Toxicological aspects of Carbon Nanotubes

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Introduction

Deposition of particles/fibres in the respiratory tract

Factors influencing biological activity of CNTs

Toxicity testing

Biodegradation

Summary / Conclusion
Introduction: Many Different Carbon Nanotubes (Single-; Double-; Multi-Walled)

- DWCNT spun into fibers (www.lanl.gov)
- MWCNT as agglomerates (www.arkema.com)
- Forest of MWCNT (www.me.mtu.edu)
- Agglomerates of Baytubes®
- Bamboo-like CNTs (www.nanotechweb.org)
- SWCNT (www.nano-lab.com)
Introduction: Differentiated Morphology for MWCNTs

- „Long and Thick“ versus „Short and Thin“ MWCNT
  - Morphology of tubes (diameter and length) examined with electron microscopy

**WHO fiber definition for carcinogenic fibers:**
Length > 5 μm; Ø < 3 μm, ratio L : A > 3 : 1

IARC (2002) Mane-made vitreous fibers. IARC Monogr. 81
Deposition of (nano)materials

Oberdörster et al., EHP (2005)

Nasen-Rachen-Raum: 5-10 µm
Trachea: 3-5 µm
Bronchien: 2-3 µm
Bronchiolen: 1-2 µm
Alveolen: 0,1-1 µm
Lung surfactant and Clearance

Protein containing lung surfactant
Nel et al., Nature Materials (2009)

Retained dose is of importance

Deposited dose minus cleared dose = retained dose

⇒ Biologically effective dose

Lung clearance

Lung macrophages (10 - 20 µm)

Frustrated phagocytosis

Fiber Pathogenicity Paradigm

Asbestos

Carbon nanotubes

Clearance
Inflammation

Donaldson et al., PFT (2010)
Effects depending on shape

Donaldson et al., (2006)
Factors influencing biological activity

- Agglomeration
- Surface
- Adsorption
- Reactivity

Nel et al., Nature Materials (2009)
Most applied toxicity tests

*In vivo*

- Intraperitoneal injection
- Inhalation studies (acute, subchronic)

*In vitro* cellular assays

- DNA damage
- Cell growth
- Inflammation
- Oxidative stress
Examples of toxicity testing results

**in vivo**

**Intraperitoneal injection** (Poland et al., 2008; Muller et al., 2009; Varga and Szendi, 2010; Takagi et al., 2008, 2012)

- investigating biological activity of CNTs in peritoneal cavity of rodents
- Results dependent on fiber type and shape as well as animal model

**Subchronic inhalation studies (90 days)** (Ma-Hock et al., 2009; Pauluhn, 2010)

- inflammation at highest concentrations, no systemic toxicity

**OEL:** 0.05 mg/m³ (8h TWA) for Baytubes ®

Standardized experimental settings necessary for toxicity testing \textit{in vitro}

**DNA damage, inflammation, oxidative stress**

- Many \textit{in vitro} models, many endpoints, mainly not standardized methods
- Effects depending on fiber shape, size, application, culture medium, cell line
- Controlled experimental settings necessary
- Selection of an appropriate dose range on the basis of available human exposure data
- Extensive characterisation of CNT under the applied assay conditions
CarboTox-Project

- Project sponsored by BMBF with ca. 1Mil€ budget to develop screening tests for possible carcinogenic potential of MWCNTs
- Project lead: Fraunhofer ITEM
- Partner: Bayer MaterialScience

Leibniz Institute for Solid State and Materials Research Dresden

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CarboTox-Project: Different types of CNTs were tested
CarboTox-Project: first results

- Percentage of CNT > 20 µm (L) varies between 2 % and 14 %
- i.p. injection (rats), examination after 3, 6 and 12 months
- Two concentrations: $1 \times 10^9$ and $5 \times 10^9$ Fibers
- Thickening of peritoneum at high concentrations
- No changes of peritoneum after MWCNT1-exposure (Hackbarth et al., 2013)
- In vitro-studies with mesothelial cells for comparison
Summary CarboTox Project

- Effects are dependent on type and shape of CNTs
- Surface of CNTs seems to influence biological activity (more studies are needed)
- Fiber shape is crucial for biological effects in vivo
- Primary mesothelial cells can be used as a suitable cell culture model
Nanorelease: Project Biodegradation

Nanorelease Project
ILSI Research Foundation
with US-EPA, 2011-2013
http://www.nanotechia.org/global-news/the-nanorelease-project

Possible scenarios of CNT-composite degradation

(Wohlleben et al., 2011)
Biodegradation depends on functionalization of CNTs

(Liu et al, Carbon, 2010)

- Changed physico-/chemical properties
- Changed biopersistence?

(Li et al, 2009)
Biodegradation of SWCNTs at low pH

Degradation and morphological instability of SWCNT-COOH upon 90 day exposure to oxidative phagolysosomal simulant. (Liu et al., Carbon, 2010)

Interesting for biomedical applications (e.g. drug delivery)

Lysosomal internalization of „cellular waste“ (Stern et al. 2012)

-acidic pH and hydrolytic enzymes in lysosomes allow biodegradation
Hypothesis of mode of action (I)

1.) Possible interaction with cell organelles

- size-dependent uptake of CNT
- deposition

(Peretz and Regev, 2012)
Hypothesis of mode of action (II)

2.) Oxidative stress

- Dependent on surface of nanomaterial

Nel et al., Nature Materials (2009)
Summary/Conclusion (I)

- Deposition of NM in the respiratory tract is size dependent.
- Lung clearance is possible if macrophages are able to phagocytose the NM (size).
- Effects of inhaled fibers depend on fiber shape (long, thin, biopersistant).
- Surface properties of NM play a role (surface reactivity, protein binding, radical formation).
- *In vitro* findings are controversial, standardized methods are needed.
Summary/Conclusion (II)

- OEL of 0.05 mg/m³ for Baytubes® as result of inhalation study
- Surface, size and structure of CNT seem to be of importance for biological activity (CarboTox)
- No release of NM embedded in materials (Nanorelease-Project)
- Biodegradation of functionalized SWCNTs possible by lysosomal fluid (enzymatic digestion)
Thank you!