

Reproductive and developmental toxicity of particles

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In vivo studies – inhalation+oral (2020)

111 studies on 19 nanomaterials

- Titanium dioxide 29%
- Silver 21%
- Zinc oxide 10%
- Silicon oxide 5%
- Carbon-based 19%
- Other 14%

- Mainly fetal development and male fertility
 - female fertility almost not studied
- Predominantly oral exposure

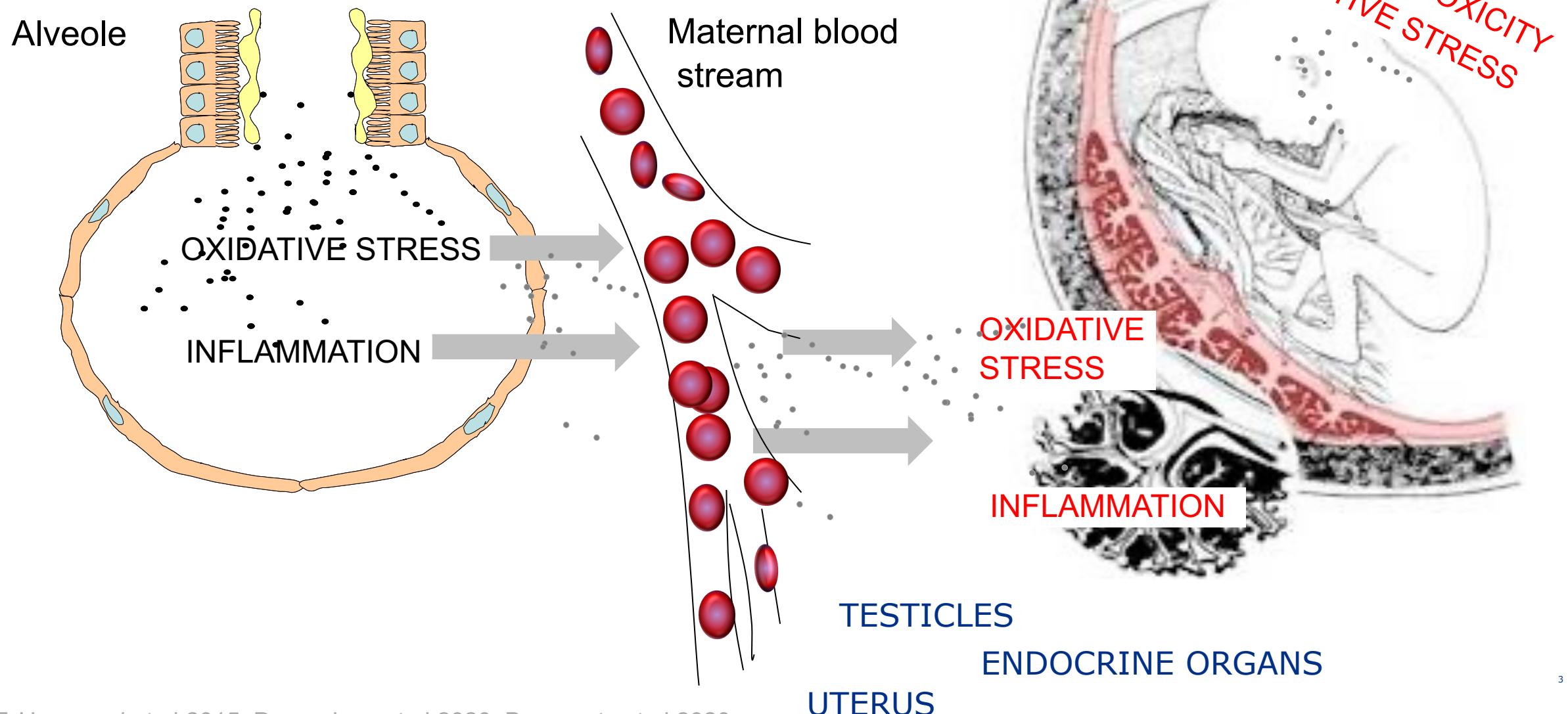


*A critical review of studies
on the reproductive and developmental
toxicity of nanomaterials*

Reference: Framework contract
ECHA/2015/50 Lot 1
Specific contract no 16 - ECHA/2019/153



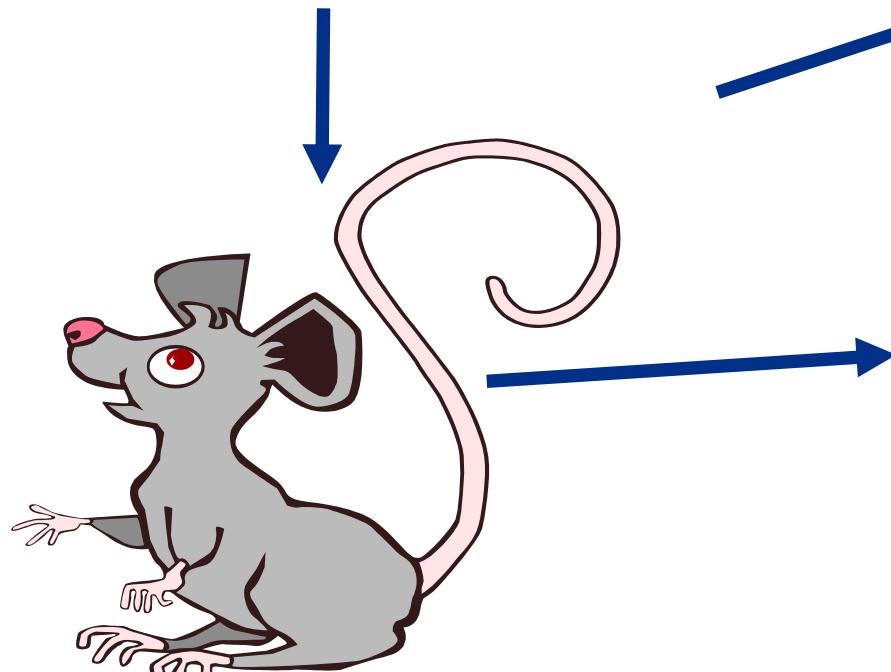
Why study particles in pregnancy



Design: Developmental toxicity studies

Exposure:

Whole body inhalation
Intracheal instillation



Outcomes

Maternal lung inflammation
Pregnancy/growth
Genotoxicity
Nervous system
Male reproduction
Immune system

Exposure periods

	Days of mouse gestation																			Birth ↓	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
TiO ₂ 42 mg/m ³																					
DEP 19 mg/m ³																					
CB 42 mg/m ³																					
4*67 µg																					
4.8 / 33.5 mg/m ³																					
MWCNT 1*67 µg																					

*Instillation

Inhalation: ~1 hour/day

Exposure periods

	Days of mouse gestation																			Birth ↓	
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MWCNT 1*67 µg																					

*Instillation

Inhalation: ~1 hour/day

Most exposures induced maternal lung inflammation

Nano-material	Exposure		Effect on organ system in <u>offspring</u>					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Maternal lung inflammation					-
MWCNT	1*67 µg	0*	Maternal lung inflammation					
	1*67 µg	0*	Maternal lung inflammation			-	-	
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-					-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Maternal lung inflammation			-	-	-
	4*67 µg	7,10, 15,18*	Maternal lung inflammation					-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)					-



Hougaard et al. 2008; 2010, 2013; Hemmingsen et al. 2009;
 Jackson et al. 2011, 2012a, b, 2013; Kyjovska et al. 2013;
 Barfoed et al. 2015; Johansson et al. 2017; Umezawa et al.
 2018; Skovmand et al. 2019, Hansen et al. 2020.

No effects on gestation and lactation parameters

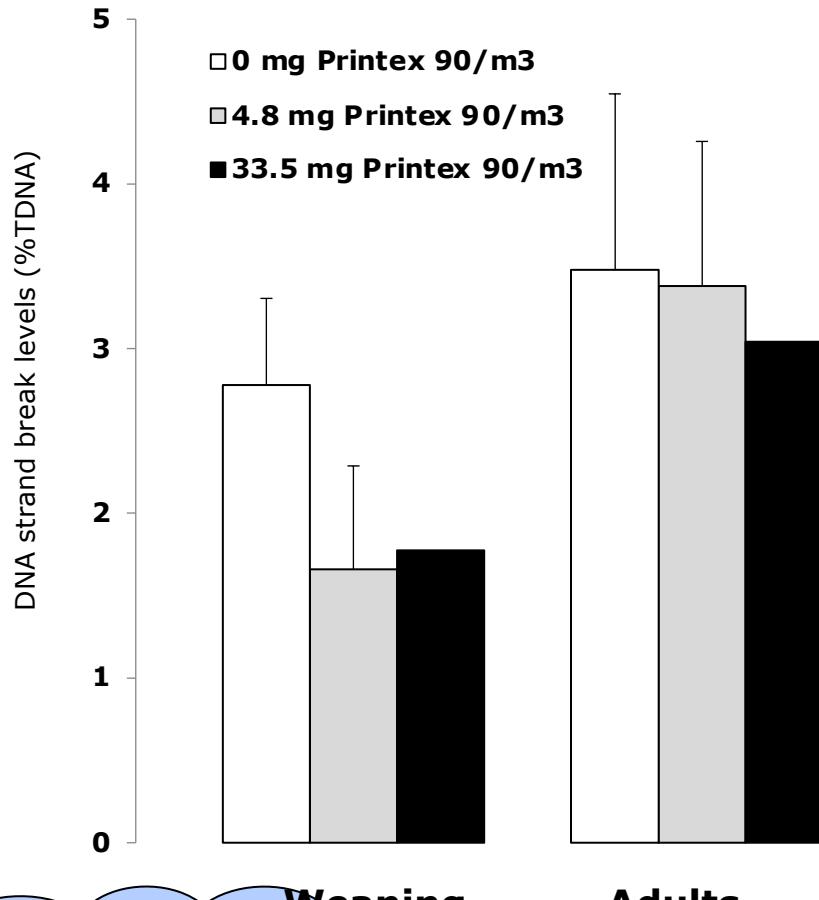
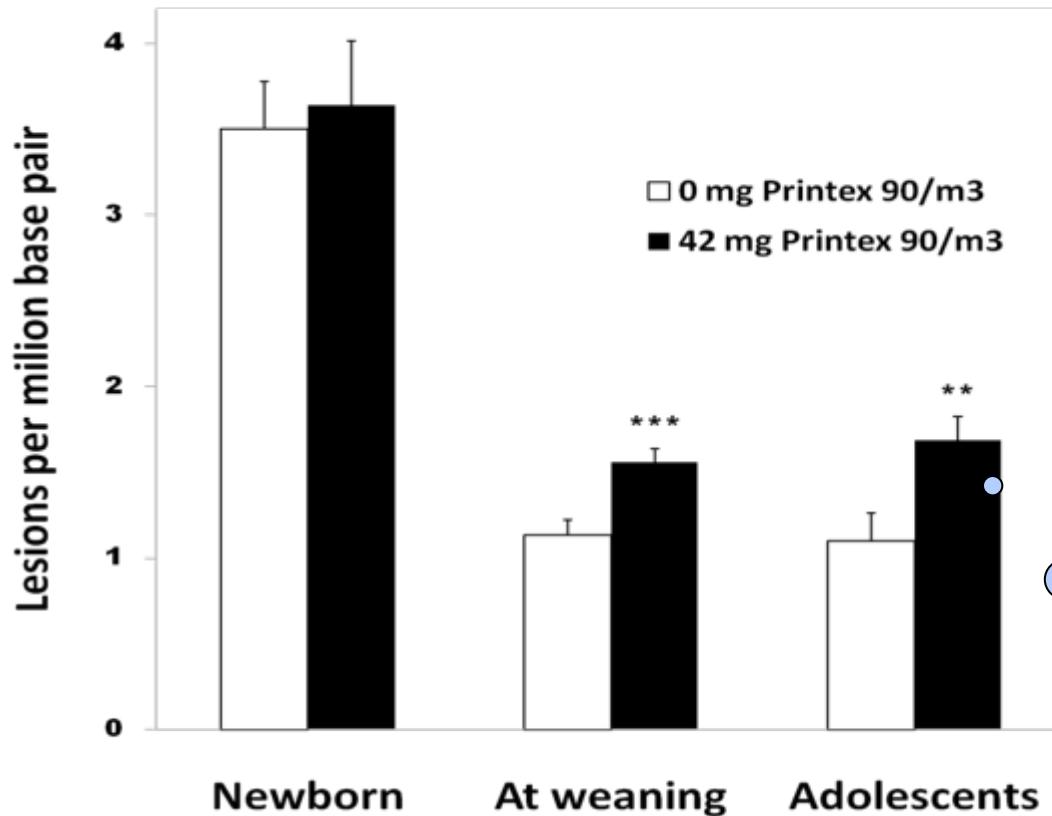
Nano-material	Exposure		Effect on organ system in offspring					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey				-
MWCNT	1*67 µg	0*	Red	Grey				
	1*67 µg	0*	Red	Grey		-	-	
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-	Red				-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey		-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey				-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey				-



Hougaard et al. 2008; 2010, 2013; Hemmingsen et al. 2009;
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 2018; Skovmand et al. 2019, Hansen et al. 2020.

Genotoxicity in offspring liver - carbon black

Comet assay: DNA strand breaks



No comet effects of:
-instilled carbon black
-inhaled TiO₂

No (?) effect on offspring DNA damage level

Nano-material	Exposure		Effect on organ system in offspring					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey	Grey	White	White	-
MWCNT	1*67 µg	0*	Red	Grey	Grey	White	White	White
	1*67 µg	0*	Red	Grey	Grey	-	-	White
Diesel-particles	18 mg/m ³	7-19 (1 hour)	White	Red	Grey	White	White	-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey	Red	-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey	Grey	White	White	-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey	Grey	White	White	-



Hougaard et al. 2008; 2010, 2013; Hemmingsen et al. 2009;
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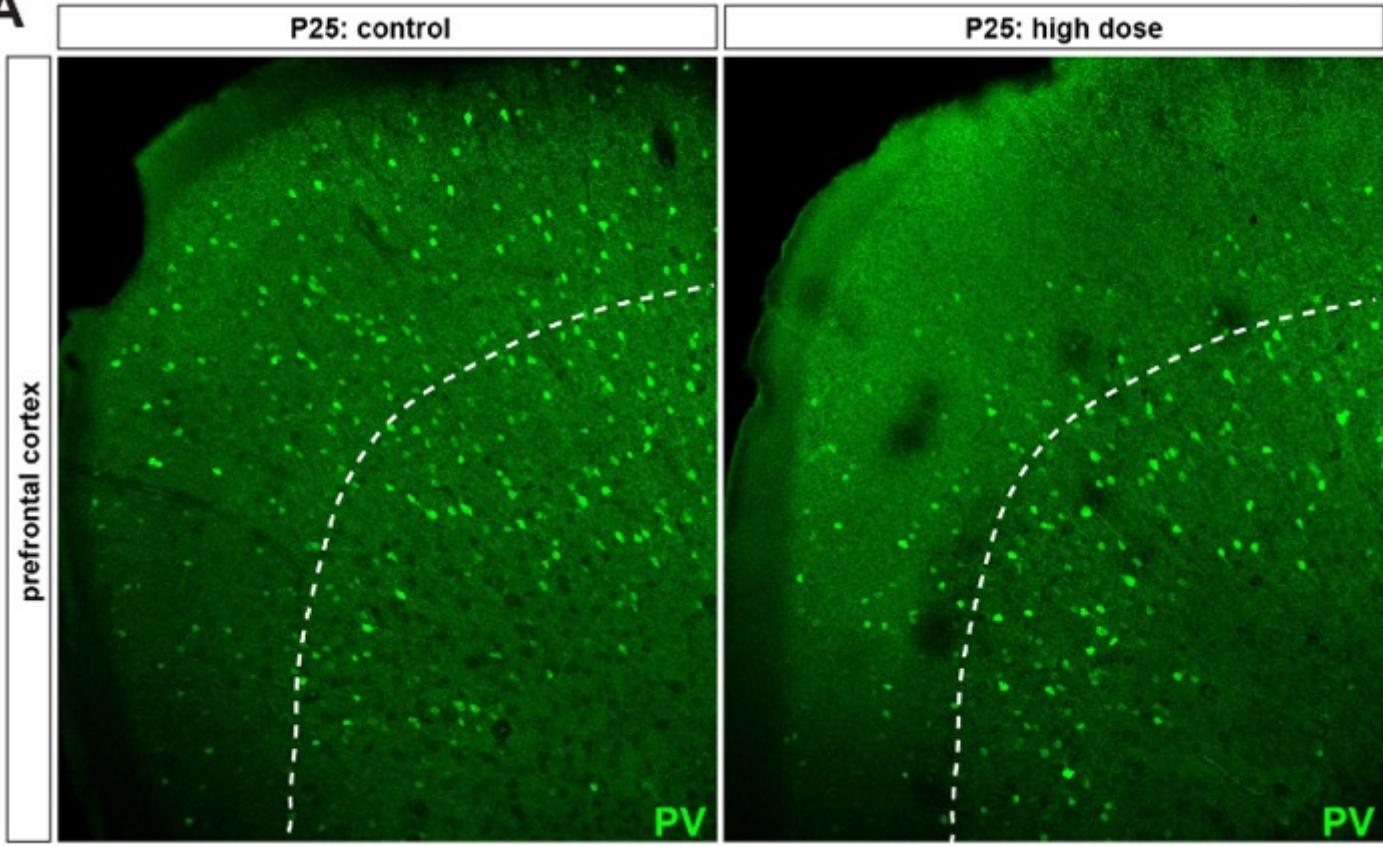


Carbon black affect CNS

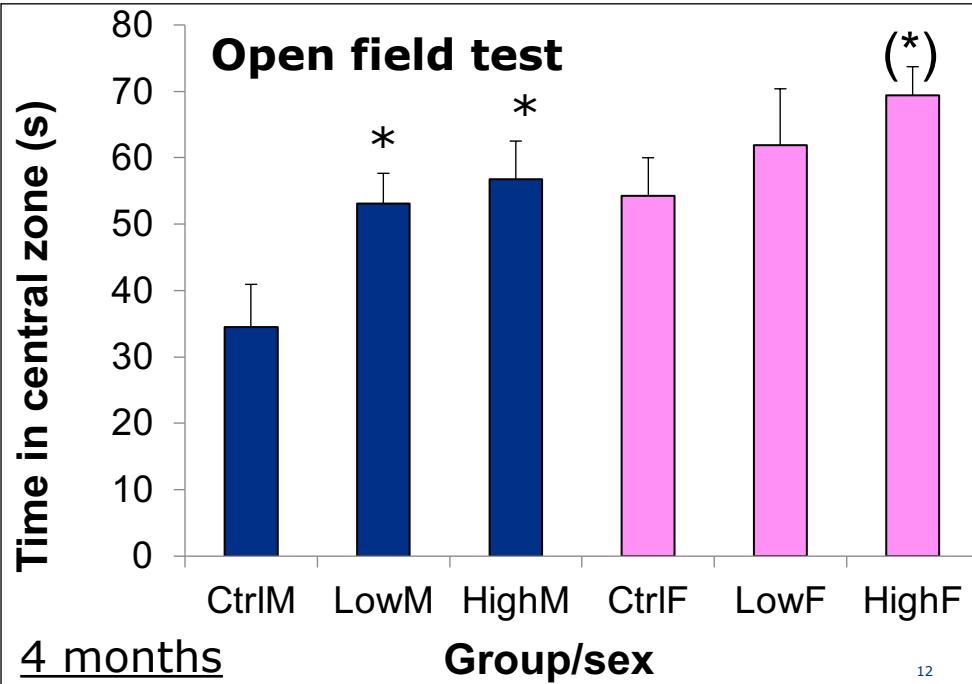
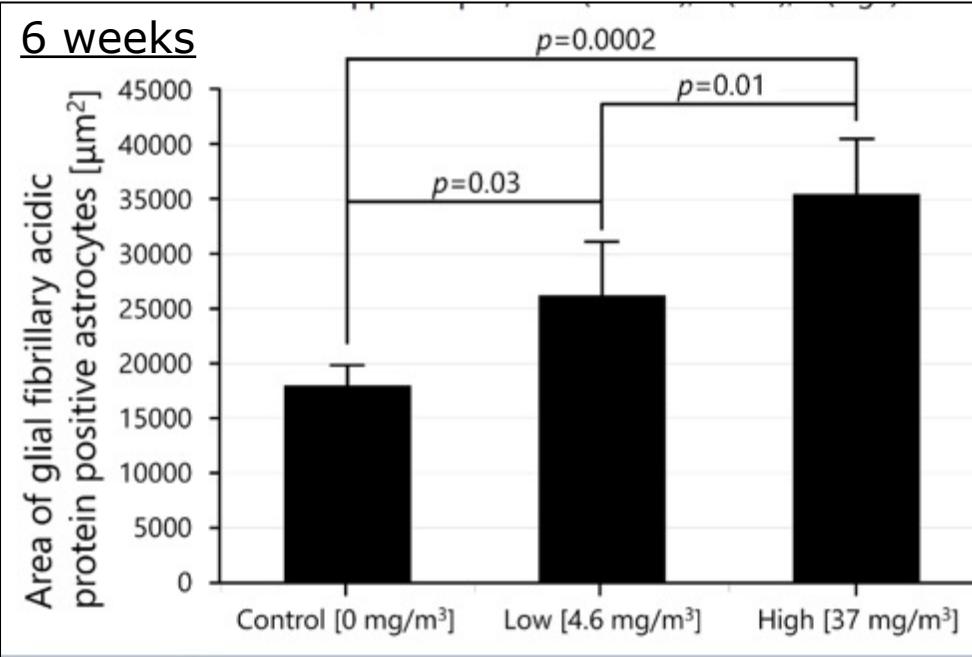
Age: 6 weeks and 4 months



A



Decrease in Parvalbumin-positive interneurons in cortex - would indicate increased risk for psychiatric disorders in animal models of neurodevelopmental disorders



Consistent histological findings across studies



Increased GFAP expression in astrocytes seems a consistent finding:

- Several different studies
- Two different labs
- ICR and NMRI mice, i.e. two outbred strains
- Inhalation and instillation exposure

!! Similar changes have been observed in
airpollution particles

Does particle exposure increase risk of
neurodegeneration?

(Onoda, 2014, 2017, 2020; Umezawa 2018)

CNS seems sensitive to maternal particle exposure

Nano-material	Exposure		Effect on organ system in offspring					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey	-	Red	-	-
MWCNT	1*67 µg	0*	Red	Grey	-	Grey	-	-
	1*67 µg	0*	Red	Grey	-	-	-	-
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-	Red	Grey	Red	-	-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey	Red	-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey	Red	Red	-	-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey	Grey	Red	-	-



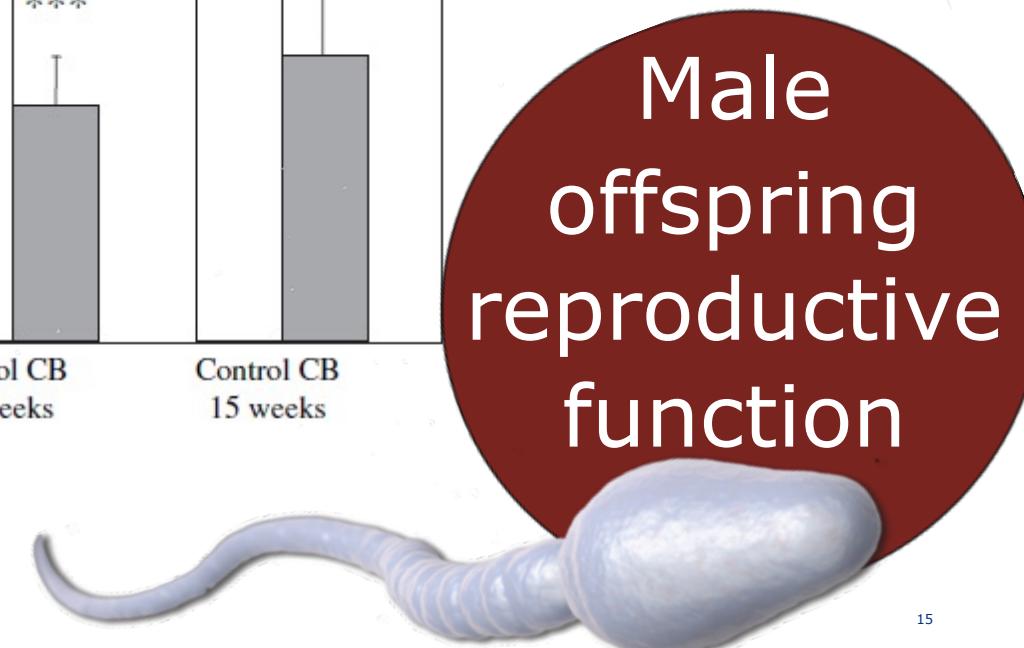
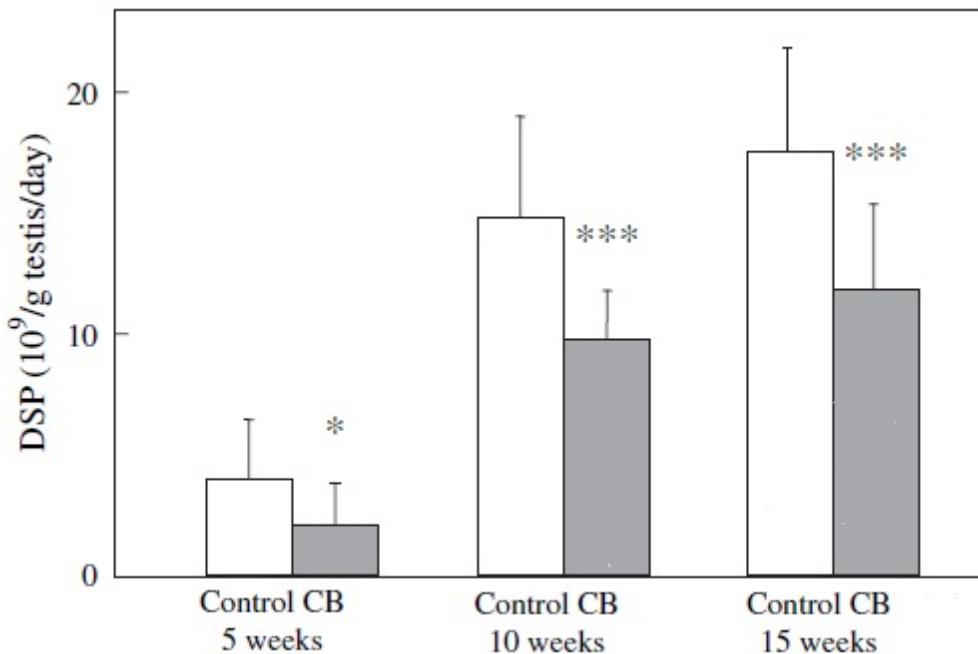
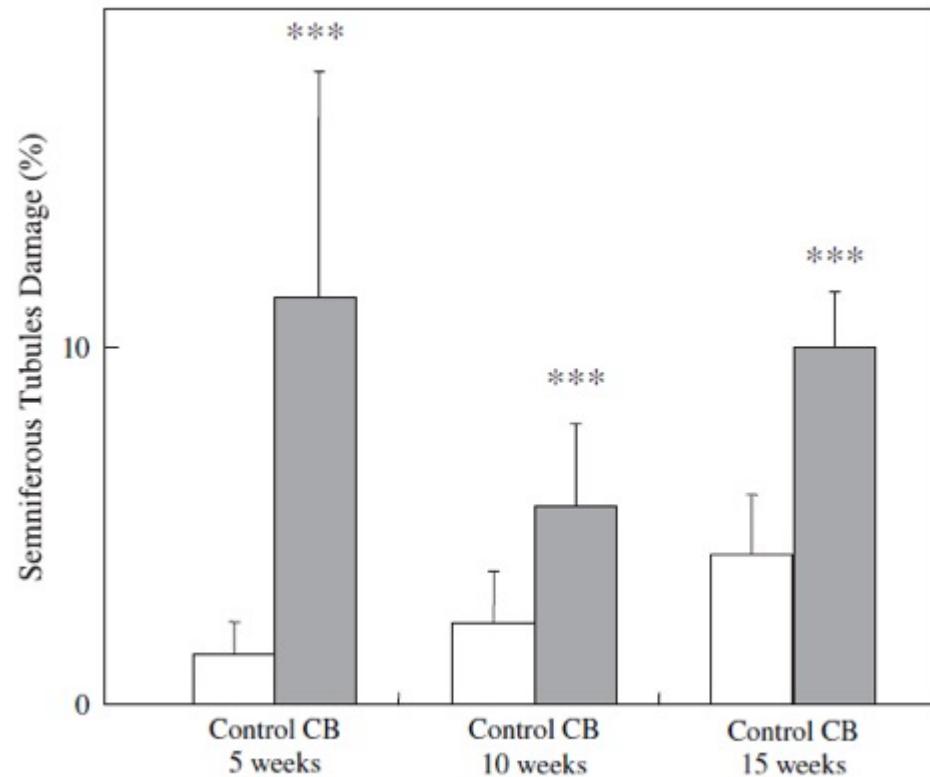
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2010: Airway exposure to carbon black affects reproduction in male offspring (Yoshida et al.)

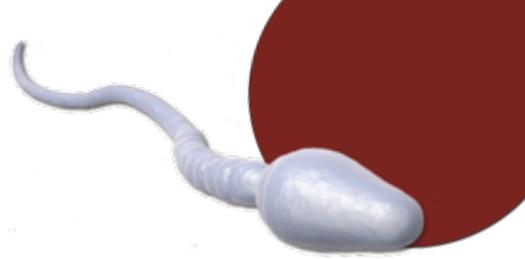
Maternal intratracheal instillation of 14 nm carbon black particles

Gestation day 7 and 14 (total 0.4 mg/mouse), ICR mice

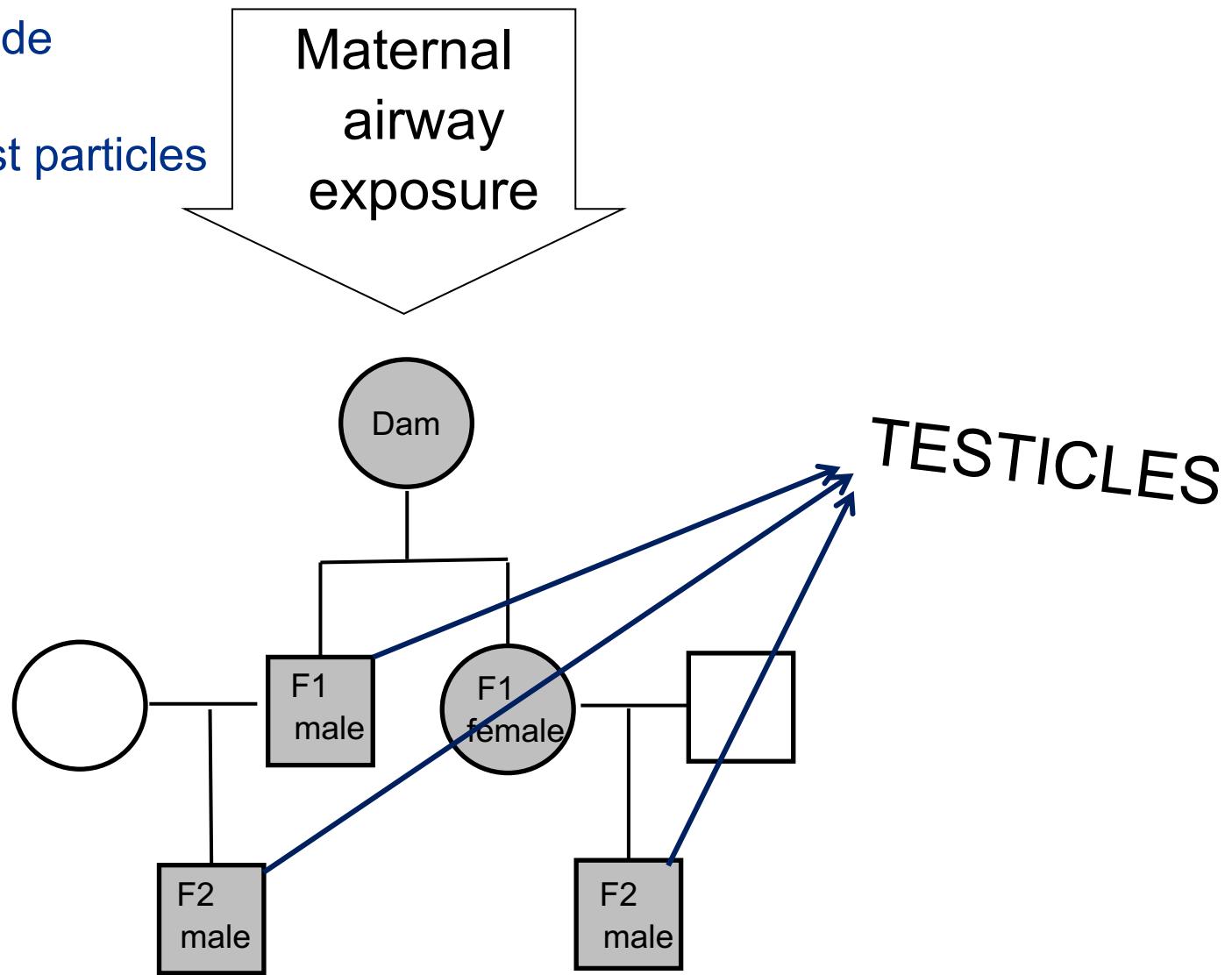
Similar effects after maternal s.c. titanium dioxide and inhalation of diesel exhaust particles
(Ono 2007, Watanabe 2005)



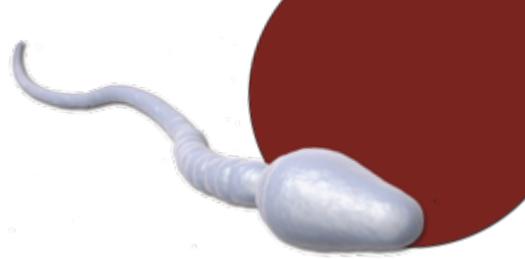
Offspring reproduction: daily sperm production



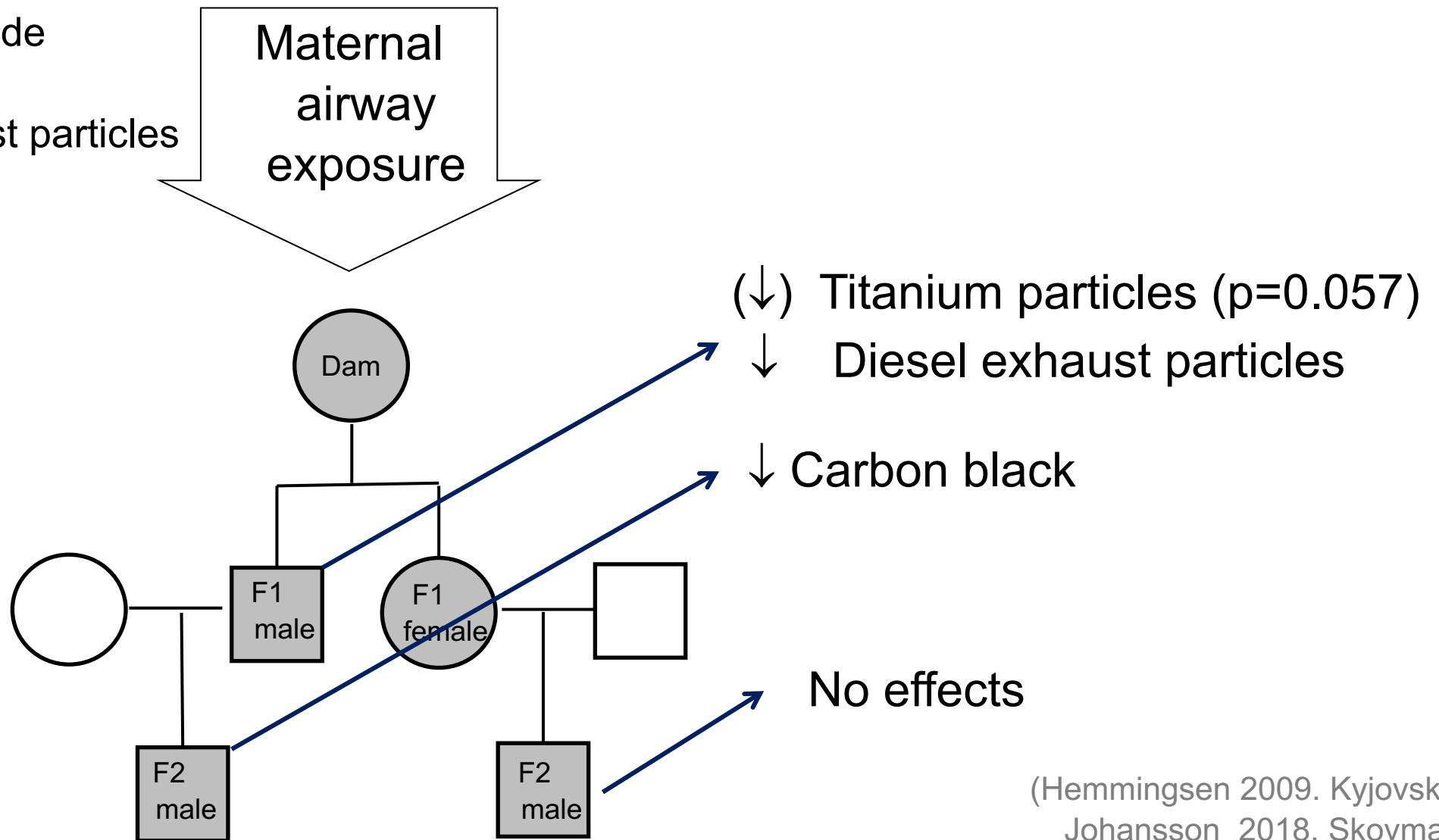
Titanium dioxide
Carbon black
Diesel exhaust particles
MWCNT



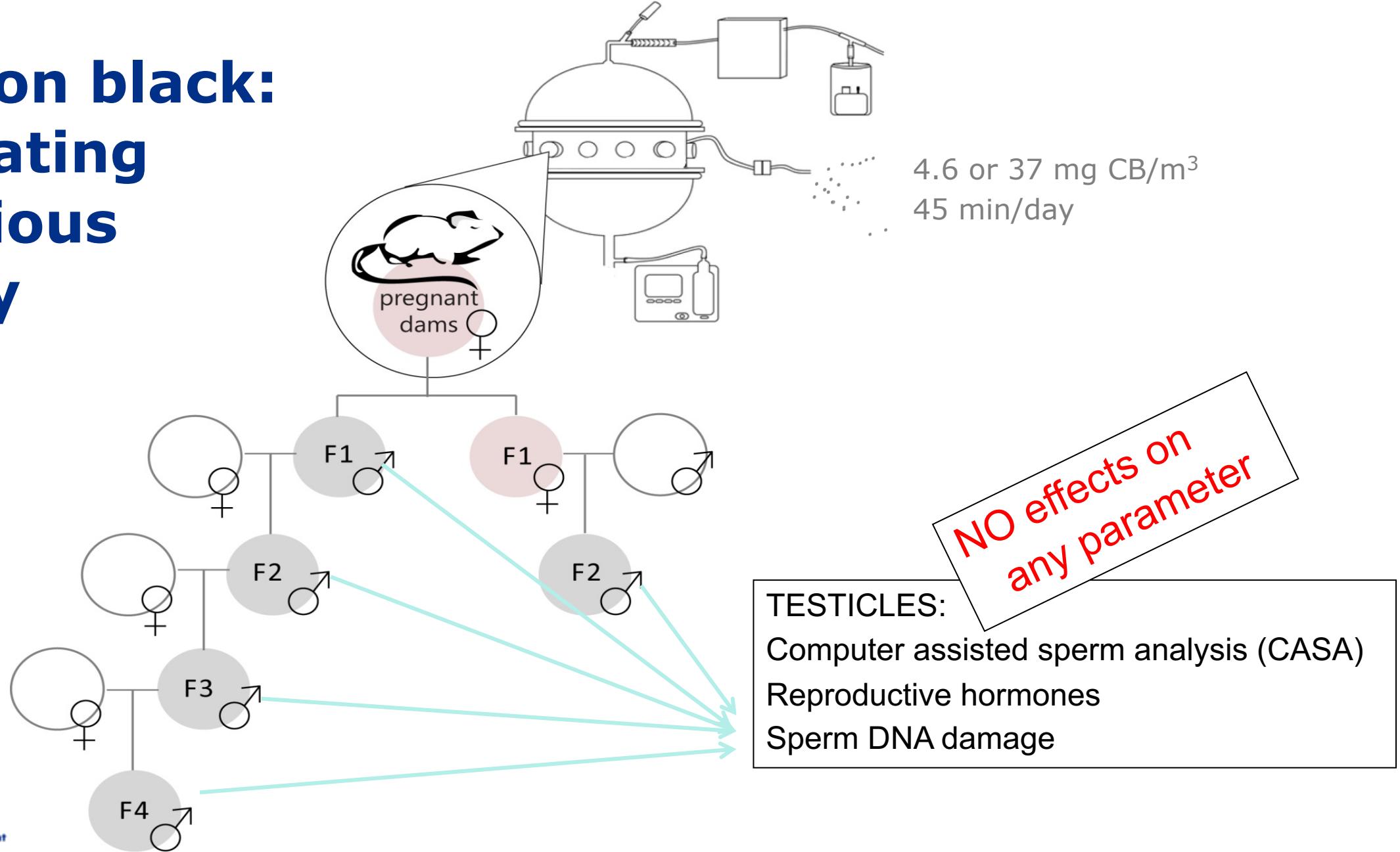
Offspring reproduction: daily sperm production



Titanium dioxide
Carbon black
Diesel exhaust particles
MWCNT



Carbon black: repeating previous study

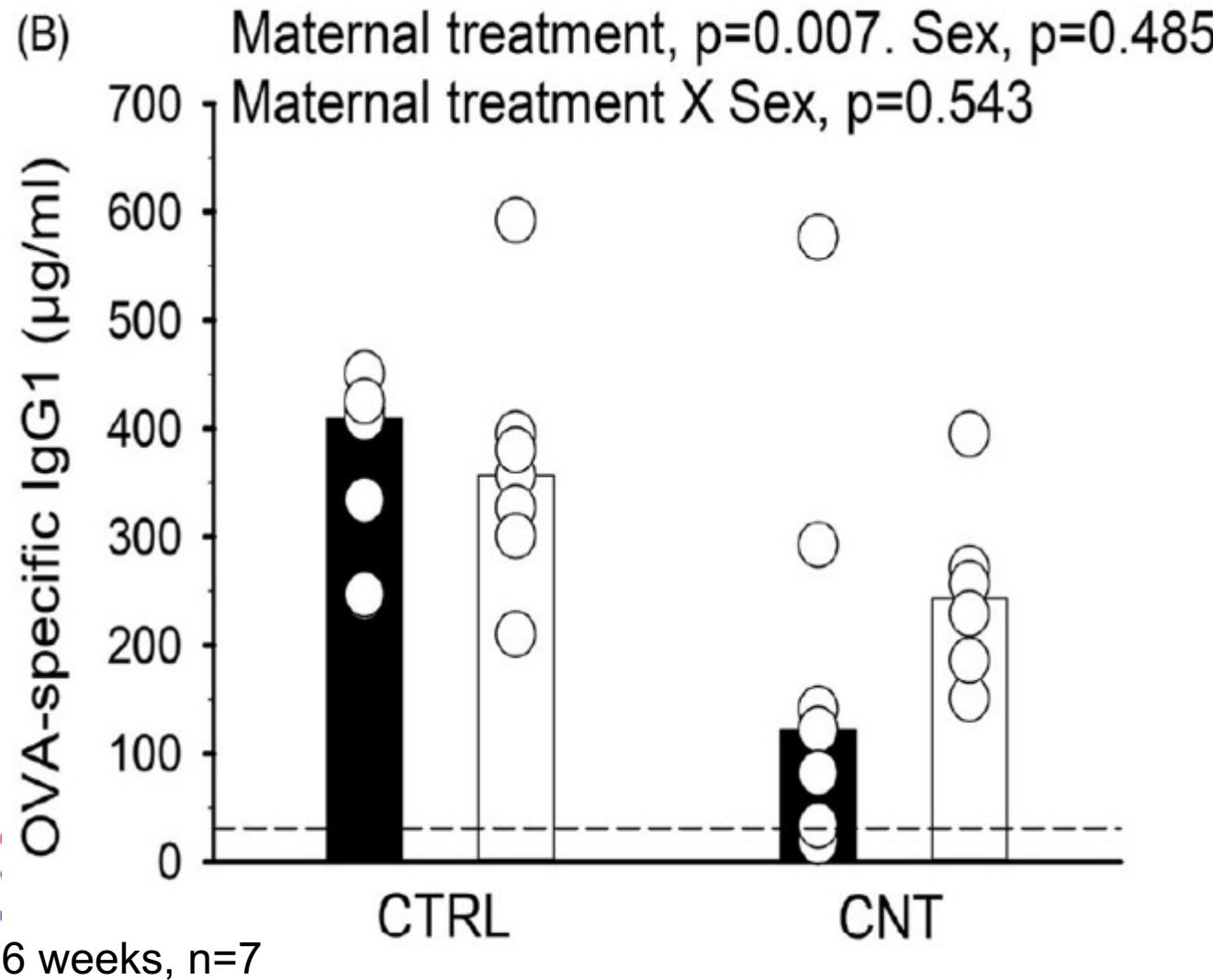


Inconsistent male reproductive effects

Nano-material	Exposure		Effect on organ system in offspring					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey	Grey	Red	Yellow (p=0.057)	-
MWCNT	1*67 µg	0*	Red	Grey	Grey	Grey	-	-
	1*67 µg	0*	Red	Grey	Grey	-	-	-
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-	Red	Grey	Red	Red	-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey	Red	-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey	Grey	Red	(2nd generation)	-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey	Grey	Red	Grey	-

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 Barfoed et al. 2015; Johansson et al. 2017; Umezawa et al.
 2018; Skovmand et al. 2019, Hansen et al. 2020.

MWCNT exposure prior to mating: Consistently decreased immune reactivity (after OVA allergen challenge)



- Two separate studies
- Four different immunisation protocols
- Four different offspring ages

MWCNT consistently decreased immune reactivity

Nano-material	Exposure		Effect on organ system in offspring					
	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey	Grey	Red	Red	-
MWCNT	1*67 µg	0*	Red	Grey	Grey	Grey	-	Red
	1*67 µg	0*	Red	Grey	Grey	-	-	Red
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-	Red	Grey	Red	Red	-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey	Red	-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey	Grey	Red	Red	-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey	Grey	Red	Grey	-

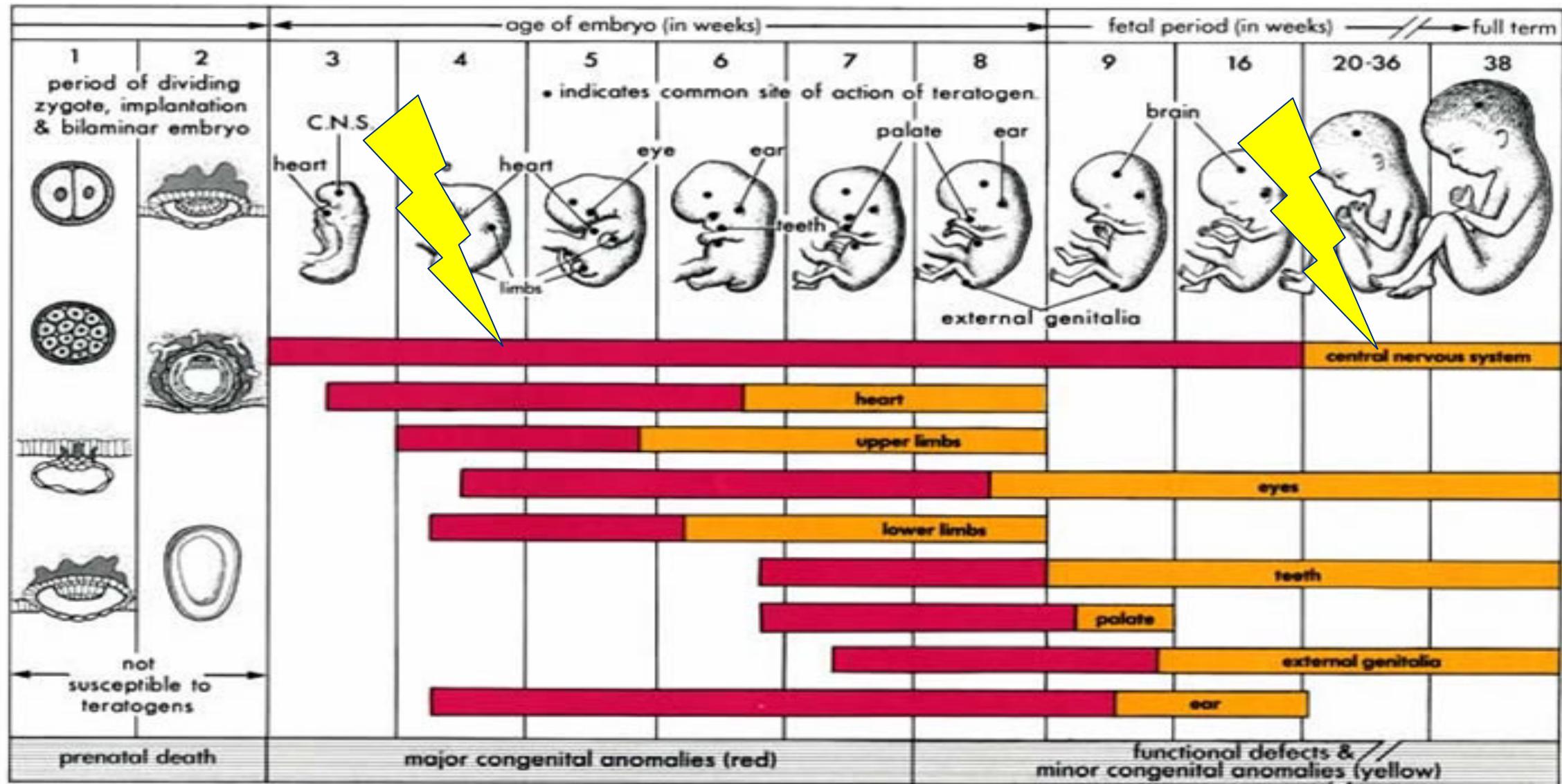


MWCNT consistently decreased immune reactivity

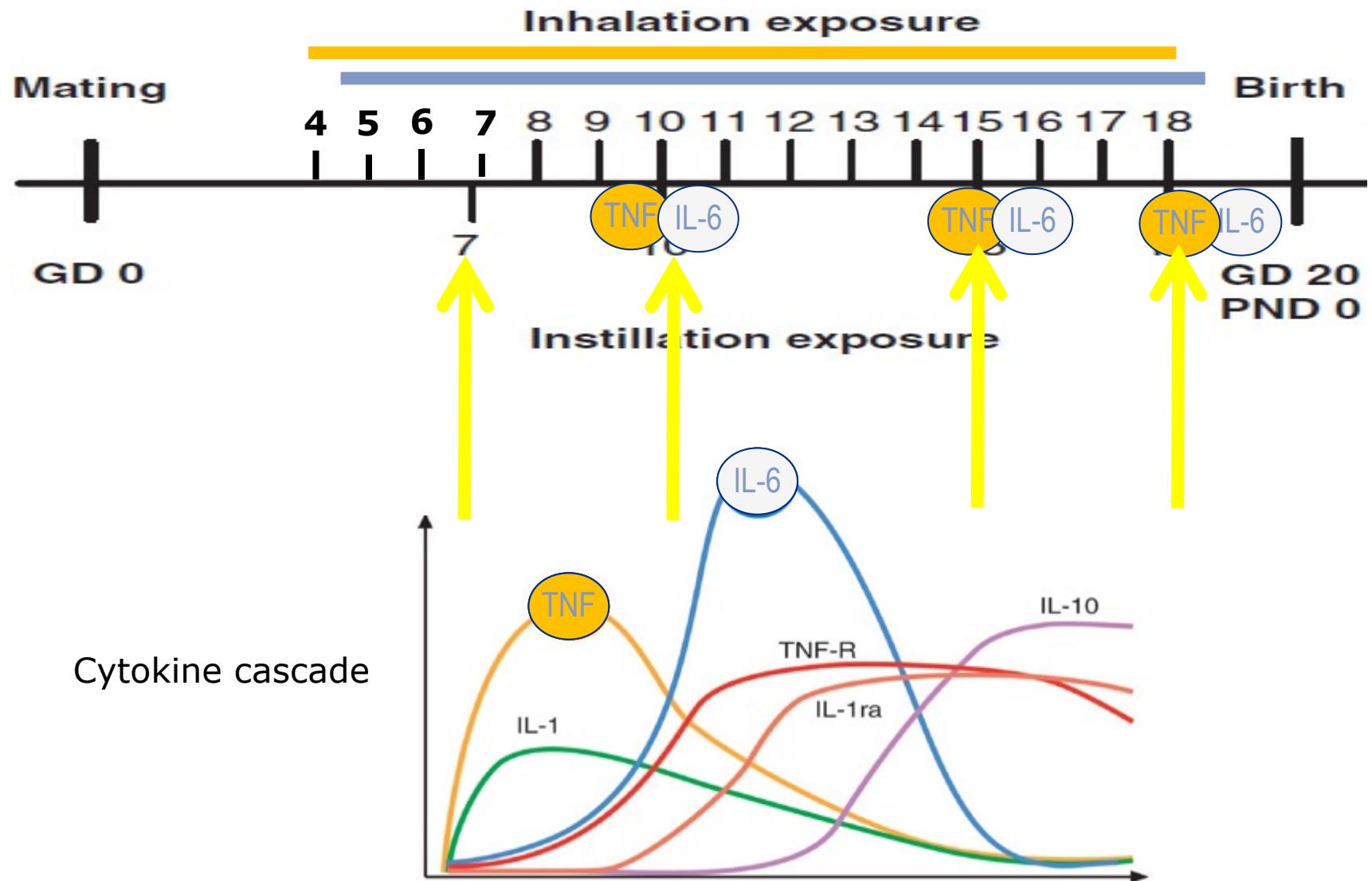
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	Level	Days in gestation	Maternal lung inflammation	Gestation/lactation	Geno-toxicity	Central nervous system	Male fertility	Immune system
TiO ₂ NP	42 mg/m ³	8-18 (1 hour)	Red	Grey	Grey	Red	Red	-
MWCNT	1*67 µg	0*	Red	Grey	Grey	Grey	-	Red
	1*67 µg	0*	Red	Grey	Grey	-	-	Red
Diesel-particles	18 mg/m ³	7-19 (1 hour)	-	Red	Grey	Red	Red	-
Carbon black NP (Printex 90)	42 mg/m ³	8-18 (1 hour)	Red	Grey	Red	-	-	-
	4*67 µg	7,10, 15,18*	Red	Grey	Grey	Red	-	-
	5 mg/m ³ 34 mg/m ³	4-18 (0.75 hour)	(9 days after birth)	Grey	Grey	Red	Grey	-



Fetal development is a sequence of events...



Influence of exposure timing



To conclude: Offspring sensitive to maternal particle exposure



- Central Nervous System
 - Risk of neurodegenerative disease?
- Immune system
- Cardiovascular system (Phoebe Stapleton, Tim Nurkiewicz)
- (Male reproduction)
- ?

Stepping forward...

- Test robustness of findings - repeat studies!
- Identify (most) sensitive organ(s)
- Underlying mechanisms (direct vs. indirect)
- Strain differences in sensitivity to particles

Life course perspective – especially relative to CNS effects



Parents'
exposure
prior to
pregnancy

Mothers'
exposure
during
pregnancy

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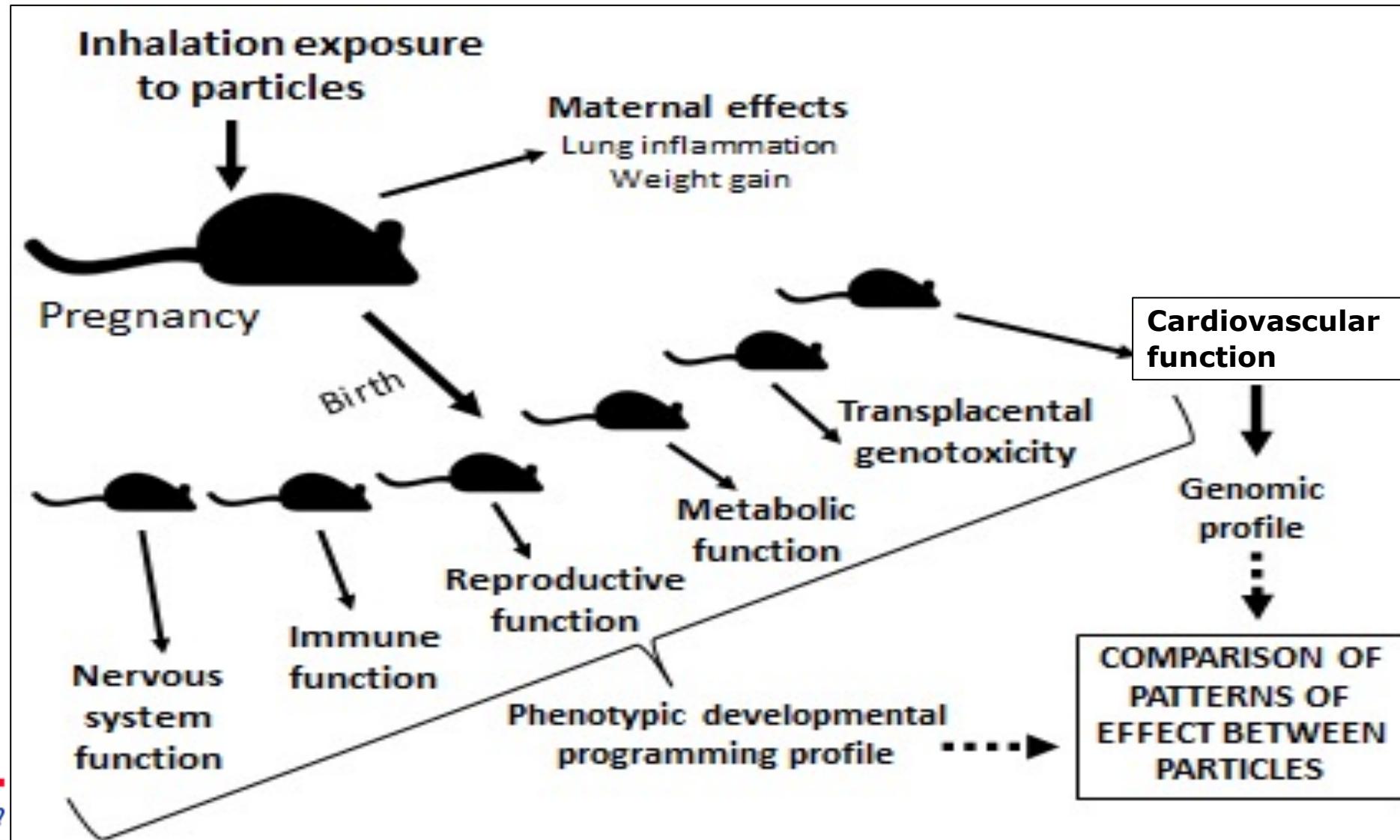
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Atsuto Onoda
Masakazu Umezawa

Sandra Goericke-Pesch²⁸

Does a "particle pattern" exist?



Influence of exposure timing

