



Rijksinstituut voor Volksgezondheid  
en Milieu  
Ministerie van Volksgezondheid,  
Welzijn en Sport



## Cerium Oxide: inhalation studies

Symposium 'De milieu-effecten  
van nanoproducten

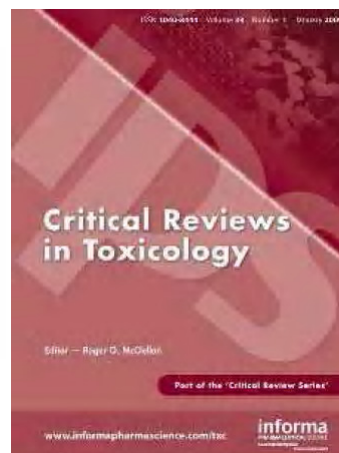
4 november

## Cerium critical review on toxicology

### Exposure and Health Effects Review of Engineered Nanoscale Cerium and Cerium Oxide Associated with its Use as a Fuel Additive

Journal:	Critical Reviews in Toxicology
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In press, 2010





## Conclusions in the review

- CeO<sub>2</sub> nanoparticles are mainly designed to be used in a dispersive manner as a diesel fuel combustion catalyst.
- The potential environmental, health and ecological effects of cerium nanoparticles remain uncertain and require additional research.
- This assessment is based on a limited environmental and health effects database, much of which is derived from controlled (combustion) studies and in vitro toxicology studies.
- Currently, there are no in vivo toxicological assessments derived from acute, sub chronic or chronic inhalation exposures to CeO<sub>2</sub> nanoparticles or to diesel emissions generated with the use of these particles as a fuel additive.



## Conclusions in the review

- Therefore, there is an absolute critical need for integrated exposure and toxicological studies in order to accurately assess the environmental, ecological and health implications of nanotechnology enabled diesel fuel additives with existing as well as new engine designs and fuel formulations.
- In line with “nano-ceriumoxide als brandstofverbeteraar”



## Studies

- Sub-acute (28-day) inhalation studies with 3 cerium oxides differing in size of which at least 2 with primary particle size <100 nm according to OECD TG 412
- Sub-acute inhalation studies with **cerium oxides as applied in diesel**







## Three 4-week studies pure cerium oxide

- All 28-day studies
  - 3 dose levels
  - 3 sizes of particles of which 2 <100 nm
  - Effects determined right after the last exposure day and 14 days post exposure (recovery).
    - > Pathology lung, BALF parameters such as inflammation, oxidative stress, cytotoxicity
    - > Cardiovascular endpoint (blood, tissue, aorta tissue (ApoE only))
    - > Brain (not started)
  - Pilot data on kinetics (not analysed)
- Moderate effects in highest dose → recovery groups will be analysed

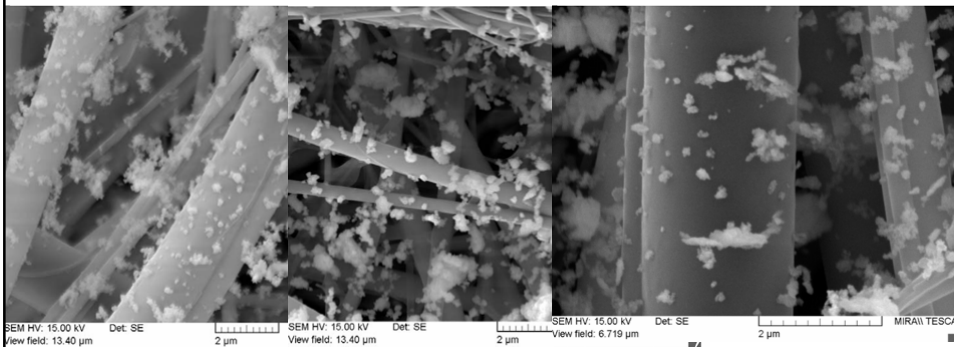


## Characteristics in air

	Dose	Size	Primary size
	2.5, 6.7 and 20.0 mg/m <sup>3</sup>	0.5 - 0.8 µm	40 nm
	5.9, 18.7 and 55.0 mg/m <sup>3</sup>	0.7 - 0.8 µm	< 5 µm
	1.2, 3.5 and 10.8 mg/m <sup>3</sup>	1.0 - 1.2 µm	50 nm 

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## Effects

- **Increased number of macrophages** involved in the clearance of particles
- **Increased neutrophil** content and increased biochemical parameters in broncho-alveolar lavage fluid indicated that exposure to Ceria Dry CeO<sub>2</sub> resulted in inflammation in the lungs at all dose levels.
- The inflammation, although at a lower level, was **still present** after a recovery period of 28 days.
- No-observed-adverse-effect level will be below the exposure level of 1.2 mg/m<sup>3</sup> equivalent 6-hour exposure concentration due to use **sensitive markers**.



## Diesel & cerium ENVIROX



### Exposure data: physical characteristics

	Mass (gravimetric) µg/m <sup>3</sup>	Mass (optical) µg/m <sup>3</sup>	Surface area µm <sup>2</sup> /cm <sup>3</sup> per TB region	Number counts #/cm <sup>3</sup> (x 10 <sup>10</sup> )	Diameter nm
1	1602 ± 98	1872 ± 132	4064 ± 114	5.7 ± 0.6	70 ± 1.8
2	1810 ± 36	1956 ± 26	4856 ± 113	5.8 ± 0.3	82 ± 1.8
3	1778 ± 252	1900 ± 32	4863 ± 213	4.8 ± 2.4	81 ± 1.8
4	1772 ± 65	1972 ± 43	3160 ± 429	4.4 ± 2.6	95 ± 1.8
Σ	1741 ± 153	1925 ± 79	4018 ± 605	5.3 ± 0.1	82 ± 1.8
1	1525 ± 105	1456 ± 747	3488 ± 484	3.1 ± 0.5	72 ± 1.8
2	1772 ± 73	1922 ± 131	4840 ± 169	3.6 ± 0.3	83 ± 1.8
3	1804 ± 137	1894 ± 89	3948 ± 350	3.0 ± 1.5	81 ± 1.8
4	1816 ± 159	1986 ± 9	3132 ± 450	2.9 ± 1.6	86 ± 1.8
Σ	1740 ± 162	1817 ± 408	3636 ± 517	3.6 ± 0.5	83 ± 1.8
ice	100%	84%	90%	69%	101%



## Diesel & cerium ENVIROX

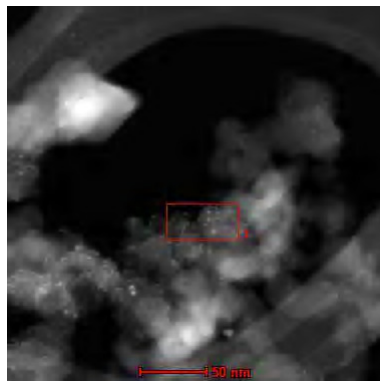


Table 2: Exposure data gases

Week	CO ppm	NO ppm	NO <sub>x</sub> ppm
<b>DE</b>			
1	10.4 ± 0.9	37.2 ± 3.9	39.4 ± 4.3
2	11.4 ± 1.1	37.6 ± 4.5	40.2 ± 4.3
3	11.0 ± 1.4	41.2 ± 5.8	43.4 ± 4.6
4	7.2 ± 1.9	17.0 ± 1.2	18.2 ± 1.3
<b>Average</b>	<b>10.0 ± 2.1</b>	<b>33.3 ± 10.5</b>	<b>35.3 ± 10.8</b>
<b>DGeE</b>			
1	10.6 ± 1.1	37.8 ± 5.8	39.8 ± 6.4
2	12.4 ± 1.1	42.4 ± 3.6	45.2 ± 3.3
3	11.6 ± 0.5	45.0 ± 2.9	47.0 ± 2.3
4	7.8 ± 1.3	19.2 ± 3.3	20.8 ± 3.7
<b>Average</b>	<b>10.6 ± 2.0</b>	<b>36.1 ± 11.0</b>	<b>38.2 ± 11.3</b>
<b>Difference</b>	<b>106%</b>	<b>109%</b>	<b>108%</b>

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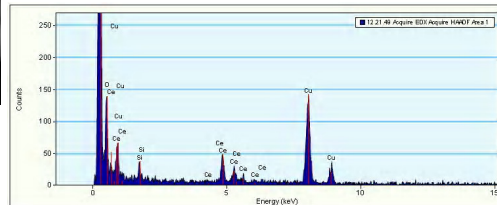
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The small white dots are Cerium containing particles.

Typical size of the nano-particles: 1-2 nm.

Up to 6 ug/mg particle mass is cerium



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## Diesel & cerium ENVIROX



Table x Histopathological observation in males after a 4-week exposure. Values indicate the number of animals

	DCeE				DE			
	Control	Low	Mid	High	Control	Low	Mid	High
Total number of animals per group	5	5	5	5	5	5	5	5
Brain -meningeal pigmented cells	0	0	0	0	1			2
Heart								
-adhesion to thoracic cavity	0	0	0	0	0	1	0	0
-foam-cell inflammation	0			3	0			0
Kidney								
-basophilic tubules	0			0	0		0	1
-hydronephrosis	0	0	0	0	0	0	1	0
-mononuclear cell infiltrate	1	0	0	0	1	0	1	1
Liver								
- increased hepatocellular vacuolation	2			1	1			2
-mixed inflammatory cells)	5			4	5			5
Lungs -pigmented macrophages	0			5*	0			5
Nasal cavity								
-dermatitis	2			2	0			0
-mononuclear cell infiltration	1			0	0			0

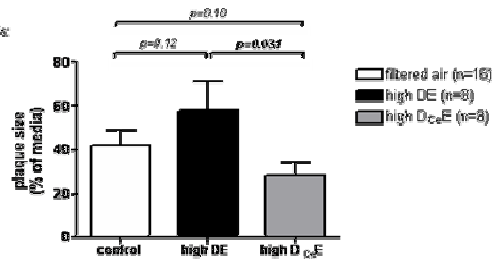
No signs for attributable (adverse) effects of cerium oxide



## Effect of diesel exhaust on the size of atherosclerotic plaques in the brachiocephalic artery



unpaired t-test:



unpaired t-test/ANOVA:

Mean±S.E.M. (n=8-16), p-values shown are results from the corresponding statistical test indicated to the left of the value).



## Exposure and Toxicology



- Reduction of surface area and particle number at same mass concentration.
- Claimed reduction of particle mass seems true
- If any, then only mild effects on pathology, heamatology, biochemistry
- Increase in plaques in aorta due to diesel and decrease due to cerium, though not stat. Significant
- 



## Opmerkingen t.a.v. Nano-ceriumoxide als brandstofverbeteraar

- Afname van CO<sub>2</sub>, CO, NO<sub>x</sub> en fijn stof niet evident
- Maak onderscheid tussen de stof CeO<sub>2</sub> en deze stof na verbranding in dieselmotor =>> nano-cerium in roet
- Reden recent werk mee tox => andere vorm, gevoeliger parameters, gevoeliger testsysteem (in vitro)
- Risk = hazard x exposure. En 'these makes the poison'
  - Mogelijk toxiciteit diesel+cerium verhoogd maar blootstelling totaal lager
- Concept cost-benefit interessant maar lastig
- Maak onderscheid tussen acute en chronische blootstelling
  - Fall et al, heel acuut, RIVM langere periode (28 dgn)





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Thank you

Flemming R Cassee