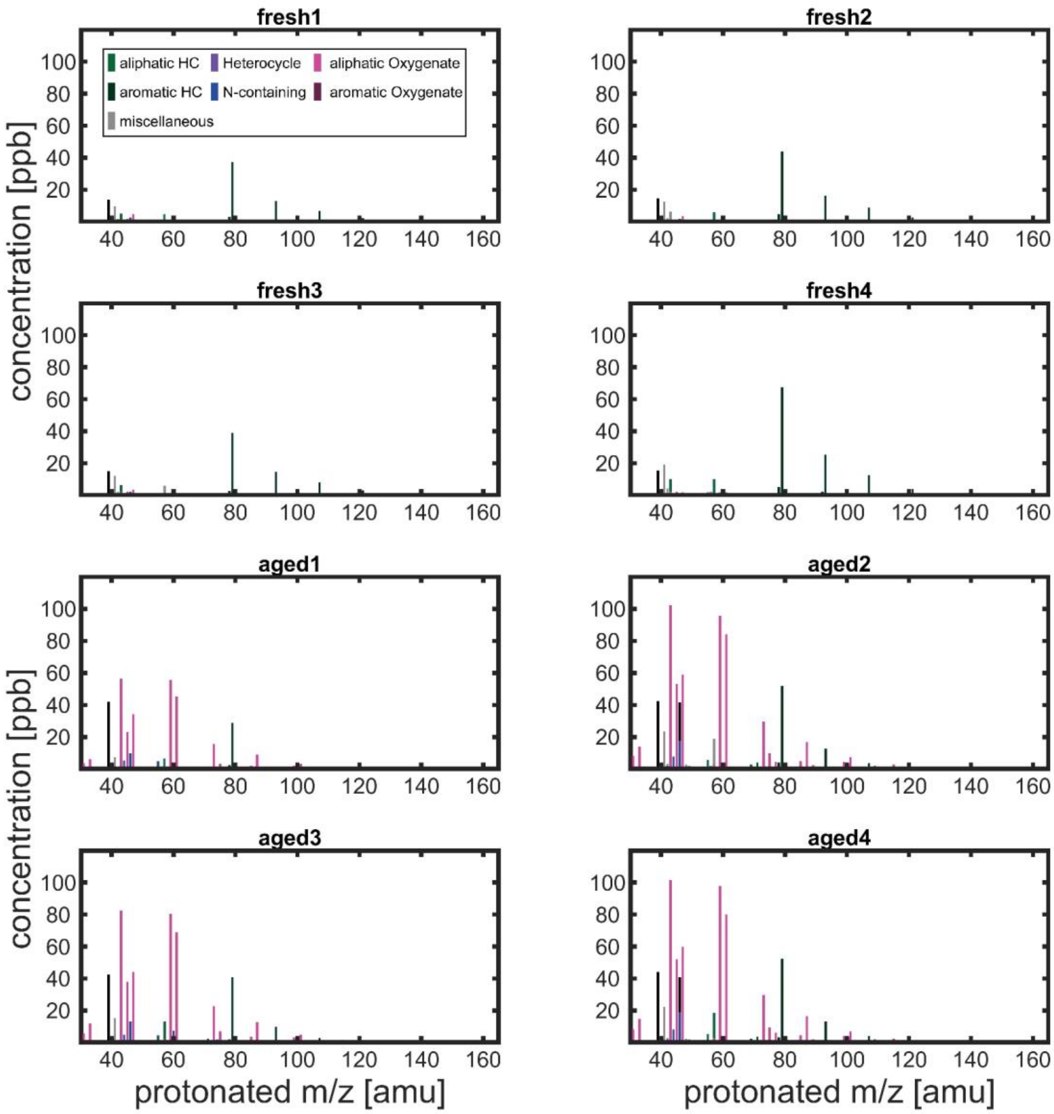

Fig. S5. PTR mass spectra of all individual fresh and aged gas phase emissions. *Analysis of VOC with compound classification in fresh and aged exhaust of all individual experiments.*

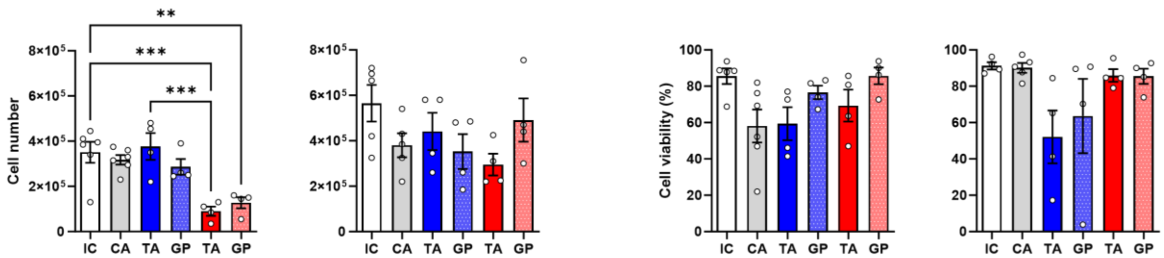

Fig. S6. Cell viability and cell number evaluated with Trypan blue staining, after exposure to fresh and aged aerosols.

*Alveolar A549 and bronchial BEAS-2B cells were exposed for 4 hours at the air-liquid interface (ALI) to total aerosol (TA) or Gas-phase only (GP) of the fresh or aged emissions of gasoline car exhaust (diluted 1:17). Directly after exposure, cells were harvested, centrifuged, and stained with Trypan Blue. Cell number and percent of live and dead cells were evaluated by counting. Results are presented as means ± standard errors of the means (s.e.m.). N≥3. One-way ANOVA with Tukey's multiple comparison as post hoc test was used for statistical comparison. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; and **** $p<0.0001$.*

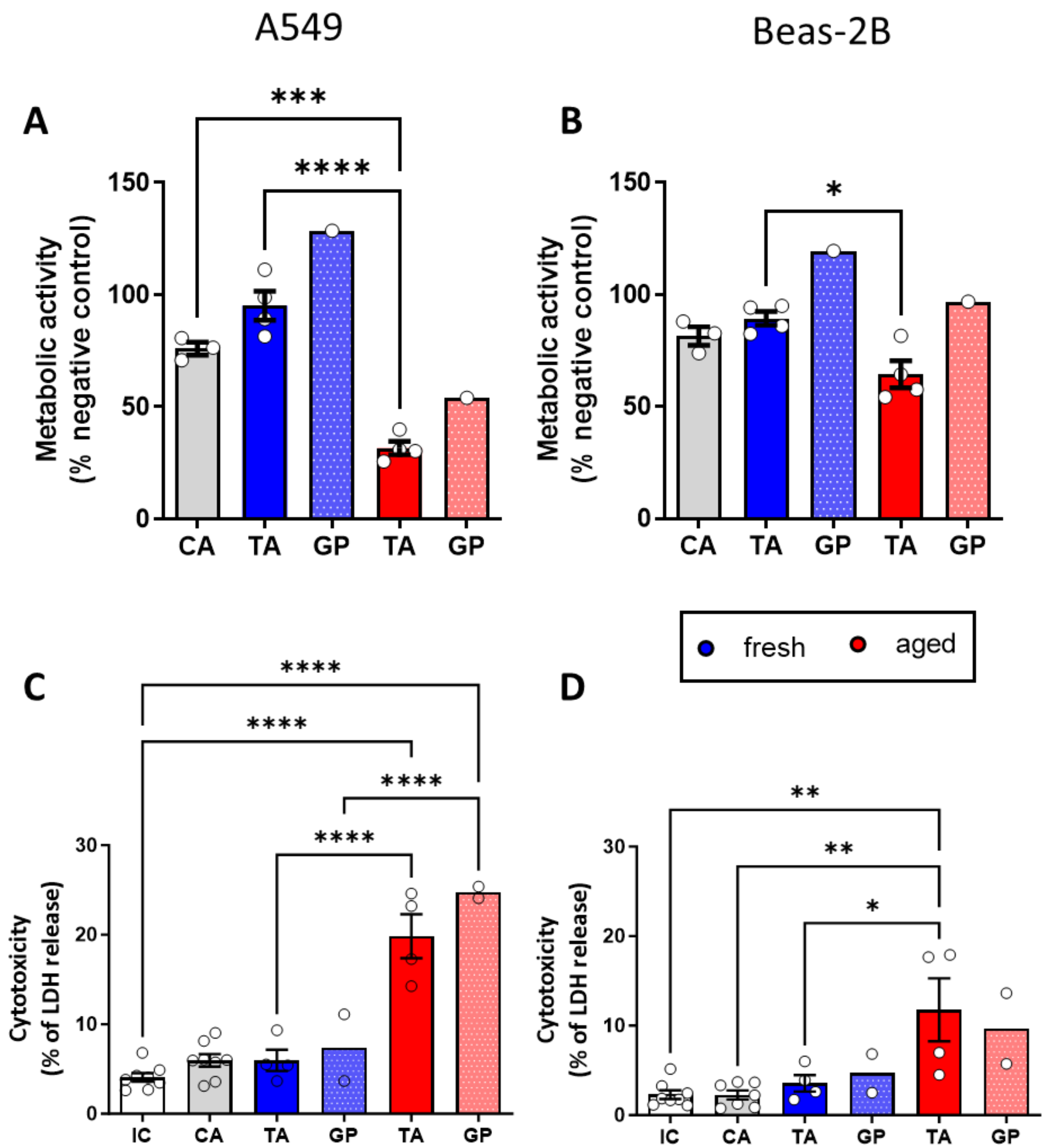

Fig. S7. Cytotoxic effects of fresh and aged gasoline car exhaust emissions on lung epithelial cells exposed either to the total aerosol or to the gas-phase only.

*Alveolar A549 (A-C) and bronchial BEAS-2B (B-D) cells were exposed for 4 hours at the air-liquid interface (ALI) to total aerosol (TA) or Gas-phase only (GP) of the fresh or aged emissions of gasoline car exhaust (diluted 1:17). Metabolic activity (A-B) and LDH release (C-D) were measured and compared to cells exposed to clean air (CA) or to cells left in the incubator (IC) as negative controls. Results are presented as means ± standard errors of the means (s.e.m.). N≥3 for controls and total aerosol exposed samples; n= 1-2 for gas phase only exposed cells. One-way ANOVA with Tukey's multiple comparison as post hoc test was used for statistical comparison. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; and **** $p<0.0001$.*

Table S1. Car specifications. *Gasoline car emissions were generated by a turbocharged light-duty gasoline direct injection (GDI) passenger vehicle (Skoda Scala 1.0 TSI, model year 2021,). The car was equipped with a three-way catalyst and a gasoline particle filter (GPF) and was compliant with the EURO-6 exhaust emission standard.*

Car specification	Turbocharged petrol engine
Make	Skoda
Model	Scala
Year	2021
Weight	1359 kg
Mileage	19921 km (6000 km driven during measurements)
Fuel	gasoline (95E10)
Engine size	0.999 L
Cylinders	3
Power	70 kW
Torque	175 Nm
Exhaust Emission Standard	EURO-6 AP (EURO-6 ISC-FCM)
Gearbox	5-spd manual

Table S2. Sorbent material (Merck, Germany) of the thermal desorption glass tubes for gas phase sampling. *The glass tubes consist of three different sorbent layers, which were separated by glass wool. Thermal desorption direction was reversed to sampling direction.*

Sorbent sampling order in direction of airflow	Sorbent	Weight [mg]
1	Carbotrap® B (20-40 mesh)	60
2	Carbotrap® Y (20-40 mesh)	60
3	Carboxen® 569 (20-45 mesh)	60

Table S3. **Internal standards used for quantification.** *Target list of aromatics with isotope-labelled internal standard and their concentration.*

Internal Standards	Concentration [g L^{-1}]	Targets quantified
Toluene d8	0.2344	Ethylbenzene, m-Xylene, o-Xylene, Styrene, Phenol
Naphthalene d8	0.0797	Indene, Indane, Naphthalene, 2-Methylnapthalene, 1-Methylnaphthalene
Acenaphthylene d8	0.0288	Acenaphthylene
Acenaphthene d10	0.0149	Acenaphthene
Fluorene d10	0.0310	Fluorene, Biphenyl, 2-ethyl-Naphthalene, 1-ethyl-Naphthalene, 2-ethenyl-Naphthalene
Phenanthrene d10	0.0217	Phenanthrene, Fluoranthene, Pyrene
Anthracene d10	0.0241	Anthracene

Table S4. Calculated lung deposition estimates (aged emission) in percent of total emission. *Relative deposition of particles of in percent of total emission in the alveolar (ALV), tracheobronchial (TB), and head airways (HA).*

Region	Unit	Exposure 1	Exposure 2	Exposure 3	Exposure 4	Average	Standard deviation (±%)
Number							
ALV	#/cm^3	2,23E+06 (36.8)	1,90E+06 (36.6)	1,93E+06 (36.9)	3,96E+06 (39.9)	2,51E+06 (37.6)	8,51E+05 (1.4)
TB	#/cm^3	6,09E+05 (10.0)	5,18E+05 (10.0)	5,27E+05 (10.1)	1,18E+06 (11.9)	7,08E+05 (10.5)	2,73E+05 (0.8)
HA	#/cm^3	3,48E+05 (5.7)	2,96E+05 (5.7)	3,01E+05 (5.8)	6,87E+05 (6.9)	4,08E+05 (6.0)	1,62E+05 (0.5)
ALV + TB+ HA	#/cm^3	3,19E+06 (52.6)	2,71E+06 (52.2)	2,76E+06 (52.8)	5,83E+06 (58.7)	3,62E+06 (54.1)	1,29E+06 (2.7)
Total emission	#/cm^3	6,07E+06 (100.0)	5,20E+06 (100.0)	5,22E+06 (100.0)	9,92E+06 (100.0)	6,60E+06 (100.0)	1948199
Surface area							
ALV	µm^2/cm^3	11133 (27.0)	9670 (26.5)	9482 (27.4)	14646 (29.5)	11233 (27.6)	2072 (1.1)
TB	µm^2/cm^3	2587 (6.3)	2236 (6.1)	2211 (6.4)	3607 (7.3)	2661 (6.5)	566 (0.4)
HA	µm^2/cm^3	1596 (3.9)	1399 (3.8)	1354 (3.9)	2174 (4.4)	1631 (3.9)	327 (0.2)
ALV + TB+ HA	µm^2/cm^3	15316 (37.1)	13305 (36.5)	13048 (37.8)	20428 (41.1)	15524 (38.1)	2964 (1.8)
Total emission	µm^2/cm^3	41290 (100.0)	36481 (100.0)	34545 (100.0)	49722 (100.0)	40510 (100.0)	5858
Mass							
ALV	µg/m^3	0,17 (20.9)	0,15 (20.3)	0,14 (21.4)	0,20 (22.7)	0,16 (21.3)	0,02 (0.9)
TB	µg/m^3	0,04 (4.4)	0,03 (4.3)	0,03 (4.6)	0,04 (5.0)	0,04 (4.6)	0,01 (0.3)
HA	µg/m^3	0,03 (4.0)	0,03 (4.3)	0,03 (4.1)	0,04 (4.2)	0,03 (4.1)	0 (0.06)
ALV + TB+ HA	µg/m^3	0,24 (29.4)	0,21 (28.7)	0,20 (30.1)	0,28 (31.9)	0,23 (30.0)	0 (1.2)
Total emission	µg/m^3	0,81 (100.0)	0,74 (100.0)	0,66 (100.0)	0,87 (100.0)	0,77 (100.0)	0

Table S5. Mean concentrations (ng/m^3) and standard deviation (SD) of carbonyl compounds (n=4). *All concentrations are significantly different (two-sided t-test) between fresh and aged aerosol with a p-value < 0.005.*

	Fresh Aerosol		Aged aerosol	
	Mean concentration	**SD (±)**	**Mean concentration**	**SD (±)**
Formaldehyde	1300	580	30000	8000
Acetaldehyde	5500	900	27000	1300
Acetone 2,4	3600	1700	10000	2900
Butyraldehyde	220	5.0	3600	880
Benzaldehyde	550	80	1400	510
Propionaldehyde	89	26	3200	650
Crotonaldehyde	96	18	2200	1300
Isovaleraldehyde	130	75	3700	610
Valeraldehyde	110	5.0	1100	390
m-Tolualdehyde	110	27	1200	310
o-Tolualdehyde	73	25	820	360