Supplementary file: Modrzynska et al. Effect on mouse liver morphology of CeO_2, TiO_2 and carbon black nanoparticles translocated from lungs or deposited intravenously

Particle detection in lung

In the lungs 1 day after intratracheal (IT) exposure to TiO_2 NPs, CeO_2 or carbon black (CB) nanoparticles (NPs), small agglomerates of foreign material were dispersed mainly in the alveolar region, either phagocytized by macrophages or free in alveolar lumen or at alveolar walls (Figure S1C and E and Figure S2G). 180 days after IT, the foreign material appeared less dispersed and was further accumulated in laden macrophages (Figure S1D and F and Figure S2I), and occasionally near blood vessels. The foreign material was also present in some but not all infiltrations of inflammatory cells in the lungs (Figure S1D and F).

Figure S1

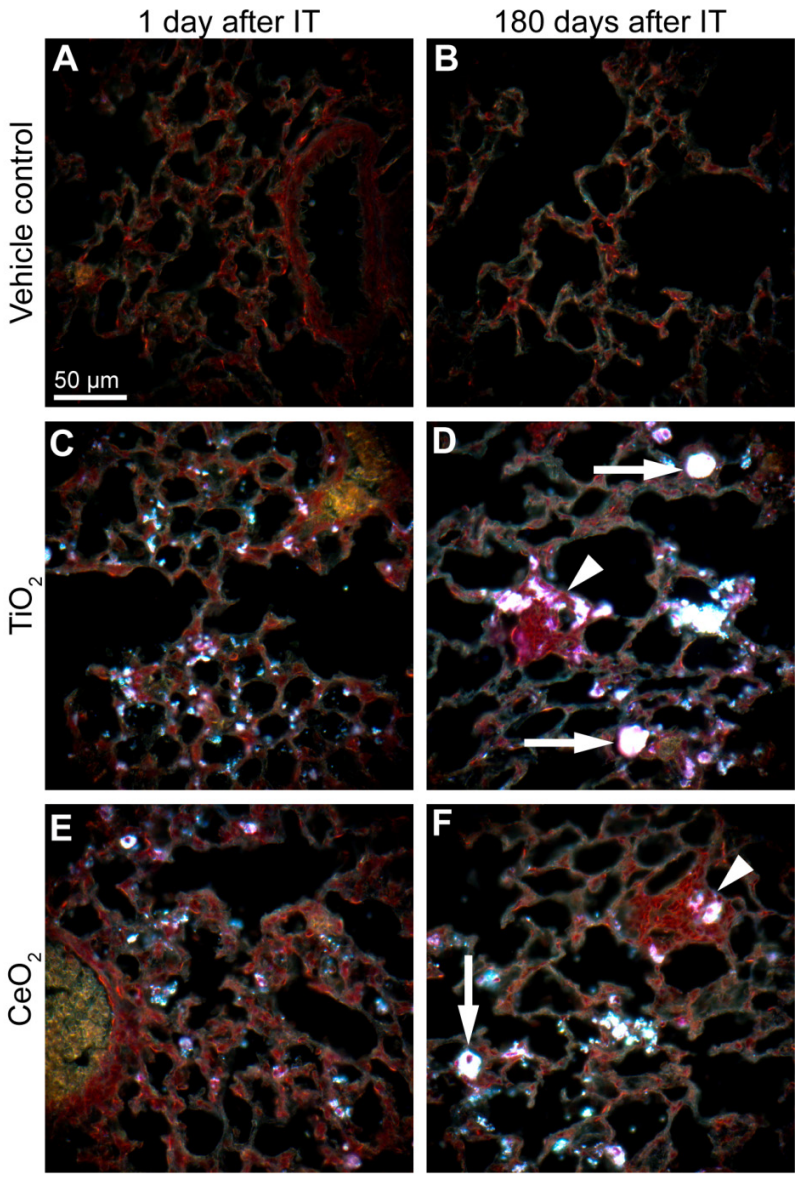

Figure S1. Lung tissue 1 and 180 days after IT exposure to vehicle control (A-B), TiO_2 (C-D) and CeO_2 (E-F) NPs. Enhanced darkfield microscopy. Light scattering agglomerates of foreign material (white) in the alveolar region. D and F: Agglomerates accumulated in laden macrophages (arrows) and occasionally in inflammatory cell infiltrates (arrow heads). Scale bar in A applies to all images.

Lung histology

The microscopic changes observed in the lungs after IT and intravenous (IV) exposures indicated inflammation (Table S2 and Figure S2 and Figure S3). One day after IT exposure, extravasation, oedema of connective tissue and peribronchial and/or perivascular inflammatory cell infiltration were recorded in all NPs instilled groups and single macrophages or single laden macrophages in the lumen or at the surface of alveoli were recorded in the TiO_2 and in CeO_2 NPs instilled groups (Table S2). These changes were also observed 28 and 180 days after IT exposure to all type of NPs. Small aggregates of laden macrophages were observed on day 28 in all NP-exposed groups. Aggregates of macrophages appeared bigger in size after exposure to TiO_2 NPs (Figure S2B) on day 28 and were also observed 180 days after exposure. Aggregates of inflammatory cells 180 days after IT exposure were both small and big in TiO_2 NP group (Figure S2C), big in CeO_2 NP group (Figure S2F), and relatively small in CB group (Figure S2I) as compared to the two other treated groups.

One day after IV exposure, inflammatory cell infiltrations in the vehicle control and TiO_2 NP groups, single macrophages in all groups, extravasation in the CB group (Table S2) and oedema of connective tissue between bronchioles and blood vessels in the vehicle control and NP treated groups were found (Figure S3A and B). Oedema was the most prevalent in the CeO_2 NP group as indicated by its presence in all examined animals (Table S2). In the CB group, foreign material was observed in laden macrophages (as indicated by the larger and dark appearance), in the lumen of some blood vessels (Figure S3E) and in the alveolar lumen, which could indicate migration of CB NPs to the alveolar space. 28 days after IV exposure oedema of connective tissue was still present in all groups and 180 days in the TiO_2 NP and CB groups. Single macrophages were recorded in the control group on day 180, and in the TiO_2 NP and CB groups on days 28 and 180.

Other microscopic changes in lungs (not included in Table S2) in groups exposed either by IT or IV were: (a) desquamation of bronchiole epithelium following IT exposure on day 28 in the vehicle control (1/3), following IV exposure on day 1 and 28 in the vehicle control (1/3 at both time points) and in the TiO_2 NP group (2/3 and 1/3 respectively); (b) hyperplasia of connective tissue (visible as single fibroblasts of supportive tissue surrounding bronchioles) after IV exposure on 1 day in the CeO_2 NP (1/3) and CB (1/3) groups and on day 28 in the TiO_2 NP group (1/3) and (c) emphysema after IV exposure in CB group (1/3).

Furthermore, congestion was seen in all groups regardless of exposure route and time and therefore it was not considered treatment related but as a consequence of insufficient exsanguination of the carcass.

Figure S2

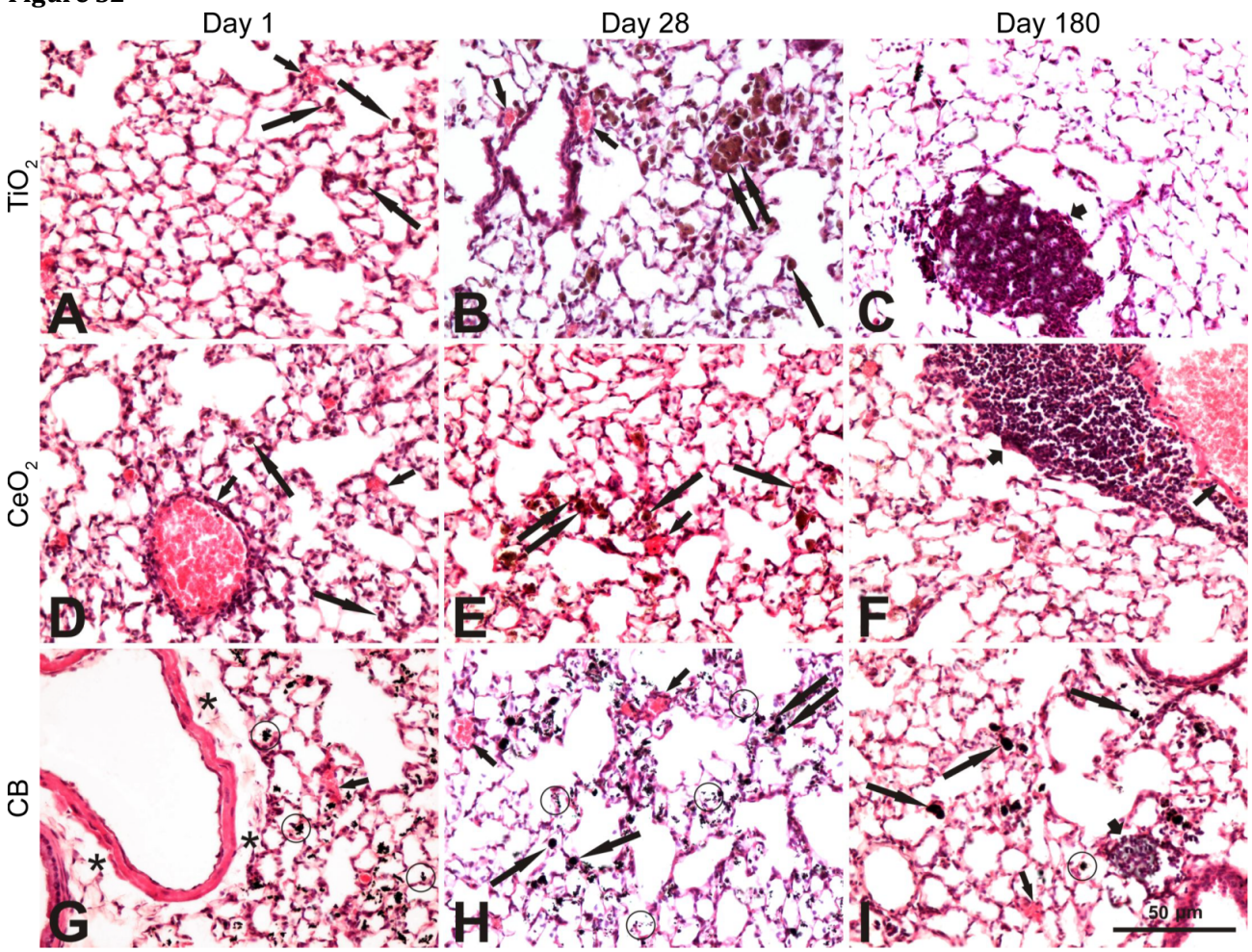

Figure S2. Microscopic changes in the lungs (N=3) of mice after intratracheal instillation (IT) with TiO_2 (A-C), CeO_2 (D-F) and CB (G-I) NPs 1, 28, or 180 days respectively. A-C: congestion (short arrows), single macrophages (arrows), big aggregates of macrophages (double arrows), inflammatory cell aggregate (short wide arrow); D-F: congestion (short arrows), single (arrows) and small aggregates (double arrows) of macrophages, perivascular inflammatory cell aggregate (short wide arrow); G-I: congestion (short arrows), oedema (asterisks), single macrophages (arrows), aggregate of inflammatory cells (short wide arrow), test material (circle). HE stain, scale bar in I applies to all images.

Figure S3

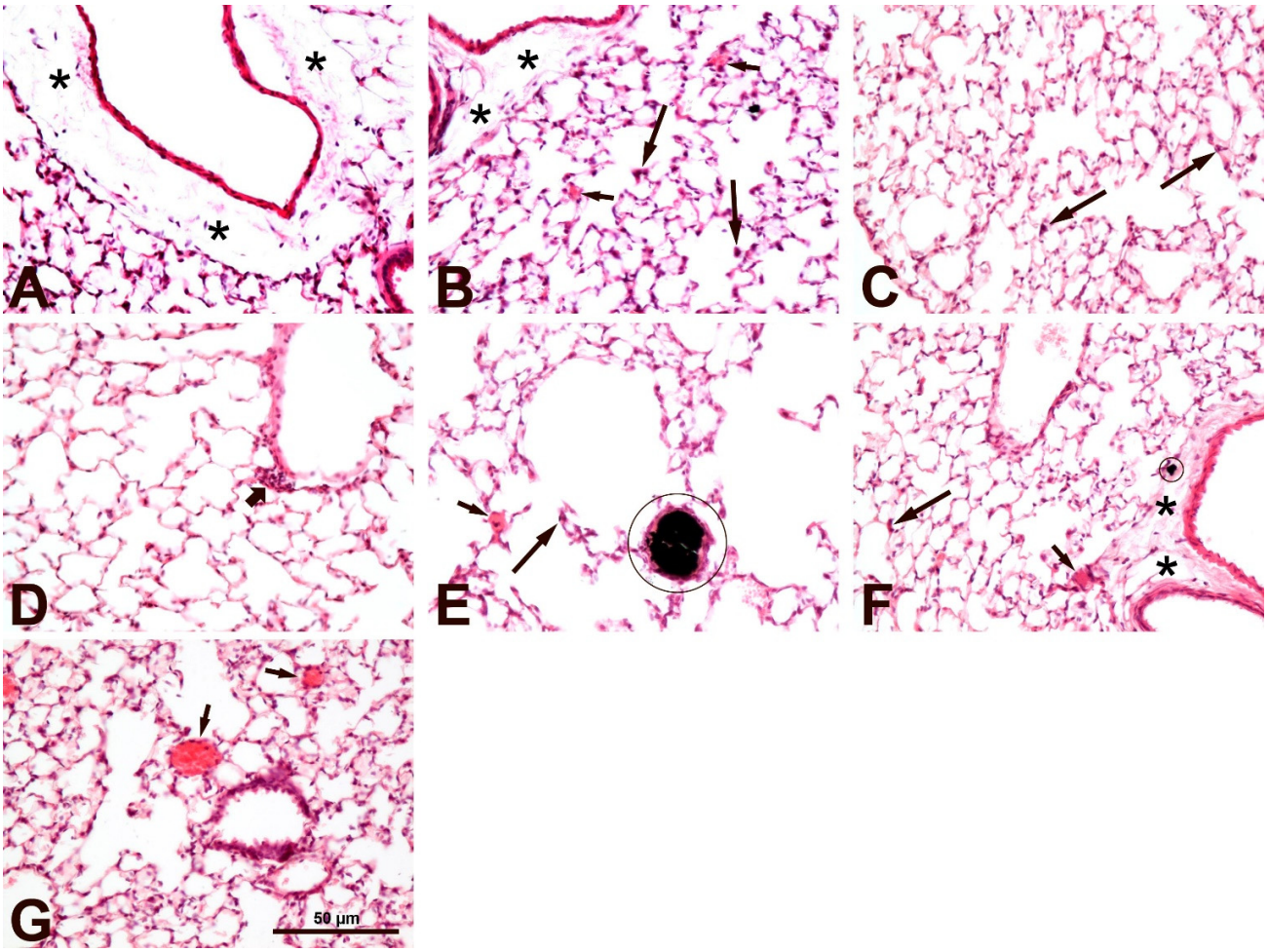

Figure S3. Microscopic changes in the lungs (N=3-4) of mice sacrificed at different times after intravenous (IV) exposure to the test nanomaterials or a vehicle. A-C: TiO_2 1, 28 or 180 days after IV exposure respectively: congestion (short arrows), single macrophages (arrows), perivascular oedema (asterisks); D: CeO_2 IV 180 days after exposure: perivascular small infiltration of inflammatory cells; E-F: CB 1 or 28 days after IV exposure: small congestion (short arrows), oedema (asterisks), single macrophages (arrows), test material (circle); G – vehicle control 28 days after IV exposure: normal structure of the lungs with congestion (short arrows). HE stain, scale bar in G applies to all images.

Table S1. Haematology results (mean ± SD) of mice 1, 28 or 180 days after a single exposure to 162 μg of TiO_2, CeO_2 or CB NPs administered by intratracheal instillation or intravenous injection.

	1 day				28 days				180 days			
	Control	TiO_2	CeO_2	CB	Control	TiO_2	CeO_2	CB	Control	TiO_2	CeO_2	CB
Intratracheal instillation												
WBC	3.7 ± 1.2	3.8 ± 1.3	4.7 ± 1.3	3.6 ± 0.8	5.7 ± 1.0	5.4 ± 1.4	5.4 ± 1.9	5.5 ± 1.5	5.4 ± 2.5	4.4 ± 1.1	5.1 ± 1.9	6.4 ± 2.2
RBC	10.7 ± 0.5	10.3 ± 1.5	9.9 ± 1.9	10.9 ± 0.2	10.5 ± 0.4	10.3 ± 0.3	9.5 ± 2.5	10.6 ± 0.4	10.2 ± 0.4	10.5 ± 0.5	10.2 ± 0.4	10.3 ± 0.3
HGB	16.3 ± 0.6	15.6 ± 2.1	14.9 ± 2.8	16.2 ± 0.3	15.6 ± 0.6	15.3 ± 0.4	14.0 ± 3.5	15.5 ± 0.4	15.5 ± 0.7	15.4 ± 0.6	14.9 ± 0.4	15.0 ± 0.3
HCT	51.9 ± 2.0	49.4 ± 7.2	47.5 ± 9.2	52.0 ± 1.1	49.7 ± 1.7	48.9 ± 1.1	45.2 ± 12.2	50.2 ± 1.5	47.5 ± 2.2	47.9 ± 2.4	46.8 ± 1.6	47.4 ± 1.4
MCV	48.4 ± 0.5	48.0 ± 0.5	48.1 ± 0.6	47.9 ± 0.6	47.6 ± 0.5	47.7 ± 0.9	47.2 ± 0.7	47.3 ± 0.7	46.7 ± 0.9	45.8 ± 0.4	46.2 ± 1.0	46.1 ± 0.4
MCH	15.2 ± 0.2	15.2 ± 0.3	15.0 ± 0.2	14.9 ± 0.3	15.0 ± 0.2	14.9 ± 0.3	14.8 ± 0.6	14.6 ± 0.2	15.2 ± 0.4	14.7 ± 0.3	14.7 ± 0.3	14.6 ± 0.2
MCHC	31.3 ± 0.3	31.6 ± 0.4	31.4±0.3	31.1±0.5	31.5±0.5	31.2±0.4	31.3±1.4	31.9±3.5	32.6±0.2	32.2±0.5	31.9±0.3[a]	31.6±0.3[a]
PLT	714 ± 163	656 ± 177	678±133	750±63	743±82	718±80	688±114	685±93	756±95	771±198	815±133	856±213
Intravenous injection												
WBC	4.2 ± 1.4	4.5 ± 1.4	4.2 ± 1.1	3.6 ± 1.2	5.8 ± 1.6	5.2 ± 1.8	4.6 ± 1.5	4.8 ± 0.9	6.6 ± 3.0	4.5 ± 1.8	4.6 ± 1.7	4.3 ± 1.5
RBC	10.1 ± 1.5	10.5 ± 0.4	10.3 ± 0.7	10.4 ± 0.7	10.3 ± 0.5	9.9 ± 1.3	10.4 ± 0.3	10.2 ± 0.4	10.5 ± 0.6	10.9 ± 0.4	10.1 ± 0.5	10.2 ± 0.4
HGB	15.6 ± 2.2	16.0 ± 0.6	15.8 ± 0.8	15.9 ± 1.0	15.6 ± 0.8	14.8 ± 1.7	15.5 ± 0.3	15.3 ± 0.6	15.6 ± 0.5	15.6 ± 0.5	14.9 ± 0.7	15.1 ± 0.5
HCT	48.5 ± 7.4	50.6 ± 2.1	49.5 ± 3.6	49.9 ± 3.2	49.0 ± 2.4	47.5 ± 6.2	49.5 ± 1.1	48.4 ± 2.1	48.6 ± 2.0	49.7 ± 1.5	46.8 ± 2.4	47.5 ± 1.4
MCV	48.0 ± 0.9	48.0 ± 0.5	48.0 ± 0.0	48.1 ± 0.6	47.9 ± 0.4	47.8 ± 0.7	47.8 ± 0.4	47.7 ± 0.7	46.3 ± 0.7	45.8 ± 0.7	46.2 ± 0.7	46.3 ± 1.1
MCH	15.5 ± 0.2	15.2 ± 0.1	15.3 ± 0.5	15.4 ± 0.2	15.2 ± 0.2	14.9 ± 0.5	15.0 ± 0.3	15.0 ± 0.3	14.8 ± 0.4	14.4 ± 0.4	14.7 ± 0.4	14.8 ± 0.5
MCHC	32. ±0.6	31.6±0.4	32.1 ± 1.2	31.9 ± 0.2	31.8 ± 0.4	31.3 ± 0.9	31.3 ± 0.4	31.5 ± 0.4	32.0 ± 0.4	31.4 ±0.4[a]	31.8 ± 0.6	31.9 ± 0.2
PLT	828±50	483±224[a]	796 ± 46	686 ± 218	676 ± 81	673 ± 197	714 ± 48	765 ± 42	795 ± 144	743 ±240	892 ± 156	808 ± 120

WBC - white blood cells ($x10^3$/μl), RBC – red blood cells ($x10^6$/ μl), HGB – hemoglobin (g/dl), HCT – hematocrit (%), MCV – mean corpuscular volume (fL), MCH – mean corpuscular hemoglobin (pG), MCHC – mean corpuscular hemoglobin concentration (%), PLT – platelets ($x10^3$/μl). ([a]) denotes $P < 0.05$ of blood parameter level in exposed groups compared to the vehicle control group (analysis of variance ANOVA followed by Tukey *post-hoc* multiple comparison test). Number of animals per group was 9 except for 8 in the IV control group after 28 days and in the IT CB, IV TiO_2 and PO CB NPs groups after 180 days.

Table S2. Type and incidence of typical microscopic changes in the lungs of mice 1, 28 or 180 days after a single exposure to 162 µg of TiO_2, CeO_2 or CB NPs administered by intratracheal instillation or intravenous injection.

Change, lung	Intratracheal instillation				Intravenous injection			
	Control	TiO_2	CeO_2	CB	Control	TiO_2	CeO_2	CB
Inflammatory cell infiltration peribronchial and/or perivascular								
day 1	0/3	1/3	1/3	1/3	1/3	1/3	0/3	0/3
day 28	1/3	1/3	1/3	1/3	0/3	0/3	0/3	1/3
day 180	0/3	3/3	2/3	2/3	1/3	0/3	2/3	0/3
Macrophages single								
day 1	0/3	3/3	1/3	0/3	1/3	1/3	1/3	0/3
day 28	1/3	2/3	1/3	0/3	0/3	0/3	0/3	1/3
day 180	0/3	2/3	2/3	0/3	1/3	1/3	0/3	0/3
Macrophages single laden (with the test material)								
day 1	0/3	3/3	0/3	0/3	0/3	0/3	0/3	1/3
day 28	0/3	3/3	3/3	3/3	0/3	1/3	0/3	0/3
day 180	0/3	1/3	1/3	3/3	0/3	1/3	0/3	1/3
Macrophages (laden), aggregates small[a]								
day 1	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
day 28	0/3	3/3	1/3	1/3	0/3	0/3	0/3	0/3
day 180	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
Macrophages (laden) aggregates big[b]								
day 1	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
day 28	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
day 180	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
Oedema of connective tissue peribronchial and/or perivascular								
day 1	0/3	2/3	3/3	1/3	1/3	2/3	3/3	2/3
day 28	3/3	2/3	3/3	1/3	1/3	3/3	2/3	1/3
day 180	0/3	3/3	1/3	2/3	0/3	1/3	0/3	1/3
Congestion								
day 1	2/3	3/3	3/3	2/3	2/3	1/3	2/3	1/3
day 28	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
day 180	2/3	1/3	3/3	2/3	3/3	3/3	0/3	0/3
Extravasation								
day 1	2/3	3/3	1/3	1/3	0/3	0/3	0/3	1/3
day 28	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
day 180	2/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3

Changes are expressed by their incidence: the number of animals with a given change of the total number of animals examined in the group. The data were not subjected to a statistical analysis due to the low number of samples per group. [a]: 1-4 macrophages at one site close to each other; [b]: more than 5 macrophages at one site close to each other.

Table S3. Incidence and number of inflammatory cell infiltrations in the liver of mice 1, 28 or 180 days after a single intratracheal or intravenous exposure to 162 µg /animal of TiO_2 NPs, CeO_2 NPs or CB.

Inflammatory cell infiltrations	Intratracheal exposure				Intravenous exposure			
	Control	TiO_2	CeO_2	CB	Control	TiO_2	CeO_2	CB
Big								
Day 1								
Incidence[a]	3/9	4/9	3/9	3/9	6/9	2/9	6/9	1/9*
Total number[b]	5.0	5.0	8.0	3	10	2	11	3*
Mean[c]	0.56	0.56	0.89	0.33	1.11	0.22	1.22	0.33
(SD)	(1.01)	(0.73)	(1.69)	(0.50)	(1.27)	(0.44)	(1.30)	(1.00)
Multiplicity[d]	1.67	1.25	2.67	1.00	1.67	1.00	1.83	3
Day 28								
Incidence	4/9	4/9	3/9	5/9	4/8	7/9	3/9	6/9
Total number	6	5	4	13	4	11	3	15
Mean	0.67	0.56	0.44	1.44	0.50	1.22	0.33	1.67
(SD)	(0.87)	(0.72)	(0.72)	(1.51)	(0.53)	(0.83)	(0.50)	(2.06)
Multiplicity	1.5	1.25	1.33	2.60	2.00	1.57	1.00	2.5
Day 180								
Incidence	6/9	3/9	6/9	2/8	4/9	4/8	4/9	5/9
Total number	11	6	10	2	8	10	9	9
Mean	1.22	0.67	1.11	0.25	0.89	1.25	1	1.00
(SD)	(1.30)	(0.32)	(1.05)	(0.46)	(1.67)	(1.75)	(1.50)	(1.32)
Multiplicity	1.83	2.00	1.67	1.00	2.00	2.50	2.25	1.80
Small								
Day 1								
Incidence	5/9	2/9	6/9	1/9	3/9	7/9	8/9*	6/9
Total number	8	2	12	1 *	6	11	12	10
Mean	0.89	0.22	1.33	0.11	0.67	1.22	1.33	1.11
(SD)	(1.05)	(0.44)	(1.32)	(0.33)	(1.32)	(0.97)	(1.00)	(1.55)
Multiplicity	1.6	1	2	1	2	1.57	1.50	1.67
Day 28								
Incidence	9/9	9/9	8/9	4/9*	5/8	8/9	6/9	1/9*
Total number	25	17*	25	11*	13	12	15	1*
Mean	2.78	1.89	2.78	1.22	1.62	1.33	1.67	0.11
(SD)	(1.09)	(1.69)	(2.54)	(1.72)	(1.77)	(0.87)	(1.41)	(0.33)

Multiplicity	2.78	1.89	3.13	2.75	2.6	1.50	2.50	1
Day 180								
Incidence	6/9	5/9	5/9	2/8	5/9	3/8	6/9	3/9
Total number	11	9	8	2	10	9	13	3
Mean	1.22	1.00	0.89	0.25	1.11	1.13	1.44	0.33
(SD)	(1.30)	(1.12)	(1.05)	(0.46)	(1.90)	(1.81)	(1.42)	(0.50)
Multiplicity	1.83	1.80	1.60	1	2.00	3	2.17	1

[a]: Incidence of lesion is expressed by the number of animals in the group with a given liver lesion of total number examined. The incidence data were analysed using Fisher' exact test. *: $p<0.05$.

[b]: Number of inflammatory cell infiltrations of a given type from all liver samples examined in the group. For each animal a liver sample from the left lobe was used for counting of inflammatory cell infiltrations. The data were analysed using Mann Whitney U test. *: $p<0.05$.

[c]: Mean number of inflammatory cell infiltrations of a given type / number of liver samples examined.

[d]: Mean number of inflammatory cell infiltrations of a given type / number of liver samples with the given inflammatory cell infiltration in the group.

Table S4. Microscopic liver changes with incidence not statistically significantly different from that in control groups 1, 28 or 180 days after a single exposure of mice to 162 μg of TiO_2 NPs, CeO_2 NPs and CB administered by intratracheal instillation or intravenous injection.

Change, liver	Intratracheal instillation				Intravenous injection			
	Control	TiO_2	CeO_2	CB	Control	TiO_2	CeO_2	CB
Necrotic hepatocytes adjacent to infiltrations of inflammatory cells								
day 1	3/9	4/9	5/9	4/9	8/9	9/9	8/9	8/9
day 28	5/9	6/9	7/9	6/9	5/8	7/9	7/9	9/9
day 180	4/9	5/9	6/9	3/8	2/9	1/8	3/9	4/9
Hyperplasia (increased number) of Kupffer cells								
day 1	0/9	1/9	1/9	0/9	0/9	1/9	0/9	4/9
day 28	1/9	1/9	1/9	2/9	2/8	0/9	6/9	1/9
day 180	0/9	0/9	1/9	0/8	0/9	0/8	0/9	0/9
Hyperplasia of oval cells								
day 1	0/9	0/9	0/9	0/9	9/9	7/9	0/9	6/9
day 28	1/9	2/9	1/9	3/9	2/8	2/9	4/9	6/9
day 180	3/9	1/9	3/9	0/8	0/9	1/8	0/9	0/9
Hyperplasia of connective tissue near bile ductules or venules (fibrosis)								
day 1	0/9	0/9	0/9	0/9	2/9	7/9	1/9	0/9
day 28	0/9	2/9	0/9	0/9	0/8	0/9	1/9	3/9
day 180	0/9	0/9	2/9	1/8	1/9	1/8	1/9	0/9
Sinusoidal dilatation								
day 1	0/9	0/9	0/9	0/9	3/9	8/9	3/9	2/9
day 28	1/9	0/9	2/9	0/9	0/8	0/9	0/9	0/9
day 180	0/9	2/9	0/9	0/8	0/9	0/8	0/9	0/9
Congestion								
day 1	0/9	0/9	0/9	0/9	1/9	5/9	1/9	4/9
day 28	6/9	0/9	0/9	0/9	3/8	0/9	6/9	0/9
day 180	0/9	1/9	1/9	0/8	0/9	2/8	0/9	1/9

Changes are expressed by their incidence: the number of animals with a given change of the total number of animals examined in the group. The incidences in the exposed groups were not significantly different from the controls ($p>0.05$, Fischer's exact probability test).