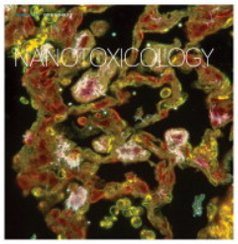

Nanotoxicology

ISSN: (Print) (Online) Journal homepage: informahealthcare.com/journals/inan20

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To cite this article: Henrik Wolff & Ulla Vogel (2024) Recent progress in the EU classification of the health hazards associated with certain multiwall carbon nanotubes (MWCNTs): what about the other MWCNTs?, Nanotoxicology, 18:2, 119-121, DOI: 10.1080/17435390.2024.2321885

To link to this article: https://doi.org/10.1080/17435390.2024.2321885

Published online: 27 Feb 2024.

NANOTOXICOLOGY
2024, VOL. 18, NO. 2, 119–121
https://doi.org/10.1080/17435390.2024.2321885

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COMMENT

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Recent progress in the EU classification of the health hazards associated with certain multiwall carbon nanotubes (MWCNTs): what about the other MWCNTs?

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ARTICLE HISTORY Received 14 February 2024; Revised 15 February 2024; Accepted 15 February 2024

In nanotoxicology, many of the studies on high aspect nanomaterials (HARNS) have been focused on particles that have been perceived to have similarities with asbestos fibers. As a result of these studies, IARC classified a particular MWCNT, MWNT-7 (also known as Mitsui-7) as 'possibly carcinogenic in humans (2B)' (IARC 2017).

In the larger context, the experiences with asbestos and other fiber-like materials including MWCNTs, have led to the so-called fiber pathogenicity paradigm (FPP) (Donaldson et al. 2011) This can be seen as a structure/toxicity model that identifies physio-chemical properties related to fiber pathogenicity. These properties are biopersistence and a set of dimensions based on WHO asbestos counting rules for occupational hygiene (length ≥ 5 µm, diameter ≤ 3 µm and an aspect ratio of ≥ 3:1) (WHO 1985). Importantly, the FPP also encompasses materials with a diameter larger than 100 nm, i.e. not filling the criteria for nanomaterials. Recently, the FPP was used directly for the classification of health risks. The risk assessment committee (RAC) of European Chemicals Agency (ECHA) has adopted a proposal to classify FPP compatible MWC(N)Ts as carcinogenic, category 1B (CLH-0-0000007108-75-01, adopted 18.03.2022). The classification proposal was submitted by the German Federal Institute for Occupational Safety and Health (BAuA). The adopted proposal contains a dimensional criterion not included in the FPP dimensions, stating that the diameter of the MWCNT should be ≥ 30 nm. This is based on the findings that the smallest diameter MWCNT known to cause mesothelioma when injected intraperitonially is 37 nm (Rittinghausen et al. 2014) and the largest diameter of MWCNTs that known *not* cause mesotheliomas is 15 nm (Xu et al. 2014). The RAC also found that a classification for 'Specific organ toxicity after repeated exposure' (STOT RE 1) is warranted for the type of materials included in the proposal adopted buy RAC/ECHA, The 'specific organ toxicity' mentioned refers to pulmonary fibrosis. The adopted proposal is still being further processed by the European Commission.

The RAC noted a need to evaluate MWCNTs, not filling the dimensional criteria in the proposal adopted by the RAC, i.e. MWCNTs shorter and/or thinner than those addressed in the BAuA classification proposal. The RAC discusses a study of these other MWCNTs (Saleh et al. 2020), this consisted of the comparison of rats exposed by intratracheal instillation to a long and rigid MWCNT (MWCNT-A), a short and thin (length 1.04 µm and diameter 7.4 nm in the exposure vehicle) MWCNT (MWCNT-B) and crocidolite asbestos. Surprisingly MWCNT-B caused more tumors than the others, and MWCNT-B exposure was more strongly associated with hyperplastic changes as compared to the other materials in the study.

In an additional study from the same group (Saleh et al. 2022), a double walled carbon nanotube (DWCNT) of a tangled type with a diameter of around 14 nm was used. The results of the DWCNT exposure were similar to the exposure to MWNT-7 regarding the causation of pulmonary neoplasia and bronchoalveolar hyperplasia. The diameter of this DWCNT is obviously under the limit for being addressed by the adopted proposal, and moreover it is unclear whether DWCNTs are considered in the proposal at all.

Barthel et al investigated (Barthel et al. 2023) if transgenic P53−/+ rats could be used to speed up animal studies on carcinogenesis. In this study, rats

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were exposed to NM-403, a MWCNT with a length of 0.4 µm and a diameter of 12 nm and the classic MWCNT-7 with a length of 5.7 µm and a diameter of 74 nm. The main result was that NM-403 caused more bronchioalveolar hyperplasia than MWNT-7. In addition, the NM 403 exposure resulted in pulmonary alveolar proteinosis (PAP), which was also reported earlier for rats exposed to the same material (Gaté et al. 2019). In two additional studies, PAP was reported after exposure to short and/or thin HARNs, in an inhalation exposure to rats of a MWCNT (Ma-Hock et al. 2009) and in mice exposed to a rod-shaped titanium oxide (Danielsen et al. 2020).

For asbestos and other materials, capable of causing fibrotic and malignant changes in exposed humans and animals, the ability to cause a persistent inflammatory response seems central as described in several nano-relevant adverse outcome pathways (Halappanavar et al. 2020, 2023). Any material reaching the lungs of test animals can cause inflammation for a period, however studies on MWCNTs where persistence of inflammation is determined are relatively rare. In a study by Pauluhn (2010), rats were exposed to a a MWCNT with a high tendency to form' low density assemblage structures'. The diameter of the MWCNTs was 10 nm and the length (in liquid after rigorous ultrasonification) was 200-300 nm The inhalation exposure lasted for 13 weeks, and the rats were followed for up to 6 months after exposure. In the histological evaluation of the lung from these rats, inflammation was detected in the bronchioalveolar region together with increased interstitial collagen staining. This persisted up to 6 months post-exposure in a dose-dependent manner. The authors did not attribute this finding to a fiber effect but rather an overload type of effect caused by the 'high displacement volume of the low density MWCNT assemblage structure'. Somewhat similar findings were obtained, with regards to inflammation, in an inhalation study using a MWCNT with a diameter of ca. 12 nm and a length of 1.07 µm ± 1.10 µm (Pothmann et al. 2015).

In a study by Knudsen et al. (2018), 11 MWCNTs were intratracheally instilled in mice and followed for a year. The thin and entangled MWCNT still showed variable degrees of inflammation related changes in the lungs one year after the instillation of the material, while the two thick and long fibers (MWNT-7 and NM-401) did not.

Taken together, there is reason to believe that MWCNTs, which are non-FPP compliant and not included in the BAuA proposal adopted by the RAC, that can cause persistent inflammation, pulmonary neoplasia, PAP and fibrotic changes. In addition, the short and/or thin fibers seem to have a propensity to cause bronchoalveolar hyperplasia to a larger extent than the FPP compliant fibers. There is so far no evidence for the causation of mesotheliomas by these non-FPP compliant MWCNTS (Nel 2023).

For FPP compliant HARNs, 'frustrated phagocytosis' (Donaldson et al. 2011) i.e. the inability to envelope long fibers within the macrophage, is a central hypothesis for the mechanism-of-action of the material to cause persistent inflammation and thus carcinogenic and other pathogenic processes. It would not seem feasible for short and flexible fibers to be able to cause this frustrated phagocytosis. The somewhat different spectrum of histological changes seen with non-FPP as compared to FPP MWCNTs (PAP and hyperplasia) and the lack of evidence that FPP non-compliant fibers could cause mesotheliomas (Nel 2023) provide evidence for a difference in the pathogenic mechanisms of action of FPP compliant and non-compliant MWCNTs.

Pauluhn (2010) has suggested that the material they studied caused overload by forming three-dimensional low-density structures, it is however not clear that all the materials discussed here form similar structures. Another possibility is indicated by the studies with two MWCNTs and a rod-shaped TiO_2 mentioned above, that caused PAP. Quartz exposure can cause silicosis, defined by the presence of fibrotic nodules in the lungs. Silicosis in turn is a risk factor for pulmonary cancer. Quartz exposure is also associated with PAP, the mechanism by which quartz causes its effects is thought to be related to destructive effects on lysosomes and this could perhaps be another possible mechanism of action for the pathogenic effects of the short and thin MWCNTs.

In conclusion, as all MWCNTs consists of synthetic graphite in a tubular form, and they mainly differ from each other in their physical dimensions, it is understandable that the dimensions, in the form of the FPP, plays a central role in the evaluation of the risks associated with MWCNTs. However there also appears to be a clearcut need to evaluate the risks of carbon nanotubes not encompassed by the adopted proposal. For this evaluation, additional long-term animal experiments are of a central importance. An additional, open question is the extension of the FPP to non-MWCNT types of HARNs and considering this in the hazard classification of these materials.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by FFIKA and FFIKA2, Focused Research Effort on Chemicals in the Working Environment, from the Danish Government and the Nummela Foundation, Finland.

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