A strategic approach to assess workplace exposure to high-aspect ratio (nano)-materials (HARM)

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Content

What are High-Aspect Ratio Materials (HARM)?

German Standards of the Assessment of Workplace Exposure to HARM

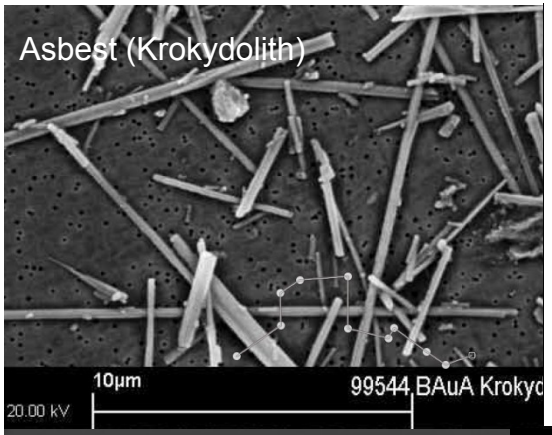
Particle collection,
Identification and
counting of hazardous fibers

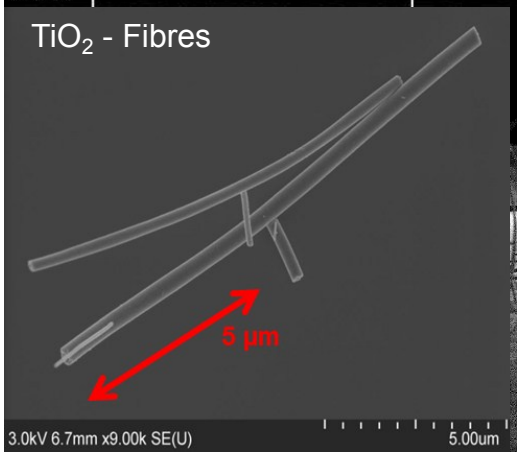
Can it be applied to nanoscale HARM?

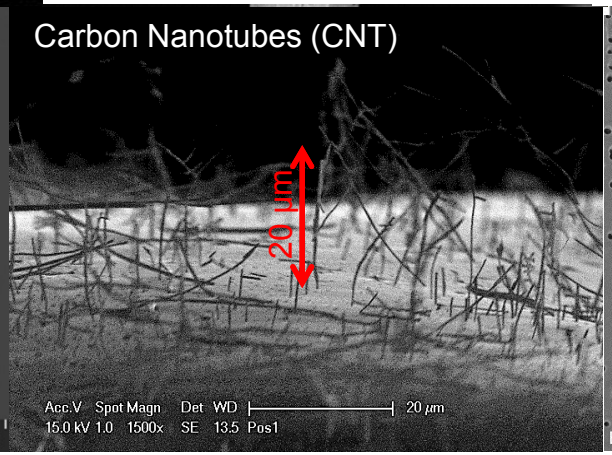
Extension of German Standards

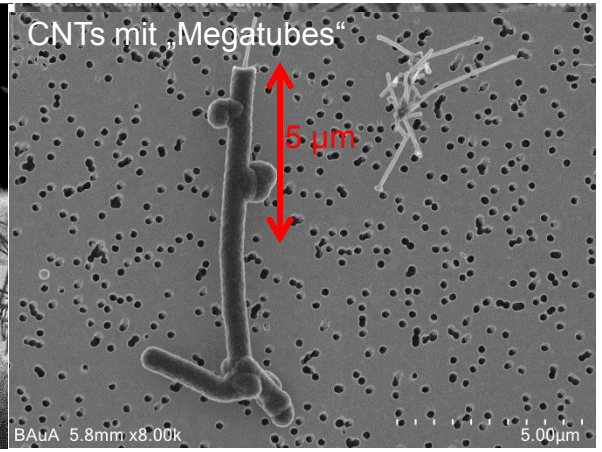
Field Study at workplace handling Nanotubes

High Aspect-Ratio Materials (HARM)









High Aspect-Ratio Materials (HARM)

Risk of HARM is associated with…

… respirable biopersistent fibrous morphologies

Small & airborne

Insoluble & non-cleared

High-aspect ratio

Assessment of Workplace Exposure to Inorganic Fibers

Personal sampling of workplace atmosphere on nuclear pore filters

Sampling inside beathing zone

„PGP“-type sampler with gold-sputtered nuclear pore filter

Microscope-aided Particle counting

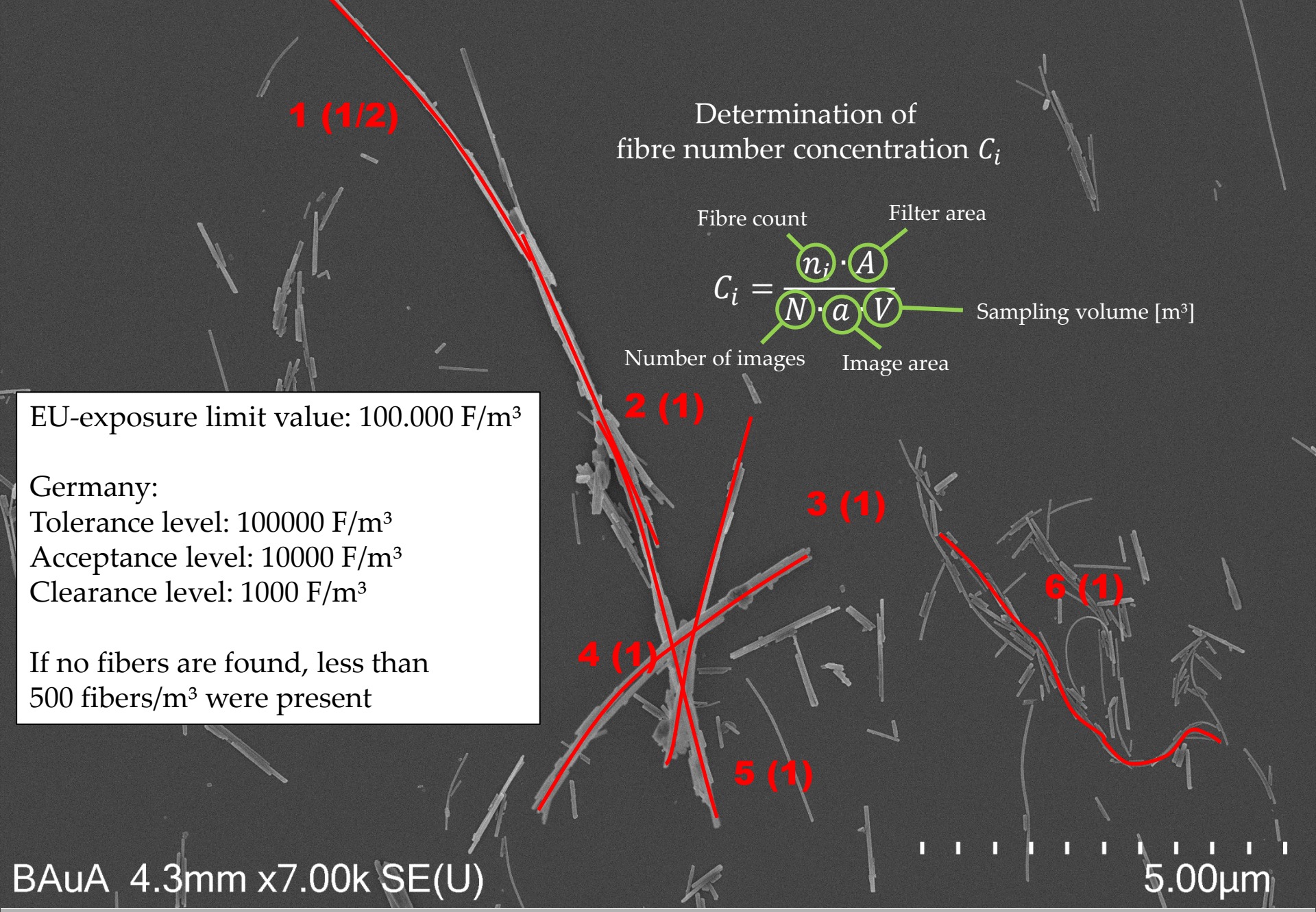
Counting rules following the German Standard VDI 3492

German VDI 3492 (originally from 1991, new version from 2013)
Manual, visual quantification with microscope (here: SEM example image)

(Some) counting rules:

1. Minimum evaluation area: 0.5 mm^2
2. Convention for hazardous fibers
   - length > 5000 nm
   - aspect ratio 3:1
   - 200 nm < diameter < 3000 nm

Due to technical resolution limit of phase-contrast microscopes!
(American Standard NIOSH Method 7402: diameter limit 250 nm)



EU-exposure limit value: 100.000 F/m³

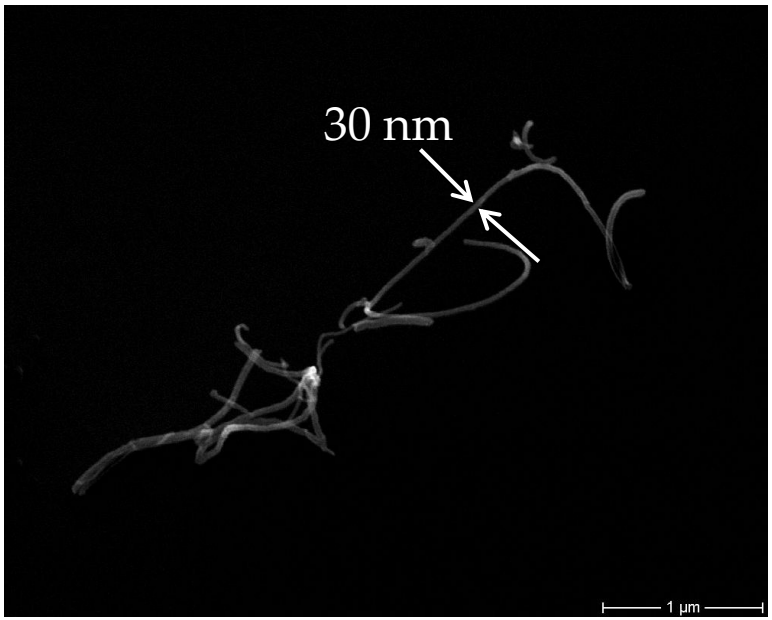
Germany:
Tolerance level: 100000 F/m³
Acceptance level: 10000 F/m³
Clearance level: 1000 F/m³

If no fibers are found, less than
500 fibers/m³ were present

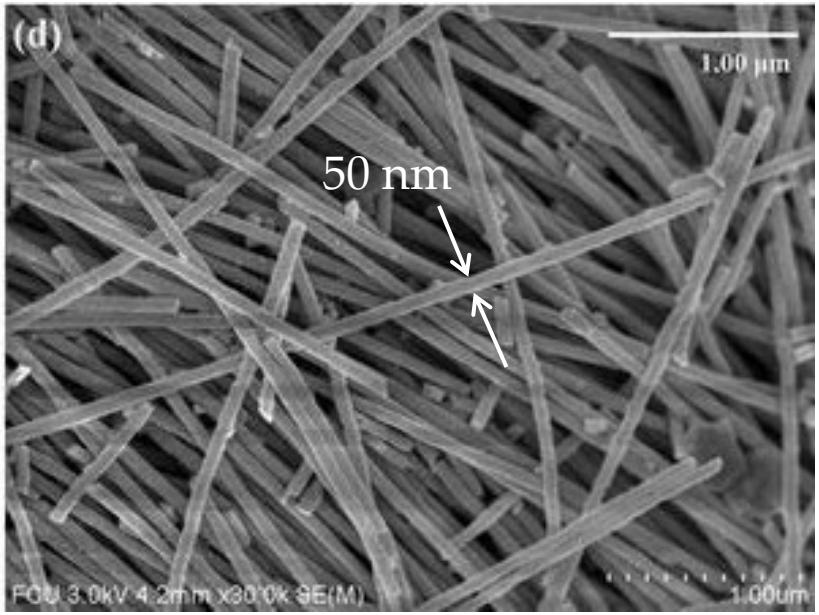
Application to Nanoscale HARM

Main issue:

Due to lower diameter detection limit of 200 nm,
the German VDI 3492 would not allow counting of relevant nanoscale HARM



Multi-walled Carbon Nanotubes
(MWCNT)

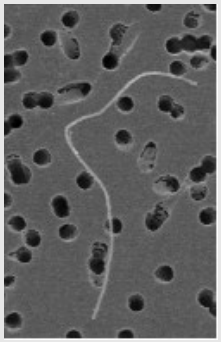


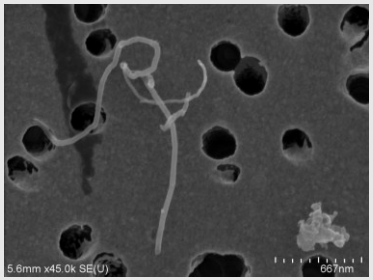
β-In_2S_3 Nanowires

Classification of nanoscale HARM-particles

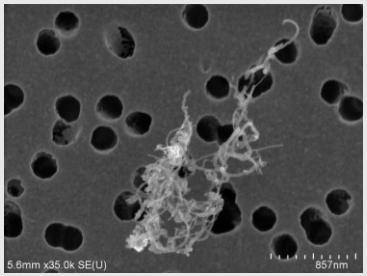
Morphologies typical for nanoscale HARM-particles

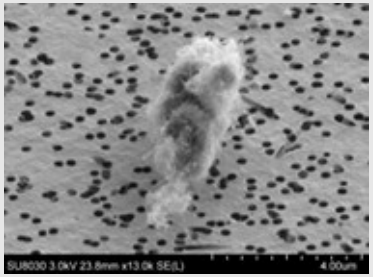
Category I:
Single fibers identifiable with 1 µm < L < 5 µm



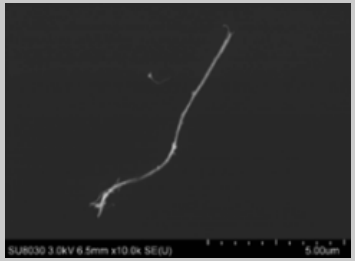


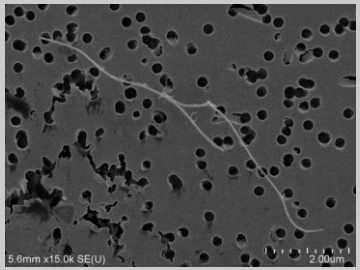
Category II:
closed clusters or agglomerates with L:W > 3:1 and 1 µm < L < 5 µm



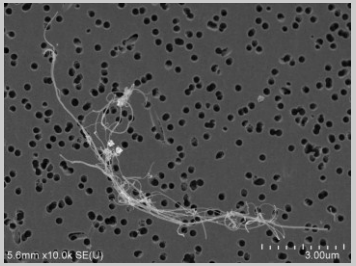


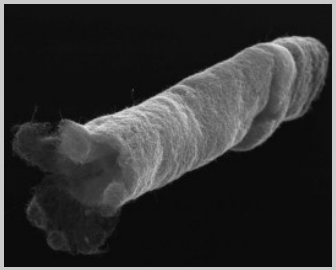
Category III (WHO):
Single fibers identifiable with L > 5 µm





Category IV (WHO):
closed clusters or agglomerates with L:W > 3:1 and L > 5 µm





Application of electron microscopy (EM)

By using EM: No technical limitation like maximum resolution!

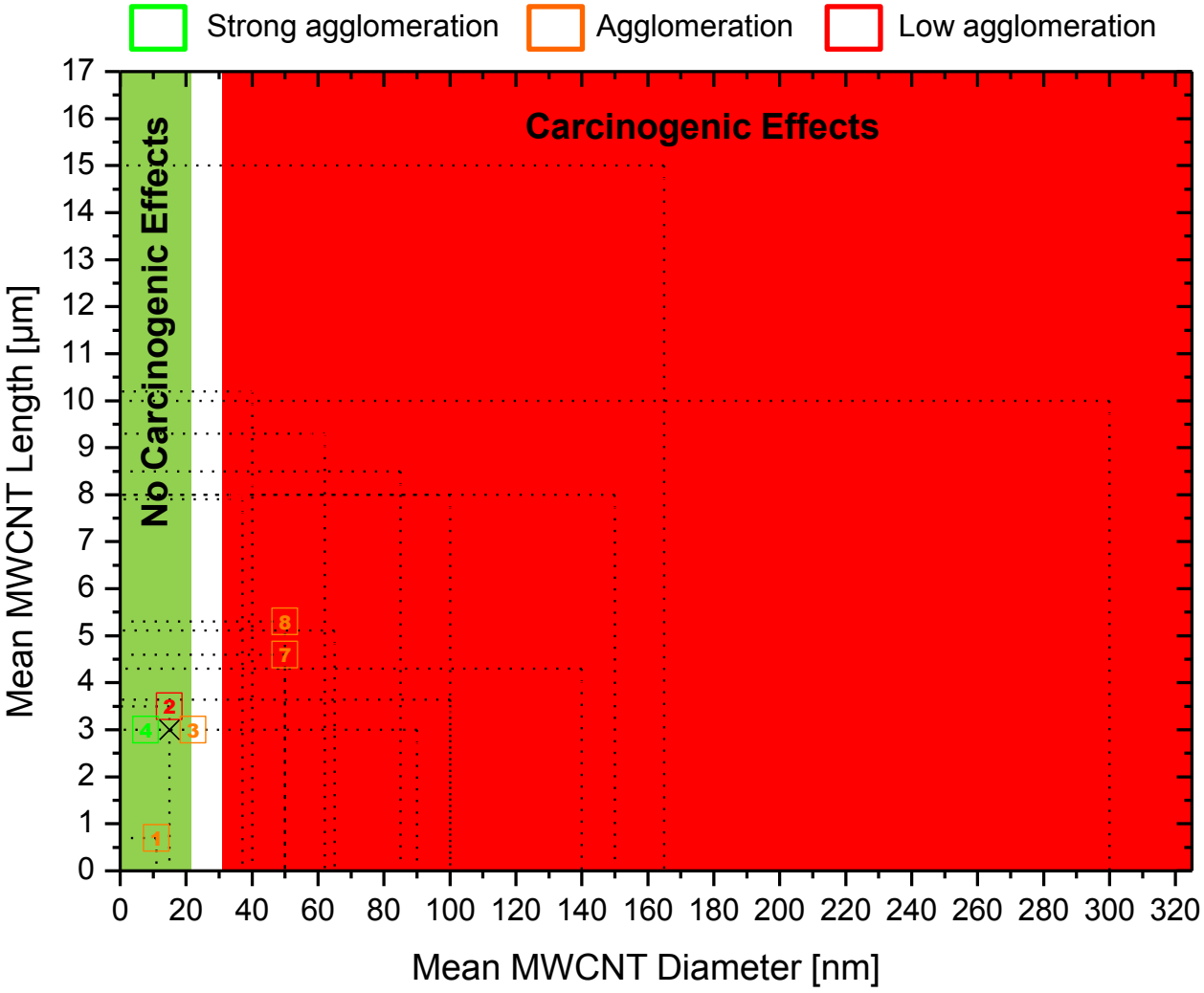
SEM image resolution necessary to cover 0.5 mm² area

Image Pixel Size	Images of 1.2 Mega Pixels	Images of 20 Mega Pixels
1 nm	400 000	25 000
3 nm	44 400	2 750
5 nm	16 000	1 000
10 nm	4 000	250
20 nm	1 000	63
50 nm	160	10
100 nm	40	3
200 nm	11	2

Manageable number of SEM images

Covering all known HARM during classification would require far too much effort

Review on MWCNT toxicity (IP tests)



[1] Muller, J., et al. (2009) Toxicol. Sci. 110(2): 442-448

[2,17] Murphy, F. A., et al. (2011) Am. J. Pathol. 178(6): 2587-2600

[3,16] Xu, J., et al. (2014) Cancer Sci. 105(7): 763-769

[4,7-8,15] Nagai, H., et al. (2011) Proc. Natl. Acad. Sci. USA 108(49)

[5-6,9,11] Rittinghausen, S., et al. (2014) Part. Fibre Toxicol. 11: 59

[10,13] Xu, J., et al. (2012) Cancer Sci. 103(12): 2045-2050

[12] Sakamoto, Y., et al. (2009) J. Toxicol. Sci. 34(1): 65-76

[14] Takagi, A., et al. (2008) J. Toxicol. Sci. 33(1): 105-116

[18] Sargent, L. M., et al. (2014) Part. Fibre Toxicol. 11: 3

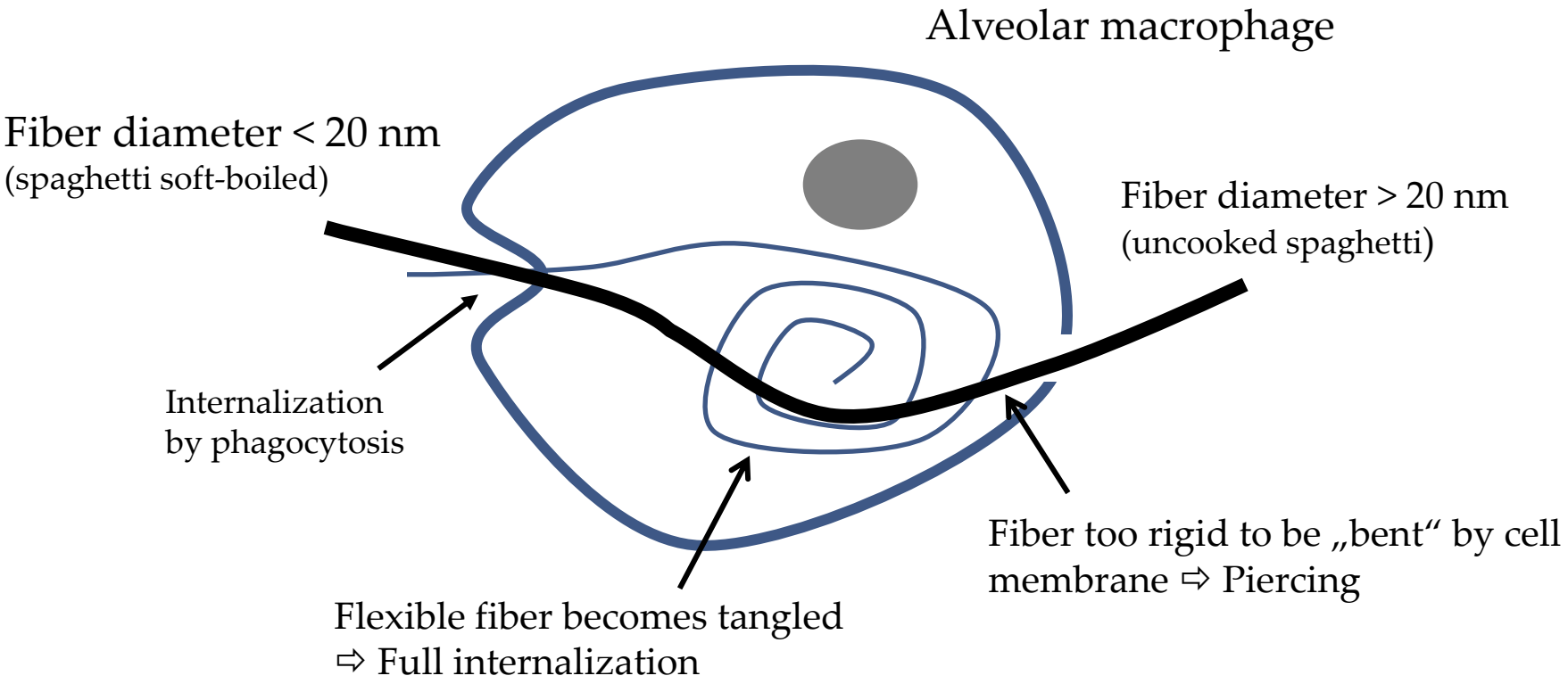
[F. Herzberg, BfR]

Hypothesis: Fibers with diameters smaller than ca. 20 nm are not carcinogenic

Hypothesis: High Fibre-Rigidity Impairs Lung Clearance Mechanism?

Lung clearance: Removal of particle by macrophages

MWCNTs with diameter >20 nm are too rigid to be internalized by alveolar macrophages



Proposal to extend VDI 3492 for Nanoscale HARM

Adapted counting rules, when nanoscale HARM might be present on filter samples:

1. Use EM to take images from filter samples

- 20-megapixel images
- X-nm pixel size
- number of images: ca. 60

⇒ X=20, but can change depending on properties of fibers expected at workplace

2. Count hazardous fibres adapted counting rules

- length > 5 µm,
- 20 nm < diameter < 3000 nm
- aspect ratio 3:1

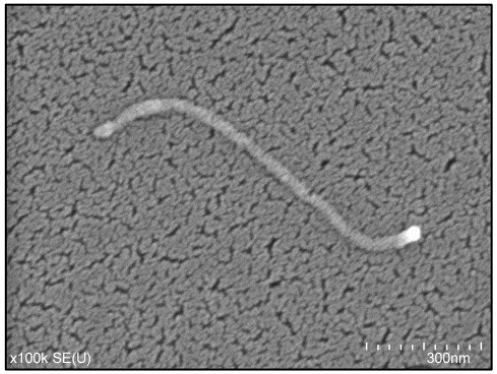
Field study: Workplace handling MWCNT (1)

Assessment of workplace exposure during dispersion of a MWCNT-containing masterbatch

Method: Sampling on gold-sputtered nuclear pore filters (0.2 µm pore size)

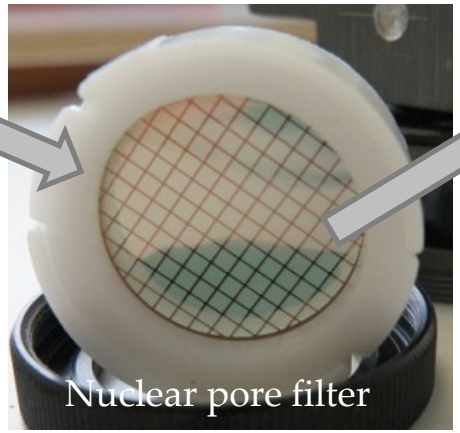
Questions for assessment strategy:

- Were fibers released?
- Were hazardous fibers (new convention) released?
- If yes – how high was the concentration?



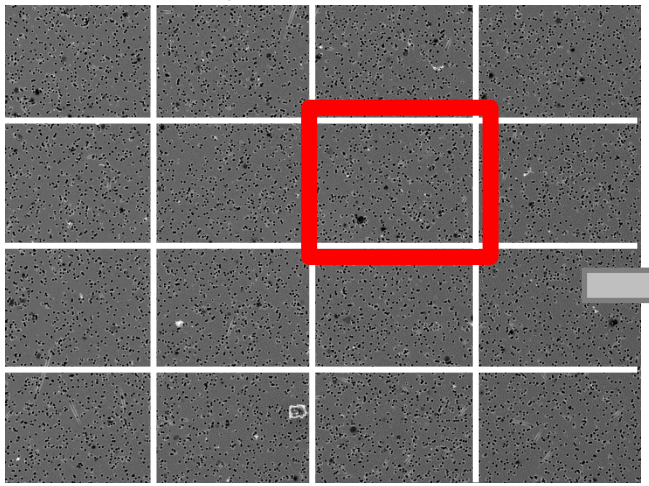
Field study: Workplace handling MWCNT (2)


Dispersion process

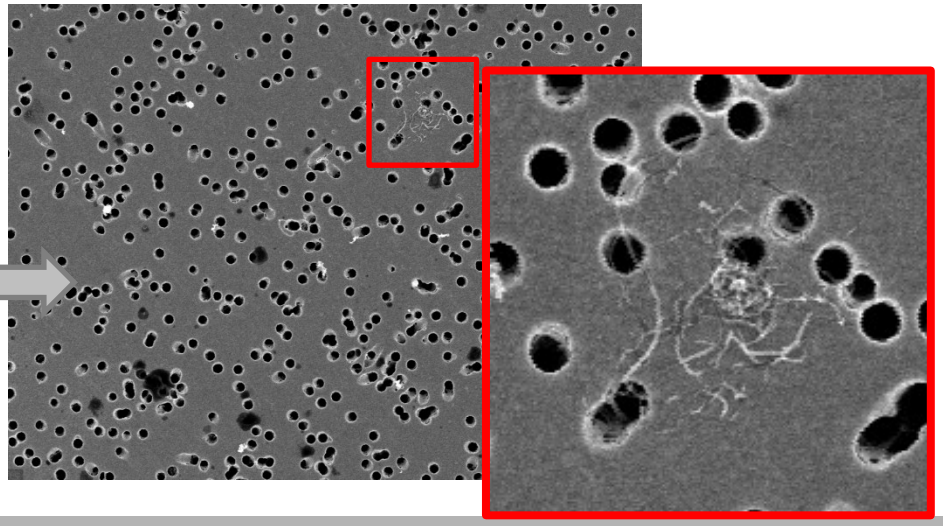

Nuclear pore filter

- Sample volume: 300 ml
- SEM-images on 170 randomly chosen locations on filter
- 20 megapixel-images at 2000x magnification (1 pixel = 12 nm) gives 3022 µm² area per image
- 170 x 3000 µm² ≈ 0.5 mm²

1 of 170 images

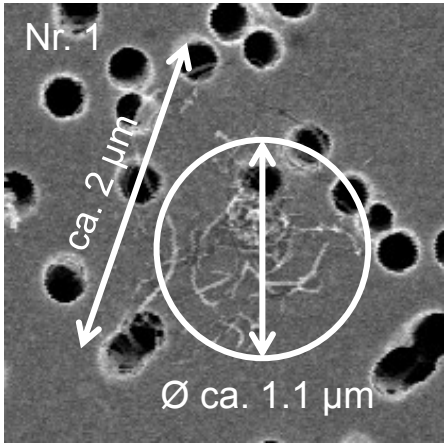


1 of 2720 image sections

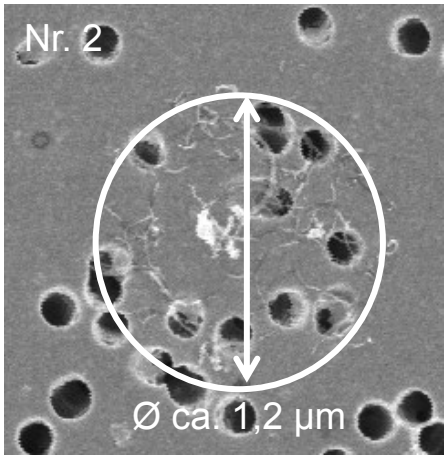


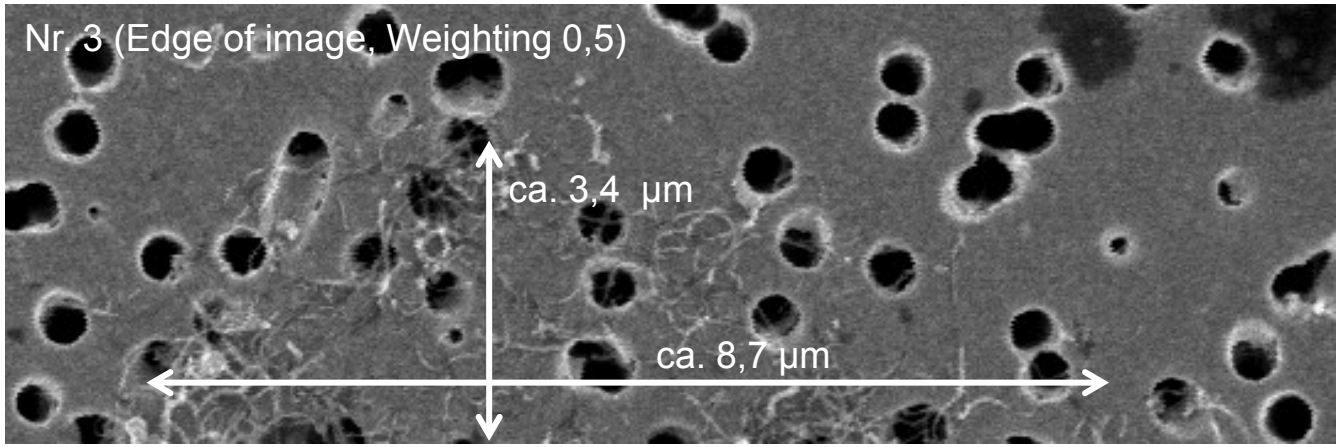
Field study: Workplace handling MWCNT (3)

Results: 46 fibre-like objects, but only 3 fibres were found in the 170 images that were clearly CNTs



- Calculated concentration: 8600 F/m³
- CNT-particle don‘t show morphologies fitting the criteria of hazardous HARM length >5µm, 20 nm < diameter < 3000 nm, aspect ratio 3:1





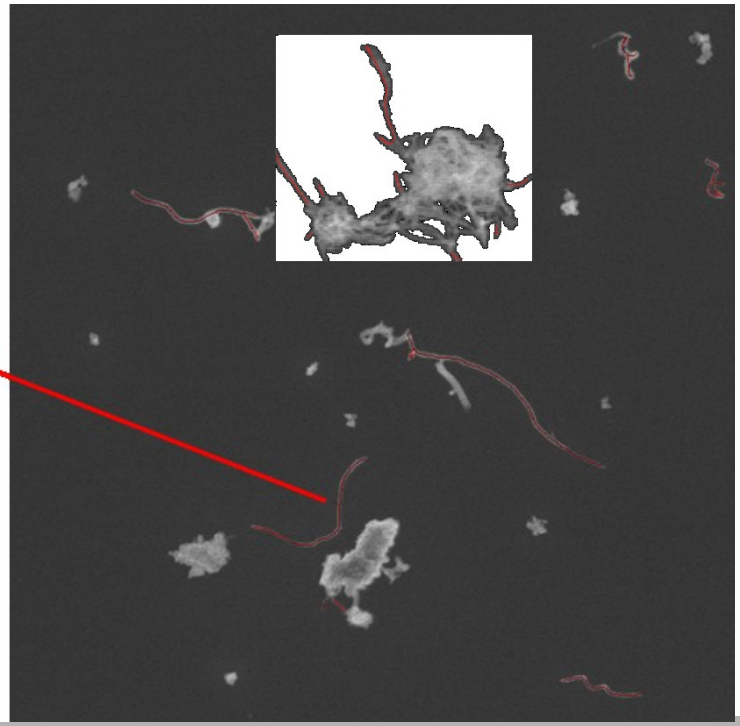
Software-aided classification of SEM images

The need to evaluate >2000 image sections hinders frequent application of assessment strategy

Solution

Automatic fiber identification and classification of

- All morphology classes: Fibers, hazardous fibres, agglomerates, hazardous
- Determination of numbe



Summary

- An extension of the German standard VDI 3292 for assessment of workplace exposure to inorgnanic fibers for nanoscale HARM was suggested .
- A new lower fiber diameter detection limit (20 nm) was defined on the basis of the scientific theory of rigidity-mediated fiber toxicity.
- The new assessment strategy was tested on a worksite handling MWCNT.
- CNTs were found, but hazardous fibers were not identified.
- Software-aided particle counting could sifnificantly lower the effort to conduct this assessment strategy

Thank you

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