A Nano-Mesothelioma False Alarm

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ABSTRACT

In May 2008, a scientific study (the "Poland Study") was published in Nature Nanotechnology*—which sparked a rash of popular media claims that like asbestos, exposure to carbon nanotubes may cause mesothelioma. In this article, a team led by lawyer John Monica evaluates the Poland Study in a potential litigation context to determine its significance, if any, in legally establishing that the inhalation of multiwalled carbon nanotubes ("MWCNTs") causes mesothelioma. After first considering the reliability of the Poland Study's design and execution, they conclude that it would not be admissible in a court of law because it fails* Daubert *standards. Specifically, they argue that: (i) the design and execution of the Poland Study are not generally accepted in the scientific community for the purposes offered; (ii) in order to reach the conclusion that inhalation of MWCNTs may cause mesothelioma, an expert would have to use the Poland Study in such a manner as to extrapolate from an accepted premise to an unfounded conclusion; and, (iii) the Study's authors failed to adequately account for obvious alternative explanations (confounders), including surface chemistry, sample contamination, sample commingling, spontaneous formation of granulomas, and possible mouse colony infections.*

I. INTRODUCTION

The May 2008 scientific study published in *Nature Nanotechnology*[1] (the "Poland Study") sparked a rash of popular media claims that the nano sky is falling; i.e., that like asbestos, exposure to carbon nanotubes may cause mesothelioma—a pernicious form of cancer. The Poland Study is the second *in vivo* study this year to investigate the "asbestos-like, pathogenic behavior" of multi-walled carbon nanotubes (MWCNT) .[2] Popular media coverage of the Poland Study has been hyperbolic, and

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[1] C. Poland, et al., *Carbon Nanotubes Introduced Into The Abdominal Cavity Of Mice Show Asbestos-Like Pathology In A Pilot study*, NATURE NANOTECHNOLOGY, May 20, 2008 ("Poland Study"). Along with their paper, the authors also published a document entitled "Supplementary Information" which describes in detail how they conducted the research.

[2] The authors define "nanotube" as "a graphene cylinder, typically a few nanometers in diameter, which can range in length from a few nanometers to millimeters." Poland Study, at 1. Single-walled nanotubes consist of one cylinder,

the business community is taking note. For example, one major insurance carrier, Continental Western Insurance Group, recently issued and then quickly withdrew what appears to be one of the first commercial insurance exclusions in the United States aimed specifically at nanotubes.[3]

With the recent attention by the news media and business community, the plaintiffs' bar may not be far behind. This article evaluates the Poland Study in a potential litigation context. When appropriate, reference is made to a recently published scientific article by Kane & Hurt that is also critical of the Poland Study.[4]

II. ANALYSIS OF THE POLAND STUDY

The Poland Study illustrates the challenge of understanding and applying the applicable science to the important legal requirement of "causation;" i.e., proving to a legal sufficiency the allegation that the inhalation of MWCNTs causes mesothelioma or other disease.

The primary basis for the Poland Study appears to be an asserted similarity between the "needle-like" shape of some MWCNTs and the shape of asbestos fibers.[5] Shape of the fibers is all important. The Poland Study claims to have shown that exposing the mesothelial lining of the body cavity of mice to long straight MWCNTs results in asbestos-like, pathogenic[6] behavior including inflammation and the formation of lesions known as granulomas.[7] The authors used the peritoneal (abdominal) cavity of mice as a surrogate for the mesothelial lining of the chest.[8] They incorrectly infer that injection of fibers into the peritoneal cavity of mice mimics the inhalation of fibers by humans. The ultimate implication of the Poland Study is that the inhalation of long straight MWCNTs by humans will result in mesothelioma.[9] The study does not, however, prove this assumption for a number of reasons discussed below.

A. Hypothesis and Methodology

The Poland Study explains that a "superficial resemblance" between certain carbon nanotubes and asbestos[10] has led some scientists to assess whether fiber-shaped nanoparticles present a unique "health risk" similar to that posed by asbestos.[11] Others, such as Kane & Hurt, have questioned the validity and

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while the MWCNTs used in the Poland Study were comprised of "2 to 50 cylinders concentrically stacked with a common long axis." *Id.*

[3] The stated intent of the exclusion issued on September 24, 2008 by Continental Western Insurance Group was to "remove coverage for the yet unknown and unknowable risks created by products and processes that involve nanotubes." The posting of the exclusion together with two explanatory documents on Continental's website was quickly removed the same day after BNA published an article about the exclusion. These documents can be found at http://www.nanolawreport.com/2008/09/articles/first-commercial-insurance-exclusion-for-nanotechnology/.

[4] A. Kane, et al., *The Asbestos Analogy Revisited*, NATURE NANOTECHNOLOGY, Vol. 3, 378-379 (July 2008) ("Kane & Hurt").

[5] Poland Study, at 1.

[6] Pathogenesis is the origination and development of a disease—called also pathogeny. (Medline Plus Dictionary).

[7] A granuloma is a mass or nodule of chronically inflamed tissue with granulations that is usually associated with an infective process. A granulation is a minute mass of tissue projecting from the surface of an organ; one of the minute red granules made up of loops of newly formed capillaries that form on a raw surface (as of a wound) and that with fibroblasts are the active agents in the process of healing. (Medline Plus Dictionary).

[8] Poland Study, at 1.

[9] The authors make a point of leading off by reiterating "concerns that widespread use of carbon nanotubes may lead to mesothelioma. Poland Study, at 1.

[10] Asbestos consists of a group of minerals that occur naturally as masses of strong, flexible fibers that can be separated into thin threads and woven. (National Cancer Institute web definition).

[11] Poland Study, at 1.

usefulness of the "nanotube and asbestos analogy" due to differences in the chemical composition and surface properties of the two substances.[12] The Poland Study hypothesizes that the high aspect ratio (ratio of length and width) of certain MWCNTs coupled with their nanoscale diameter and needle-like shape make them comparable to asbestos in their ability to cause a particular type of injury to the mesothelium.[13] In particular, the authors predict that exposure to long straight MWCNTs will cause asbestos-like pathogenic behavior resulting in inflammation and the formation of granulomas in the peritoneal mesothelium.[14] They further hypothesize (not prove) that the granulomas will eventually progress to mesothelioma.[15]

The authors of the Poland Study speculate that long straight MWCNT's cause granulomas as a result of "frustrated phagocytosis": "[I]n attempting to phagocytose or engulf a fiber longer than the length they can completely enclose, the specialized 'engulfing cells,' macrophages, are chronically stimulated to release mediators that cause inflammation" which in turn leads to giant cell formation (granulomas).[16]

The test methodology used by the Poland Study was to inject five separate substances[17]—including asbestos and MWCNTs—into the abdominal cavity of mice and then compare the reaction of each in the mouse mesothelium.[18] Each test sample was placed into a separate saline solution and then injected into the peritoneal cavity of an eight week old female mouse at a dose of 50 μg.[19] A different mouse was used for each sample.[20]

B. Findings

The Poland Study found that long straight MWCNTs and long asbestos fibers caused the same type of inflammation and granulomas in the peritoneal abdominal cavity of mice but that short/tangled fibers of both substances did not, nor did the non-fibrous ultrafine carbon black.[21] This led the authors to

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[12] Kane & Hurt, at 378. While acknowledging that the nanotube/asbestos "analogy" exists due to the similarity of the small fiber diameter, long length, and biopresence characteristics of the two substances, Kane & Hurt point out that there are also important differences between these two fibrous materials such as their chemical composition and surface properties.

[13] The mesothelium is a "sac-like membrane that protects most of the body's internal organs that is divided into two distinct protective layers of cells: the visceral (the layer directly surrounding the organ) and the parietal (a sac surrounding the body cavity). By releasing a lubricating fluid, the mesothelium allows the organs to move more freely within the body cavity; for example, the contraction and expansion of the lung." (National Cancer Institute web definition).

[14] Poland Study, at 2.

[15] *Id.* at 4. Mesothelioma is a relatively rare cancer with approximately 2,000 new cases diagnosed in the United States each year. A history of occupational asbestos exposure is reported in about 70 percent to 80 percent of all cases. (National Cancer Institute web definition).

[16] Kane & Hurt in their review of the Poland Study suggest that the normal defense mechanism against foreign materials is accumulation of activated marcrophages and multinucleated giant cells to form a granuloma. They further assert that if the foreign materials are resistant to degradation and cause persistent generation of tissue-damaging free radicals, granulomas can become sites for recruitment of fibroblasts, deposition of collagen scar tissue and in-growth of new blood vessels. Continuing in their attempt to explain the mechanism of disease, Kane & Hurt assert that the free radicals also cause DNA damage and mutations in proliferating cells that are the precursors of mesothelioma. Kane & Hurt, at 379.

[17] The following substances were injected into separate mice: long fiber amosite asbestos; short fiber amosite; MWCNTs consisting of a "substantial portion" of long straight fibers; MWCNTs consisting of long tangled MWCNTs; and ultrafine carbon black. Poland Study, at 2.

[18] *Id.* at 2.

[19] *Id.*

[20] *Id.*

[21] *Id.* at 4-5.

conclude that the shape (long straight) of the injected substance was determinative of its adverse health consequences and that long straight MWCNTs acted the same as long straight asbestos fibers.[22]

C. Limitations and Considerations

A close reading of the Poland Study reveals a number of limitations.

1. There Is No Showing That Granulomas Progress to Mesothelioma

First, and foremost, the authors do not even purport to show that granulomas progress to mesothelioma. The Poland Study clearly states that it does not address whether the mice that developed granulomas would go on to develop mesotheliomas.[23] Furthermore, no other study is cited in the paper for the proposition that these types of granulomas are likely to progress to mesothelioma.

2. There Is No Showing That the MWCNTs Tested Caused the Granulomas

It is not unusual for granulomas to appear as a result of inflammation caused from various sources. Granulomas are formed as a result of inflammatory reactions caused by biologic, chemical, or physical agents.[24] Kane & Hurt in their review of the Poland Study observe that the normal defense mechanism against foreign materials is accumulation of activated macrophages and multinucleated giant cells to form a granuloma.[25] Logically, any material not naturally found within the body should be classified as "foreign" and may, if it causes persistent inflammation, result in granulomas being formed at the inflammation site.[26] Thus, the formation of granulomas is not unique to the presence of MWCNTs. Rather, it may be expected when any foreign substance which causes persistent inflammation is introduced into the body.[27]

Thus, the Poland Study does not conclusively establish that the suspected MWCNTs caused the granulomas. At best the Poland Study raises a question as to the possible cause(s) of the granulomas, one of which may have been the MWCNTs. Other possible causes such as contaminating metals found in the test samples are also mentioned in the Poland Study. This will be further discussed below.

3. The Method of Administering the Test Samples Raises Questions—Injection vs. Inhalation

The authors of the Poland Study admit their hypothesis that MWCNTs may cause mesothelioma is premised on the unproven assumption that MWCNTs **inhaled** into the lungs will reach the lung mesothelium in sufficient numbers (fiber burden) to cause the disease.[28] The authors acknowledge that "for there to be any adverse effect, the numbers of such [long] fibers must reach a sufficient level to cause chronic activation of inflammatory cells, genotoxicity, fibrosis and cancer in the target tissue."[29] (Citing Mossman)."[30]

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[22] *Id.*
[23] *Id.* at 5.
[24] Steadman's Medical Dictionary, Williams & Wilkins (1972).
[25] Kane & Hurt, at 379.
[26] *Id.*
[27] *Id.*
[28] Poland Study, at 1 & 5.
[29] *Id.* at 1.
[30] *Id.* at 6, n.11 (citing with approval Mossman & Churg, *Mechanisms In The Pathogenesis Of Asbestos And Silicosis*, AM. J. RESPIR. CRIT. CARE MED., Vol. 157, pp. 1666-1680 (1998) ("Mossman Article")).

Achieving the requisite fiber burden is premised on two important factors, both of which the Poland Study admits[31] are presumed, but not proven to exist; i.e., inhalation of sufficient MWCNTs from ambient air, and sufficient biopersistence of the MWCNTs once inhaled to enable them to travel from the lung to the mesothelium over a long period of time. The authors concede that it unknown whether there will be sufficient inhalation of MWCNTs to cause mesothelioma: "[I]t remains unknown whether there will be sufficient exposure to long CNT's in the workplace or the environment to reach the threshold dose in the mesothelium." Biopersistence, the second key element, is also admittedly unestablished: "Exposure of the mesothelium, however, is based on the caveat that all materials tested shared a high level of biopersistence, allowing sufficient time for migration through the lung to the mesothelium. As such, our study does not address whether CNT's would be able to reach the mesothelium in sufficient numbers to cause mesothelioma following inhalation exposure."[32]

These concerns are echoed by Kane & Hurt who, after identifying fiber length and biopresence as key physical properties that may be relevant for potential toxicity and carcinogenicity, express concern about the paucity of data on the biopresence of MWCNTs. [33] They believe it is "unclear" whether MWCNTs will reach the mesothelial lining in sufficient numbers following inhalation as this "requires initial penetration to the deep lung followed by translocation across the air sacs into the pleura.[34]"

All of this highlights the problem of using injection of MWCNTs directly into the mesothelium cavity of the abdomen as a surrogate for inhalation of MWCNT's into the lung. The authors fail to explain how inhaled nanotubes of the size and shape tested would have the biopersistence necessary to travel from the lung to the mesothelium in the first place, and do so in such numbers as to cause the requisite inflammation and development of tumors over many years.

4. Results From This Particular Animal Study Have Limited Application to Humans

The use of animal studies is well accepted and is important to understanding potential carcinogenesis. However, animal studies are inconclusive and are at best an important source of indicative information. There can be legitimate disputes as to the applicability of a particular animal study to human carcinogenesis.[35]

Moreover, Kane & Hurt point out that the "gold standard' for testing fiber carcinogenicity is a chronic **inhalation** assay using a **range of doses** in **two rodent species**.[36] The Poland Study used injection instead of inhalation, and only one dose and one type of rodent.

i. The Type of Mouse Used Is Questionable

The Poland Study used eight-week-old female C57BL/6 mice.[37] It is not explained why this particular animal model was selected. Kane & Hurt point out the problems that can arise when genetically engineered mice that may be susceptible to induction of foreign body tumors are used for a study to investigate the pathogenic behavior of fibers.[38] They found this to be a problem with the

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[31] Poland Study, at 1 & 5.
[32] Poland Study, at 5.
[33] Kane & Hurt, at 378-79.
[34] *Id*.
[35] *See Toxicity Tests in Animals: Extrapolating to Human Risks*, ENVIRONMENTAL HEALTH PERSPECTIVES, Vol. 101, No. 5 (Oct. 1983); P. Shubik, *The Validity of Animal Studies with Chemical Carcinogens*, CA CANCER J. CLIN., 1981; 31; 120-123.
[36] Kane & Hurt, at 378-379 (July 2008).
[37] Poland Study, Supplementary Information, "Experimental Animals", at 4.
[38] Kane & Hurt, at 379.

Japanese study by Takagi, *et al*. with regard to confirming the diagnosis of mesothelioma as distinct from foreign body tumors.[39]

Additionally, the fact that the Poland Study found granulomas in mice that were administered short/tangled MWCNTs as well as in mice injected with long straight MWCNTs at least raises a question as to whether these particular mice are capable of displaying granulomas spontaneously. The Poland Study suggests that in fact the granulomas observed in some of these mice may have arisen "spontaneously by chance."[40]

Finally, infections which can cause granulomas[41] are notorious in mouse colonies.[42] There is no showing that the subject mice were tested for infection before being used in the Poland Study.

ii. The Number of Mice Used Was Inadequate

The authors admit there is also a question as to whether the number of mice used in the Poland Study was too small to properly draw conclusions: "a similar study in a larger group of animals would provide greater confidence."[43]

iii. Use of an Animal Model in This Instance May Be Inappropriate

Importantly, the validity of using an animal model to predict human reaction to foreign fibers entering the body was raised by Mossman which is cited with approval by the Poland Study.[44] Mossman explains that "there are few available data on the relationship of fiber size measures and asbestosis, and these data are difficult to reconcile with animal studies."[45] Going to the heart of the Poland Study's findings, Mossman questions whether it has been established that long fibers found in the human body are more fibrogenic than short fibers. In fact, Mossman suggests that the **converse** may be true:

> [D]avis and colleagues and Adamson and Bowden both observed that long fibers were considerably more fibrogenic than short (<5 μ.m.) fibers in rats and mice. But in humans, we were unable to show that lungs with asbestosis had longer fibers than lungs with other types of asbestos-induced disease and, in fact, we found an inverse correlation between fibrosis grade and mean fiber length ….[46]

Thus, animal studies such as the one used in the Poland Study may not be predictive of results in humans—an issue the authors do not address.

5. The Dose of Nanotubes Injected Directly Into the Abdominal Mesothelium of the Test Animals Is Not Representative of the Dose to Which a Human Would Be Exposed Through Inhalation—The Dose Was Too Rapid and Too High

The Poland Study further acknowledges that the body must receive a minimum threshold dose of the cancer-producing substance for mesothelioma to occur: "Above all, for there to be any adverse effect, the

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[39] *Id.*
[40] Poland Study, at 3.
[41] Infections granulomas are defined as any granulomatous lesion caused by a living agent including bacteria, fungi, helminths, etc. Stedman's Medical Dictionary, Williams & Wilkins (1972).
[42] M. Mahler, et al., "Health Monitoring in the Laboratory Mouse," edited by Hans Elsevier, Academic Press (2004).
[43] Poland Study, at 3.
[44] *Id*. at 1.
[45] Mossman Article, at 1670.
[46] *Id*. at 1670-1671.

numbers of such fibers must reach a sufficient level to cause chronic activation of inflammatory cells, genotoxicity, fibrosis and cancer in the target tissue."[47] In the real world, MWCNTs would have to be slowly accumulated in the lung mesothelium over a period of time as they migrate from the lung itself after inhalation. The latency period for mesothelioma to develop from asbestos exposure requires continual and gradual exposure until the threshold[48] dosage necessary to cause disease is accumulated, not a sudden injection of a large dose of fibers directly into a confined area of the mesothelium. Mossman points out that it is entirely possible that the proliferation of cells causing fibrogenesis and possibly carcinogenesis may initially occur at sites of accumulation of inhaled materials, and/or later at distal sites where particles or fibers are translocated over time.[49]

An additional problem exists regarding the dosage of MWCNTs administered to the test animals. As a part of the research, each mouse was injected with a total of 50 μ.m. of MWCNTs over a seven day period.[50] There is no suggestion, much less a showing, that the high dose used (maximum tolerable dose?) accurately approximates what would be accumulated in the body through inhalation.[51] Furthermore, only one total dose was used, not a range of doses[52] as suggested by Kane & Hurt.

6. The Poland Study Fails To Properly Consider The Surface Chemistry Of The Fiber Samples

The authors paid little attention to the surface chemistry of the MWCNTs. Instead, the Poland Study states in conclusory fashion that "fibrous shape dominates over simple graphene chemistry in effects on the mesothelium."[53] The long straight MWCNT was declared to be the one that caused granulomas. However, Mossman points out that chemical and surface properties of the fibers also are quite important in cellular responses including toxicity to cells because they (iron and transitional metals) may catalyze the formation of reactive oxygen species.[54] Other surface chemicals such as magnesium may also render the fiber with a positive charge which has been shown to cause injury to cells by reacting with the external cell membrane.

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[47] Poland Study, at 1.

[48] Mossman observed: "Epidemiologic studies indicate very clearly that the development of asbestosis requires heavy exposure to asbestos and provide strong evidence that there is a threshold fiber dose below which asbestos is not seen." Further: "[T]he lower the exposure, the longer it takes to reach this threshold in workplace settings." Mossman Article, at 1667.

[49] Mossman Article, at 1672.

[50] Poland Study, at 2.

[51] The dose-response relationship between a substance and the reaction it causes in the human body is very important. Dose-response analysis usually involves an extrapolation from the generally high doses administered to experimental animals or exposures noted in epidemiologic studies to the much lower exposure levels expected from normal human contact with the agent. Positive studies above the maximum tolerated dose (MTD) must be carefully reviewed to ensure that the responses are not due to factors inoperative at exposure levels below the MTD.

[52] Poland Study, at 2.

[53] *Id.* at 4.

[54] Mossman observed:

> The geometry and dimensions of these (asbestos) materials may govern their deposition and clearance kinetics, biologic reactivity, and dissolution in the lung, but chemical and surface properties, including sorption, oxidation/reduction reactions, and charge, also play important roles in biopersistence, cellular responses, and pathogenicity.
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> ….
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> [S]urface chemistry is important in driving oxidant production and possibly other deleterious reactions in the lung that are linked to the advent of inflammation and fibrogenesis."

Mossman Article, at 1668, 1675.

7. The Long Fiber MWCNT Test Specimens Were Contaminated With Short/Tangled Fibers

The Poland Study describes the "long" MWCNTs in the test as containing a "substantial portion" of long straight fibers longer than 20 μ.m.[55] Thus, the Poland Study uses 20 μ.m. as the demarcation of what it considers to be a "long" fiber. Interestingly, Table 1 of the paper states that only 11.54% of the fibers in the sample designated "NT (long 1)" and 76.85% of those constituting sample "NT (long 2)"[56] were greater than 20 μ.m.[57] Conversely, 88.46% and 23.15% respectively of these two "long" fiber samples were composed of something **other** than long fibers.[58] It thus appears that the two granuloma-producing MWCNTs did not consist solely or even "predominantly" of **long straight** fibers. Instead, they contained substantial, and in one case predominantly, short/tangled fibers. This calls into question the authors' conclusion that only long straight fibers cause granulomas.[59]

Additionally, the Poland Study's Supplementary Information when discussing the "physico-chemical" characteristics of the fibers, points out that the suspension media used to inject the fibers into the mice was different from that used to measure the geometry of the fibers.[60] A protein solution was used for injection while a solvent was used to suspend the particles for measurement. This was evidentially done because other studies have found problems with using organic solvents for the dispersion of nanotubes. Solvents are stated as often unsuitable for use in a biological system due to their inherent toxicity.[61] One might legitimately question, however, whether suspending the fibers in different solutions might affect their geometry through absorption or some other factor resulting in a different size MWCNT being injected than was measured. The Poland Study does not address this issue.

8. The MWCNT Test Specimens Were Inadequately Controlled And Were Not Uniform

Difficulties can arise when a study fails to adequately specify or characterize the sources and properties of the test fibers used. Mossman observes:

> [M]any epidemiologic and experimental studies fail to specify or characterize the source and properties of minerals used. Thus, defining the exact properties of minerals important in the causation of asbestosis or silica-induced lung diseases is problematic.[62]

The classification of the MWCNTs used in the Poland Study is set out in Table 1 of the article. Several important differences between the MWCNT samples are obvious which call into question the conclusions of the study.

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[55] Poland Study, at 2.

[56] Equally curious is the fact that this sample did not truly consist of nanoscale materials. The mean diameter of the particles in this sample was 165.02 ± 4.68 nanometers. Approximately 85% of those particles were greater than 15 micrometers in *length*. Poland Study, at 2, figure 1. Commonly accepted standards require at least one dimension of the particle be less than 100 nanometers in length for the material to be deemed "nanoscale."

[57] Poland Study, at 2, Table 1.

[58] *Id.*

[59] The Poland Study concludes that only long-fiber nanotubes cause granulomas but admits that a "small insignificant" granuloma response was also found in one of the three mice injected with the tangled/short sample. The authors speculate that this may have been caused by either contamination of the short-fiber sample with long fibers, spontaneous occurrence of granulomas by "chance," or an unidentified substance such as a metal contaminating the nanotube test samples. The Poland Study also observes that "transitional metals" were implicated in CNT-mediated stress in an earlier study published on nanotubes.

[60] Poland Study, Supplementary Information, at 3.

[61] *Id*.

[62] Mossman Article, at 1669.

First, the MWCNT samples are not pure but instead are comprised of long, short and intermediate nanotube fibers with some described as "bundles" and others as "ropes" of MWCNTs.[63]

Second, the samples originated from three different sources: NanoLab, Inc.; Mitsui & Co.; and the University of Manchester.[64] It is imprudent to assume that samples are of the same dimension, quality and composition when their sources are different. They may in fact have structural and/or chemical features which vary. This makes accurate comparisons of test results using the different samples difficult, if not impossible.

The Poland Study apparently attempted to deal with part of this problem by examining and reclassifying the various samples by diameter and length.[65] However, no classification by chemical content appears in Table 1 even though the possible impact of various chemicals contained in the samples is acknowledged. The authors seem to give little significance to chemical properties and instead base their conclusions almost completely on the physical dimensions of the nanotube fibers.

Interestingly, the fiber diameter of the test specimens stated by the supplier is in some instances significantly different than that determined by the authors.[66] Presumably the authors used their own determination of diameter and length as controlling. Some of the diameters of the long MWCNTs were determined to be approximately twice that stated by the supplier.[67] The lengths also appear to vary from that stated by the supplier. No explanation is given. This leaves a question as to what caused the significant difference between the measurements by the suppliers and the authors, and which is correct. Since the physical properties of the MWCNTs are so important to the Poland Study's results, one would expect this issue to have been thoroughly addressed.

Most of the samples are contaminated with soluble metals (Fe, Cu, V, Ni, Zn, and Co) which vary according to sample and supplier.[68] This makes comparisons problematic and also raises the issue of the role of these metals in causing the "asbestos-like" inflammation and granulomas. Mossman points out that elements such as magnesium and iron that are leached from asbestos test fibers may mediate the toxicity of the fibers through surface change or redox reactions.[69] The same is true of foreign substances contained in the nanotube fibers used for testing purposes. The Poland Study admits a potential problem in this regard:

> Transitional metals have been implicated in CNT-mediated stress in a earlier published study on nanotubes in vitro, but neither soluble nor total metals can explain the differences in peritoneal response seen here with long and short MWTN's. There is a remote possibility that some unmeasured metal or other component could contribute to this difference but, in the opinion of the authors, it is unlikely.[70]

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[63] Poland Study, at 2, Table 1.
[64] *Id.*
[65] *Id.*
[66] *Id.*
[67] *Id.*
[68] There was some attempt to remove the contaminating metals contained within the samples which the study first states produced "no significant' inflammatory effects 24 hours after injection" but then admits a "remote possibility" that some unmeasured metal or other component could have contributed to the difference in the peritoneal response observed between long and short MWCNTs. One might legitimately raise a question as to whether 24 hours is long enough to wait for inflammation to develop.
[69] Mossman Article, at 1669.
[70] Poland Study, at 3-4.

Where then do the above variations in the test samples leave us? The authors admit these issues might present an "attendant problem."[71] Summing up the significance of the differences in the test samples, the authors freely admit that the "clear differences" that their study showed between long and short/tangled MWCNTs might have been influenced by differences in the "source, preparation and purification of different commercial CNT's and therefore potential differences in physicochemistry and contaminating metals."[72] The fact that the alleged granuloma-producing MWCNTs did not consist solely of long straight fibers is quite important as only these type of fibers were claimed to cause granulomas. Instead, they appear have been contaminated with either long tangled and/or short nanotube fiber in addition to certain metals.

Additionally, the test fibers alleged to have caused the granulomas were not shown to replicate those that would be inhaled by the human body. The test samples were obviously specifically "manufactured" for testing purposes and are not necessarily what a human being might be expected to inhale in a "normal" situation. This is made clear in the Supplementary Information portion of the Poland Study which explains that the two long fiber test CNT's were created in different manners.[73] The first sample (NT long1) was produced by Mitsui & Co. Ltd., Japan, by "catalytic chemical vapor synthesis using the floating reaction method."[74] The second test sample (NT long2) was produced by a University of Cambridge academic research laboratory "using catalytic vapour discharge (CVD) method using a ferrocene-toluene feedstock to grow nanotubes from iron catalysts held on a silica plate."[75] It is explained that "residual iron" remained within these nanotube samples which the scientists conducting the Poland Study attempted to removed."[76] Thus, the MWCNT samples used in the Poland Study may not replicate true exposure scenarios.

Kane & Hurt comment on the importance of the test samples replicating those that will be inhaled by the human body. They observe that it is unknown whether the Poland Study test samples meet this requirement:

> [T]he biological mechanism in question is triggered by geometry, but the "effective" nanotube geometry sensed by cells is determined by aggregation state, which may include bundles, ropes, spherical balls or free tubes. Moreover, the actual geometry is experiment-dependent and governed by the environmental and processing history of the samples… In real human exposure scenarios, the actual physical form of nanotubes that are presented to internal target tissues such as the mesothelium remains unknown.[77]

Thus, while the test samples were arguably MWCNTs, it must be recognized that there is a wide variation in what is commonly referred to as a "nanotube." There was no showing that these particular types of MWCNTs would be encountered by inhalation either in the workplace or in using a commercial product.

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[71] *Id.* at 4.
[72] *Id.*
[73] Poland Study, Supplementary Information, at 1.
[74] *Id.*
[75] *Id.*
[76] *Id.*
[77] Kane & Hurt, at 379.

III. LEGAL ANALYSIS

A. Scientific v. Legal Causation

Tort suits sounding in warranty, negligence, and/or strict liability can be expected if a plaintiff claims injury such as mesothelioma caused by inhalation of MWCNTs. To succeed in such a law suit the plaintiff must be able to prove that inhalation of the specific MWCNTs in question caused his/her disease.

It is important to distinguish "legal" from "scientific" causation. Science uses a probabilistic method to determine causation whereas the law requires proof by a preponderance of the evidence; i.e. proof that it is "more likely than not" that the substance caused the disease. The law does look to the testimony of scientific experts in the appropriate field of science to help the finder of fact (usually a jury) decide whether causation has been proven. However, to be accepted in a court of law, expert testimony must meet certain criteria which are closely examined by the trial court before permitting the expert to testify and subsequently by the appellate court when the admission of the expert testimony is claimed by the losing party to constitute reversible error. The U.S. Supreme Court has handed down a series of decisions that establish the criteria for accepting testimony from any expert in federal court, including those claiming expertise on scientific subjects such as whether the inhalation of a substance such as MWCNTs caused a plaintiff's mesothelioma or other disease.

The key question is often whether the judge will permit a scientific expert to testify that causation has been established; i.e., that the substance in question caused the plaintiff's disease. This in turn will involve a decision by the judge concerning whether the probabilistic causation opinion offered by the expert is sufficient under law to permit the jury to hear it. If the jury is permitted to hear the testimony, it must then must decide whether the expert testimony together with any other relevant evidence received, establishes that the substance in question caused the specific disease of the plaintiff. It is not enough for the evidence to prove "general causation'; i.e., that the substance is capable of causing the disease. Instead, for the plaintiff to successfully establish that he is entitled to compensation for his injury, he must prove that it is more likely than not that the substance under scrutiny caused his specific disease. In terms of probability, this means showing that there is at least a 51% likelihood that the substance caused the plaintiff's disease. This is the plaintiff's burden of proof and if it is not established at the trial, the plaintiff will lose the law suit.

Although the law is frequently more conservative than scientists in finding that causation has been established, neither science nor the courts require that the actual "mechanism of disease" be shown before causation can be deemed established. Thus, it is both scientifically and legally possible to establish causation without showing how the disease occurred. A scientist is able to say with confidence that a substance causes cancer without knowing how the cancer is caused because the scientist's conclusion is based on probabilistic reasoning, not on a conclusive understanding of the mechanism of disease. This probabilistic reasoning often uses the mathematical devices of statistics and epidemiology to prove general causation; i.e., that the substance in question is capable of causing a certain type of disease, for example that cigarette smoking causes lung cancer. However, it is generally recognized that these mathematical devices are incapable of proving specific causation; i.e., that cigarette smoking caused this specific plaintiff's lung cancer. Something more than statistics and epidemiology is needed to satisfy the law. A physician or other scientist must testify that they are of the opinion that the substance caused this specific disease in this specific plaintiff. This will no doubt be based on personal examination of the plaintiff, tissue samples, or at least a pathological report written by a respected pathologist.

As one legal commentator explains, the "intertwining of law and science has created several interesting issues that trial judges must face in determining whether expert testimony gives sufficient assurance of trustworthiness to be admissible under Federal Rule of Evidence 702. Today, federal courts

must be equipped to digest top-notch scientific theories and data, including human and animal testing, the weight given to each in determining toxicity, the extrapolation of results between similar substances and species, the establishment of minimal dose response relationships, the temporal relationships between exposure and onset of disease, and the differential diagnosis and its meaning and role in causation within the legal and medical fields."[78]

B. Federal Rule of Evidence 702

The federal rules of evidence require the judge in charge of a case to determine whether expert scientific testimony and the evidence upon which it is based passes a threshold test before it is admitted. If it "will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise, if the testimony is based upon sufficient facts or data, the testimony is the product of reliable principles and methods, and the witness has applied the principles and methods reliably to the facts of the case."[79]

C. The *Daubert* Standard

Applying Rule 702 standards, the U.S. Supreme Court has held that judges must act as gatekeepers to determine whether scientific evidence is reliable and relevant before it is admitted in a case. Some of the more important factors in the court's reliability analysis are whether: (1) the theory or technique has been or can be empirically tested;[80] (2) the theory or technique has been subject to peer review and publication;[81] (3) the researchers adequately considered the known or potential rate of error of test methods used;[82] (4) the science and test methods used are generally accepted by the scientific community;[83] (5) the opinions and research were created independent of litigation;[84] (6) the study extrapolates from an accepted premise to unfounded conclusion;[85] and (7) the experts adequately accounted for obvious alternative explanations.[86] These factors are not exhaustive. Courts have wide discretion in considering any factor they deem important when making reliability determinations regarding scientific evidence. This methodology is commonly referred to as the "*Daubert*" standard or test after the leading U.S. Supreme Court case on the issue.[87]

Importantly, in applying these factors, a court "will not look at the actual opinion held by the expert, but merely examine his or her methodology to determine whether the procedure used would be expected to lead to trustworthy results."[88] "If an expert relies on unreliable foundational data or his methodology is not reliable, then his entire opinion is likewise unreliable and should be excluded from the jury."[89]

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[78] C. Smith, *Peering into the Microscope: the Rise of Judicial Gatekeeping After Daubert and Its Effect on Federal Toxic Tort Litigation*, 13 B. U. J. SCI. & TECH. L. 218, 218 (2007).
[79] Fed. R. Evid. 702.
[80] *See Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 (1993).
[81] *See id.*
[82] *See id.*
[83] *See id.*
[84] *See id.*
[85] *See General Elec. Co. v. Joiner*, 522 U.S. 16, 146 (1997).
[86] *See Claar v. Burlington N.R.R.*, 259 F. 3d 499 (9th Cir. 1994).
[87] *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 (1993).
[88] S. Loomis, *The Daubert Test of Reliability: Fighting 'Junk Science' in the Courtrooms*, http://www.skepticreport.com/skepticism/dauberttest.htm (citing *North Dallas Diagnostic Center v. Dewberry*, 900 S. W. 2d 90, 95 (Tex. App.—Dallas 1995, writ denied)).
[89] *Id*. (citing *Merrell Dow Pharmaceuticals, Inc. v. Havner*, 953 S.W.2d 706, 714 (Tex. 1997)).

D. Application of the *Daubert* Standard to the Poland Study

Remembering that at the outset we stated our objective as being the evaluation of the Poland Study in a potential litigation context, it is appropriate to consider the significance of the study, if any, in legally establishing that the inhalation of MWCNTs causes mesothelioma. The appropriate place to begin this inquiry is to consider whether the results of the Poland Study would even be admissible in a court of law. To complete this analysis we will assume that the results of the Poland Study are sought to be admitted at time of trial either through the expert testimony of one of the study's authors or the testimony of another expert witness relying on the study to support his or her opinion testimony. The proffer of testimony based on the study would, no doubt, prompt a *Daubert* challenge.

Under *Daubert*, "any step that renders the analysis unreliable . . . renders the expert's testimony inadmissible. This is true whether the step completely changes a reliable methodology or merely misapplies that methodology."[90] Our conclusion is that the Poland Study fails the *Daubert* test because it is unreliable in both design and execution.

First, the design and execution of the Poland Study are not generally accepted in the scientific community for the purposes offered. Both Mossman and Kane & Hurt question the use of Poland's animal model as the predicate for this study. Kane & Hurt point out that the "gold standard' for testing fiber carcinogenicity is a chronic **inhalation** assay using a **range of doses** in **two rodent species**.[91] The Poland Study used injection instead of inhalation, and only one dose and one type of rodent. Furthermore, the validity of even using an animal model to predict human reaction to foreign fibers entering the body was raised by Mossman who explains that "there are few available data on the relationship of fiber size measures and asbestosis, and these data are difficult to reconcile with animal studies."[92] Going to the heart of the Poland Study's findings, Mossman questions whether it has been established that long fibers found in the human body are more fibrogenic than short fibers. In fact, Mossman suggests that the **converse** may be true.[93]

Federal courts have closely scrutinized whether the results of a particular animal model used can be properly extrapolated to humans.[94] Such evidence is often rejected unless the plaintiff can show "what happens in an animal would . . . necessarily happen in a human being."[95] In one leading case, the court held that "[w]hether animal studies can ever be a proper foundation for an expert's opinion [is] not the issue. The issue [is] whether these experts' opinions were sufficiently supported by the animal studies on which they purportedly rely. The studies were so dissimilar to the facts presented in this litigation that it was not an abuse of discretion for the [Court] to have rejected the experts' reliance on them."[96] Similarly courts have rejected the use of inconclusive animal studies which are not backed by epidemiological evidence, noting the "very limited usefulness of animal studies when confronted with questions of toxicity."[97] The Poland Study would not pass these standards.

Second, in order to reach the conclusion that inhalation of MWCNTs may cause mesothelioma, an expert would have to use the Poland Study in such a manner as to extrapolate from an accepted premise to an unfounded conclusion. The Poland Study's authors admit that there was no showing that the MWCNTs caused the granulomas in question or that granulomas progress to mesothelioma. At most, the

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[90] *In Re Paoli R. R. Yard PCB Litigation*, 35 F.3d 717, 745 (3d Cir. 1994).
[91] Kane & Hurt, at 378-379.
[92] Mossman Article, at 1670.
[93] *Id*. at 1670-1671.
[94] *Rider v. Sandoz Pharmaceuticals Corp.,* 295 F.3d 1194, 1202 (11th Cir. 2002).
[95] *Id.*
[96] *Moore v. Ashland Chemical, Inc.*, 151 F.3d 269, 272 (5th Cir. 1998).
[97] *Allen v. Pennsylvania Engineering Corp.*, 102 F.3d 194, 197 (5th Cir. 1996).

Poland Study claims to have shown that exposing the mesothelia lining of the body cavity of mice to long straight MWCNTs results in asbestos-like, pathogenic behavior including inflammation and the formation of lesions known as granulomas. Simply put, "[a] court may conclude that there is simply too great an analytical gap between the data and the opinion proffered."[98] The gap is simply too great to bridge in this instance.

Additionally, the materials, exposure methods, and doses used in the Poland Study do not replicate realistic human exposure scenarios. The study also presumes biopersistence once the MWCNTs are inhaled with no supporting evidence or authority for that conclusion. Courts have rejected scientific studies that fail to present realistic possible human exposure scenarios.[99] The Poland Study does not meet these requirements.

Finally, the Poland Study's authors failed to adequately account for obvious alternatives explanations [confounders], including surface chemistry, sample contamination, sample commingling, spontaneous formation of granulomas, and possible mouse colony infections. These defects alone would render the Poland Study inadmissible as a matter of law under *Daubert* standards.[100]

Because of these defects, federal courts[101] will find the Poland Study inadmissible as a matter of law under Federal Rule of Evidence 702.

IV. CONCLUSION

The Poland Study is certainly insufficient to establish legal causation; i.e., that inhalation of MWCNTs causes mesothelioma or any disease. In reality, the study's actual findings, as opposed to its suggested implications, are quite limited. When carefully reviewed, the authors claim—at most—to have established their hypothesis that long rigid MWCNTs, when injected into the abdominal (not lung) mesothelium-lined cavity of mice, cause a reaction similar to that experienced when long asbestos fibers are injected into the same type of mouse mesothelium, i.e., inflammation and the formation of granulomas. The Poland Study does not purport to show that the granulomas progress to mesothelioma. Nor do the authors claim to have proven that inhalation of nanotubes would cause the same inflammation and granulomas in lung mesothelium, mouse or human. Certainly the Poland Study cannot be understood to show that inhalation of nanotubes by humans will cause mesothelioma of the lung.

Additionally, even if the Poland Study is said to have proven its stated hypothesis, which is questionable, its application is quite limited. First, it would apply only to nanotubes, not all nanomaterials. Second, it would apply only to a limited type of nanotube consisting of long straight fibers, not short and/tangled fibers.[102] Third, it would apply only to multiwalled nanotubes, not single-

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[98] *General Electric Co. v. Joiner*, 522 U.S. 136, 143-44 (1997) (citing *Turpin v. Merrell Dow Chemical, Inc*, 959 F.3d 1349, 1360 (6th Cir. 1992)); *see also Moore v. Ashland Chemical, Inc.*, 151 F.3d 269, 272 (5th Cir. 1998); *Mitchell v. Gencorp Inc.*, 165 F.3d 778, 782 (10th Cir. 1999).

[99] *General Electric Co. v. Joiner*, 522 U.S. 136, 143-44 (1997).

[100] *See Karte v. Exxonmobil Coal USA, Inc.*, 164 Fed. Appx. 553, 556 (7th Cir. 2006) (excluding evidence because expert failed to properly consider and exclude possible alternative causes "such as seasonal allergies, pesticides, or cigarette smoking.").

[101] While many state courts have adopted the same analysis as federal courts, some use an older, more general test. In those jurisdictions, scientific evidence is admissible if it is based on science generally accepted as reliable in the scientific community. *Frye v. United States*, 293 F. 1014 (D.C. Cir. 1923). We reach the same conclusions applying this broader test.

[102] One of the Poland Study's findings was that short and tangled nanotubes did not cause either inflammation or granulomas. Poland Study, at 4. Thus, it might arguably be used to absolve short and tangled nanotubes from

walled nanotubes or fullerenes, since only the former were used. Fourth, it would at best apply only to nanotubes made of carbon, not from other materials, since only carbon nanotubes were used.

Other than sharing at least one dimension on the nanoscale, there is little in common between diverse nanoscale materials such as nanosilver, quantum dots, nanoscale pharmaceuticals, and carbon nanoscale materials for environmental, health, and safety (EHS) purposes. Even within the broad family tree of carbon-based engineered nanoscale materials, there is huge variety. Our unscientific survey located over 280 existing EHS-related studies involving various types of carbon nanoparticles. These particles include functionalized and unfunctionalized MWCNTs, functionalized and unfunctionalized single-walled carbon nanotubes, doubled-walled carbon nanotubes, functionalized and unfunctionalized fullerenes, fullerols, nitrogen-doped carbon nanotubes, carbon nanofibers, hat-stacked carbon nanofibers, nanohorns, and a variety of naturally occurring or incidental carbon nanoparticles. Most of these materials have several variants. Results from the studies were mixed. Many other carbon nanoparticles have not yet been the subject of rigorous studies.

The simple point—implicit in the Poland Study but lost in the media translation—is that the huge variety of nanoparticles that exists should not be lumped together for EHS purposes. Simply put, "[t]here are just too many types of nanoparticles all under the broad umbrella of nanotechnology to make any blanket statements about their interactions with, say, a cell or tissue…. Even if we focus on one type of nanoparticles, those that are carbon-based, there is still a tremendous variety of nanostructures that can be created."[103]

In concluding, reviewers of the Poland Study are left asking a fundamental question: Even if everything the authors claim is true, what is the legal significance of establishing that long rigid MWCNTs when injected into the abdominal mesothelium of mice cause a reaction similar to that experienced when long asbestos fibers are injected into the same type of mouse mesothelium? This finding may be interesting and may suggest a need for further scientific investigation, but it certainly does not establish causation regarding mesothelioma or any disease, either scientifically or legally.

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having an adverse health impact on mice (or humans). The difficulty of using the Poland Study to extrapolate to other nanotubes due to the limited nature of its findings is also recognized by Kane & Hurt.

[103] Susan R. Morrissey, *Challenge of Risk-Based Nanotech Research*, CHEMICAL & ENGINEERING NEWS (Oct. 15, 2007) (quoting Dr. Kristen Kulinowski, Director of the International Council on Nanotechnology at Rice University).