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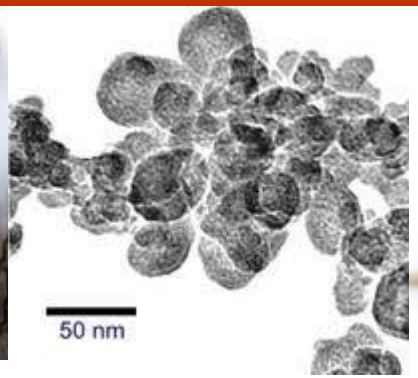

Which toxicological aspects support the classification of diesel engine exhaust as carcinogenic in humans?

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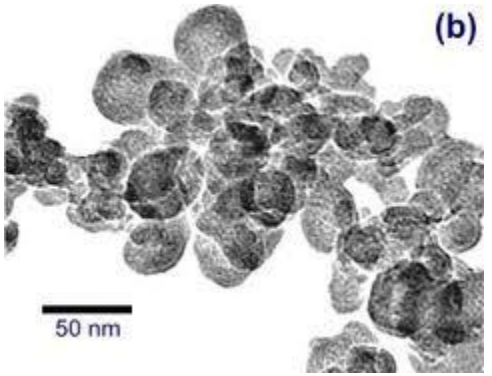
Content

- Conventional technology diesel engines
  - Mechanistic evidence
  - Evidence from animal studies
- After-treatment technology in diesel engines
  - Mechanistic evidence
  - Changes in emissions of carcinogens
- New technology diesel engines
  - Mechanistic evidence
  - How will the toxicity change
- Conclusions

Exhaust from conventional technology diesel engines

- Carbon particles
  - Particle size submicron 0.124 ± 0.025 µm CMD
  - Aggregate (grape)
  - High specific surface (30-50 m^2/g)
- Adsorbed substances
  - Semivolatile organic compounds (SVOC)
  - Metals and metal oxides
- Gas phase
  - Volatile organic compounds
  - Inorganic gases (NOx)



Deposition, retention and clearance of DEP in humans

Properties relevant to biological fate:

- Deposition: in non-ciliated part of the lungs
- Retention:
  - Particles: long during to poor water solubility
  - Organic substances slow lodging/leaching
- Clearance: half life of 100-300 days
- Lodging of organic substances: slow

Tumors by (any) inert ultrafine particles in rats in six steps

At (extremely) high doses of inert UFPs such as TiO_2 and carbon black lead to so-called overload conditions:

1. Phagocytosis of excessive quantities of DEP
2. Sequestration of particles in interstitial space
3. Influx of leucocytes → chronic inflammation
4. Reactive oxygen species (ROS) → oxidative damage
5. Proliferating lung cells have increased dividing activity
6. High expression of DNA-mutations →tumors

Suggestion: this does NOT happen at low doses!

But: rats are unlike humans uniquely sensitive for overload

Human volunteer studies

“Scandalous Misconduct by EPA: Lawsuit Filed for Exposing Subjects to Diesel Exhaust”

“EPA parked a truck's exhaust pipe directly beneath an intake pipe on the side of a building. The exhaust was sucked into the pipe, mixed with some additional air and then piped directly into the lungs of the human subjects. EPA actually has pictures of this gas chamber, a clear plastic pipe stuck into the mouth of a subject, his lips sealing it to his face, diesel fumes inhaled straight into his lungs.” source: www.sott.net

Non-cancer effects observed in humans

Volunteers

- Accumulation of DEP in macrophages
- Changes in lung cell populations
- Fibrotic effects
- Impaired pulmonary clearance

Workers

- Inflammation
- Increased susceptibility to infections
- Exacerbations of allergic response
- Cardiovascular response

Cancer-related effects observed in humans

Volunteers

- Increased expression of genes involved in oxidative stress and inflammation responses
- Inflammation in blood lymphocytes and cells in bronchoalveolar liquid (BAL)

Workers

- Bulky DNA-adducts (indicating involvement of PAH)
- Oxidative DNA and protein damage
- Putative mutations
- Chromosome breaks, sister chromatid exchanges, micronuclei, aneuploidy (abnormal no. of chromosomes)

Cancer-related effects observed in experimental systems

Rodents exposed to whole diesel exhaust, DEP suspensions, DEP extracts:

- Up-regulation of genes in pathways related oxidative stress inflammation, DNA damage,
- Up-regulation of genes related to defense responses: antioxidant responses, cell cycle, cell transformation and apoptosis

Mechanistic considerations: carcinogenic hazards

Known human carcinogens in the gas phase

- Acetaldehyde
- Acrolein
- Benzene
- 1,3-butadiene
- Formaldehyde
- Naphthalene

Known human carcinogens in the particle phase

- PAHs
- Nitro-PAHs
- Metals

Mechanistic considerations: genotoxic activities

DEP extracts show genotoxic activity *in vitro* and *in vivo*:

- Mutations in transgenic animals
- Bulky DNA-adducts
- Oxidative DNA damage
- DNA-strand breaks
- Unscheduled DNA synthesis
- Sister chromatide exchanges
- Chromosomal hydroxyl radicals

DEP extracts show co-carcinogenic activity in mice in vivo:

- Angiogenesis and vasculogenesis in mice

Mechanistic considerations: oxidative stress by ROS

Diesel exhaust components can generate reactive oxygen species (H_2O_2, O_2^-, $OH^\bullet$):

- Fresh and washed DEP
- Quinones formed by atmospheric or metabolic processes
- Inflammation → up-regulation of COX-2 → cell proliferation
- Metals
- Phagocytosis and

- ROS →oxidative DNA damage
- ROS →lipid peroxidation products →DNA adducts
- ROS →lipid peroxidation product aldehyde → cytotoxicity

Mechanistic considerations: workers studies

Workers exposed to diesel exhaust have enhanced levels of:

- 1-hydroxypyrene in urine (from gas and particle-phase of diesel exhaust but also possible other sources)
- 1-aminopyrene in urine (specific biomarker for DEP formed after reduction from 1-nitropyrene)
- 3-aminobenzanthrone in urine (specific biomarker for DEP formed after reduction from 3-nitrobenzanthrone)
- DNA adducts, DNA strand breaks, oxidative DNA damage and micronuclei in peripheral blood lymphocytes
- Up-regulation of genes related to oxidative stress and inflammation

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Classified as probable human carcinogens in 2012

Mechanistic considerations: volunteer studies

Human volunteers exposed to diesel exhaust in controlled chamber studies have demonstrated increases in:

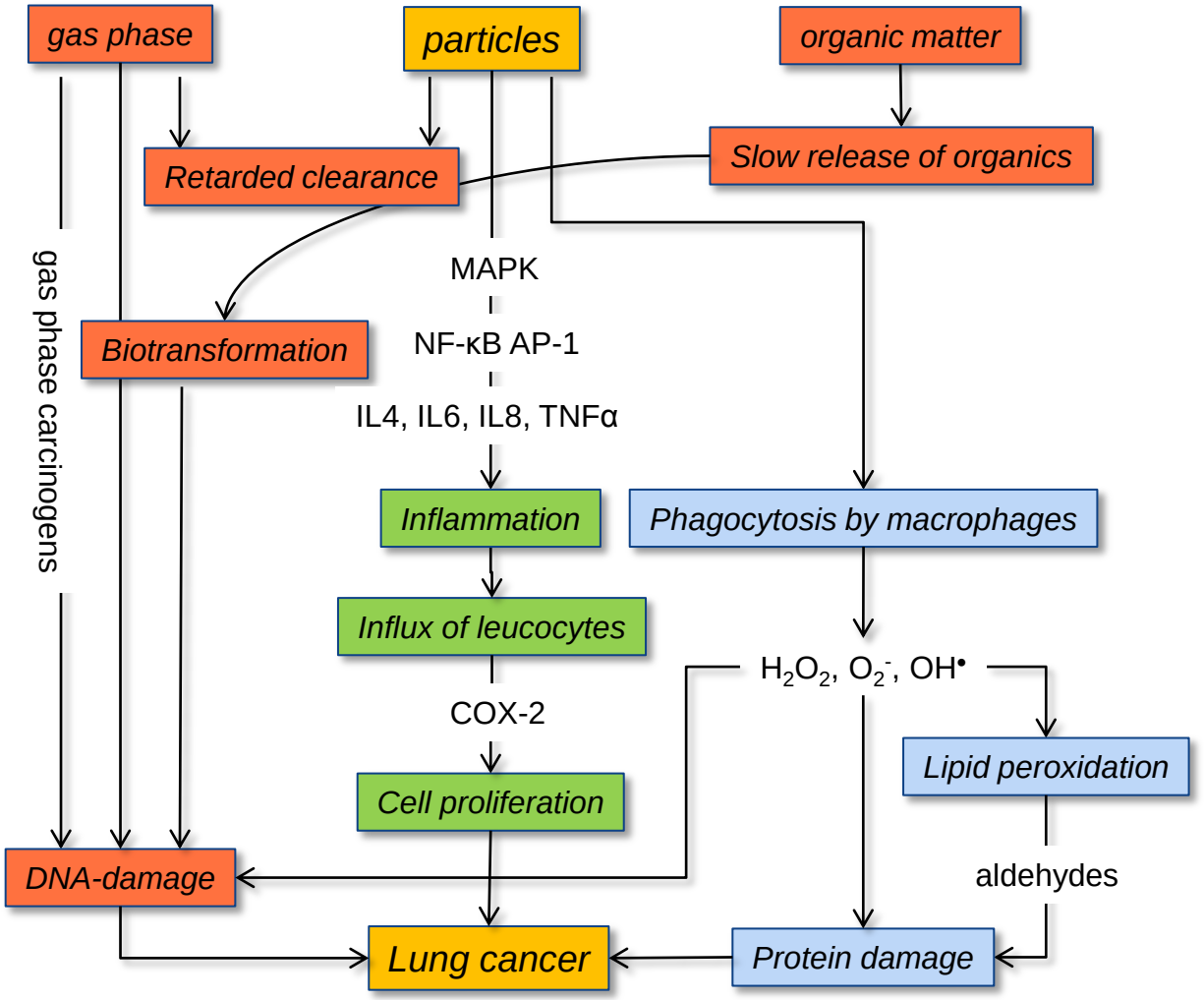
- Proteins and lymphocytes in brocheal lavage fluid (BAL)
- Interleukin-8 (IL-8) and increased IL-8 gene transcription →up-regulation of genes with key oxidative stress protein degradation and coagulation pathways
- Up-regulation of endothelial adhesion molecules
- Oncogene-α (GRO-α) protein growth expression
- Cascade of MAPK signaling pathways NF-κB and AP-1 transcription factors →pro-inflammatory mediators → airway leukocyte infiltration and inflammation

Conclusions from mechanistic studies

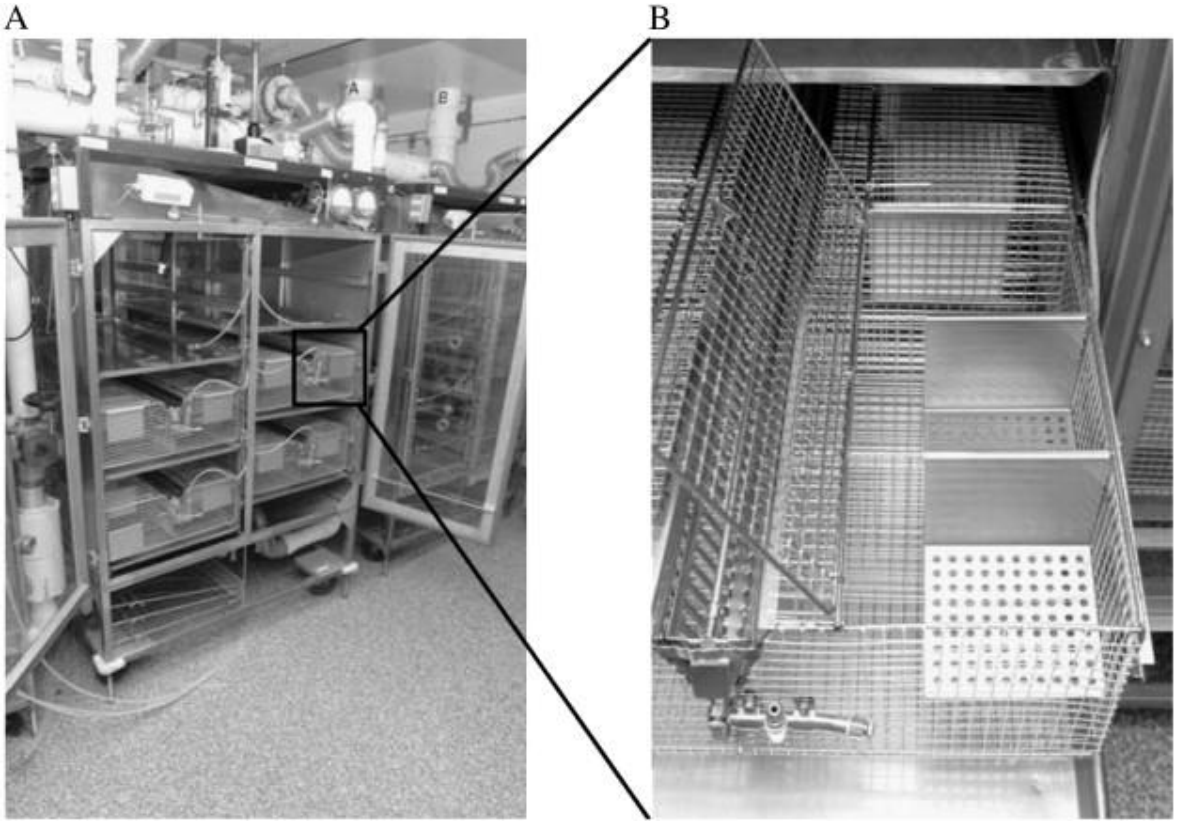
1. Strong mechanistic evidence for the ability of diesel engine exhaust – as well as many of it components - to induce lung cancer in humans inducing induction of:
   - DNA damage
   - Gene and chromosomal mutations
   - Changes in relevant gene expression
   - Production of reactive oxygen species
   - Inflammatory responses
2. Likely contribution to induce lung cancer in humans due to effects by other known human or suspected carcinogens:
   - Co-carcinogens, cell-proliferators, tumor-promotors

Mechanisms of carcinogenesis

(Source: Scheepers PTJ and Bos RP (1992) Combustion of diesel fuel from a toxicological perspective. II. Toxicity. Int Arch Occup Environ Health 64:163-177



Inhalation studies



Animal studies:

Rats are kept in cages in an atmosphere of diluted diesel exhaust.

Exposure during ~1 year; observation during ~2 years

Control animals breathe filtered ambient air

Animal studies

- Whole diesel exhaust
- Gas-phase diesel exhaust (with particles removed)
- Diesel exhaust particles (DEP)
- Organic extracts of diesel exhaust particles

Whole diesel exhaust

Inhalation studies of diluted diesel exhaust

Species	Total	New 2012	No. of positive	Induced tumors	Conclusion
Mouse	4	2	1	Adenocarcinomas only in highest dose group	Positive
Rat	20	7	11	Benign + malign lung tumors	Positive
Hamster	3	1	-	n/a	Negative
Monkey	1	1	-	n/a	Inconclusive

Diesel exhaust with particles removed

Inhalation of filtered diesel exhaust

Species	Total	New 2012	No. of positive	Induced tumors	Conclusion
Mouse	5	3	1	Lung	False positive finding
Rat	7	3	-	n/a	Negative
Hamster	3	1	-	n/a	Negative

Diesel exhaust particles

Intratracheal instillation of concentrated DEP

Species	Total	New 2012	No. of positive	Induced tumors	Conclusion
Mouse	1	3	1	Lung tumors	Non-significant result
Rat	3	3	2	Benign and malign lung tumors	Postive
Hamster	1	1	-	n/a	Negative

Diesel exhaust particle extracts

Different administrations of DEP extracts

Species	Administration method	No. of studies	No. of positive	Induced tumors	Conclusion
Mouse	Subcutaneous injection	3	1	Sarcomas at injection site	1 positive and 2 inadequate studies
Mouse	Skin painting	1	-	n/a	Negative
Mouse	Initiation promotion study	2	1	Skin papilloma	Positive
Rat	Intrapulmonary implantation	1	1	Lung carcinoma	Postive

Conclusions from animal studies

Diesel exhaust	Evidence for carcinogenicity
Whole	Sufficient
Particulates	Sufficient
Extracts from particles	Sufficient
Gas-phase	Inadequate

Conventional diesel technology and after-treatment

- Oxidation catalysts produce exhaust that can augment the toxicity in vitro and in vivo.
- Also extracts of DEP and semi-volatile organic compounds (expressed per unit extractable organic matter or per mass unit DEP) augment in vitro activity.

However, at the same time:

- … there is evidence that after-treatment (filter, oxy-cat) can result in substantial reductions in activity of DEP extract activity or activity of semi-volatile organic compounds
- … there is no data to compare genetic and related effects of conventional to new technology engines

Emissions from conventional technology engines (ng/hp-hr)

(Source: Sharp CA, Howell SA and Jobe J (2000) SAE Technical Paper Series)

Vehicle (engine)	Substance	100 % Biofuel[a]			20 % Biofuel[a] in conventional diesel		100 % Conventional diesel fuel	
	Oxidation Catalyst:	--	--	+	--	+	--	+
Urban transit bus (DDC Series 50, 205 kW)	2-nitrofluorene	48	40	14	70	67	88	90
	1-nitropyrene[b]	8.5	5.2	37	19	249	83	76
	7-nitrobenz(a)anthracene	<0.5	nd	<0.5	0.9	0.7	1.4	1.4
	6-nitrochrysene[b]	<0.5	nd	1.8	0.8	4.0	0.8	5.8
	6-nitrobenz(a)anthracene	0.8	<0.5	<0.5	2.4	1.8	11.1	4.2
Full size pickup truck (Cummins B5.9, 119 kW)	2-nitrofluorene	142	122	73	-	365	257	478
	1-nitropyrene[b]	34	20	325	265	1644	210	2171
	7-nitrobenz(a)anthracene	0.6	<0.5	4.5	9.8	44	34	60
	6-nitrochrysene[b]	<0.5	<0.5	6.2	1.9	58	11	56
	6-nitrobenz(a)anthracene	1.9	0.7	2.2	8.6	11	6.1	6.8

[a] Methyl ester from virgin soybean oil (AG Environment Products); [b] Classified by IARC as 2A probable carcinogens

1-Nitro-PAH emissions in on-road vehicles

Source: Zielinska B, et al. 2004 J Air Waste Manag Assoc 54:1138-1150

Vehicle make and type	Fuel	N	Year	Pyr ug/m	1-NP ug/m	Chr ug/m	6-NC ug//m	BaP ug/m	6-NBP ug/m
Mazda Millenia, Ford Explorer, Nissna Maxima, GMC 1500 Pickup, Mercury Sable	Normal gasoline at 72 F	18	1982-1996	27.9	0.10	7.77	0.07	1.82	0.01
Ford F-150 pick-up	Gasoline black smoker	5	1976	110.6	0.12	29.79	0.57	12.34	0.04
Mitsubishi Montero	Gasoline white smoker	2	1990	337.6	0.08	206.86	0.08	81.65	0.07
Mazda Millenia, Ford Explorer, Nissna Maxima, GMC 1500 Pickup, Mercury Sable	Normal gasoline	12	1992-1996	254.5	0.02	69.06	0.02	30.09	0.02
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	Current technology diesel vehicles	9	1998-2000	11.0	1.94	3.78	1.50	1.47	0.04
Dodge Ram 2500 Pickup	Diesel high PM emitter	6	1991	45.2	1.10	6.01	0.74	1.47	0.53
Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TD	Current technology diesel vehicles	6	1998-2000	250.8	3.85	104.2	1.29	9.87	2.42

Effect of new technology

Genetic end-points

- New technology DEP extracts induced skin papillomas and adenocarcinomas in mouse skin
- There are data on mutations in bacteria but there is no comparative data to evaluate the genetic an related effects of new-diesel technology

Co-carcinogen end-points increase:

- Expression of xenobiotic metabolism
- Oxidative damage and anti-oxidant response
- Expression of cell cycle genes in mammalian cells in culture

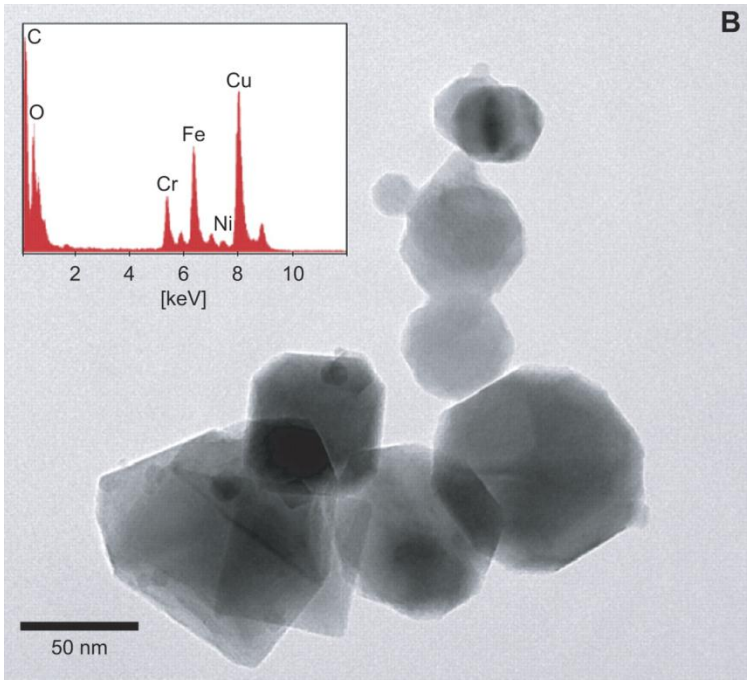
Effects of new technology

Induction of non-cancer endpoints:

- Lung function
- Lung inflammation
- Immunology and infection
- Systemic inflammation and cardiovascular aberrations
- Morphological cell transformation in mammalian cells

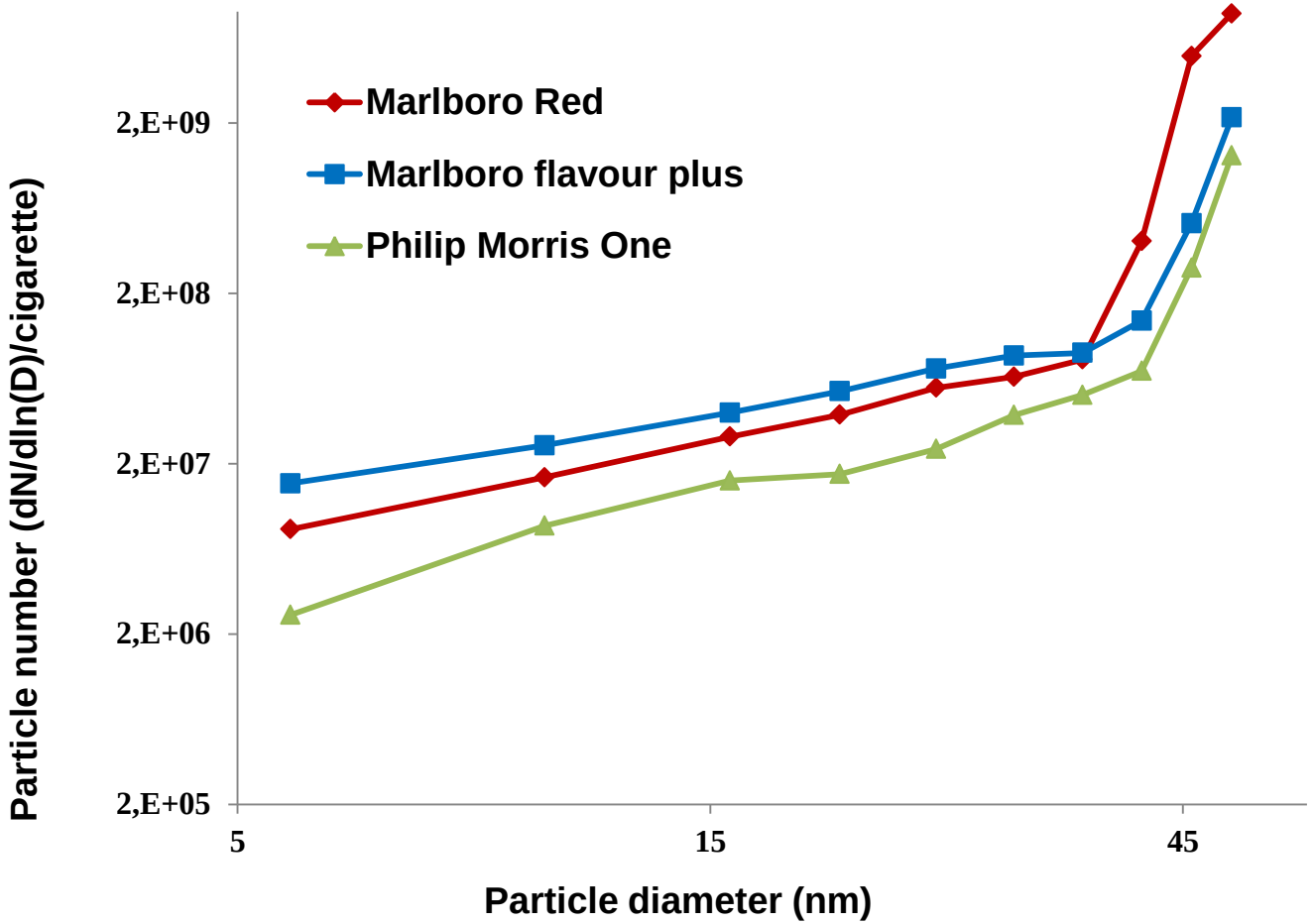
Effects of new technology

- If carbon is further reduced soot will disappear from the exhaust. Presumably solid metals and metal oxide particles will remain.
- Semivolatile organic compounds (SVOC) will no longer absorb to carbon cores
- SVOC including PAH and nitro-PAH will likely form ultrafine condensates (like in cigarette smoke)
- How does this change the toxicology of diesel exhaust?



Cigarette smoke condensate contains nanosize particles

(Source: van Dijk WD, Gopal S, Scheepers PTJ (2011) Nanoparticles in cigarette smoke; real-time undiluted measurements by a scanning mobility particle sizer. Anal Bioanal Chem. 399:3573-8)



Conclusions

- “No one mechanism seems to predominate for diesel engine exhaust in humans”. Direct DNA-damage, reactive oxygen species, inflammation and co-carcinogens play a role.
- There is sufficient evidence for the carcinogenicity of diesel exhaust particle fractions in animal studies
- The evidence for carcinogenicity of diesel exhaust gas phase in animal studies is inadequate
- Oxidation catalyst on a conventional technology diesel engine can increase emissions of sufficient evidence carcinogenic nitro-PAH tenfold.
- The low contribution of soot particles will likely change the toxicity of new technology diesel exhaust.

Further reading

- Benbrahim-Tallaa L, Bouvard V, Carel R, El Ghissassi F, Grosse Y, Guha N, Lajoie P, Lauby-Secretan B, Loomis D, Moore S, Müller K, Olsson A, Straif K, Vlaanderen.J Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes.International Agency for Research on Cancer Monograph Working Group. Lancet Oncol. 2012 Jul;13(7):663-4.
- Scheepers PTJ, Vermeulen RCH (2012) Diesel engine exhaust classified as a human lung carcinogen. How will this affect occupational exposures? Occup Environ Med 2012 Oct;69(10):691-3
- Scheepers PTJ, Vermeulen RCH (2012) Dieselmotoremissies bewezen humaan carcinogeen (editorial). Wat verandert er? Tijdschrift voor Toegepaste Arbowetenschap. No. 2 38-39.

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