“Toxic effects of brake wear particles on epithelial lung cells in vitro”

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Important note: This work has recently been published in *Particle and Fibre Toxicology*: http://www.particleandfibretoxicology.com/content/pdf/1743-8977-6-30.pdf.

Background: Fine particulate matter originating from traffic correlates with increased morbidity and mortality. An important source of traffic particles is brake wear of cars which contributes up to 20% of the total traffic emissions. Recently correlations were found between PM2.5 originating from speed-changing traffic and the modulation of the autonomic control of the heart rhythm, increase in the frequency of premature supraventricular beat and pro-inflammatory and pro-thrombotic responses. The aim of this study was to evaluate potential adverse effects of human epithelial lung cells exposed to freshly generated brake wear particles.
Investigation methods: An exposure box was mounted around a car's braking system. Lung cells cultured at the air-liquid interface were then exposed to particles emitted from two typical braking behaviours („full stop” and „normal deceleration”). The particle size distribution as well as the brake emission components like metals and carbons was measured on-line, and the particles deposited on grids for transmission electron microscopy were counted. The tight junction arrangement was observed by laser scanning microscopy. Cellular responses were assessed by measurement of lactate dehydrogenase activity (cytotoxicity), by investigating the production of reactive oxidative species and the release of the pro-inflammatory mediator interleukin-8.
Results: Brake wear particles show a wide variety in shape and size (Figure 1) with the highest number of particles at 90nm. From the measured metals, iron was found in the highest concentrations (up to 5000µg/m3) followed by copper (up to 200µg/m3) and manganese (up to 30µg/m3).
There was no increase of the extracellular lactate dehydrogenase after exposure. This indicates no cytotoxicity from brake wear particles. However the density of the tight junction protein occluding decreased significantly ($p < 0.05$) with increasing metal concentrations (iron, copper and manganese, which were all strongly correlated with each other). Occludin was also negatively correlated with the intensity of reactive oxidative species. The concentrations of interleukin-8 were significantly correlated with increasing organic carbon concentrations. No correlation was observed between occludin and interleukin-8, nor between reactive oxidative species and interleukin-8.
Conclusions: These findings suggest that the metals of brake wear particles damage tight junctions with a mechanism involving oxidative stress. Brake wear particles also increase pro-inflammatory responses. However, this might be due to another mechanism than via oxidative stress.

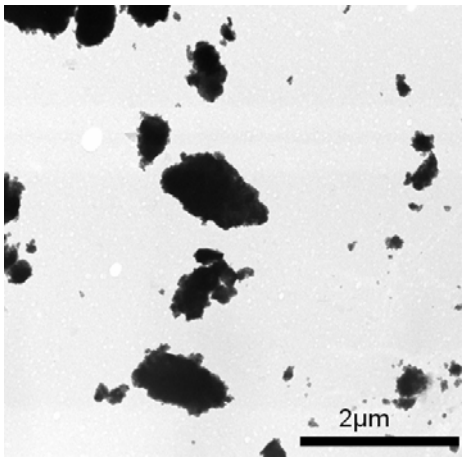

Figure 1: Deposited particles produced during full stop braking. The image was taken by transmission electron microscopy (TEM).

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Toxic effects of brake wear particles on epithelial lung cells *in vitro*

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Introduction: Traffic particles over time

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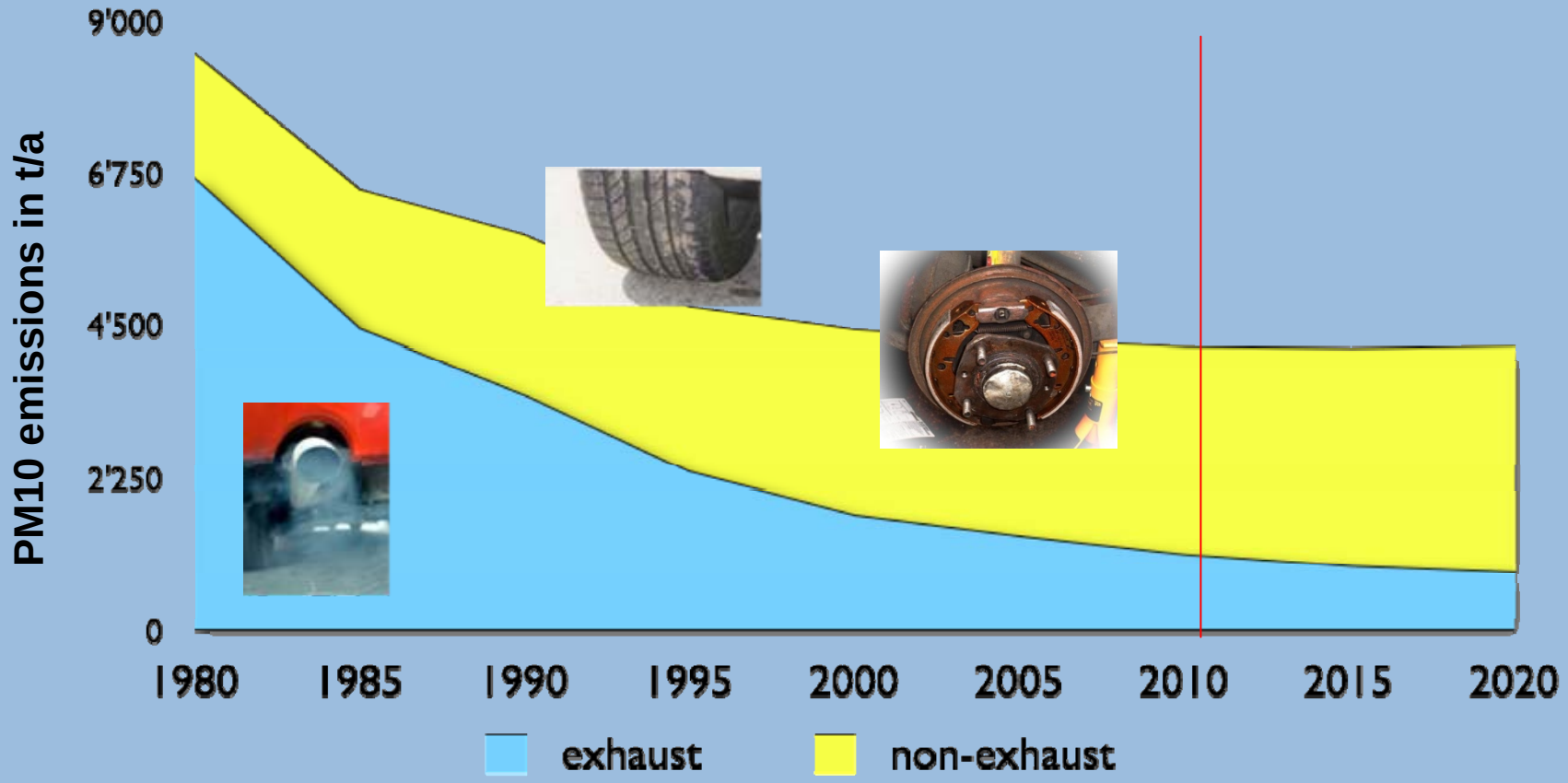
Traffic PM_{10} emissions in Switzerland from 1980 to 2020



After BUWAL Report Nr. 355, Swiss UVEK, BUWAL, 2004, p.40/57

Introduction: In-vehicle traffic particles and health effects

- Increased metal concentrations inside cars
- In-vehicle exposure to $PM_{2.5}$ is supposed to cause changes in inflammation, coagulation, and cardiac rhythm
- Speed changing traffic was identified as a key factor

Do the brakes play a role?

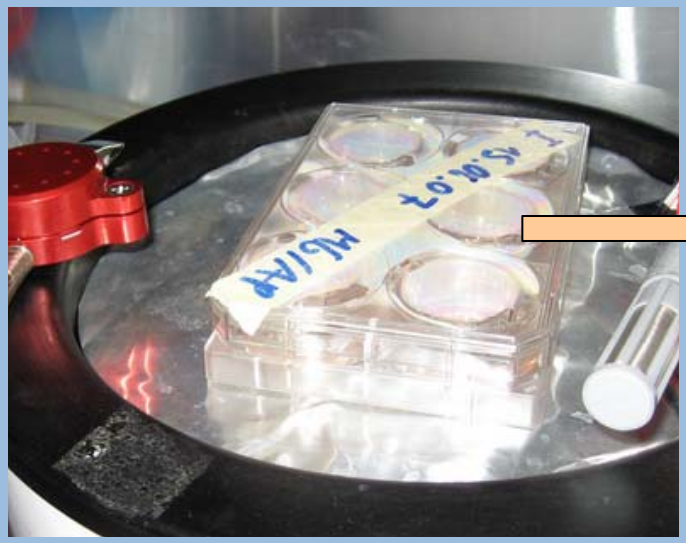
Riediker, et al. 2003. Environ Sci Technol. 37:2084-2093; Riediker, et al. 2004. Am J Respir Crit Care Med. 169:934-940; Riediker et al. 2004. Part Fib Toxicol. 1:2.

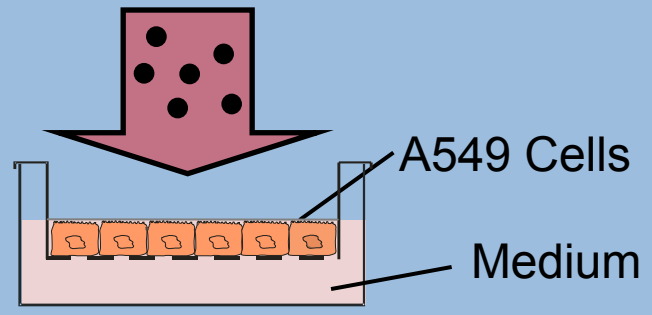
Goals of the study

- Establish an *in situ* exposure system that provides stable environmental conditions for cultured lung cells
- Collect and characterize brake wear particles
- **Investigating adverse effects of brake particles on cultured lung cells**
- Examine different braking behaviors and various repetitions
  - Normal deceleration (8 repetitions)
  - Full stop (4 repetitions, 8 repetitions)
  - No stop

The experimental setting

Cell cultures inside the box

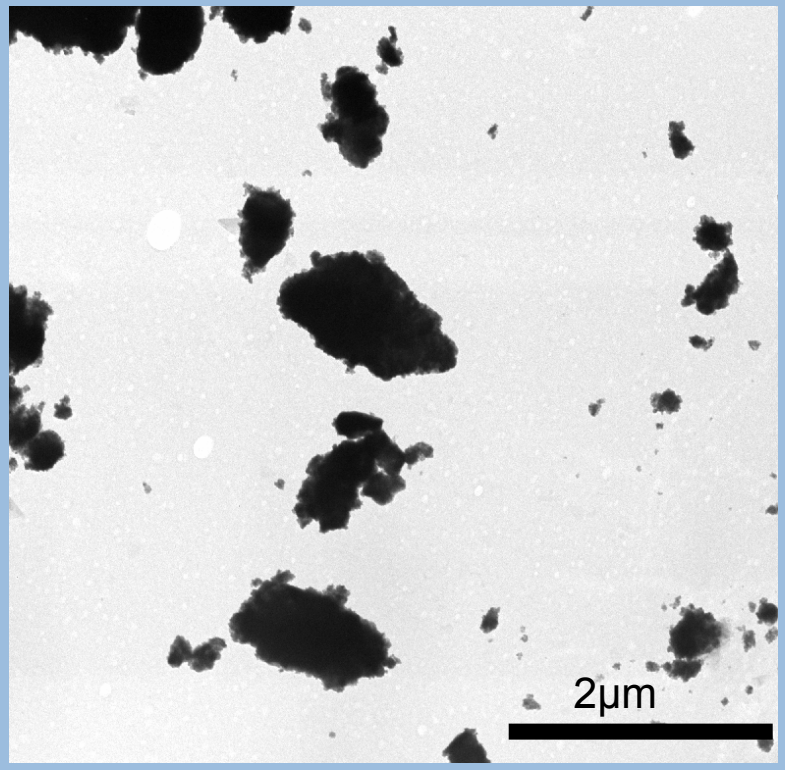




Modified from Rothen-Rutishauser et al. 2005

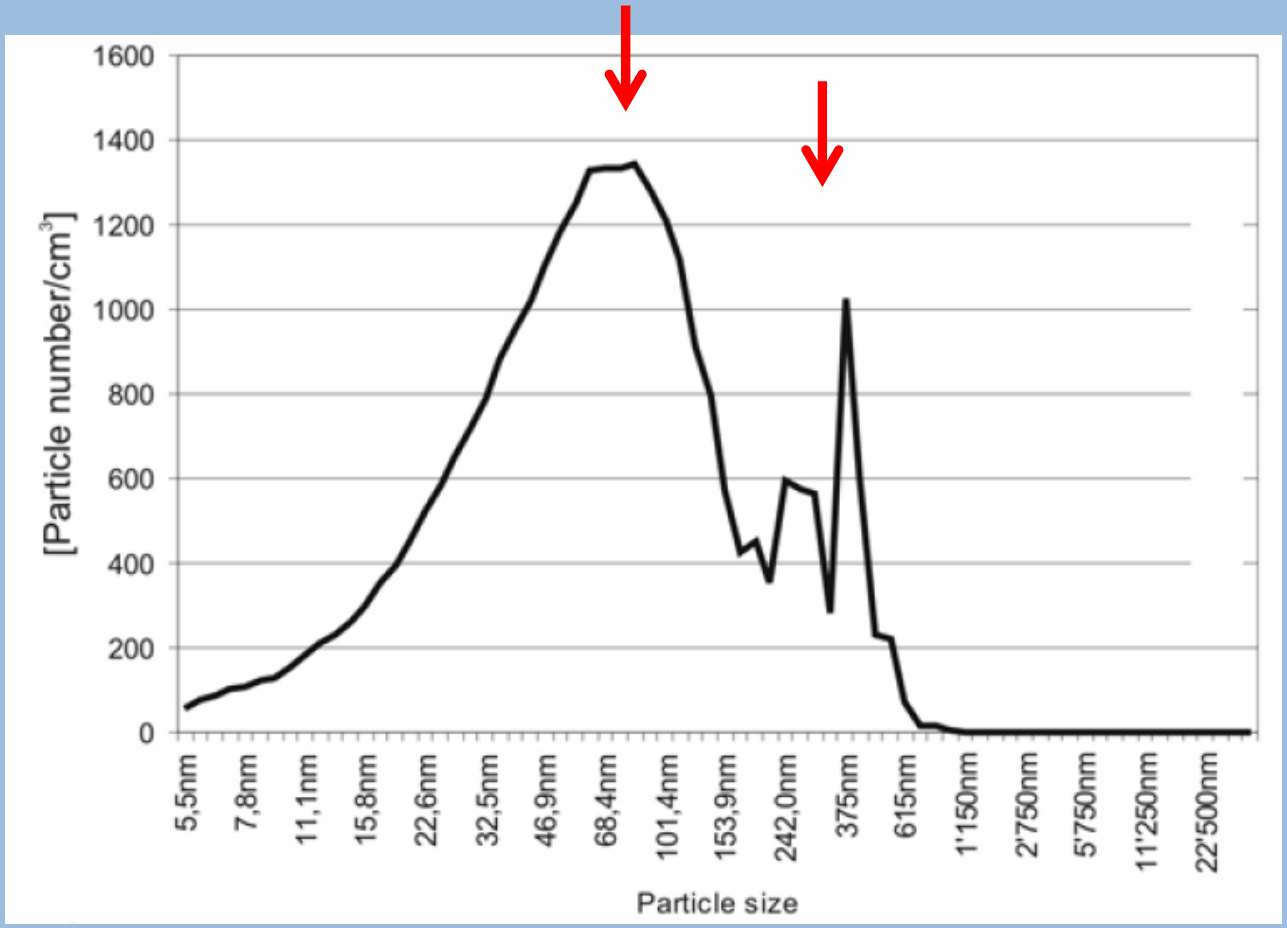
> Monocultures of lung epithelial cells (A549)

Characterization: Morphology of deposited brake wear particles



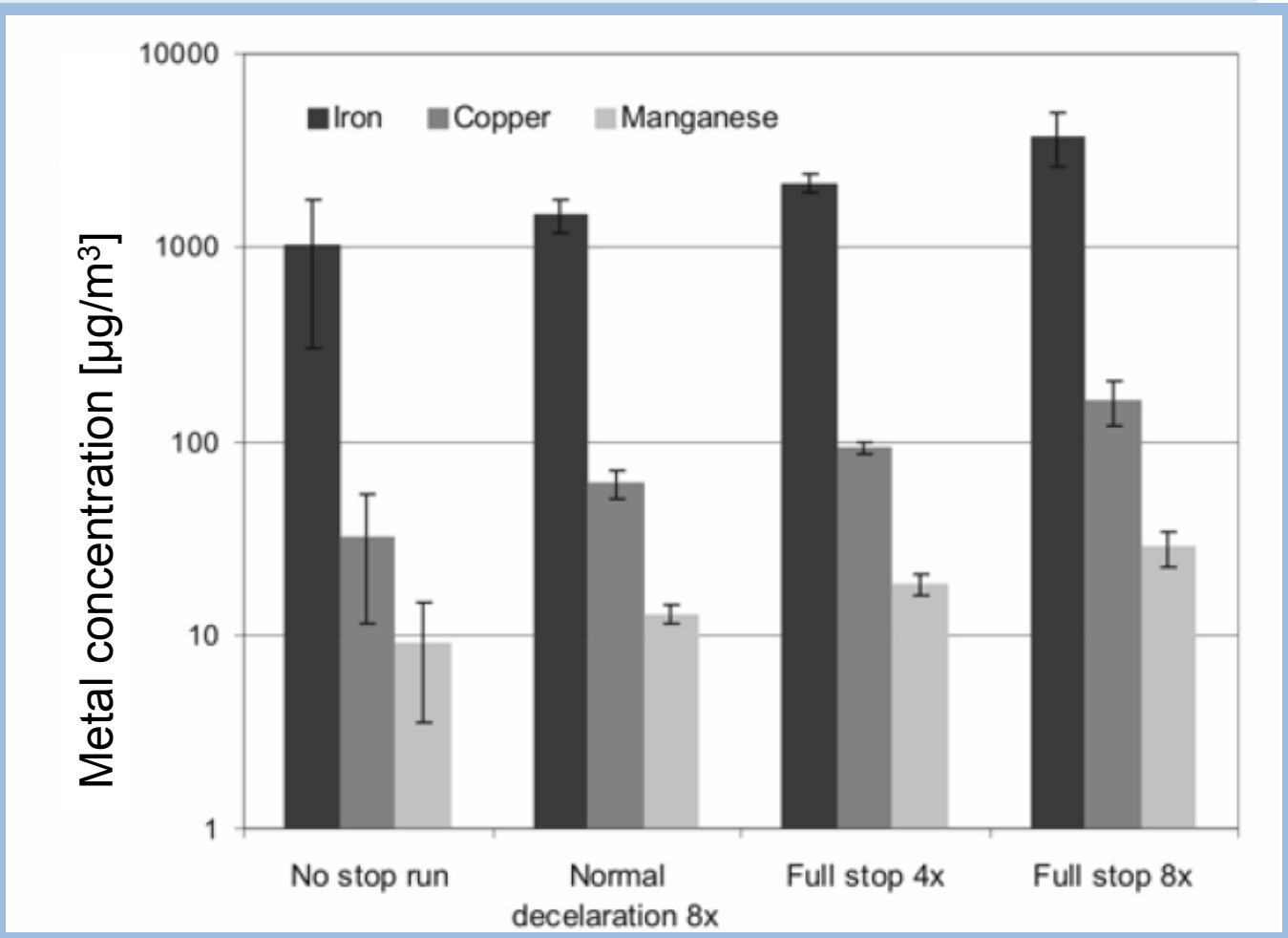
Deposited particles from a full stop 2x. Image was taken by transmission electron microscopy (TEM).

Characterization:
The particles size distribution



Particle size distribution of a “full stop” braking measured with a Wide Range Aerosol Sampler (WRAS, Grimm Aerosol Technik GmbH & Co., Ainring, Germany)

Characterization:
Metal compounds of particles



Investigating adverse effects of brake particles on lung cells

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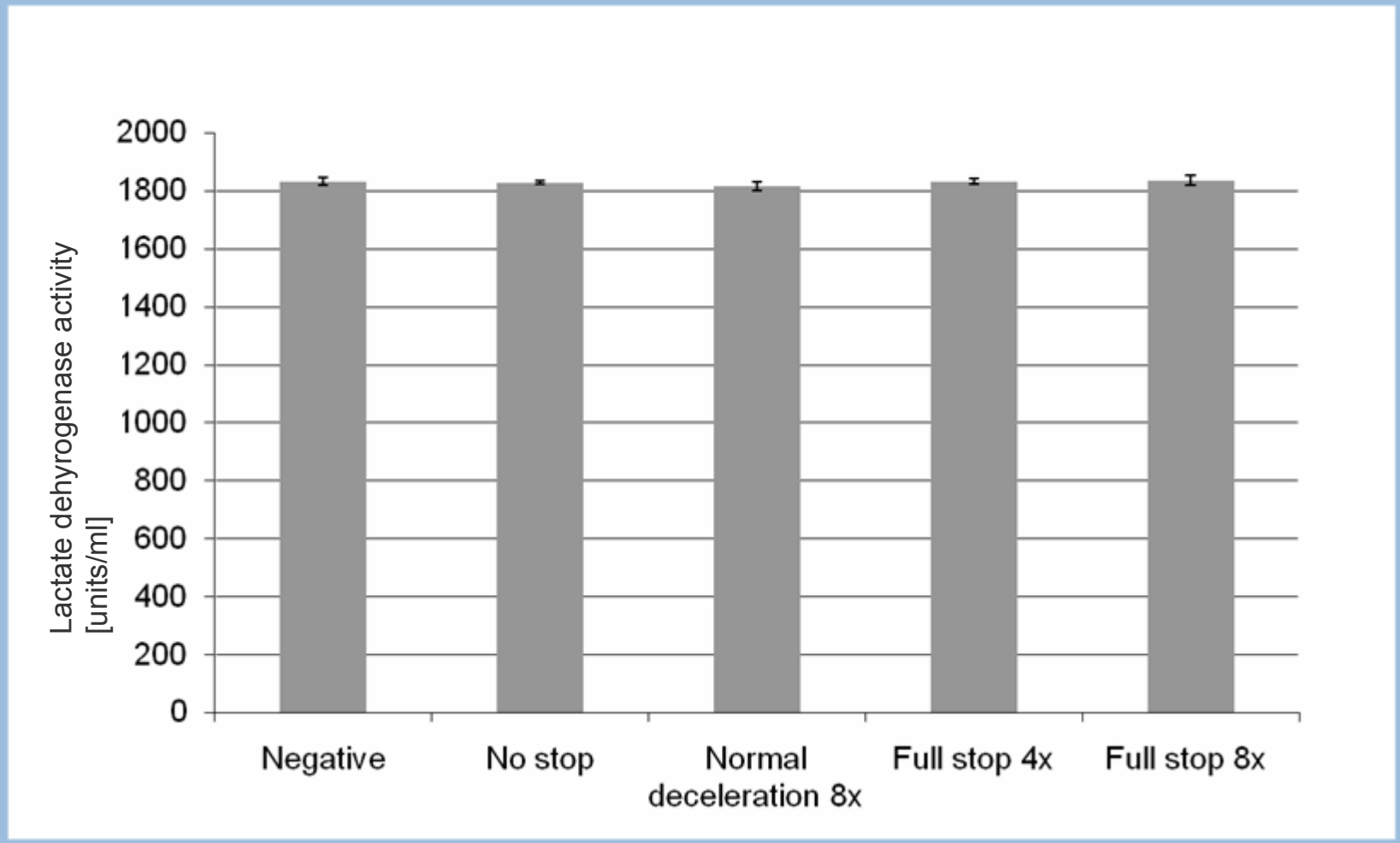
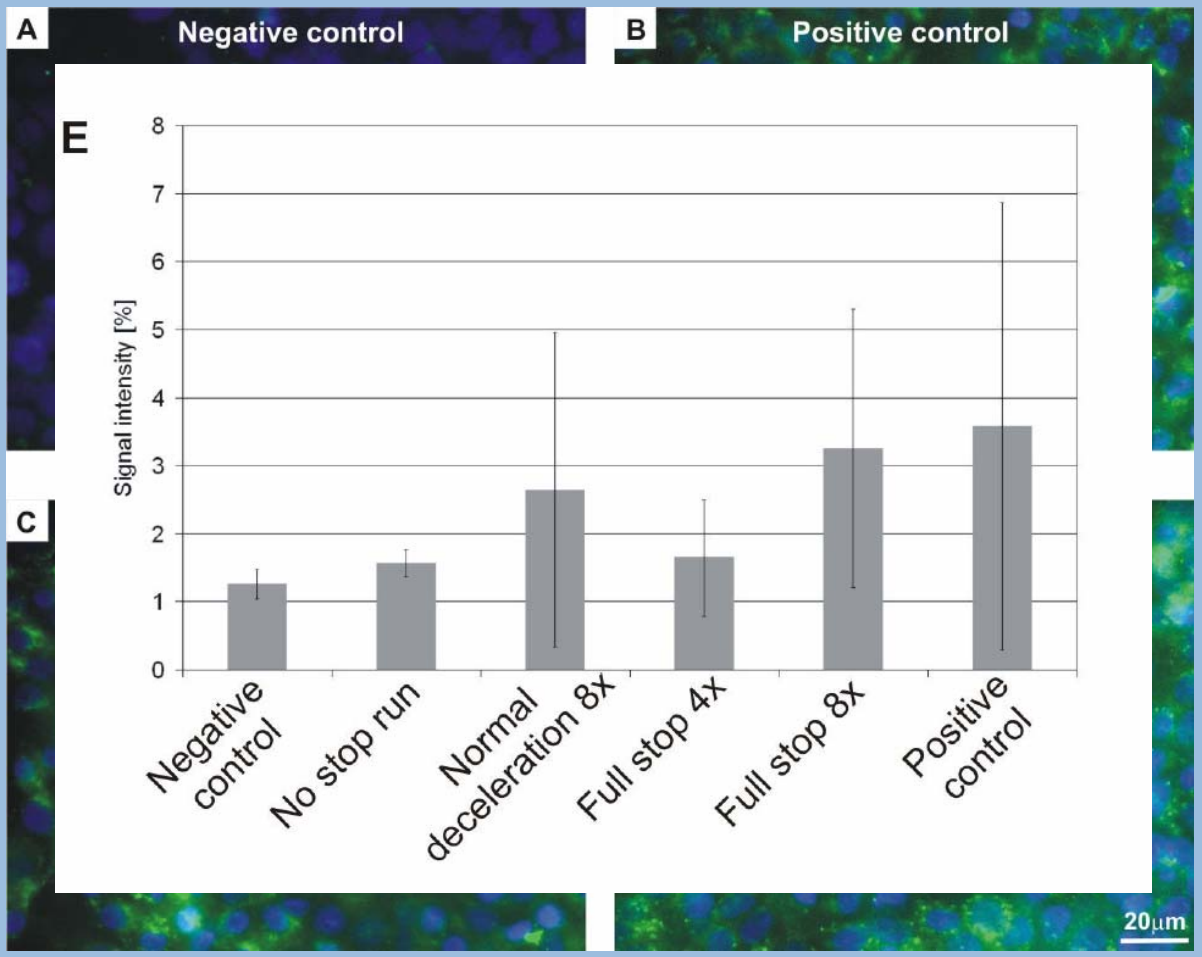
- **Endpoints**
  - Cytotoxicity (lactate dehyrogenase release)
  - Reactive oxygen species
  - Chemokine release (IL-8)
  - Tight junction arrangement (occludin)

Biological effects: Cytotoxicity (lactate dehyrogenase release)



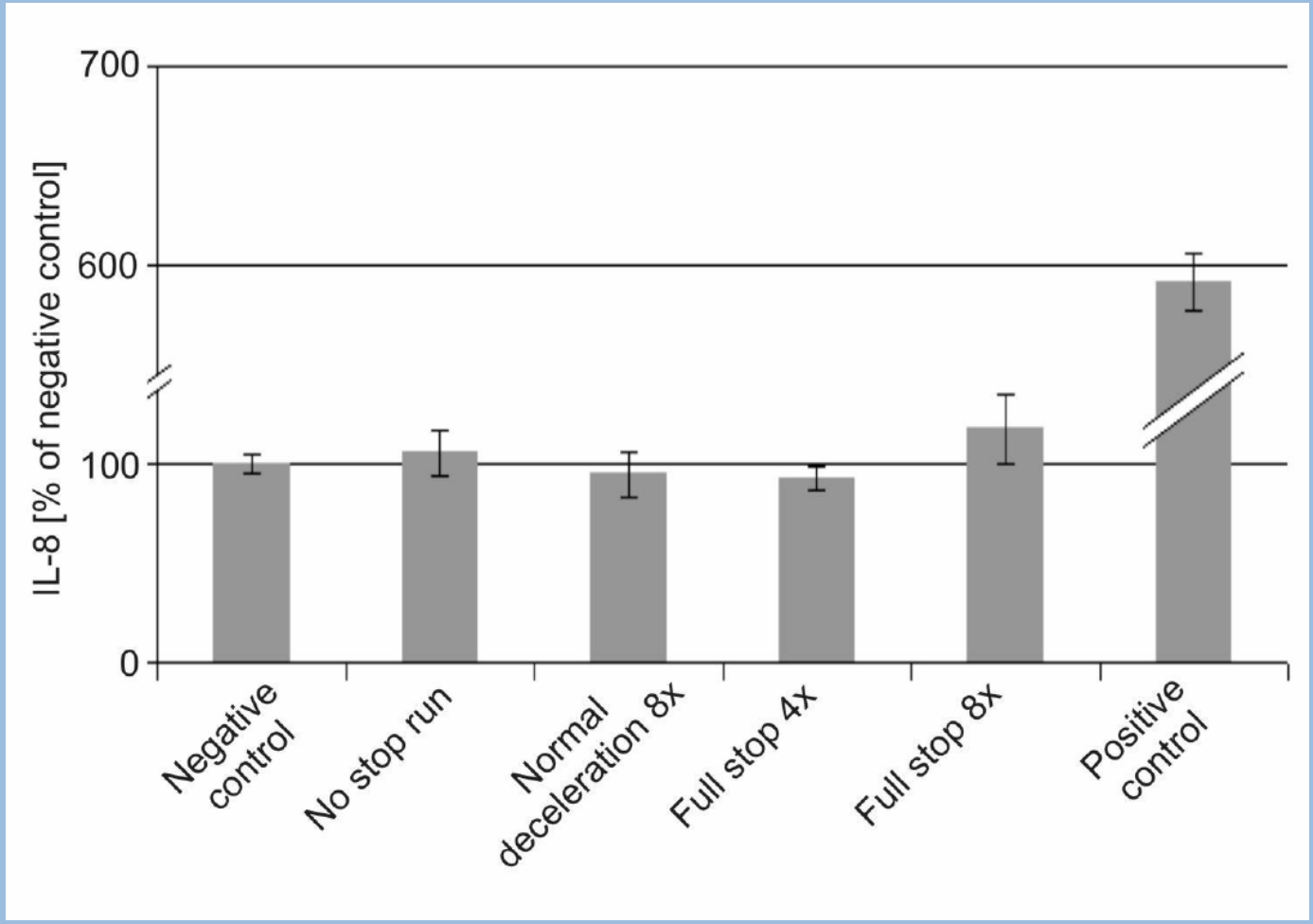
Biological effects: Reactive oxygen species



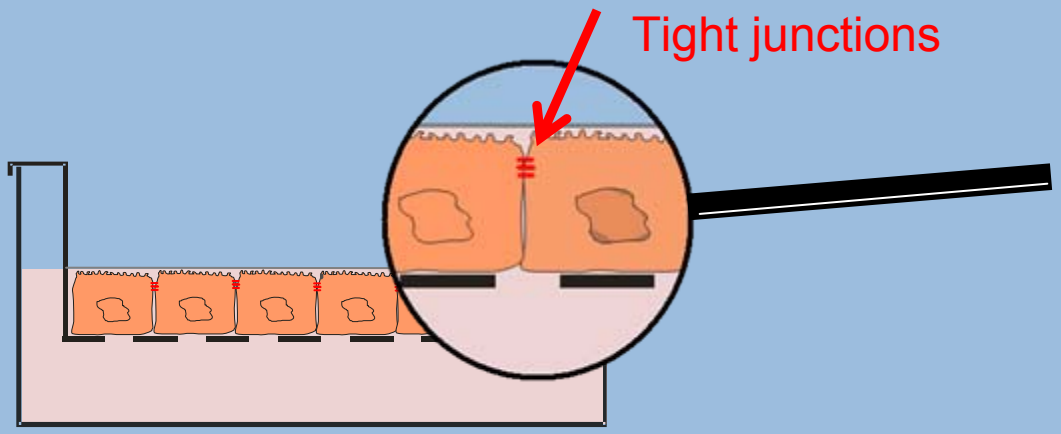
Blue: Cell nucleus

Green: Reactive oxygen species

Biological effects: Chemokine release (IL-8)

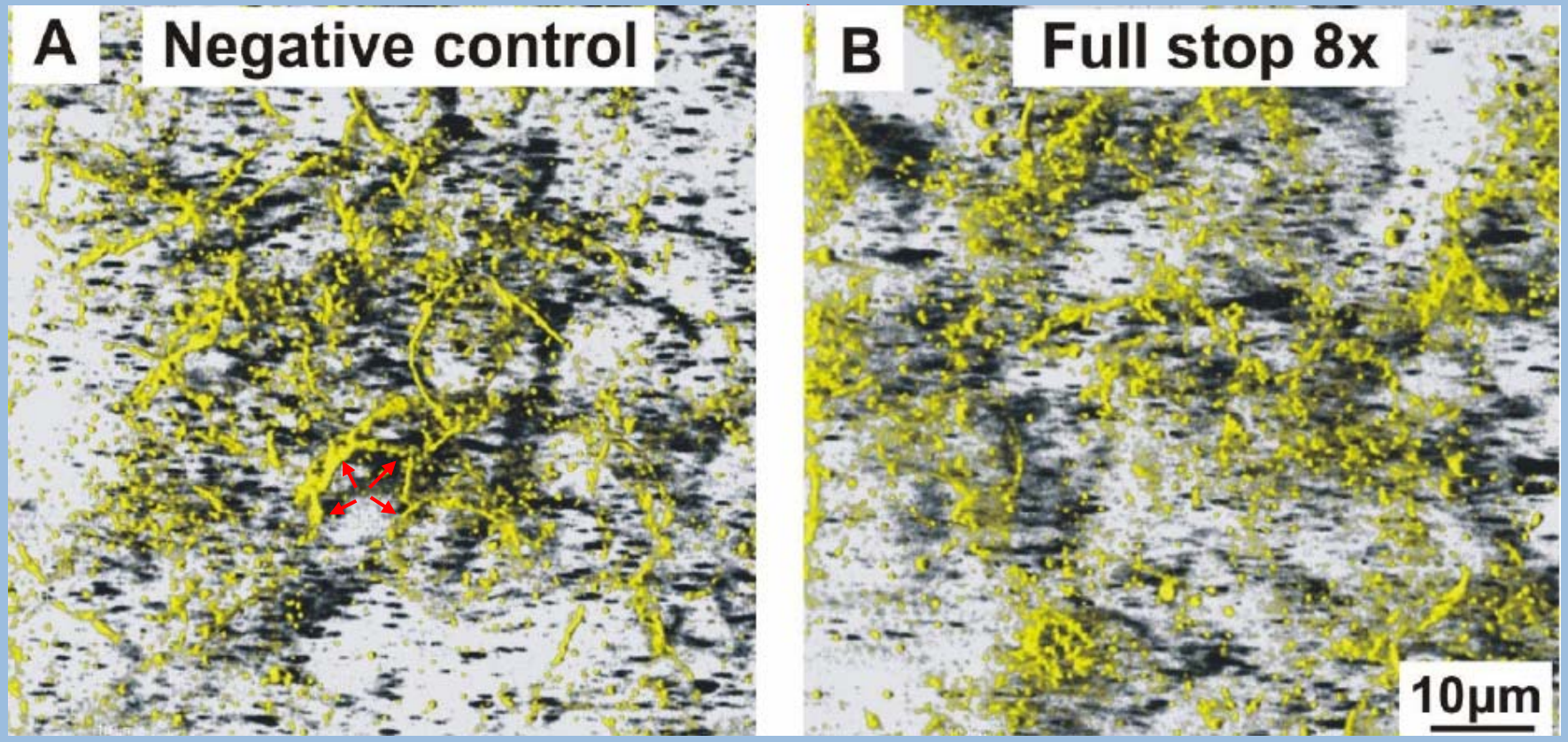


Biological effects:
Tight junctions (occludin)

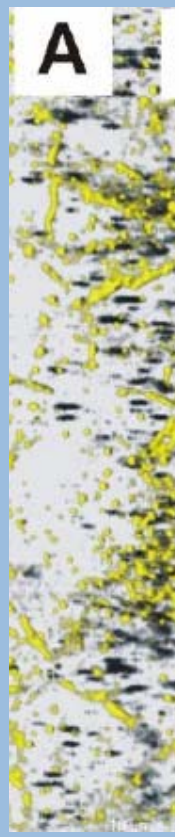


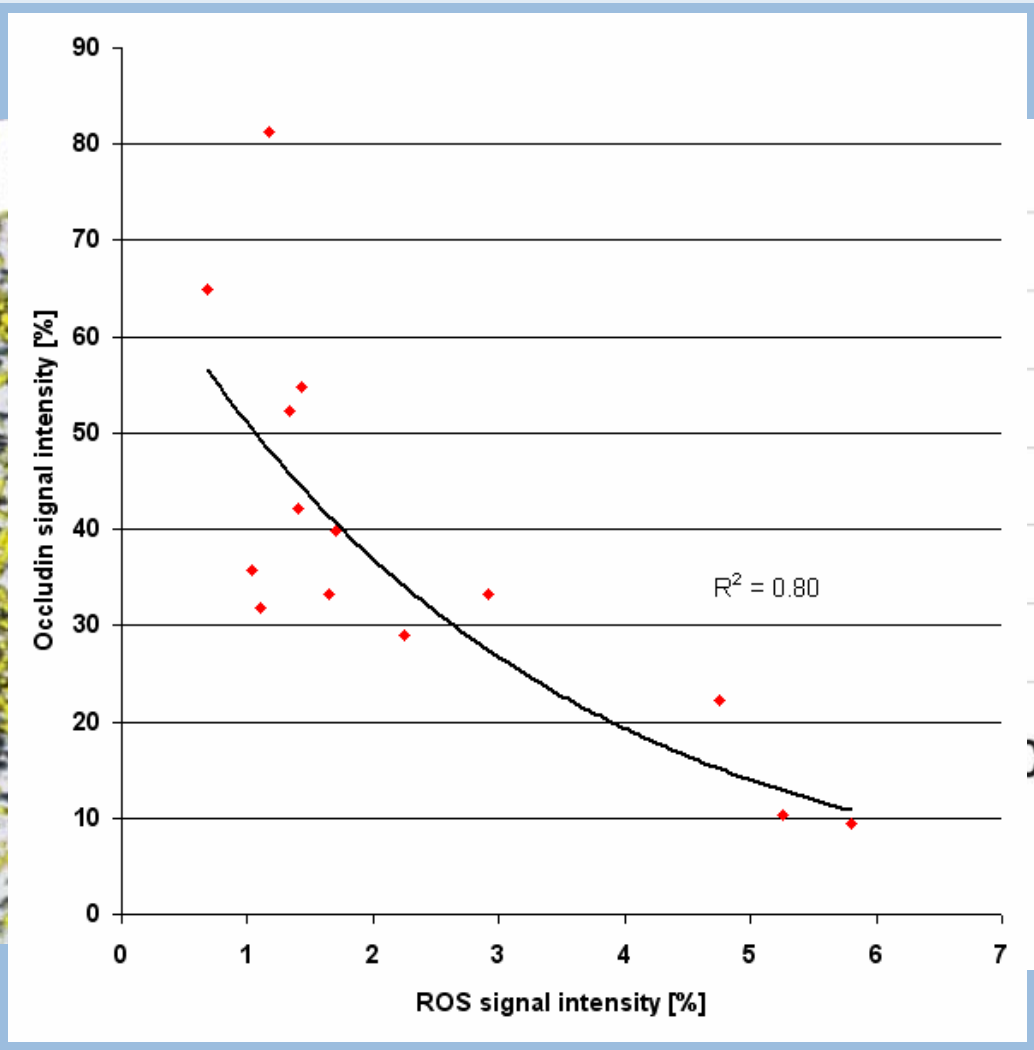
Modified from Rothen-Rutishauser et al. 2005

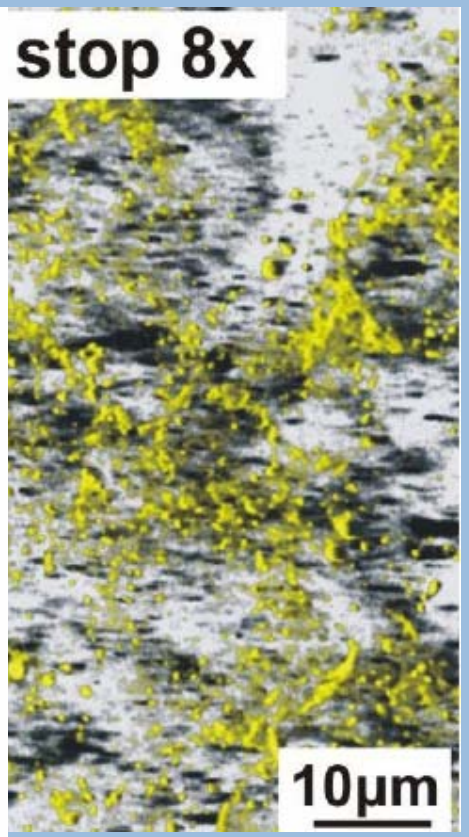
Biological effects:
Tight junctions (occludin)



Biological effects:
Tight junctions (occludin)







Summary and conclusion (1)

- Reliable approach to investigate the cellular effects of directly emitted brake wear particles
- No increase in lactate dehyrogenase release
  →No cytotoxicity trough brake wear particles
- „Full stop“ with most repetitions provoked the strongest adverse effects (reactive oxygen species↑ and IL-8↑)

Summary and conclusion (2)

- Negative correlation between metals and the amount of occludin
- Negative correlation of the amount of occludin and reactive oxygen species

  →Metals of brake wear particles may damage tight junctions with a mechanism involving oxidative stress

→ Several indications for an impairment of the airway epithelium

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For providing equipment/lifts: École de Métiers Lausanne, Garage Mirabile Lausanne, Garage Lehmann Herzogenbuchsee.

Braking protocols

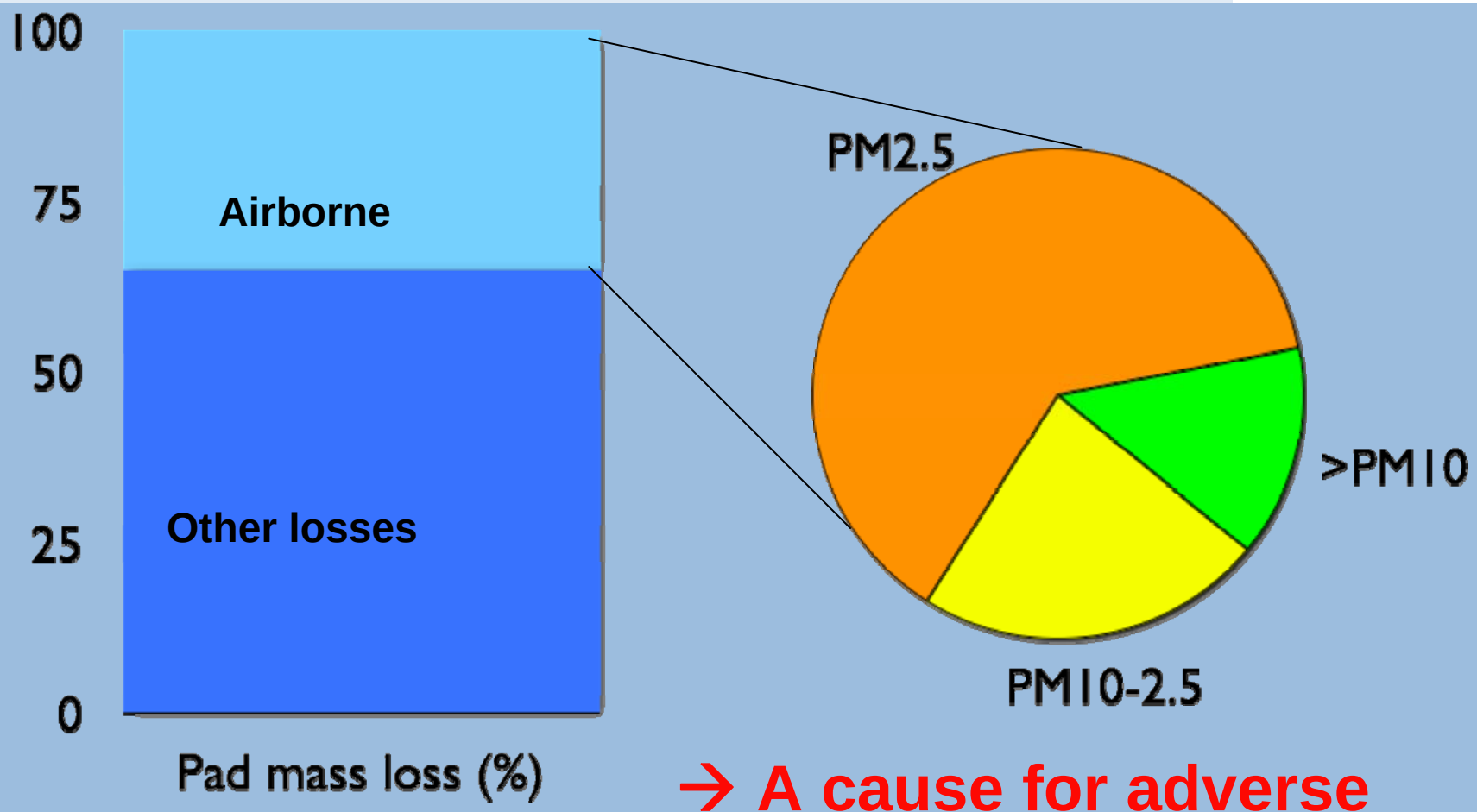
Normal braking: Two minutes in 3rd gear at 2'000 RPM, then 10 sec deceleration to 1750 RPM (gas position unchanged).

Full stop: Two minutes in 3rd gear at 3'000 RPM, then full stop until the engine stalled (gas pedal position unchanged).

No stop: 3rd gear at 2'000 RPM (control to measure the particles released from the turning wheel).

Modified from Sanders et al. Brake dynamometer measurement of airborne brake wear debris, SAE Technical Paper Series, 2001-01-1280, 2002

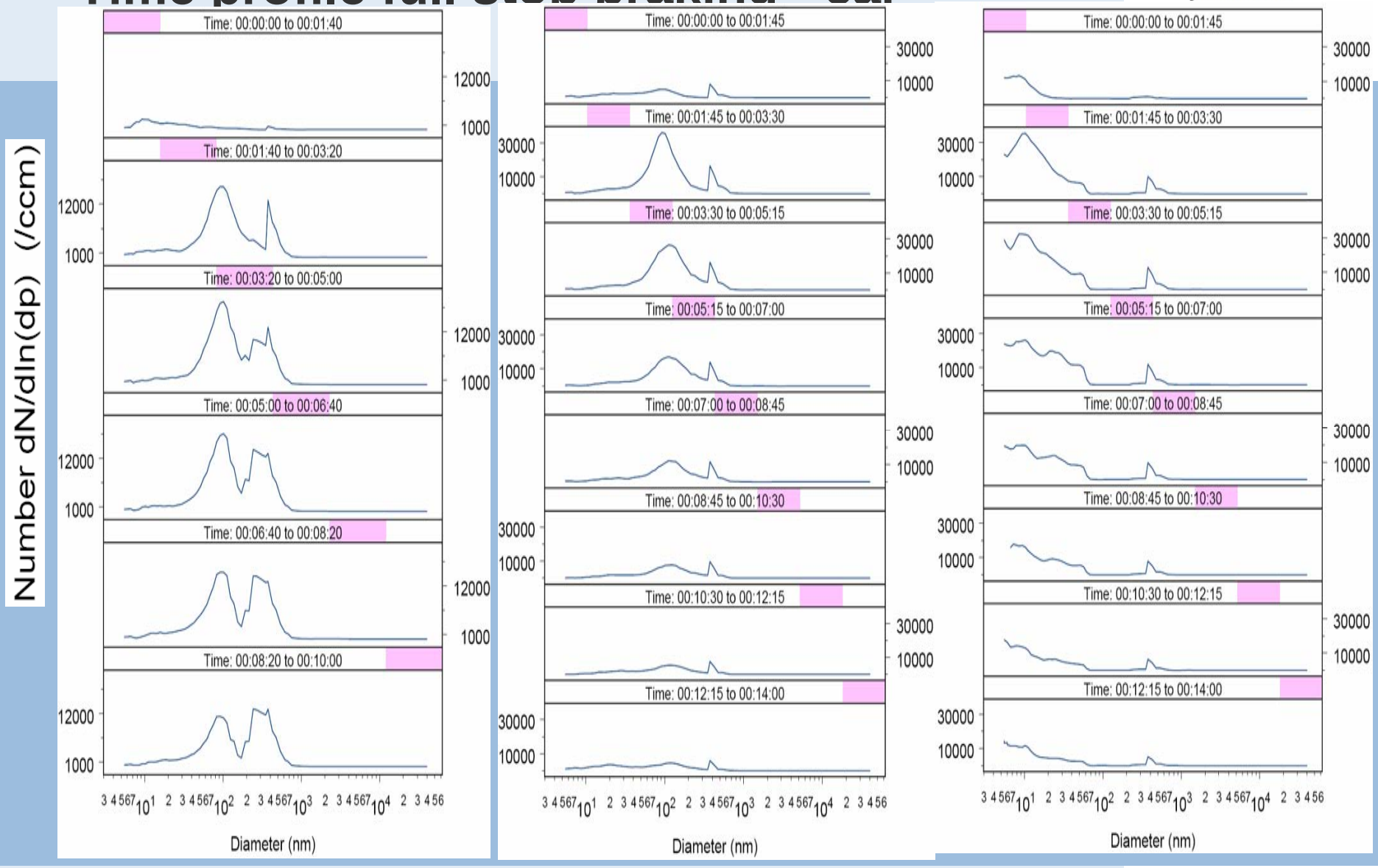
Brake wear particles



→ A cause for adverse effects to cells ?

Test bench brake wear emissions - after Garg et al. ES&T 2000

Time profile full stop braking - car



Estimated emissions

- Exhaust: 10 mg/km (car) to 500 mg/km (truck)
- Tire loss:
  = 120 mg/km, airborne ca. 5 mg/km (1 - 100, disputed)
- Brake pad loss:
  = airborne 10 - 50 mg/km

> **CAVE**: Emissions are dynamic! Speed *change* matters!

After Garg et al. ES&T 2003, Gehrig et al. IJEP 2004, BUWAL Nr. 355 and other sources

Cars tested = frequent on Swiss streets

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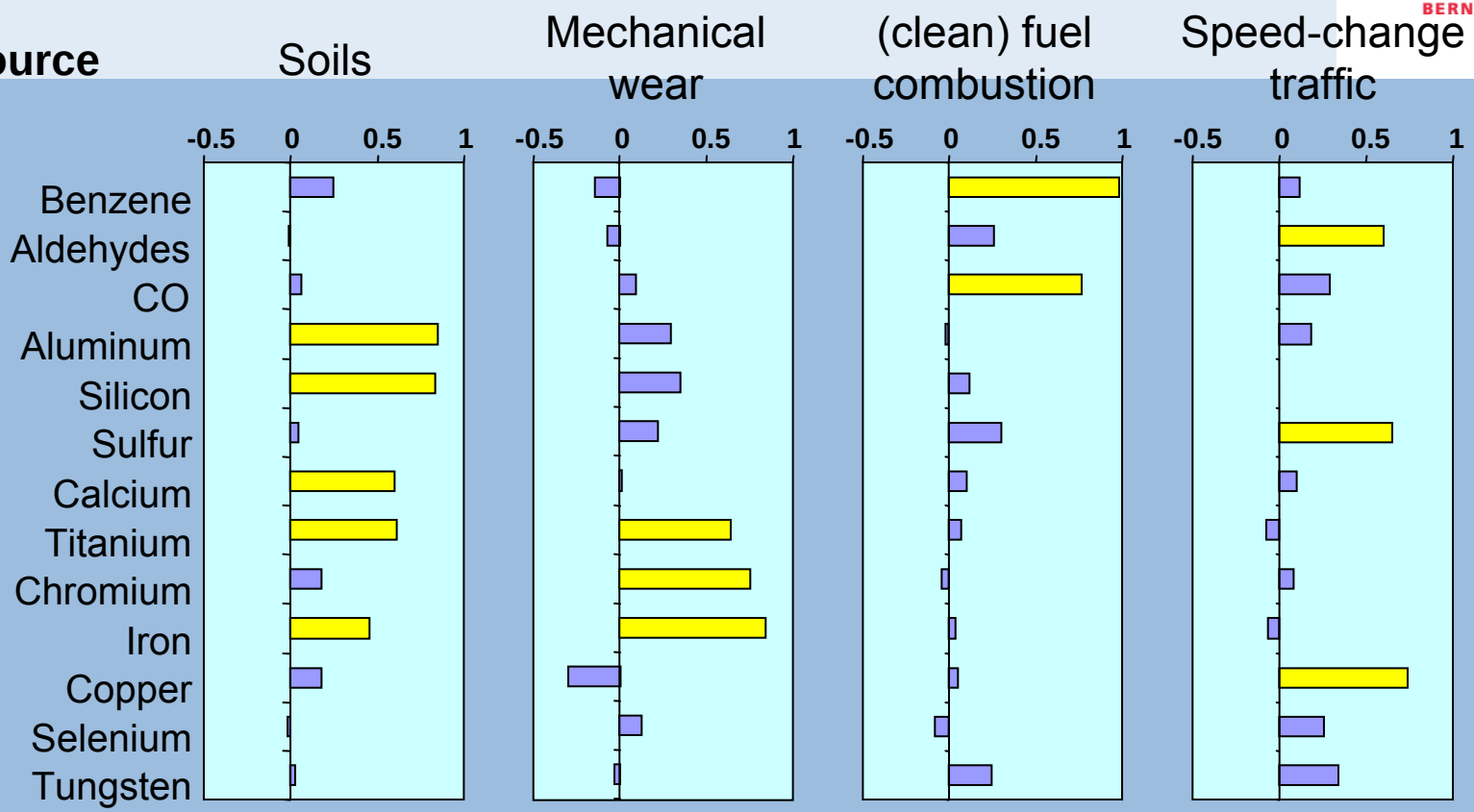
Brand	Brand Rank #	Model	# New registrations in Switzerland (Model)	Kilometrage of the tested car
Opel	2	Astra 1.6 Si Automatic	6’017 (Astra total)	84'000 (Brake pads changed 1x)
Renault	4	Modus 1.2 16V	727	530
VW	1	Golf Variant	10’016 (Golf total)	102’000 (Brake pads changed 1x)
Fiat	13	Panda 1.2	1’784	30’700
Opel	2	Astra 1.4 G14 Cvan	6’017 (Astra total)	62’700
Renault	4	Laguna 2.0 Limousine	1’384	221’000 (Brake pads changed 3x)

Strassen-fahr-zeuge in der Schweiz - Eingeführte Strassenfahrzeuge 2004 Bundesamt für Statistik (BFS)

Speed-change traffic effects - could it be the brakes?

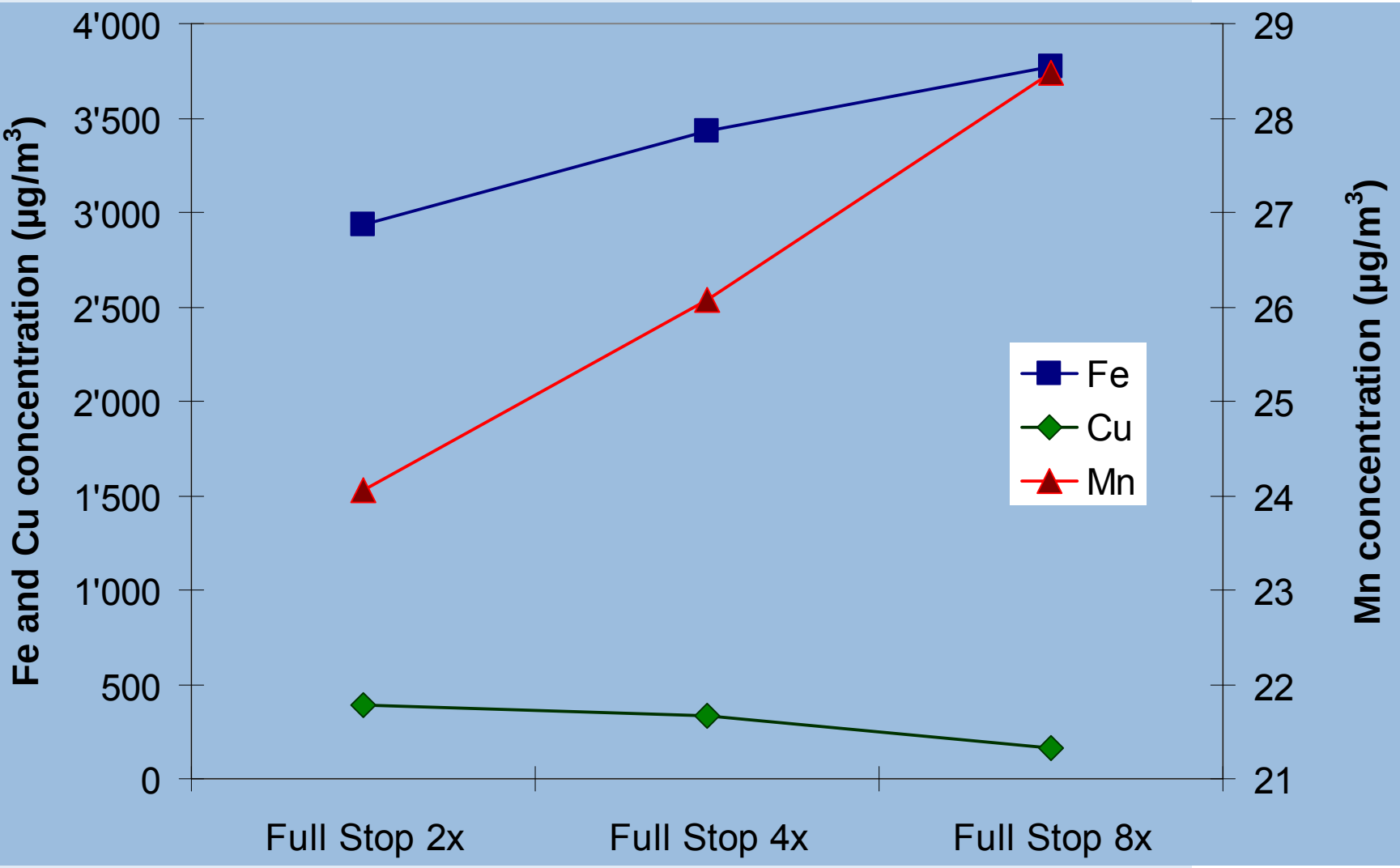


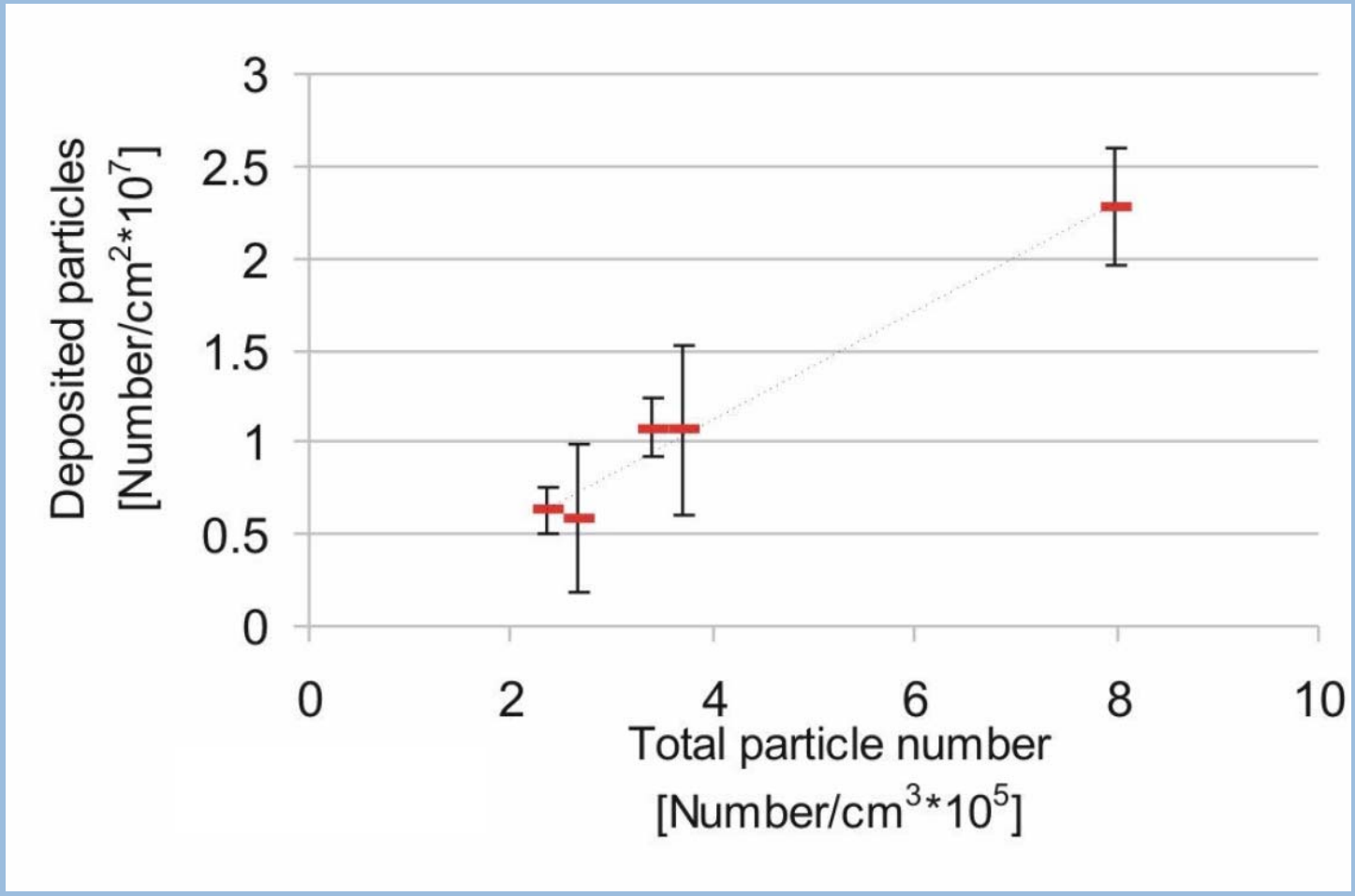
+ uric acid

+ BUN, +MCV
+ vWF, - protein C
+ neutrophils, - lymphocytes
+ MCL, + SDNN, + pNN50, + ectopy

Riediker et al, PFT 2004

Influence of repeated full stop braking




Deposited particles [Number/cm2*107]
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
Total particle number [Number/cm3*105]

Correlations

	IL-8	Occludin	ROS
Iron	0.217	-0.678*	0.476
Copper	0.259	-0.615*	0.392
Manganese	0.224	-0.636*	0.434
TC	0.608*	0.014	0.028
OC	0.594*	0.049	0.000
EC	0.342	-0.577	0.523
Number	0.364	-0.301	0.196
Mass	0.231	-0.469	0.490
Surface	0.217	-0.385	0.385

Spearman's rank correlations (2-sided significance) for fixed factors and endpoints.

*$p<0.05$.

Table overview

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		PAK*	PAK**	Eisen	Kupfer	Mangan	TC	OC	EC	Anzahl*	Masse*	Oberfläche*	Anzahl**	Masse**	Oberfläche**
		[pA]	[pA]	[ug/m3]	[ug/m3]	[ug/m3]	[ug/m3]	[ug/m3]	[ug/m3]	[/cm3]	[ug/m3]	[mm2/m3]	[/cm3]	[ug/m3]	[mm2/m3]
Bremstyp 1	Vollbremsung 2x I	152	1.05	1657	≤12	12.1	428	399	nd	nd	nd	nd	nd	nd	nd
31.05.2007	Normalbremsung 8x I	166	0.15	893	≤6	5.1	395	392	nd	nd	nd	nd	nd	nd	nd
	Normalbremsung 8x II	191	0.24	469	≤6	3.9	139	142	nd	nd	nd	nd	nd	nd	nd
	Nulldurchgang	31	0.05	163	≤6	0.9	106	105	nd	nd	nd	nd	nd	nd	nd
Bremstyp 3	Vollbremsung 2x	280	2.14	2716	203	20.9	230	213	nd	147197	8391	21143	25833	1600	3583
14.06.2007	Vollbremsung 4x	616	2.97	4723	395	29.0	317	275	42	277624	14314	54978	27564	1848	5573
	Normalbremsung 8x	503	1.42	2695	230	22.9	311	296	nd	204502	15250	35056	12501	976	1913
	Normalbremsung 16x	770	0.50	3052	265	21.1	252	212	40	349568	23094	73002	6686	487	1049
	Nulldurchgang	270	0.67	1558	105	9.5	147	127	20	162514	9261	23315	9771	518	1179
Bremstyp 4	Vollbremsung 2x	249	1.56	1608	110	18.2	340	285	nd	131586	7935	15548	16627	1100	1733
21.06.2007	Vollbremsung 4x	504	1.35	2144	154	21.6	484	430	nd	187725	11607	28635	10609	799	1585
	Normalbremsung 8x	162	0.19	509	27	7.7	286	277	nd	154883	8010	14858	6601	561	678
	Normalbremsung 16x	304	0.24	933	47	9.6	174	151	nd	220088	11401	26920	6292	342	607
	Nulldurchgang	171	0.45	561	39	8.0	56	47	nd	116472	6434	15889	5627	426	697
Bremstyp 5	Vollbremsung 2x	1047	7.58	5747	850	45.0	719	523	196	370292	16921	66797	43816	1757	6938
28.06.2007	Vollbremsung 4x	964	4.96	4501	872	33.7	622	475	147	nd	nd	nd	15825	1134	2647
	Vollbremsung 10x	1534	2.42	5826	926	44.1	265	224	41	338717	31429	87006	9289	985	2029
	Normalbremsung 8x	1658	4.48	3371	385	27.1	nd	nd	nd	797600	36431	132287	34244	1930	5813
	Normalbremsung 16x	1030	1.52	1731	308	13.1	nd	nd	nd	264535	33889	65954	9253	810	1538
	Nulldurchgang	1285	2.85	7612	1219	55.6	nd	nd	nd	236867	33226	70254	10517	1554	2755
Bremstyp 6	Vollbremsung 2x	317	2.25	nd	nd	nd	3920	3799	nd	220704	14747	44287	30461	2207	5805
09.07.2007	Vollbremsung 4x	682	3.75	4884	396	35.5	1497	1310	72	350571	17765	56016	43566	2064	6443
	Vollbremsung 10x	901	0.76	3619	310	25.5	nd	nd	nd	439201	27252	79650	6861	583	1315
	Normalbremsung 8x	714	0.73	3699	280	25.0	1564	1545	nd	440106	27663	87627	4531	455	867
	Normalbremsung 16x	739	0.17	1763	120	11.9	3239	3160	29	nd	nd	nd	2886	261	523
	Nulldurchgang	115	0.07	nd	nd	nd	5379	5296	nd	79447	6495	15243	1771	91	162

> IL-8: Comparison with DEP & Scooter exhaust