PATROLS

Advanced Tools for NanoSafety Testing

In vivo benchmarking (chronic exposure studies)

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A number of nanomaterials are classified as carcinogenic or possibly carcinogenic by IARC

- Diesel exhaust particles (1)
- Carbon black (2B)
- Titanium dioxide nanoparticles (2B)
- Mitsui-7 carbon nanotubes (2B)

Nanosize increases the carcinogenic potency in chronic inhalation studies of TiO_2

	Air concentration	Total number of lung tumors	
Fine, rutile TiO_2	250 mg/m^3	39/151 rats= 26%	Lee et al (1985)
Nano TiO_2 (P25)	10 mg/m^3	32/100 rats= 32%	Heinrich (1995)

Fine, rutile TiO_2 did not cause cancer in 2-year inhalation studies at 5, 10 or 50 mg/m^3
(Muhle et al 1991, Lee et al , 1985)

Cancer risk for ultrafine TiO2: 250 mg/m3 gives 26% cancer
Divide by 22.5 to normalise to 45 years instead of 2 years
Divide by 260 to normalise to 0.1% risk : 43 ug will give 1:1000 cancer risk

Animal models of particle-induced lung cancer

- If no epidemiological evidence is avaiable, animal studies are used for risk assessment
- Only inhalation studies can be used for risk assessment
- 2 year chronic inhalation studies in rats (no cancer in mice and hamsters)
- We want to estimate human lung cancer risk (0.1% - 0.001%) during 40 years of exposure based on groups of 50-100 rats exposed for 2 years (the detection limit is ca. 5% cancer), so the air concentrations should at least be 50 (5%/0.1%) x 20 (40 years/2 years) = 1000 fold higher in the animal studies
- Concern has been raised that impaired clearence (overload) will lead to over-estimation of cancer risk

Proposed key characteristics of carcinogens

Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Release of toxic substances; fx PAH, metals

Surface-dependent ROS generation

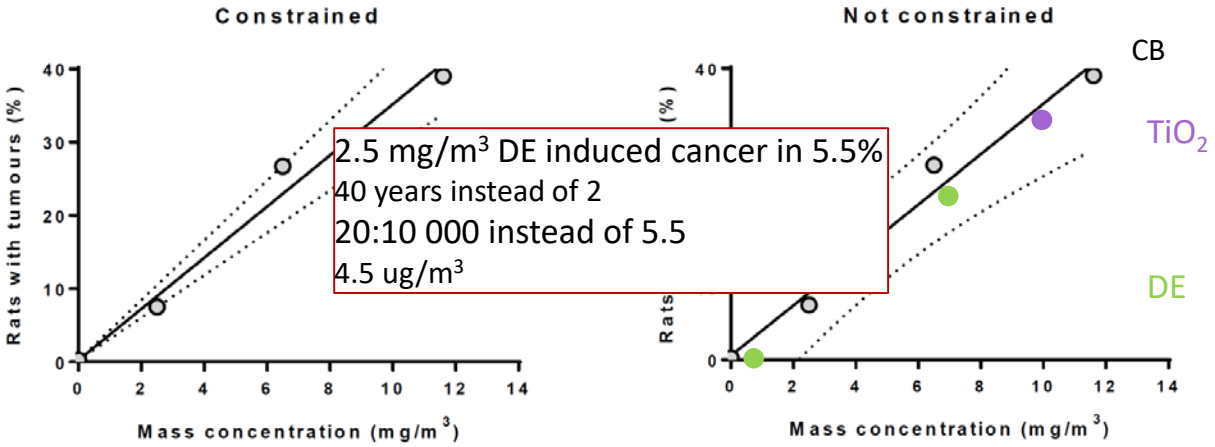
Deposited total surface area
Shape (HARN)

Smith MT EHP, 2016, PMID: 26600562

Mechanisms of action of diesel exhaust-induced cancer

- Both DEP and NO_x induces inflammation
- Diesel exhaust induces lung cancer in chronic inhalation studies in rats
- 2 year cancer studies in rats: Diesel Engine Exhaust induces lung cancer, but not filtered DEE. Thus, **the particulate fraction is the carcinogenic component (Brightwell 1989)**.
- Both inhalation of DEE and instillation of DEP and DEP extracts induced mutations in lungs of mice (Hashimoto 2007)
- Evidence that both carbon core and DEP extracts (PAH, OC) contribute to carcinogenicity (Hashimoto, 2007, Heinrich 1995)
- PAH adduct formation and particle **surface-induced ROS: primary genotoxicity and non-threshold effects**

Cancer frequency in female rats in chronic inhalation studies; the carcinogenic potency is the same for 3 insoluble NMs (DEP, TiO_2 and CB)



Two year inhalation study in female (and male) rats exposed to diesel exhaust (DE), TiO_2 (P25) and CB (Printex90) (**Heinrich et al., 1995**).

	Average particle exposure (mg/m³)					
	Clean air	DE			CB	TiO_2
Exposure concentration	0	0.8	2.5	7.0	11.6	10
Number of rats with tumours						
with benign tumours	1/217	0/198	11/200	22/100	39/100	32/100
without benign tumours			4/200	9/100	28/100	19/100

Table 4. Lung cancer incidence in rats exposed to diesel exhaust particles (DEP), carbon black (CB) and titanium dioxide (TiO_2) after 30 months (24 months of exposure followed by 6 months in clean air) (Heinrich et al. 1995).

Concern: the overload hypothesis

- Rats develop lung cancer after particle inhalation (2 years)
- Mice and hamsters do not
- Dose-dependent differences in particle retention
- Overload is seen for CB Printex90 at 50 mg/m^3 (13 weeks)
- It is argued that rats therefore overestimate human cancer risk

TABLE 4
Particle Retention Half Times for Rats, Mice, and Hamsters following 13 Weeks of Exposure to Carbon Black

Nano-sized carbon black particles (P90) at different exposure levels

	Rats	Mice	Hamsters
HSCb, 1 mg/m^3	64[a]	133	42
HSCb, 7 mg/m^3	115	343	53
HSCb, 50 mg/m^3	*No significant clearance*	322	309

[a]Times are reported in days and were calculated from the retention curves using a two-parameter monoexponential decay function. For the mice, the half times were estimated from the retention curves in Figure 4b.

Elder et al, 2005, Tox Sci, 88(2) 614

Clearence half-times for CB (Printex90), TiO_2 (P25) and diesel exhaust particles in the two year inhalation study

TABLE 9. Half-Times of Pulmonary Tracer Clearance (^{59}Fe)

	Half-time of alveolar clearance (days)			
Exposure	3 mo	12 mo	18 mo	18 mo Recovery
Control	61	72	96	93
Diesel soot, 0.8 mg/m^3	94[a]	121[a]	221[a]	n.d.[b]
Diesel soot, 2.5 mg/m^3	119[a]	254[a]	272[a]	n.d.[b]
Diesel soot, 7.0 mg/m^3	330[a]	541[a]	687[a]	1068[a]
Carbon black	244[a]	368[a]	363[a]	591[a]
TiO_2	208[a]	403[a]	357[a]	368[a]

[a]Significant at $p < .01$ (Dunnett's test).
[b]n.d., Not determined.

Heinrich et al, 1995

Cumulative dose-response relationship between diesel exhaust exposure and lung cancer risk in three epidemiologial studies

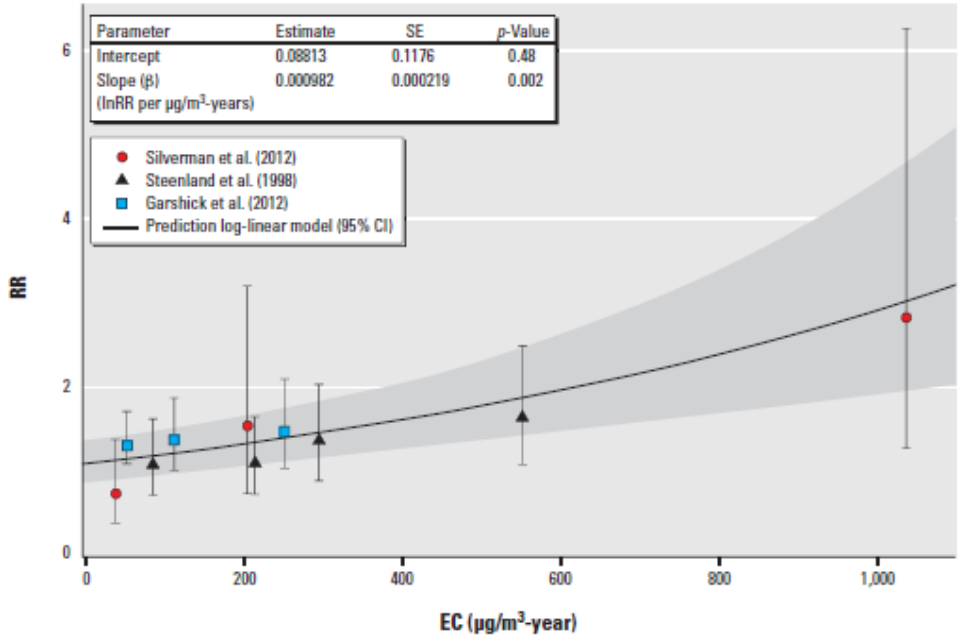


Figure 1. Predicted exposure–response curve based on a log-linear regression model using RR estimates from three cohort studies of DEE and lung cancer mortality. Individual RR estimates [based on HRs reported by Garshick et al. (2012) or ORs reported by Silverman et al. (2012) and Steenland et al. (1998)] are plotted with their 95% CI bounds indicated by the whiskers. The shaded area indicates the 95% CI estimated based on the log-linear model. The insert presents the estimates of the intercept and beta slope factor, the SE of these estimates, and the associated *p*-values.

Vermeulen et al, 2014, EHP

Risk estimate for DEP based on epidemiological evidence

Table 1. Exposure–response estimates (lnRR for a 1-μg/m^3 increase in EC) from individual studies and the primary combined estimate based on a log-linear model.

Model[a]	Intercept	β (95%CI)
All studies combined	0.088	0.00098 (0.00055, 0.00141)
Silverman et al. (2012) only	–0.18	0.0012 (0.00053, 0.00187)
Steenland et al. (1998) only	–0.032	0.00096 (0.00033, 0.00159)
Garshick et al. (2012) only	0.24	0.00061 (–0.00088, 0.00210)

[a]Log-linear risk model (lnRR = intercept + β × exposure). Exposure defined as EC in μg/m^3-years.

Table 2. Excess lifetime risk per 10,000 for several exposure levels and settings, United States in 2009.

Exposure setting	Average EC exposure (μg/m^3)	Excess lifetime risk through age 80 years (per 10,000)
Worker exposed, age 20–65 years	25	689
Worker exposed, age 20–65 years	10	200
Worker exposed, age 20–65 years	1	17
General public, age 5–80 years	0.8	21

Based on linear risk function, lnRR = 0.00098 × exposure, assuming a 5-year lag, using age-specific (5-year categories) all cause and lung cancer mortality rates from the United States in 2009 as referent.

The EU OEL for DEP is 50 ug/m^3

Vermeulen et al, EHP

Comparable risk estimates from chronic inhalation studies and epidemiological studies on DEP

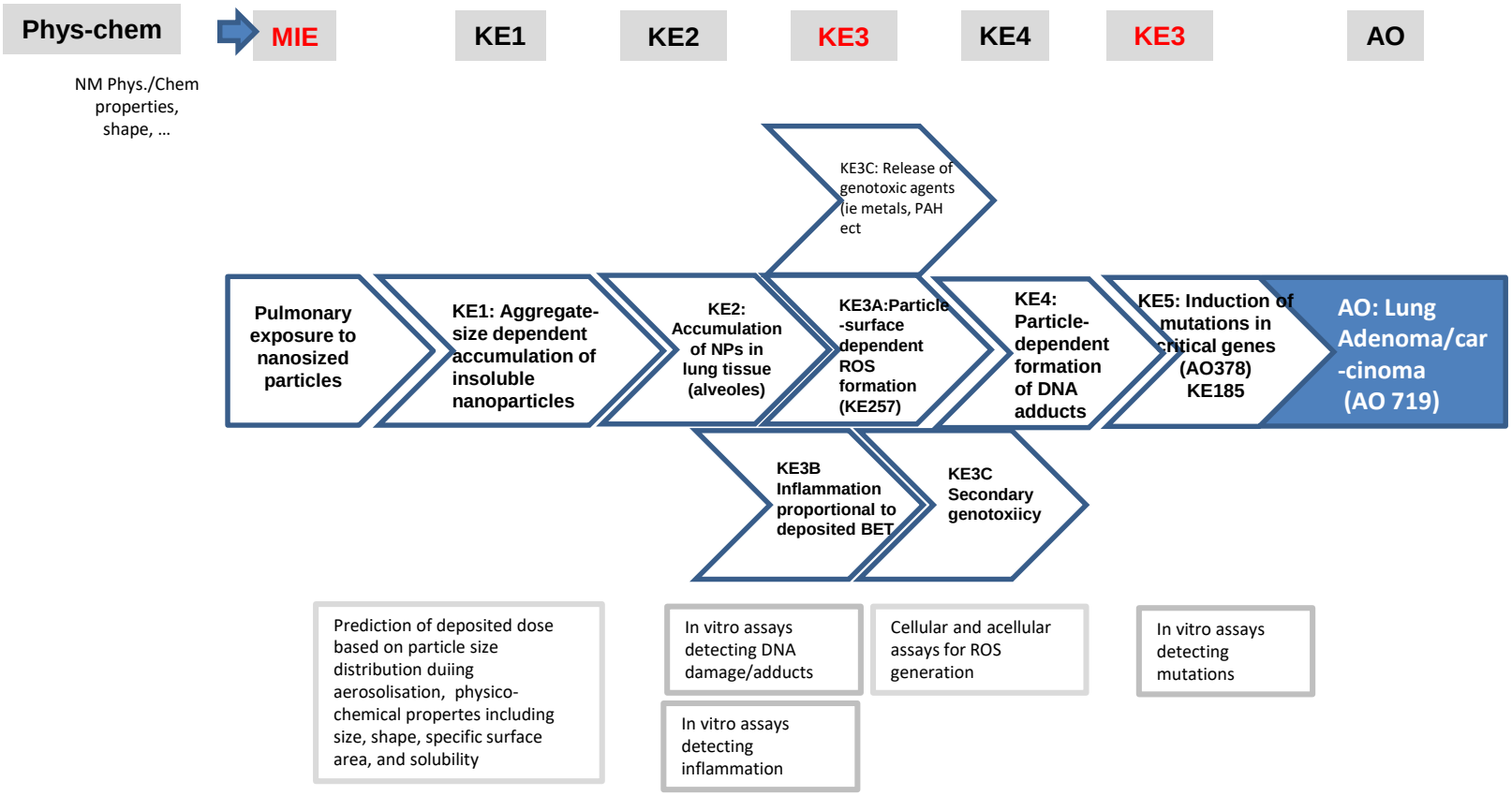
Diesel exhaust as a case:

Diesel exhaust (DE) cause cancer, filtered DE does not.

In chronic (2 year) inhalation studies in rats,

- 2.5 mg/m^3 DE induced cancer in 5.5% of the exposed rats
- 40 years instead of 2
- 20:10 000 instead of 5.5%:
- 4.5 ug/m^3 will induce 20 excess lung cancer cases
- Epidemiological meta-analysis: 1 ug/m^3 induce 17 lung cancers:10 000 exposed
- the chronic inhalation in rats does not over-estimate lung cancer risk for DE

AOP: NP-induced lung cancer



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Thank you for your attention!