Toxics Use Reduction Institute Science Advisory Board Meeting Minutes
December 8, 2022
Virtual Zoom Meeting
10:00 AM

Members Present: Robin Dodson (Chair), Christine Rioux (Vice Chair), Heather Lynch, Wendy Heiger-Bernays, Rich Gurney, Denise Kmetzo, Lisa Cashins, Helen Poynton

Members not present: Christy Foran

Program staff present: Liz Harriman (TURI), Heather Tenney (TURI), Karen Thomas (TURI), Caredwen Foley (OTA), Sandy Baird (Mass DEP)

Others present: Carol Holahan (Foley Hoag ACC), Christina Bramante (Nano-C), Raza Ali (ACC), Tom Lada (Nano-C), Katherine Robertson (MCTA), Jerome Lang (Nano-C), John Monica (Offit Kurman), Clint Richmond (Mass Sierra Club) , Laura Spark (Clean Water Massachusetts)

Welcome & Introductions

The chair noted that this meeting is being conducted remotely, consistent with *An Act Relative to Extending Certain State of Emergency Accommodations* signed by Governor Baker on June 16th 2022. This allows the extension of the remote meetings under the Open Meeting Law until March 31, 2023**.** Board members introduced themselves, program staff were announced, and attendees were asked to put their name and affiliation in the chat.

Approve September Meeting Minutes

A motion was made to approve the September meeting minutes, and there was a second. A roll call vote was conducted and five members voted in favor, one abstaining.

Single Walled Carbon Nanotubes (vote)

At the March meeting the Board made a recommendation to list multi-walled carbon nanotubes based on the evidence of pulmonary toxicity, lung cancer, mesothelioma, and environmental persistence. There are additional concerns for genotoxicity and toxic environmental degradation products. The nano petition also asked the Board to consider carbon nanofibers and single-walled carbon nanotubes. The Board discussed carbon nanofibers at the April meeting, yet did not come to a conclusion. The Board began discussion of single-walled carbon nanotubes at the May meeting, continued in June and September. At the past single-walled carbon nanotubes meetings,

very high persistence, overall inflammatory effects and generation of reactive oxygen species and DNA damage were noted.

Pulmonary Toxicity

Draft summary statement from previous meeting:

There is evidence that SWCNTs cause pulmonary toxicity in animals, including inflammation, fibrosis, and granulomas. There is a lack of understanding, however, whether the high doses used in the animal studies exceed particle overload conditions, which are common with poorly soluble particles administered via inhalation. Particle overload overwhelms particle clearance mechanisms, resulting in tissue irritation, release of inflammatory cells, and generation of reactive oxygen species (ROS). Persistent lung inflammation can cause cell proliferation and tissue remodeling, which when insufficiently repaired can lead to non-neoplastic effects such as fibrosis, DNA damage, and possibly, tumor formation. Particle overload conditions are not a response to the agent but rather to the overload conditions.

New information since the last meeting is primarily the additional comments from Nano-C received November 22nd and the pulmonary toxicity table for single-walled carbon nanotubes. Also important to note for this meeting are the Vietti, Saxena, and Magnum studies shared previously.

A member asked if there was consensus on what dose causes overload. It was noted that it is difficult to determine which studies are not overload and therefore show actual pulmonary effects. It was also noted as shown in Zhang 2011, perhaps 0.06 mg/kg is consistent with occupational exposure. Tracheal aspiration studies are still valid for proof of principle. A member noted that there are too many variables to determine a cut off. The effects of prolonged inflammation are the same with overload and non-overload doses. It was noted that the Board must focus on hazard. There is inflammation at very low doses but it is non-specific and might or might not be clear hazard. A member reached out to another expert in the field and author of one of the studies. This person agreed that there is no simple way to determine if it is lung particle overload. The question is whether it meets the standard of concern enough to put it on the list even with this uncertainty. The NIOSH bulletin basically says that there are effects in animals and while it's not confirmed that this is relevant to humans, NIOSH is concerned enough to create an Occupational Exposure Limit. It was suggested that we characterize the uncertainty even though the decision to list is based on the evidence in the studies.

A member commented that the question of short-term inhalation versus aspiration for single-walled carbon nanotubes is similar to that of multi-walled carbon nanotubes. Based on the Oberdorster study, the degree of response to aspiration and short-term inhalation showing similar effects suggests that aspiration is appropriate for hazard identification.

A member offered that the Song, 2009 study on exposure to nanoparticles (not SWCNT) in polyacrylate paint, 30 nm in diameter, of a small group of women in China showed pulmonary fibrosis and granuloma. Two died of respiratory failure. The particles had been made more hydrophobic and perhaps more fibrous-looking. This shows strong effects from nanoparticles in general.

A member notes that the Board should be considering this through the lens of an accumulation of data. In addition to the pulmonary effects, the Board should use the other data, in vivo rodent, in vitro, molecular (Teeguarden, 2011). All this data tells a story. The environmental data is clear -- persistence, presence in benthic invertebrates, and environmental movement - - and that coupled with the hazard information is a body of evidence. A member offered that the Board should use an AOP approach as rodent studies become less available, that the molecular data and in vitro studies are important.

A member offered that there is sufficient evidence for fibrosis with SWCNTs.

Heather shared the summary statement from the previous meeting. Board members worked to incorporate today's discussion into the statement. A member offered that the Board should focus on the volume of hazard data and less on lung particle overload. A member stated that the Board should include the fibroblast evidence (Vietti 2016). A member noted the Dahm epi study, 12 facilities, 108 participants (CNT/F) where there was a broad range of exposures and 4 of the 12 facilities used single-walled carbon nanotubes. This shows occupational exposure. A member noted the Maynard 2003 study of exposure to single-walled carbon nanotubes at 4 facilities. This suggests that deposition is taking place.

A member pointed out that there is extrapolation relevance in what is known about other substances: asbestos, silica, carbon black. Some of the effects for single walled carbon nanotubes are comparatively greater than for asbestos. There was a short discussion on the relevance of comparing to other substances. It was noted that potency is not as relevant for hazard.

A member noted that there is weight of evidence here with the fibrosis and because collagen is involved it is not reversible. Heather noted that there will be an opportunity for visitor comments before a vote is taken.

The members took a five minute break to work on the language independently. Following this five minute break and some back and forth on language, the following was proposed with input from all Board members.

There is evidence that SWCNT cause pulmonary toxicity in animals, including inflammation, fibrosis, and granulomas. This evidence is supported by in vitro studies in which cellular changes and molecular events occur along the Adverse Outcome Pathway for fibrosis, irrespective of dose and route of administration. There is a lack of understanding, however, whether the high doses used in some of the animal studies exceed particle overload conditions, which may cause rodent-specific effects with limited relevance to humans. However, some of the same effects, such as fibrosis, may be caused by the substance itself. While human evidence is

limited, exposures to SWCNT in occupational settings have reported deposition and internal exposures are occurring. Overall, taken together, the weight of evidence indicates that SWCNTs may cause pulmonary effects in animals and humans

Genotoxicity

The Board moved on to genotoxicity. Following is the statement from a previous meeting. *SWCNTs have been shown to generate reactive oxygen species (ROS) in a dose-response manner. The generation of ROS is considered a major factor in the genotoxicity. ROS are able to cause the oxidation of DNA, DNA strand breaks or lipid peroxidation-mediated DNA adducts. Evidence for genotoxicity is not well supported, but there is evidence of ROS and DNA damage. Evidence for mutagenicity is not well supported by the evidence, which includes studies that lack adequate chemical and physical characterization. Jiang, 2020 was noted as a well-done study and helpful for this endpoint. Molecular toxicity of SWCNTs is characterized well and is concentration and structure dependent. The materials in this study were well characterized with varying lengths and functionalization, and all except a semiconducting SWCNT exhibited positive genotoxicity*.

One member summarized: there is evidence of ROS production in a variety of assays; there is one study with evidence of genotoxicity on micronuclei in response to SWCNTs; do not have direct evidence of genotoxicity though the effects are indicative of genotoxicity; no direct evidence of mutagenicity; SWCNTs generate ROS in dose-response manner. After further discussion, the following statement was proposed:

SWCNTs have been shown to generate reactive oxygen species (ROS) in a dose-response manner. The generation of ROS is considered a major factor in the genotoxicity. ROS are able to cause the oxidation of DNA, DNA strand breaks or lipid peroxidation-mediated DNA adducts.

There is evidence that SWCNTs cause induction of micronuclei and DNA stand breaks, indicative of genotoxicity, however mutagenicity is not well supported by the evidence, which includes studies that lack adequate chemical and physical characterization.

Environment

The statement on the environment is that there is very high persistence with no degradation.

Very high persistence with no degradation under typical environmental conditions

The Chair asked for visitor input.

Visitor Questions/Comments

Christina Bramante commented in the chat: “In weighing the evidence, how is the Morimoto 2012 inhalation study being considered as this study most closely meets the guidelines recommended by Oberdorster, et al.?” and “In a non-overload study, Morimoto 2012, demonstrates SWCNT are not intrinsically hazardous.”

Christina offered that the Morimoto study is the most well-designed and significant study and the dosing is realistic. When comparing it to Shvedova, there is a huge difference in dosing. She does understand the weight of evidence but aspiration and inhalation studies use massive doses. Why would one not expect an impact with doses like that? She also wanted to note on the environmental statement that enzymes do exist that can break the SWNCTs down.

Tom Lada thanked the group for a very considerate discussion over the last several months.

Katherine Robertson asked to circle back to beginning of conversation, when the Board talked about studies and findings. Katherine asked for clarification about the role of uncertainty, and whether it is or is not a barrier to listing. Is the preponderance of evidence decision made only on inconclusive and uncertain studies?

Clint Richmond commented that he would like to see the environmental section expanded. In addition to being persistent, SWNCTs are very mobile and there are no scale mitigation strategies. He would hope that for any class of synthetic materials, the Board could be precautionary. These remarks apply to CNTs and CNFs. He would like to look at air and water transport.

Continued Board Discussion related to visitor comments

A member offered that the Board is looking at hazard at any exposure. There are fibroblast effects in human lung cells and effects at various does. While Morimoto is a good study, the doses are very low.

A member stated that there is always some level of uncertainty and the uncertainties are not always expressed in the statements made by the Board. A member offered that it is the sum of all the evidence that leads the Board to a recommendation. There is sufficient evidence that SWCNTs cause pulmonary toxicity even though some studies did not show these same effects. A member listed the following: studies show collagen deposition and fibrosis at in-between doses; the Board errs on the health-protective side in this regulatory environment; there are enough studies that show hazard. A member offered that they want to consider all the studies, not throw out the ones with high doses. There is also the in vitro evidence. It is a qualitative consideration of all the studies.

With regard to environmental degradation, the studies of enzymes breaking them down were incubated studies, not indicative of the natural environment, and the CNTs were functionalized in a way that promotes degradation. Nanoribbons are the breakdown products and the toxicity of those is unknown.

Addressing the airborne and respirable qualifier, one member offered that any chemical in a facility may be airborne at any point. Even when it is bound, one cannot guarantee that it will not be unbound again. The respirable range is less than 4 microns. None of these particles are above respirable size.

Regarding mobility, the Board looked at transport in water but not in air. No air fate and transport studies could be identified. A member stated that the incineration of CNTs will result in the residual likely being in the ash, not aerosol.

Motion to list SWCNT based on evidence of pulmonary toxicity and environmental persistence. There are additional concerns for reactive oxygen species (ROS) production and DNA damage. The motion was seconded. All eight members present voted in favor.

Next Meeting

At the January meeting, flame retardants will be discussed. Note that the flame retardants discussion is completely different than the TURA work. Nanofibers will also be discussed.

Adjourn

Visitor Comments (inserted verbatim from zoom chat)

From Jerome Lang to Everyone 10:04 AM

Jerome Lang Nano-C

From Katherine Robertson to Everyone 10:04 AM

Katherine Robertson, MCTA

From Tom L to Everyone 10:05 AM

Tom Lada, Nano-C

From Raza Ali to Everyone 10:05 AM

Raza Ali, American Chemistry Council.

From Christina Bramante to Everyone 10:07 AM

Christina Bramante, Representing Nano-C

From Carol Holahan to Everyone 10:07 AM

Carol Holahan, Foley Hoag LLP

From Liz to Everyone 10:10 AM

please put your name and affiliation in the chat if you haven't already. thanks!

From Clint Richmond, Sierra Club to Everyone 10:11 AM

Clint Richmond, Mass. Sierra Club

From Christina Bramante to Everyone 10:30 AM

Please note our May 13th submission which states, "In-vitro and in vivo scientific studies , , , describe “that there are different enzymatic peroxidase assisted mechanisms that biodegrade single wall carbon nanotubes, finally leading to carbon dioxide by such oxidative clearance mechanism."

Scientific papers are cited within this submission

From Rich Gurney (he, his) Simmons University to Everyone 10:40 AM

Looking now

From Heather Lynch to Everyone 10:52 AM

thank you, Christina.

From Robin Dodson to Everyone 11:03 AM

There is evidence that SWCNT may cause pulmonary toxicity in animals, including inflammation, fibrosis, and granulomas. Available in vitro data support potential fibrogenic activity. We note, however, that there is a lack of understanding as to whether the high doses used in the animal studies exceed particle overload conditions, which are common with poorly soluble particles administered via inhalation. Particle overload overwhelms particle clearance mechanisms, resulting in tissue irritation, release of inflammatory cells, and generation of reactive oxygen species (ROS), which may be indistinguishable from the inflammatory response to the agent itself. Persistent lung inflammation can cause cell proliferation and tissue remodeling, which when insufficiently repaired can lead to non-neoplastic effects such as fibrosis, DNA damage, and possibly, tumor formation.

From Wendy Heiger-Bernays (she/her) to Everyone 11:04 AM

There is evidence that SWCNT cause pulmonary toxicity in animals, including inflammation, fibrosis, and granulomas. This evidence is supported by in vitro studies in which cellular changes and molecular events occur along the Adverse Outcome Pathway for fibrosis, irrespective of dose and route of administration.

From Christine Rioux to Everyone 11:07 AM

While human evidence is limited, exposures to SWCNT in occupational settings have reported deposition and internal exposures are occurring. Some animal studies using doses consistent with realistic occupational exposure report lung toxicity though other studies use higher doses that may be associated with particle overload.

From Heather Lynch to Everyone 11:09 AM

There is evidence that SWCNT cause pulmonary toxicity in animals, including inflammation, granulomas, and fibrosis. In vitro studies in human and mouse lung fibroblast cells reported that

SWCNT can activate fibroblasts and initiate key events in an adverse outcome pathway including inflammation and collagen production, which could lead to fibrosis. This evidence is supported by intratracheal studies in rats that reported inflammation and fibrosis. There is a lack of understanding, however, whether the high doses used in the animal studies exceed particle overload conditions, which are common with poorly soluble particles administered via inhalation. Particle overload overwhelms particle clearance mechanisms that can lead to the same effects as those caused by the substance itself, such as fibrosis. Because rodents are more susceptible to particle overload, relative to humans, and human health studies of SWCNTs is limited, there is uncertainty regarding whether similar effects would be observed in humans

exposed to SWCNTs. Overall, taken together, the weight of evidence indicates that SWCNTs may cause pulmonary effects in animals and humans.

From Christina Bramante to Everyone 11:13 AM

In weighing the evidence, how is the Morimoto 2012 inhalation study being considered as this study most closely meets the guidelines recommended by Oberdorster, et al.?

From Christina Bramante to Everyone 11:34 AM

In a non-overload study, Morimoto 2012, demonstrates SWCNT are not intrinsically hazardous

From Katherine Robertson to Everyone 12:01 PM

Can't find raise hand function. Would like to be recognized.

From Robin Dodson to Everyone 12:20 PM

44 Allen, B.L., et al., Biodegradation of Single-Walled Carbon Nanotubes through Enzymatic Catalysis, Nano Letters,Vol. 8, 2008, pp. 3899–3903. doi: 10.1021/nl802315h45 Allen, B. L., et al. Mechanistic investigations of horseradish peroxidase-catalyzed degradation of single-walledcarbon nanotubes, J. Am. Chem. Soc., Vol. 131, 2009, pp. 17194–17205. doi: 10.1021/ja9083623

From Heather Tenney to Everyone 12:21 PM

Allen, B.L., et al., Biodegradation of Single-Walled Carbon Nanotubes through Enzymatic Catalysis, Nano Letters, Vol. 8, 2008, pp. 3899–3903. doi: 10.1021/nl802315h

Allen, B. L., et al. Mechanistic investigations of horseradish peroxidase-catalyzed degradation of single-walled carbon nanotubes, J. Am. Chem. Soc., Vol. 131, 2009, pp. 17194–17205. doi: 10.1021/ja9083623

From Clint Richmond, Sierra Club to Everyone 12:22 PM

I would also like to be recognized if possible.

From Rich Gurney (he, his) Simmons University to Everyone 12:24 PM

From the Allen 2009 abstract “Such data signify a heterolytic cleavage of H2O2 with HRP as pristine nanotubes do not degrade”

From Clint Richmond, Sierra Club to Everyone 12:39 PM

Or water

Perhaps there could be transport on the surface of the water

I think that landfilling as a source could be addressed

From Heather Tenney to Everyone 12:41 PM

Motion to list MWCNTs based on the evidence of pulmonary toxicity, biopersistence, lung cancer, mesothelioma, and environmental persistence. There are additional concerns for genotoxicity and toxic environmental degradation products.

From Rich Gurney (he, his) Simmons University to Everyone 12:53 PM

I make a motion to call a vote on the motion ”to list SWCNTs based on evidence of pulmonary toxicity and environmental persistence. There are additional evidence of concerns for reactive oxygen species (ROS) production and DNA damage.”