Proceeding of the ACVP/ASVCP Concurrent Annual Meetings

Dec.3-7, 2011
Nashville, Tennessee, USA

American College of Veterinary Pathologists

Next Meeting : **December 1-5, 2012** - **Seattle, WA, USA**

Nanoparticulate Pathology

Ann F. Hubbs, DVM, PhD, DACVP; Robert R. Mercer, PhD; Linda Sargent, PhD; Vincent Castranova, PhD; Krishnan Sriram, PhD; Lori A. Battelli, MS, RLAT; and Dale Porter, PhD

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV (ahubbs@cdc.gov)

Introduction

Carbon is a basic building block of life and is the major component of many compounds commonly used in our society, including carbon black and graphite. Surprisingly, new forms of carbon were discovered in the 1980s and 1990s.[1-3] The carbon molecules were connected by single and double bonds and arranged into hexagonal and pentagonal rings which formed the convex three dimensional structures known as buckminsterfullerene (buckyballs) and carbon nanotubes.[4, 5] The discovery of fullerenes led to the 1996 Nobel Prize in chemistry and played a major role in enabling three dimensional engineering at a molecular level. The resulting new field is nanotechnology, the technology used to synthesize particulates with at least one dimension less than 100 nm. Nanotechnology is currently a multi-billion dollar industry with growth potential.[6] Some of the promising new nanotechnology products include pharmaceuticals and medical devices engineered, or containing components which are engineered, in nanoscale dimensions, so that they may deliver improved therapies to patients. [7-12] Assuring these improved therapies do not convey undo risks to patients clearly involves understanding the potential hazards of engineered nanoparticulates (NPs).[8, 11, 13-15] In addition, the products of nanotechnology have the potential to expose the workers who produce them, researchers who study them, and the general public to newly designed structures that will often be unseen by the unaided eye.[13, 16-23] For toxicologic pathologists, nanotechnology also presents new challenges and amazing opportunities.

Challenges of Nanotechnology

The greatest challenge presented by nanotechnology is the virtually infinite and rapidly changing number of potential products.[24-26] The carbon-based NP products have been joined by numerous other elements and combinations of elements with the potential to be engineered in nanoscale dimensions. Even among the original carbon-based NPs, the ability to modify the parent structures is rapidly evolving.[4] A recent PubMed search using the term “nanotechnology” recovered 30,800 publications. More than 30,000 of these were published in the past 10 years, with more than 5,000 published in the last year. A PubMed search of the term nanomedicine recovered 2470 publications, with 2276 of those published in the past 5 years. In addition, within specific classes of NPs, the method of synthesis can alter the physical structure and chemical composition of the NP. This makes physical and chemical characterization of NPs an essential component of meaningful nanotoxicology investigations.

A second major challenge presented by nanotechnology is the complexity of characterizing, measuring and controlling exposures. Historically, environmental and occupational exposures have generally been measured on a mass basis.[27, 28] When size classifications were used, these were generally for airborne particulates. Such measures have been useful for distinguishing total dust from the dust most likely to reach different regions of the respiratory tract and for identifying the toxicologic importance of particulate matter less than 10 μm (PM10) and less than 2.5 μm (PM2.5) in diameter.[29-33] For the NPs, numerous research studies suggest that surface area, chemical composition, crystalline structure, physical shape, particulate size and particle number are all potential contributors to toxicity.[19, 26, 29, 34-41] Unfortunately, the best measures for exposure hazards from NPs are not yet established and new methods to quantify NP exposures are clearly needed. [20, 42-46] Being able to measure the exposure and incorporate exposure doses that are relevant to real world situations into experimental designs is an essential component of NP risk assessment.[13] A related challenge is that federal exposure standards are based historically on particulate composition rather than particulate dimensions. This means that NPs may be in current use while toxicology and toxicologic pathology safety evaluations are ongoing. This contributes a sense of urgency to such studies.

A unique challenge for the toxicologic pathologist is identifying NPs and the histopathologic alterations they produce in tissue sections. Specialized techniques may be required to prevent agglomeration of NPs used for respiratory toxicology studies.[47-50] NPs often can be visualized within tissues sections if they block light. Thus, the carbon nanotubes can often be seen in tissue section, particularly when they have thick walls, as in the multi-walled carbon nanotubes, or are agglomerated.[51, 52] However, identifying dispersed NPs in tissue sections with light microscopy often requires high magnification objectives, high contrast labels, specialized techniques and specialized microscopes to improve visibility.[25, 47] For example, high signal-to-noise darkfield based imaging was recently demonstrated to scatter more light from multi-walled carbon nanotubes than from the surrounding tissue, enhancing contrast and detection in tissue sections.[53] The microvasculature and lymphatics are often targets of NP-induced tissue injury and specialized techniques, such as immunofluorescence, may help to visualize them in tissue sections.[36, 48]

Opportunities for Toxicologists and Toxicologic Pathologists

NPs are useful tools for understanding environmental and occupational exposures to particulates. Importantly, particulates with a dimension less than 100 nm are an important component of combustion products and other environmental exposures.[54] Much of the scientific foundation for understanding nanotoxicology actually began with studies of ultrafine particulates, which include environmental NPs.[18] What has changed is the ability to engineer particulates with specific composition and shape. Because of these improvements in nanotechnology, nanotoxicology is now providing data relevant to engineered NPs as well as those naturally occurring NPs or NPs produced as combustion products. The ability to engineer particulates with specific dimensions provides toxicologic pathologists with critical tools for understanding the pathogenesis of diseases associated with particulate exposures. For example, long, thin, durable, respirable asbestos fibers have greater carcinogenicity and cytotoxicity than short, thick, durable, respirable asbestos fibers.[55, 56] The ability to engineer fibrous and nonfibrous particulates with specific physical dimensions, suggests that scientists will soon have much needed tools for addressing mechanisms for particulate- induced neoplasia and interstitial lung disease.[25, 55] Characterizing engineered NPs also allows comparable structures to be recognized in environmental samples.[54] Thus, characterizing the physical and chemical properties of carbon nanotubes may have enabled their subsequent identification in the dust samples from the World Trade Center attacks and from the lungs of responders with lung disease.[57]

The cytopathologic and histopathologic changes produced by well-characterized NPs are particularly important in understanding the pathogenesis of particulate-induced lung disease, cardiovascular disease, and neoplasia. Single-walled carbon nanotubes disrupt the orderly process of chromosomal segregation and cause aneuploidy through interactions with DNA, microtubules and centrosomes.[58, 59] Single-walled carbon nanotubes, fullerene NPs, and titanium dioxide NPs are all mutagenic.[51, 60] Both single-walled and multi-walled carbon nanotubes induce free radical generation, inflammation, and fibrosis in exposed lungs.[48, 51, 53] Multi-walled carbon nanotubes can cause lymphangiectasia and penetrate the pleura of exposed lungs.[48, 61] Titanium dioxide NPs are much more effective than fine particulates in causing microvascular dysfunction.[36, 37, 62-64] The translocation of NPs to the lymph nodes, vasculature and the brain all suggest the potential for extrapulmonary effects of inhaled NPs.[65-73]

Our understanding of the pathology of NPs is still emerging. However, the findings in this emerging field are incredibly exciting. Some of the greatest opportunities and the greatest challenges for toxicologic pathologists will be identifying physicochemical properties which make NPs more safe, allowing the promise of nanomedicine to be harnessed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

References

1. Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE: C60: Buckminsterfullerene, Nature 1985, 318:162-163
2. Iijima S: Helical Microtubules of Graphitic Carbon, Nature 1991, 354:56-58
3. Iijima S, Ichihashi T: Singel-shell carbon nanotubes of 1-nm diameter, Nature 1993, 363:603-605
4. Martin N: New challenges in fullerene chemistry, Chem Commun (Camb) 2006, 2093-2104
5. Hawkins JM, Meyer A, Lewis TA, Loren S, Hollander FJ: Crystal structure of osmylated c60: confirmation of the soccer ball framework, Science 1991, 252:312-313
6. Bradley J, Nordan MM, Tassinari O: The Recession's Ripple Effect on Nanotech. Edited by Boston, MA, Lux Research, Inc., 2009, p.
7. Jain KK: Advances in the field of nanooncology, BMC Med 2010, 8:83
8. Pautler M, Brenner S: Nanomedicine: promises and challenges for the future of public health, Int J Nanomedicine 2010, 5:803-809
9. Taylor E, Webster TJ: Reducing infections through nanotechnology and nanoparticles, Int J Nanomedicine 2011, 6:1463-1473
10. Teow Y, Asharani PV, Hande MP, Valiyaveettil S: Health impact and safety of engineered nanomaterials, Chem Commun (Camb) 2011, 47:7025-7038
11. Parveen S, Misra R, Sahoo SK: Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, Nanomedicine 2011,
12. Dominguez-Vera JM, Fernandez B, Galvez N: Native and synthetic ferritins for nanobiomedical applications: recent advances and new perspectives, Future Med Chem 2010, 2:609-618
13. Oberdorster G: Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology, J Intern Med 2010, 267:89-105
14. De Jong WH, Borm PJ: Drug delivery and nanoparticles:applications and hazards, Int J Nanomedicine 2008, 3:133-149
15. Linkov I, Satterstrom FK, Corey LM: Nanotoxicology and nanomedicine: making hard decisions, Nanomedicine 2008, 4:167-171
16. Groso A, Petri-Fink A, Magrez A, Riediker M, Meyer T: Management of nanomaterials safety in research environment, Part Fibre Toxicol 2010, 7:40
17. Cassee FR, van Balen EC, Singh C, Green D, Muijser H, Weinstein J, Dreher K: Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive, Crit Rev Toxicol 2011, 41:213-229
18. Oberdorster G, Oberdorster E, Oberdorster J: Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ Health Perspect 2005, 113:823-839
19. Castranova V: Overview of current toxicological knowledge of engineered nanoparticles, J Occup Environ Med 2011, 53:S14-17
20. Song Y, Li X, Wang L, Rojanasakul Y, Castranova V, Li H, Ma J: Nanomaterials in humans: identification, characteristics, and potential damage, Toxicol Pathol 2011, 39:841-849
21. Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, Lee SB, Ji JH, Cho MH, Yu IJ: Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility, Inhal Toxicol 2008, 20:741-749

22. Murashov V: Occupational exposure to nanomedical applications, Wiley Interdiscip Rev Nanomed Nanobiotechnol 2009, 1:203-213
23. Johnson DR, Methner MM, Kennedy AJ, Steevens JA: Potential for occupational exposure to engineered carbon-based nanomaterials in environmental laboratory studies, Environ Health Perspect 2010, 118:49-54
24. Bonner JC: Nanoparticles as a potential cause of pleural and interstitial lung disease, Proc Am Thorac Soc 2010, 7:138-141
25. Hubbs AF, Mercer RR, Benkovic SA, Harkema J, Sriram K, Schwegler-Berry D, Goravanahally MP, Nurkiewicz TR, Castranova V, Sargent LM: Nanotoxicology--a pathologist's perspective, Toxicol Pathol 2011, 39:301-324
26. Maynard AD, Warheit DB, Philbert MA: The new toxicology of sophisticated materials: nanotoxicology and beyond, Toxicol Sci 2011, 120 Suppl 1:S109-129
27. OSHA: 29 CFR - Occupational Safety and Health Regulations (OSHA Standards). Edited by Labor USDo. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992, Occupational Safety & Health Administration, 2006, p.
28. Hubbs A, Greskevitch M, Kuempel E, Suarez F, Toraason M: Abrasive blasting agents: designing studies to evaluate relative risk, J Toxicol Environ Health A 2005, 68:999-1016
29. Dankovic D, Kuempel E, Wheeler M: An approach to risk assessment for TiO_2, Inhal Toxicol 2007, 19 Suppl 1:205-212
30. Eftim SE, Samet JM, Janes H, McDermott A, Dominici F: Fine particulate matter and mortality: a comparison of the six cities and American Cancer Society cohorts with a medicare cohort, Epidemiology 2008, 19:209-216
31. Abbey DE, Nishino N, McDonnell WF, Burchette RJ, Knutsen SF, Lawrence Beeson W, Yang JX: Long-term inhalable particles and other air pollutants related to mortality in nonsmokers, Am J Respir Crit Care Med 1999, 159:373-382
32. Daniels MJ, Dominici F, Samet JM, Zeger SL: Estimating particulate matter-mortality dose-response curves and threshold levels: an analysis of daily time-series for the 20 largest US cities, Am J Epidemiol 2000, 152:397-406
33. Daniels MJ, Dominici F, Zeger SL, Samet JM: The National Morbidity, Mortality, and Air Pollution Study. Part III: PM10 concentration-response curves and thresholds for the 20 largest US cities, Res Rep Health Eff Inst 2004, 1-21; discussion 23-30
34. Sager TM, Castranova V: Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide, Part Fibre Toxicol 2009, 6:15
35. Sager TM, Kommineni C, Castranova V: Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: role of particle surface area, Part Fibre Toxicol 2008, 5:17
36. Nurkiewicz TR, Porter DW, Hubbs AF, Cumpston JL, Chen BT, Frazer DG, Castranova V: Nanoparticle inhalation augments particle-dependent systemic microvascular dysfunction, Part Fibre Toxicol 2008, 5:1
37. Nurkiewicz TR, Porter DW, Hubbs AF, Stone S, Chen BT, Frazer DG, Boegehold MA, Castranova V: Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling, Toxicol Sci 2009, 110:191-203
38. Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL: Correlating nanoscale titania structure with toxicity: A cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells, Toxicol Sci 2006, 92:174-185
39. Schmid O, Moller W, Semmler-Behnke M, Ferron GA, Karg E, Lipka J, Schulz H, Kreyling WG, Stoeger T: Dosimetry and toxicology of inhaled ultrafine particles, Biomarkers 2009, 14 Suppl 1:67-73

40. Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H: Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Part Fibre Toxicol 2005, 2:8
41. Boverhof DR, David RM: Nanomaterial characterization: considerations and needs for hazard assessment and safety evaluation, Anal Bioanal Chem 2010, 396:953-961
42. Balbus JM, Maynard AD, Colvin VL, Castranova V, Daston GP, Denison RA, Dreher KL, Goering PL, Goldberg AM, Kulinowski KM, Monteiro-Riviere NA, Oberdorster G, Omenn GS, Pinkerton KE, Ramos KS, Rest KM, Sass JB, Silbergeld EK, Wong BA: Meeting report: hazard assessment for nanoparticles--report from an interdisciplinary workshop, Environ Health Perspect 2007, 115:1654-1659
43. Aitken RJ, Chaudhry MQ, Boxall AB, Hull M: Manufacture and use of nanomaterials: current status in the UK and global trends, Occup Med (Lond) 2006, 56:300-306
44. Maynard AD, Kuempel ED: Airborne nanostructured particles and occupational health, J Nanoparticle Res 2005, 7:587-614
45. Methner M, Hodson L, Dames A, Geraci C: Nanoparticle Emission Assessment Technique (NEAT) for the identification and measurement of potential inhalation exposure to engineered nanomaterials--Part B: Results from 12 field studies, J Occup Environ Hyg 2010, 7:163-176
46. Methner M, Hodson L, Geraci C: Nanoparticle emission assessment technique (NEAT) for the identification and measurement of potential inhalation exposure to engineered nanomaterials--part A, J Occup Environ Hyg 2010, 7:127-132
47. Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, Shvedova AA, Castranova V: Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model, Am J Physiol Lung Cell Mol Physiol 2008, 294:L87-97
48. Porter DW, Hubbs AF, Mercer RR, Wu N, Wolfarth MG, Sriram K, Leonard S, Battelli L, Schwegler-Berry D, Friend S, Andrew M, Chen BT, Tsuruoka S, Endo M, Castranova V: Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon nanotubes, Toxicology 2010, 269:136-147
49. Porter D, Sriram K, Wolfarth M, Jefferson A, Schwegler-Berry D, Andrew M, Castranova V: A biocompatible medium for nanoparticle dispersion, Nanotoxicology 2008, 2:144-154
50. Demokritou P, Buchel R, Molina RM, Deloid GM, Brain JD, Pratsinis SE: Development and characterization of a Versatile Engineered Nanomaterial Generation System (VENGES) suitable for toxicological studies, Inhal Toxicol 2010, 22 Suppl 2:107-116
51. Shvedova AA, Kisin E, Murray AR, Johnson VJ, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Keohavong P, Sussman N, Jin J, Yin J, Stone S, Chen BT, Deye G, Maynard A, Castranova V, Baron PA, Kagan VE: Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis, Am J Physiol Lung Cell Mol Physiol 2008, 295:L552-565
52. Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, Hubbs AF, Antonini J, Evans DE, Ku BK, Ramsey D, Maynard A, Kagan VE, Castranova V, Baron P: Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice, Am J Physiol Lung Cell Mol Physiol 2005, 289:L698-708
53. Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Friend S, Castranova V, Porter DW: Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes, Part Fibre Toxicol 2011, 8:21
54. Murr LE: Microstructures and nanostructures for environmental carbon nanotubes and nanoparticulate soots, Int J Environ Res Public Health 2008, 5:321-336

55. Stanton MF, Layard M, Tegeris A, Miller E, May M, Morgan E, Smith A: Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals, J Natl Cancer Inst 1981, 67:965-975
56. Lipkin LE: Cellular effects of asbestos and other fibers: correlations with in vivo induction of pleural sarcoma, Environ Health Perspect 1980, 34:91-102
57. Wu M, Gordon RE, Herbert R, Padilla M, Moline J, Mendelson D, Litle V, Travis WD, Gil J: Case report: Lung disease in World Trade Center responders exposed to dust and smoke: carbon nanotubes found in the lungs of World Trade Center patients and dust samples, Environ Health Perspect 2010, 118:499-504
58. Sargent LM, Shvedova AA, Hubbs AF, Salisbury JL, Benkovic SA, Kashon ML, Lowry DT, Murray AR, Kisin ER, Friend S, McKinstry KT, Battelli L, Reynolds SH: Induction of aneuploidy by single-walled carbon nanotubes, Environ Mol Mutagen 2009, 50:708-717
59. Sargent LM, Reynolds SH, Castranova V: Potential pulmonary effects of engineered carbon nanotubes: in vitro genotoxic effects, Nanotoxicology 2010, 4:396-408
60. Xu A, Chai Y, Nohmi T, Hei TK: Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells, Part Fibre Toxicol 2009, 6:3
61. Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Schwegler-Berry D, Castranova V, Porter DW: Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes, Part Fibre Toxicol 2010, 7:28
62. Nurkiewicz TR, Porter DW, Barger M, Castranova V, Boegehold MA: Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation, Environ Health Perspect 2004, 112:1299-1306
63. Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao KM, Marvar PJ, Hubbs AF, Castranova V, Boegehold MA: Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure, Environ Health Perspect 2006, 114:412-419
64. Nurkiewicz TR, Porter DW, Hubbs AF, Stone S, Chen BT, Frazer A, Boegehold MA, Castranova V: Pulmonary nanoparticle exposure disrupts systemic microvascular nitri oxide signaling, Tox Sci 2009, 110:191-203.
65. Calderon-Garciduenas L, Solt AC, Henriquez-Roldan C, Torres-Jardon R, Nuse B, Herritt L, Villarreal-Calderon R, Osnaya N, Stone I, Garcia R, Brooks DM, Gonzalez-Maciel A, Reynoso-Robles R, Delgado-Chavez R, Reed W: Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults, Toxicol Pathol 2008, 36:289-310
66. Aiso S, Kubota H, Umeda Y, Kasai T, Takaya M, Yamazaki K, Nagano K, Sakai T, Koda S, Fukushima S: Translocation of intratracheally instilled multiwall carbon nanotubes to lung-associated lymph nodes in rats, Ind Health 2011, 49:215-220
67. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G: Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, Environ Health Perspect 2006, 114:1172-1178
68. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C: Translocation of inhaled ultrafine particles to the brain, Inhalation toxicology 2004, 16:437-445
69. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C: Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats, J Toxicol Environ Health A 2002, 65:1531-1543
70. Oberdorster G, Utell MJ: Ultrafine particles in the urban air: to the respiratory tract--and beyond?, Environ Health Perspect 2002, 110:A440-441

71. Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdorster G, Kreyling WG: Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs, Inhal Toxicol 2004, 16:453-459
72. Kreyling WG, Semmler-Behnke M, Seitz J, Scymczak W, Wenk A, Mayer P, Takenaka S, Oberdorster G: Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs, Inhal Toxicol 2009, 21 Suppl 1:55-60
73. Oberdorster G, Elder A, Rinderknecht A: Nanoparticles and the brain: cause for concern?, J Nanosci Nanotechnol 2009, 9:4996-5007