Appendix A – Single-Walled Carbon Nanotube Toxicity Memorandum

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To	Kathy Hart, EPA/OPPT, Mary Ann Curran, EPA/ORD
From	Jay Smith and Shanika Amarakoon, Abt Associates Inc.
Subject	Determination of a toxicity value for single-walled carbon nanotubes (SWCNTs)

Due to their unique electrical, thermal, and mechanical properties, single-walled carbon nanotubes (SWCNTs) are being embedded in an increasing number of consumer and manufactured products. These properties are the reason that an anode containing SWCNTs is being developed for possible future use in Li-ion batteries, and will be evaluated in the Lithium-ion Batteries and Nanotechnology for Electric Vehicles Partnership Life-Cycle Assessment (LCA) study. The following memorandum presents Abt's progress to date in determining an appropriate toxicity value for SWCNTs, and asks for inputs from EPA technical staff so that the process can move forward. Below we provide: (1) a summary of available literature related to carbon nanotube (CNT) toxicity and exposure potential, (2) our process and rationale for toxicity value selection, and (3) proposed next steps in the review and finalization of an appropriate toxicity value.

A.1 Literature Review of CNT Toxicity

There are two main limitations affecting the choice of appropriate occupational and public hazard values for SWCNTs: (1) the dearth of vetted toxicity values available from reputable institutions whose recommendations are preceded by a rigorous peer review process, and (2) the heavy focus in the literature on the toxicity of multi-walled carbon nanotubes (MWCNTs), which differ in macromolecular structure from SWCNTs. Despite these limitations, there is an abundance of literature addressing *in vivo* and *in vitro* carbon nanotube (CNT) toxicity, from 2004 onwards. This literature is described in detail below.

A.1.1 *In vitro* impacts

Reported *in vitro* impacts have by and large involved some form of cytotoxicity, or toxicity to cells. In mouse lung macrophage tissue, the adverse cell response to SWCNTs and MWCNTs was observed to be similar to that seen with exposure to chrysotile asbestos (Murr et al., 2005). The cytotoxic effects of rope-like aggregations of SWCNTs on human mesothelioma tumor cells were demonstrated to be greater in magnitude than those of asbestos (Wick et al., 2007). In alveolar macrophage cells, SWCNTs were found to be more cytotoxic than MWCNTs by mass (Jia et al., 2005). MWCNTs were observed to be more cytotoxic than metal oxide nanoparticles, such as titanium and aluminum oxide (Simon-Deckers et al., 2008). Different properties of the CNTs, such as their structure, length, level of aggregation, chemical modification of sidewalls, surface area, level of oxidation, and manufacturing method all influence the degree of cytotoxicity demonstrated by the material (Magrez et al., 2006; Sayes et al., 2006; Kayat et al., 2011). Taken together, the *in vitro* findings emphasize the potential for a high degree of chemical hazard.

A.1.2 *In vivo* impacts

Tests conducted on test animals have shown mixed results, but have also demonstrated that serious adverse pulmonary effects are a potential outcome of exposure to these materials. Acute intratracheal and intrapharyngeal instillation of SWCNTs has been shown to cause pulmonary granulomas, or microscopic nodules, along with inflammation, fibrosis, and other toxicological changes in the lungs of mice and rats (Warheit et al., 2004; Muller et al., 2005; Lam et al., 2006). The association with asbestos and fibrotic

toxicological endpoints has been explored using *in vivo* animal models. Injection of MWCNTs into the abdominal cavities of mice has been shown to result "in asbestos-like, length-dependent, pathogenic behaviour" (Poland et al., 2008). In addition to pulmonary impacts, some evidence of cardiovascular effects due to SWCNT exposure has been observed in mice (Li et al., 2006). Evidence of the potential for harm is not always clear cut. Other *in vivo* studies have shown no association between exposure to CNTs and adverse health endpoints, including asbestos-like endpoints. In one study, no acute or chronic toxicity was seen after 4 months of direct bloodstream injection of SWCNTs in mice (Schipper et al., 2008). In another study assessing the carcinogenicity of MWCNTs, no increase in mesothelioma (i.e., a disease typically associated with chronic exposure to asbestos) was seen over 2 years in rats that were injected in the body cavity (Muller et al., 2009).

Of the existing *in vivo* studies, some have yielded results that could be used to derive an effect threshold for MWCNTs, though none for SWCNTs. In a subchronic MWCNT inhalation study in rats, the no adverse effect level (NOAEL) for multiple pulmonary endpoints was 0.1 mg/m^3, and the lowest adverse effect level (LOAEL) was 0.4 mg/m^3, at which exposure-related lesions were observed (Pauluhn, 2010). A slightly earlier study in the same animal model resulted in observations of minor lung inflammation, even at 0.1 mg/m^3 (Ma-Hock et al., 2009). In mice, MWCNT exposures at particle concentrations of 0.3 mg/m^3 and higher caused immunosuppression after 14 days, as shown by elevated spleen enzyme levels (Mitchell et al., 2007). These three studies are important in that they demonstrate dose-related injury via the existing physiological mechanism of inhalation, as opposed to intra-cavity injections or instillations (Warheit, 2009). The National Institute for Occupational Safety and Health used the animal studies reported here to derive a suggested occupational recommended exposure limit (REL) of 0.007 mg CNT/m^3 (NIOSH, 2010). This limit was an 8-hour time-weighted average based on the upper limit of quantitation of current CNT analytical methods, and therefore, would have been lower in the presence of more sensitive analytical methods.

A.2 Selection of Toxicity Value

In choosing a toxicity value suitable for use in the calculation of an occupational hazard value, a number of factors need to be taken into account:

- First, the similarity of the material analyzed to the form it will be used in the production of lithium-ion batteries for electric vehicles should be considered. In this case, the toxicity values all correspond to MWCNTs, rather than SWCNTs. There are likely differences in toxicity between the two nanotube types; however, the exact magnitude or direction of the difference is unknown. Although this is a substantial source of uncertainty, the importance of placing the hazard of these novel materials into the context of occupational hazard over the overall battery supply chain outweighs the potential lack of quantitative accuracy at the screening level.
- In addition, the reproducibility of the toxicity value results should be factored into the level of confidence in the selection. Two studies using rats generated results that were similar in magnitude (Ma-Hock et al., 2009; Pauluhn, 2010). One found no adverse effect to MWCNT exposure at 0.1 mg/m^3, while the other found minor lung tissue inflammation at this concentration. The study in mice did not find a NOAEL, and the LOAEL was similar to that seen in rats. Thus, even with the small sample size, the degree of reproducibility in animal models is relatively good.
- Finally, the length of exposure in the toxicological studies should map as closely as possible with chronic (>1 year) exposure to the material that the hazard values are supposed to represent. The rodent studies mentioned above are subchronic, and therefore, represent shorter periods of exposure than is typically used to inform the derivation of chronic toxicity values (e.g., two-year

studies in rats). The standard EPA risk policy in such a case is to apply a factor to make up for the uncertainty in extrapolating from sub-chronic to chronic exposures. It is generally assumed that chronic non-cancer toxicity thresholds will be lower than subchronic thresholds, due to the increased length of exposure.

Taking the rat NOAEL of 0.1 mg MWCNT/m^3 from the 2010 Pauluhn study, we divide by an uncertainty factor of 100 to address the MWCNT to SWCNT extrapolation, the subchronic to chronic extrapolation, and the uncertainty surrounding the minor lung inflammation seen at the same concentration in the 2009 Ma-Hock study (factors of $10^{1/2} \times 10 \times 10^{1/2}$, respectively). **Accordingly, our selected non-cancer toxicity value—which is a synthetic (non-observed) chronic NOAEL in the most sensitive species, rats—is 0.001 mg SWCNT/m^3.** This is in the range of NIOSH's recommended occupational REL value (0.007 mg CNT/m^3), which has been adjusted to compensate for 40-hour weekly exposure duration rather than constant exposure, and insensitive analytical methods of detection. We apply a generic cancer value of 1 to SWCNTs, due to the sparseness of data on carcinogenic potential.

In terms of the actual potential for CNT exposure in the workplace or environment, little quantitative information is available, though there is no reason to assume that exposure cannot take place during the production or end-of-life stages of the lithium-ion battery life cycle. Release and exposure during the use phase is unlikely given the batteries are encapsulated within a sealed case (Köhler et al., 2007). A laboratory and field study of occupational SWCNT release showed that the agitation of the material would cause suspended concentrations of less than 0.53 mg SWCNT/m^3, and glove loadings of 0.2 to 6 mg/hand (Maynard et al., 2004; Helland et al., 2007). This study also demonstrated variable behavior in nanotubes produced using different manufacturing methods. Those produced using laser ablation resulted in a more aggregated material and lower air concentrations. However, those produced using high-pressure carbon monoxide resulted in a material that was more conducive to suspension in air.

With regard to transport in the environment, biomagnification up the food chain and atmospheric transport are possible components of complete human and ecological exposure pathways. CNTs are very persistent, insoluble in water in pure form, and lipophilic. As a result, these chemicals have significant potential to increase in tissue concentration as one moves up the food chain through biomagnification, eventually resulting in dietary exposure in humans (Lam et al., 2004; Helland et al., 2007). A risk assessment based on emissions during the CNT life cycle in Switzerland assumed that CNTs were likely to enter the environment via air emissions and landfilling (Mueller and Nowack, 2008). This study also found that even using conservative assumptions for fate, transport, and exposure, the expected concentration of the CNTs in air, water, and soil was many orders of magnitude lower than concentrations of toxicological concern. No other studies were found that estimated concentrations in environmental media or quantified human and ecological exposure. As a result, there is little background information on the potential for the general public to be exposed to emitted CNTs.

A.3 EPA Response

Based on EPA's review of the memorandum above, EPA indicated that the toxicity value selected by Abt Associates was within the range of acceptable values, and likely on the more conservative end of that range (Partnership, 2011).

A.4 Appendix A References

Helland, A.; Wick, P.; Koehler, A.; Schmid, K.; Som, C. Reviewing the environmental and human health knowledge base of carbon nanotubes. Environmental Health Perspectives 2007, 115(8), 1126-1131.

Jia, G.; Wang, H.; Yan, L.; Wang, X.; Pei, R.; Yan, T.; Zhao, Y.; Gu, X. Cytotoxicity of Carbon Nanomaterials: Single-Wall Nanotube, Multi-Wall Nanotube, and Fullerene. Environmental Science and Technology 2005, 39(5), 1378-1383.

Kayat, J.; Gajbhiye, V.; Tekade, R. K.; Jain, N. K. Pulmonary toxicity of carbon nanotubes: a systematic report. Nanomedicine 2011, 7(1), 40-49.

Köhler, A. R.; Som ,C.; Helland, A.; Gottschalk, F. Studying the potential release of carbon nanotubes throughout the application life cycle. Journal of Cleaner Production 2008, 16, 927-937.

Lam, C.-W.; James, J. T.; McCluskey, R.; Arepalli, S.; Hunter, R. L. A Review of Carbon Nanotube Toxicity and Assessment of Potential Occupational and Environmental Health Risks. Clinical Reviews in Toxicology 2006, 36(3), 189-217.

Li, Z.; Hulderman, T.; Salmen, R.; Chapman, R.; Leonard, S. S.; Young, S.-H.; Shvedova, A.; Luster, M. I.; Simeonova, P. P. Cardiovascular Effects of Pulmonary Exposure to Single-Wall Carbon Nanotubes. Environmental Health Perspectives 2007, 115(3), 377-382.

Magrez, A.; Kasas, S.; Salicio, V.; Pasquier, N.; Seo, J. W.; Celio, M.; Catsicas, S.; Schwaller, B.; Forró, L. Cellular Toxicity of Carbon-Based Nanomaterials. Nano Letters 2006, 6 (6), 1121-1125.

Ma-Hock, L.; Treumann, S.; Strauss, V.; Brill, S.; Luizi, F.; Mertler, M.; Wiench, K. Gamer, A. O.; van Ravenzwaay, B.; Landsiedel, R. Inhalation Toxicity of Multiwall Carbon Nanotubes in Rats Exposed for 3 Months. Toxicological Sciences 2009, 112(2), 468-481.

Maynard, A. D.; Baron, P. A.; Foley, M.; Shvedova, A. A.; Kisin, E.R.; Castranova, V. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. Journal of Toxicology and Environmental Health 2004, 67(1), 87-108.

Mitchell, L. A.; Gao, J.; Wal, R. V.; Gigliotti, A.; Burchiel, S. W.; McDonald, J. D. Pulmonary and Systemic Immune Response to Inhaled Multiwalled Carbon Nanotubes. Toxicological Science 2007, 100(1), 203-214.

Muller, J.; Huaux, F.; Moreau, N.; Misson, P.; Heilier, J.-F.; Delos, M.; Arras, M.; Fonseca, A.; Nagy, J. B.; Lison, D. Respiratory toxicity of multi-wall carbon nanotubes. Toxicology and Applied Pharmacology 2005, 207(3), 221-231.

Muller, J.; Delos, M.; Panin, N.; Rabolli, V.; Huaux, F.; Lison, D. Absence of Carcinogenic Response to Multiwall Carbon Nanotubes in a 2-Year Bioassay in the Peritoneal Cavity of the Rat. Toxicological Science 2009, 110(2), 442-448.

Murr, L. E.; Garza, K. M.; Soto, K. F.; Carrasco, A.; Powell, T. G.; Ramirez, D. A.; Guerrero, P. A.; Lopez, D. A.; Venzor III, J. Cytotoxicity Assessment of Some Carbon Nanotubes and Related Carbon Nanoparticle Aggregates and the Implications for Anthropogenic Carbon Nanotube Aggregates in the Environment. International Journal of Environmental Research and Public Health 2005, 2(1), 31-42.

National Institute of Occupational Health and Safety (NIOSH). Current Intelligence Bulletin: Occupational Exposure to Carbon Nanotubes and Nanofibers. November 2010.

Partnership Call on March 8, 2011. Meeting minutes from call between Abt Associates and EPA's technical experts from OPPT on nanomaterials.

Pauluhn, J. Subchronic 13-Week Inhalation Exposure of Rats to Multiwalled Carbon Nanotubes: Toxic Effects Are Determined by Density of Agglomerate Structures, Not Fibrillar Structures. Toxicological Science 2010, 113 (1), 226-242.

Poland, C. A.; Duffin, R.; Kinloch, I.; Maynard, A.; Wallace, W. A. H.; Seaton, A.; Stone, V.; Brown, S.; MacNee, W.; Donaldson, K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature Nanotechnology 2008, 3, 423-428.

Sayes, C. M.; Liang, F.; Hudson, J. L.; Mendez, J.; Guo, W.; Beach, J. M.; Moore, V. C.; Doyle, C. D.; West, J. L.; Billups, W. E.; Ausman, K. D.; Colvin, V. L. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicology Letters 2006, 161(2), 135-142.

Simon-Deckers, A.; Gouget, B.; Mayne-L'Hermite, M.; Herlin-Boime, N.; Reynaud, C.; Carrière, M. In vitro investigation of oxide nanoparticle and carbon nanotube toxicity and intracellular accumulation in A549 human pneumocytes. Toxicology 2008, 253(1-3), 137-146.

Schipper, M. L.; Nakayama-Ratchford, N.; Davis, C. R.; Wong, N.; Kam, S.; Chu, P.; Liu, Z.; Sun, X.; Dai, H.; Gambhir, S. S. A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. Nature Nanotechnology 2008, 3, 216-221.

Warheit, D. B.; Laurence, B. R.; Reed, K. L.; Roach, D. H.; Reynolds, G. A. M., Webb, T. R. Comparative Pulmonary Toxicity Assessment of Single-wall Carbon Nanotubes in Rats. Toxicological Sciences 2004, 77(1), 117-125.

Warheit, D. B. Long-term Inhalation Toxicity Studies with Multiwalled Carbon Nanotubes: Closing the Gaps or Initiating the Debate? Toxicological Science 2009, 112(2), 273-275.

Wick, P.; Manser, P.; Limbach, L. K.; Dettlaff-Weglikowska, U.; Krumeich, F.; Roth, S.; Stark, W. J.; Bruinink, A. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicology Letters 2007, 168(2), 121-131.