A Life Cycle Risk Assessment Framework of Carbon Nanotubes in the Pharmaceutical Industry

A Plan B Paper

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Abstract

The use of carbon nanotubes is an emerging method for transporting medicines. This paper reviews the research into single- and multi-walled carbon nanotubes and the human health risk studies pertaining to their pharmaceutical application. A life cycle risk assessment (LCRA) framework of carbon nanotube technology in the pharmaceutical industry was used to determine the data gaps in assessing human health risk. The framework helped identify sources where information is presently available and where additional information, resources, and research are needed. Existing literature was found to focus on laboratory testing in non-human species with very few studies in risks associated with the life cycle of production, use, and disposal. Furthermore, current studies do not focus on accidental release or on the end-of-life for carbon nanotubes. While extensive research exists, further research is necessary in order to address the complex and continually evolving nanotechnology, carbon nanotubes.

List of Acronyms

ARRA	American Recovery and Reinvestment Act
CAGR	Compound Annual Growth Rate
CNM	Center for Nanomaterials Manufacturing
CNT	Carbon Nanotube
CPSC	Consumer Product Safety Commission
CVD	Chemical vapor deposition
DOD	Department of Defense
DOE	United States Department of Energy
GIN	Global Issues in Nanotechnology
EHS	Environmental, Health, and Safety
ENM	Engineered nanomaterial
EPA	United States Environmental Protection Agency
FDA	Food and Drug Administration
GPa	Gigapascals
I.V.	Intravenous
LCA	Life Cycle Analysis
LCRA	Life Cycle Risk Assessment
MSDS	Material Safety Data Sheets
MWCNT	Multi-walled carbon nanotube
NASA	National Aeronautics and Space Administration
NEHI	Nanotechnology Environmental and Health Implications Working Group
NHDF	Normal human dermal fibroblast cells
NIH	National Institutes of Health
NILI	Nanomanufacturing, Industry Liaison, and Innovation
NIOSH	National Institute for Occupational Safety and Health
NIR	Near-Infrared Light
NIST	National Institute of Standards and Technology
NME	New Molecular Entities
NNCO	National Nanotechnology Coordination Office
NNI	National Nanotechnology Initiative
NP	Nanoparticles
NRC	National Research Council
NSET	Nanoscale Science and Engineering Subcommittee
NSF	National Science Foundation
NSTC	National Science and Technology Council
NTRD	Nanotechnology Research Directions
OMB	Office of Management and Budget
OSHA	Occupational Safety and Health Administration
PCAST	President's Council of Advisors on Science and Technology
PEN	Project on Emerging Technologies
PMN	Premanufacture Notice
PSI	Pound-force per square inch
RA	Risk Analysis
REL	Recommended Exposure Limit
SNUR	Significant New Use Rule
SWCNT	Single-walled carbon nanotube
TSCA	Toxic Substances Control Act
UC	University of California

I. Introduction

Technology and medicine have seen immense advancements while addressing world health epidemics and lengthening human life span. Research and design in the medical industry have led to the emergence of the use of nanotechnology in medicine. The Nanotechnology Research Directions (NTRD) report from the United States National Science Foundation (NSF), describes nanotechnology as having "the ability to control and restructure the matter at the atomic and molecular levels in the range of approximately 1-100 nm" (Roco M. C., 2011). Nanotechnology is able to focus on the creation of materials, devices, and systems with fundamentally new properties and functions (Roco, Williams, & Alivisatos, 1999) through human manipulation and control of matter (Ferrari, 2005). These allow for "advances in microscopy, material science, molecular-level manipulation, and scientific understanding at the borderline between classical, and quantum physics" (Mnyusiwalla, Daar, & Singer, 2003). This cross-disciplinary technology has a vast potential and is expected to change current products and create new opportunities (Donaldson, et al., 2006).

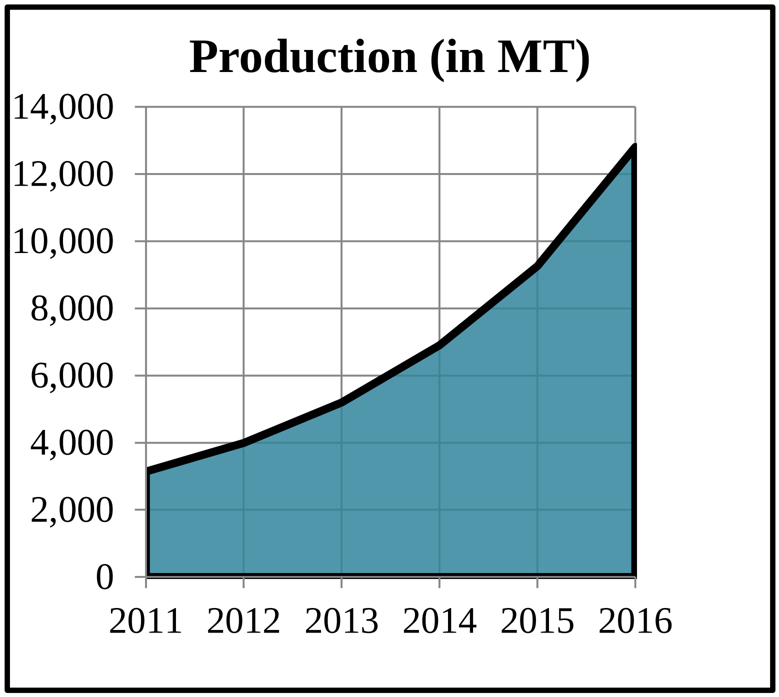
Nanotechnology has been linked to initiatives for technological competitiveness and an advanced technology revolution with an expansive scope of potential. From 2001 to 2010, roughly 150,000 contributors to nanotechnology existed in the United States with over 100 large nanotechnology research and development (R&D) facilities (Roco M. C., 2011). Within this time frame, roughly 3,000 companies emerged that utilize nanotechnology-enabled products (Roco M. C., 2011). By 2004, 60 countries had developed nanotechnologies (Roco M. C., 2011). In 2005, $9.6 billion was devoted to nanotechnology research and development in the United States (Helland, Wick, Koehler, Schmid, & Som, 2007), and by 2011, this number increased to $436 billion (Now, 2011). Nanotechnology is seeing expansion in funding for research and development, production, and applications. Purchases for nanotechnology equated to $96 billion in 2009 in the United States, while $250 billion was spent worldwide (Roco M. C., 2011). Projections for 2014 indicate that purchases of nanotechnologies are projected to be $2.6 trillion worldwide as industrial demands seek innovative ways to address issues on the nanoscale (Thayer, 2007). For the medical industry, nanotechnology is expected to result in $110 billion by 2016 (Bawa & Johnson, 2007).

One type of nanotechnology involves the production of nanoparticles (NP) called engineered nanomaterials (ENM) that are generally defined as “particles less than 100 nm in any dimension” (Donaldson, et al., 2006). An example of ENMs is carbon nanotubes (CNTs), which are built on a nanometer scale and have electronic, light-emitting, and catalytic properties with uses in high-strength materials, reinforced rods, quantum wires, mechanical memory, biomedical industry, and micro fabrication (Shvedova, et al., 2003).

The global production value for carbon nanotubes (CNTs) in 2010 was $668.3 million with multi-walled carbon nanotubes (MWCNTs) accounting for $631.5 million (Nanowerk, 2011). Worldwide growth in production value is expected to reach $1.1 billion by 2016 which equates to a 10.5 percent Compound Annual Growth Rate (CAGR) (Nanowerk, 2011). Whereas the projected growth in quantity could increase from 3,141 metric tons to 12,806 metric tons indicating a 32.5 percent CAGR (Nanowerk, 2011). As markets evolve, production value could have a 10.5 percent CAGR, while the amount of MWCNTs could have a 32.5 percent CAGR, potentially indicating that as production increases, price per MWCNT could decrease. The projected growth of CNTs worldwide in metric tons is shown in **FIGURE I** (Nanowerk, 2011). Projections indicate that research and development will continue to increase and that carbon nanotubes will be utilized more frequently as potential uses continue to unfold (Kohler, Som, Helland, & Gottschalk, 2008). This growth will inevitably increase the potential exposure of carbon nanotubes to the general human population (Kohler, Som, Helland, & Gottschalk, 2008). As of 2011, roughly one hundred companies dominated the market (Nanowerk, 2011). In 2010, the United States had the largest market share followed by Japan, China, and then Germany (Nanowerk, 2011).

FIGURE I: Carbon Nanotube Forecast (in metric tons)

The table outlines the projected global carbon nanotube forecast based on 2011 data for 2012 to 2016 (Nanowerk, 2011).



This paper will present the policy context and background information on carbon nanotube technology, and will then focus on reviewing existing research involving exposure and effects of carbon nanotubes used in pharmaceutical settings. A health-based life cycle assessment risk assessment framework of carbon nanotube technology in the pharmaceutical industry is then developed to identify data gaps for assessing human health risk. The paper serves to examine where information is presently available and where additional information, resources, and research are needed. This analysis is useful in order to prioritize future risk studies and ultimately determine ways of ensuring the safety of carbon nanotubes in medicine.

II. Background

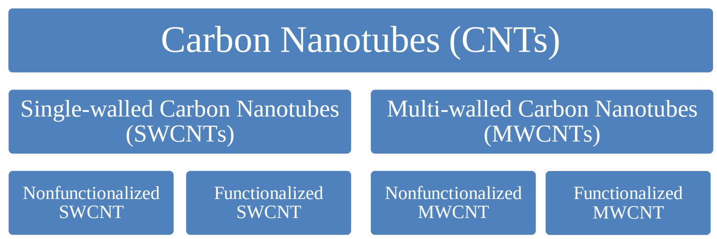
The integration of carbon nanotubes into the pharmaceutical industry has seen growth, and it is important for risk analysis (RA) and life cycle analysis frameworks (LCA) to note where advancements have been made. In the following sections, first the physiochemical properties of carbon nanotubes will be explained to provide a deeper understanding of their potential benefits. Then an overview of past and current policies in the United States helps to identify where regulatory and data gaps may exist. Finally, deeper understanding of these gaps is framed along the life cycle of the extraction, production, handling, disbursing, and disposal of carbon nanotubes in the pharmaceutical industry. This paper will review research conducted within the last thirty years and priority will be given to the most recent findings. For each stage of the life cycle, health risks affiliated with exposure of carbon nanotubes through four routes, intravenous, dermal, inhalation, and ingestion, will be discussed.

A. Physiochemical Properties

Particle size, shape, surface area, and surface chemistry play roles in determining how a carbon nanotube reacts (Tsuji, et al., 2006). The types of carbon nanotubes (CNTs) discussed in this paper are shown in **FIGURE II**. This figure depicts the hierarchical structure of CNTs. In this paper both single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are included for review.

FIGURE II: Types of Carbon Nanotubes

Carbon nanotubes (CNTs) can be either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs). Both SWCNT and MWCNT are considered nonfunctionalized, unless they are functionalized to meet a certain need.

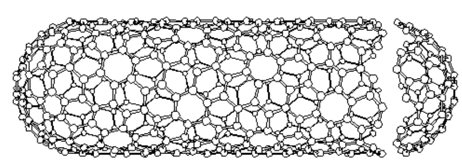


i. Structure

Carbon nanotubes (CNTs) are structured through the arrangement of carbon atoms in a condensed tubular structure (Lacerda, Bianco, Prato, & Kostarelos, 2006). The structures consist of concentrically aligned carbon hexagons, and are generally capped with fullerene[1]-like structures on the ends as shown in **IMAGE I** (Lin, Bajpai, Ji, & Dai, 2003) (Lam, James, McCluskey, & Hunter, 2004). Most CNTs have been made from transition-metal catalysts, iron and nickel (Shvedova, et al., 2003); however nickel has the potential to influence toxicity (Lam, James,

IMAGE I: Carbon Nanotube Fullerene Structure

This figure depicts a carbon nanotube with the fullerene-like structure (ASU, 2013). One end cap is shown detached in this figure (ASU, 2013).



[1] Fullerene is defined as "any of various cage-like, hollow molecules composed of hexagonal and pentagonal groups of atoms, and especially those formed from carbon, that constitute the third form of carbon after diamond and graphite; alternatively, a class of cage-like carbon compounds of fused, pentagonal, or hexagonal sp^2 carbon rings" (PTO, 2013).

McCluskey, & Hunter, 2004). To put the size of a carbon nanotube in perspective, **IMAGE II** helps to illustrate their size. A large CNT can be the size of 1,000 nm (1µm) (Lacerda, Bianco, Prato, & Kostarelos, 2006) (Liu, Tabakman, Welsher, & Dai, 2009).

IMAGE II: Carbon Nanotube Size Comparison

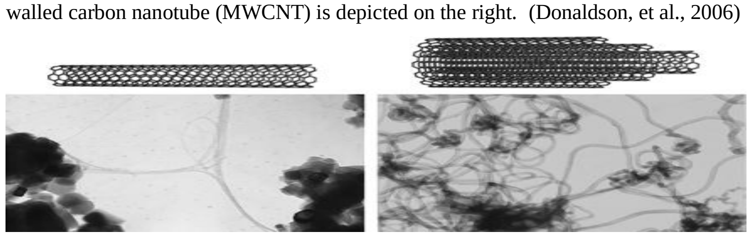
This image depicts the size comparison of a single-walled carbon nanotube to a strand of hair to a house (EPA, 2011).

ii. Single- and Multi-walled Carbon Nanotubes

Two types of carbon nanotubes (CNTs) exist: single-walled (SWCNT) and multi-walled (MWCNT) (**FIGURE II**) (Lacerda, Bianco, Prato, & Kostarelos, 2006). Single-walled carbon nanotubes consist of a single cylindrical layer of graphene while MWCNTs are formed from several concentric graphene sheets (Lacerda, Bianco, Prato, & Kostarelos, 2006) ranging from two to fifty concentrically placed cylinders (Donaldson, et al., 2006). A structural and microscopic comparison of single- and multi-walled carbon nanotubes is shown in **IMAGE III**. The diameter of a SWCNT typically ranges from 0.4 to 2.0 nanometers (nm) and they are about 20 to 1,000 nm in length, (Lacerda,

IMAGE III: Single-walled and Multi-walled Carbon Nanotube Structure

This image depicts the structure and microscopic view of single-walled and multi-walled carbon nanotubes (CNTs). A single-walled carbon nanotube (SWCNT) is depicted on the left and a multi-walled carbon nanotube (MWCNT) is depicted on the right. (Donaldson, et al., 2006)



Bianco, Prato, & Kostarelos, 2006) (Liu, Tabakman, Welsher, & Dai, 2009) while a MWCNT ranges from 1.4 to 100 nm in diameter with lengths from 1 to several micrometers (μm) (Lacerda, Bianco, Prato, & Kostarelos, 2006). Functionalization of CNTs will be discussed in the next section.

iii. Non-functionalized vs. Functionalized

When carbon nanotubes are made, they are in pristine form which is classified as non-functionalized (Lacerda, Bianco, Prato, & Kostarelos, 2006). These are inherently hydrophobic, making them insoluble in most solvents (Lacerda, Bianco, Prato, & Kostarelos, 2006). Biological and medical applications of carbon nanotubes require more solubility in aqueous solutions (Lacerda, Bianco, Prato, & Kostarelos, 2006). In order to address this, modification of the surface of the carbon nanotube occurs through a process called functionalization (Lacerda, Bianco, Prato, & Kostarelos, 2006). Functionalization techniques are applied to address issues of adsorption or electrostatic interaction, to covalently bond different molecules in order to increase water solubility (Lacerda, Bianco, Prato, & Kostarelos, 2006). Adding strong acids to a CNT can be used to oxidize it, which then functionalizes it, and improves its solubility (Bianco, Kostarelos, & Prato, 2005). Adding ammonium groups to the walls and tips also increases solubility (Bianco, Kostarelos, & Prato, 2005). Most pharmaceutical uses require functionalized CNTs so that they can be transferred in solution.

iv. Surface Area

In single-walled carbon nanotubes, every atom is exposed at the surface allowing for a large surface area (theoretically 1300 m^2/g) (Liu, Tabakman, Welsher, & Dai, 2009). However, due to their larger size and surface area, multi-walled carbon nanotubes (MWCNT) have the potential for different uses including operating as a carrier for large biomolecules such as DNA plasmids into cells (Singh, et al., 2005) (Pantarotto, et al., 2004) (Liu, et al., 2005) (Gao, et al., 2006). This suggests that MWCNTs have even more potential for the pharmaceutical industry.

v. Strength

Strength is important in ensuring that the carbon nanotube will not break when used in pharmaceutical procedures. The strength of carbon nanotubes is important in the pharmaceutical industry because they need to be able to withstand high levels of pressure as they pass through the human body. Single-walled carbon nanotubes (SWCNT) have been found to be 10 times the strength of steel and 1.2 times rigid as a diamond (Walters, et al., 1999). A multi-walled carbon nanotube (MWCNT) was tested for a tensile strength[2] of 63 gigapascals (GPa), which is over nine million pound-force/inch² or pound-force per square inch (PSI) (Yu, et al., 2000). In perspective, a standard football is inflated to withstand a pressure of thirteen pound-forces per square inch (PSI) (Utah, 2012). Multi-walled carbon nanotubes can have strength greater than one hundred GPa (Peng, et al., 2008). Even though the strength of carbon nanotubes (CNT) is high, the compression strength has been found to be much lower. Due to the hollow structure, compression strength can cause CNTs to experience buckling and contortion when placed under compressive, torsional, or bending (Jensen, Mickelson, Kis, & Zetti, 2007). The yield strength amount to 1.7 GPa, which is equivalent to 246564.154141 pound-force/inch² or pound-force per square inch (PSI) (Jensen, Mickelson, Kis, & Zetti, 2007). As carbon nanotubes

[2] Tensile strength is defined as the "force required to pull something such as a rope, wire … to the point where it breaks" (ScienceDaily, 2012).

are used in pharmaceutical applications, CNTs will experience pressure while they pass through the human body, so it is important they can withstand these forces.

B. Existing Pharmaceutical Uses

Carbon nanotubes (CNTs) have been introduced in the pharmaceutical industry because of the physiochemical properties discussed above. In the pharmaceutical industry, carbon nanotubes have a variety of applications, which are used "to achieve unique mechanical, optical, electrical, magnetic properties" (Tsuji, et al., 2006) and for thermal and imaging properties on a microscopic scale (Masciangioli & Zhang, 2003). These properties help to increase efficiency of medicines, monitor growth, and improve testing. Due to their small size, CNTs have the ability to easily cross cellular membranes (Martin, 2006) so they may enter the human body more easily and be more biologically active due to their larger surface area per mass unit (Helland, Wick, Koehler, Schmid, & Som, 2007). This increases the potential for CNTs to be used in therapeutic settings (Martin, 2006). In addition, the walls of CNTs are non-reactive to potential medicines, but their tips tend to be more reactive (Lin, Bajpai, Ji, & Dai, 2003). This increases their ability for storing medicines and attaching pharmaceutical compounds to the outside lining of the CNT walls (Lin, Bajpai, Ji, & Dai, 2003). In general, they can also work as bone substitutes, biomimetic[3] composites, chemical and genetic probes, biosensors, and pharmaceutical transporters (Shvedova, et al., 2003).

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[3] Biomimetics is synonymous with 'biomimesis', 'biomimicry', 'bionics', 'biognosis', 'biologically inspired design'. It is defined as the transfer of functions, mechanisms and principles from one field to another. It operates across the border between living and non-living systems (Vincent, Bogatyreva, Bogatyrev, Bowyer, & Pahl, 2006).

i. Cancer

As research and development on carbon nanotubes (CNT) has increased, so has the understanding of CNTs in cancer treatment. The structure for CNTs allows for them to be made with or without end caps. A CNT without an end cap is able to hold a pharmaceutical inside, allowing for the drug to more easily pass through cell membranes (Pastorin, 2008). This allows for pharmaceutical storage, reduced toxicity, higher bioavailability, protection, and controlled release of drugs (Pastorin, 2008).

One type of treatment utilizes carbon nanotubes in the Kanzius Radio wave Cancer Treatment project (KCRF, 2013). The therapy utilizes single-walled carbon nanotubes (SWCNT) that are inserted near cancerous cells (KCRF, 2013). The SWCNT are then activated using radio waves which causes them to rise in temperature, cooking the surrounding cells (KCRF, 2013). Another treatment uses carbon nanotubes as drug delivery mechanisms because they have shown the ability to target specific cancer cells with a dosage lower than conventional drug-delivery methods (Srinivasan, 2008). This targeting method has been shown to be more effective in killing cancerous cells while causing minimal damage to healthy cells, and reducing side effects (Hilder & Hill, 2008).

Advancements in carbon nanotube and cancer treatment therapy have led to the functionalization of carbon nanotubes by modifying the surface of the carbon nanotube for drug delivery and cancer therapy (Kam, O'Connell, & Wisdom, 2005). Functionalized single-walled carbon nanotubes (SWCNT) used i*n vitro* has proved successful at selective cell death (Kam, O'Connell, & Wisdom, 2005) through the use of near-infrared light (NIR). This technique was used for optical stimulation of the carbon nanotubes inside cancerous cells (Kam, O'Connell, & Wisdom, 2005). Unlike standard cancer treatments, such as chemotherapy, the use of NIR lasers results in less serious side effects and protects healthy cells.

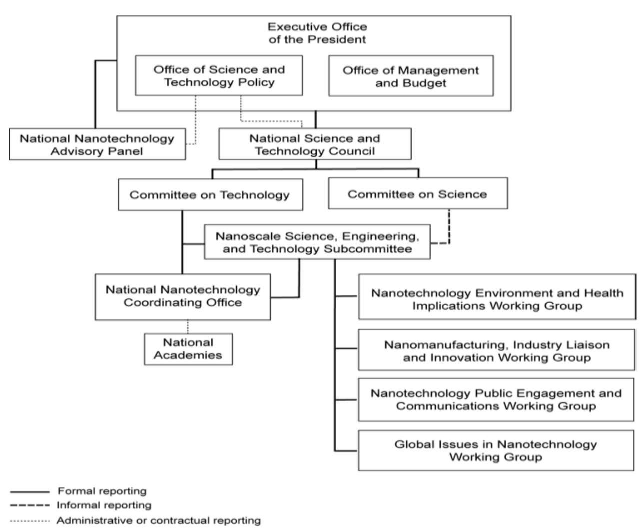
C. Policy Context

Worldwide trends indicate that growth in the nanotechnology industries will continue to occur. With the rising usage of nanotechnology, specifically carbon nanotubes and products, it will be important for policymakers to understand the potential implications of this set of emerging technologies and products. Contemporary risk characterizations are exhibiting slow response times to new emerging technologies which can complicate United States regulatory practices (Philbrick, 2010).

i. History

In 1991, the National Science Foundation (NSF) formed its first program devoted to nanoparticles research (NSF, 1997). From 1997 to 1998, a cross- disciplinary program called the "Partnerships in Nanotechnology" was formed and from 1998 to 2000 the fields of engineering and nanoscale science were merged under a single science-based definition along with a 10-year research and development (R&D) vision for

FIGURE III: National Nanotechnology Initiative Structure



nanotechnology (Roco M. C., 2011). In 2000, the United States National Nanotechnology Initiative (NNI) was formed in order to address gaps in research and to address the economic potential of nanotechnology (Roco M. C., 2011). The NNI structure is shown in **FIGURE III**. The twenty-five departments and agencies involved are shown in **TABLE I**.

Every three years the NNI prepares a Strategic Plan in order to evaluate the progress and future uses of nanotechnology (Roco M. C., 2011). In addition to the Strategic Plan, the National Research Council (NRC) of the National Academies and the President's Council of Advisors on Science and Technology (PCAST) is evaluated every third year as the National Nanotechnology Advisory Panel (Roco M. C., 2011). To ensure taxpayer funds and public interest are best represented, the Government Accountability Office and other organizations evaluate the NNI (Roco M. C., 2011).

TABLE I: National Nanotechnology Initiative Federal Departments and Agencies

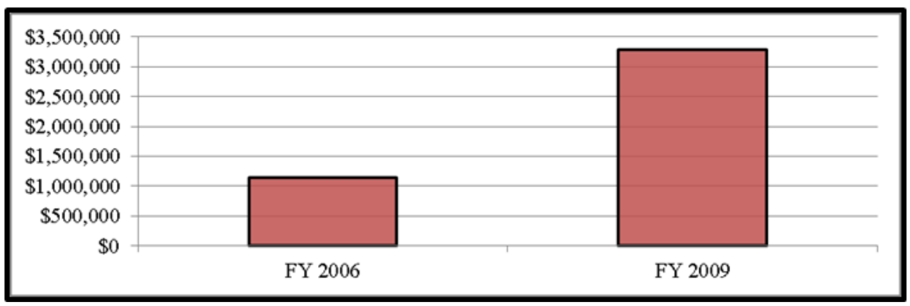
Consumer Product Safety Commission (CPSC)	Department of Defense (DOD)	Department of Energy (DOE)
Department of Homeland Security (DHS)	Department of Justice (DOJ)	Department of Transportation (DOT, including the Federal Highway Administration, FHWA)
Environmental Protection Agency (EPA)	Food and Drug Administration (FDA, Department of Health and Human Services)	Forest Service (FS, Department of Agriculture)
National Aeronautics and Space Administration (NASA)	National Institute for Occupational Safety and Health (NIOSH, Department of Health and Human Services)	National Institute of Food and Agriculture (NIFA, Department of Agriculture)
National Institutes of Health (NIH, Department of Health and Human Services)	National Institutes of Standards and Technology (NIST, Department of Commerce)	National Science Foundation (NSF)

Note: The Agricultural Research Service (ARS) within the USDA was added in 2012 (Roco M. C., 2011).

ii. Environmental Health and Safety (EHS) Concerns

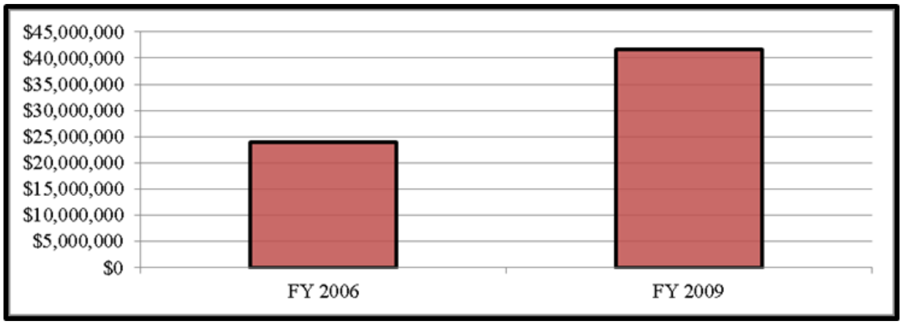
In 2011, the National Nanotechnology Initiative developed a new Environmental, Health, and Safety (EHS) Research Strategy for "ensuring the safe, effective, and responsible development and use of nanotechnology" (NNI, 2013). The NNI EHS Research Strategy utilizes risk assessment principles and life cycle analyses in order to determine human health risk (NNI, 2013). Funding for EHS primarily goes to Consumer Product Safety Commission (CPSC), Environmental Protection Agency (EPA), National Institute for Occupational Safety and Health (NIOSH), and the National Science Foundation (NSF) (**FIGURE IV)**. In the fiscal year of 2006, $1,144,000 was spent on 5 projects, while in the fiscal year of 2009; $3,289,000 was spent on 14 projects (National Nanotechnology Initiative, 2011).

FIGURE IV: Human Exposure Assessment Funding



When funding for EHS includes other big agencies like the Department of Defense (DOD) and NIH, there is a significant increase in funding. However, in these cases, it is difficult to separate funding for risk assessment or EHS implications from health applications. Funding from the DOD, CPSC, NSF, NIOSH, EPA, Food and Drug Administration (FDA), National Institute of Health (NIH), and National Institute of Standards and Technology (NIST) on projects focused on human health and nanotechnology in 2006, was $23,975,000 while in the fiscal year (FY) of 2009, $41,610,000 was spent (**FIGURE V**) (National Nanotechnology Initiative, 2011). When looking specifically at EPA and EHS research, funding is more modest. Funding (EPA) on environmental health and safety research increased from $3.6 million in FY 2006 to $17.6 million in FY 2010 (GAO, 2012). In FY 2006, this funding accounted for 10 projects, while in FY 2010, the funding accounted for 32 projects (GAO, 2012).

FIGURE V: Human Health Funding



While there has been increased funding and projects for environmental health and safety (EHS), there has been discourse on how to best focus on federal investment in EHS research, federal regulation, and international engagement. A centralized source of funding for EHS research does not exist in federal agencies (Sargent Jr., 2011). Each agency is responsible for determining their budget along with the amount of funding requested for EHS research (Sargent Jr., 2011). There is some coordination of EHS-research budgets through the Nanotechnology Environmental and Health Implications (NEHI) working group and during the Office of Management and Budget (OMB) budget development process, but overall funding is decentralized (Sargent Jr., 2011). Funding for all EHS Research Funding for nanotechnology is shown in **TABLE II**.

TABLE II: National Nanotechnology Initiative Environmental Health and Safety Funding

	EHS Research in Current Dollars	**EHS Research's Share of Total Annual NNI Budget**
FY 2006	$37.7 million	2.8%
FY 2007	$48.3 million	3.4%
FY 2008	$67.9 million	4.4%
FY 2009	$74.5 million	4.4%
FY 2010	$91.6 million	5.1%
FY 2011	116.9 million	6.6%

As **TABLE II** identifies, EHS research funding has increased steadily from 2006 to 2011. Critics argue that federal EHS nanotechnology funding is too low and that the current allocations may cause an issue when more products exist that are unable to be assessed and managed for their EHS risks (Sargent Jr., 2011). Officials in the executive branch assert that "the United States leads the world in EHS funding and, by inference, that the current funding level is adequate" (Sargent Jr., 2011). Industry groups like the NanoBusiness Alliance assert that EHS funding for nanotechnology should be at least 10% of the entire NNI total funding which in fiscal year 2010 would equate to $178.1 million (Sargent Jr., 2011).

Besides funding, there have been concerns that current laws, regulations, and authorities are inadequate to address EHS implications from nanotechnology. Increase "pace, scope, and complexity of technological innovation may pose challenges to the existing regulatory system" that affect nanotechnology research and development (Sargent Jr., 2011). Historically, chemicals are defined by its composition, without regard to particle size (Sargent Jr., 2011). This poses a problem because nanotechnology may exhibit different properties that may pose different EHS concerns (Sargent Jr., 2011). The NNI and PCAST both concluded that existing regulatory authorities were adequate for current activities; regulatory mechanisms should be used to address EHS effects of nanotechnology; and new implemented policies for nanotechnology are "rational, science-based, and consistent across the federal government."
Tension exists between the global competitiveness position of the United States and addressing the potential nanotechnology implications (Sargent Jr., 2011).

Andrew Maynard, the chief science advisor for the Woodrow Wilson Center's Project on Emerging Nanotechnologies (PEN) has stated that "the federal government needs a master plan for identifying and reducing potential risks. This plan should include a top-down risk research strategy, dedicated and sufficient funding to do the job and the mechanisms to ensure that resources are used effectively" (Sargent Jr., 2011).

Funding through federal agencies is working to support research and development on carbon nanotubes. One example is funded from the National Institutes of Health (NIH) and the American Recovery and Reinvestment Act (ARRA), which was given to researchers at the University of California (UC) Davis for $1,000,000 to study the health effects of inhaling carbon nanotubes (NIH, 2009). Studies focused on health effects of carbon nanotubes can help to advance the understanding of risks in pharmaceutical use, the focus of this paper.

iii. Regulation

a. Legislation

Congressional interest in nanotechnology safety has increased in the last 5 years. For example, under the 111th Congressional session in 2009, the National Nanotechnology Initiative Amendments Act revised the 21sts Century Nanotechnology Research and Development Act (Sargent Jr., 2011). It directed the "National Nanotechnology Coordination Office to develop and marinating a public database of National Nanotechnology Initiative (NNI) environmental health and safety (EHS) projects, including the agency funding source and funding history" (Sargent Jr., 2011). It required the development of an "annual research plan for federal nanotechnology EHS activities, monitoring and encouraging agency EHS efforts, and for encouraging agencies to engage in public-private partnerships to support EHS research" (Sargent Jr., 2011). It also required interdisciplinary research centers from the agencies involved in NNI to include EHS research to understand environmentally benign nanoscale products and processes (Sargent Jr., 2011)

b. Federal Agency Efforts

Federal agencies have taken different approaches to nanotechnology safety. Approaches range from mandatory rules specific to nanoproducts to guidelines for industry and recommendations on exposure levels. Below are some of the key agencies for nanotechnology regulation and a synopsis of their approaches.

Food and Drug Administration (FDA)

The FDA has taken steps to address nanotechnology EHS under its current product approval systems. A guidance for industry draft was released by the Food and Drug Administration (FDA) titled "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" in 2011 (FDA, 2011). When an FDA-regulated product contains nanomaterials or involves nanotechnology, the FDA will determine "whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1 nm to 100 nm)" or "whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (FDA, 2011).

In order to address nanotechnology risks, the FDA is “maintaining its product-focused, science-based regulatory policy” (FDA, 2012) because their regulatory approach is meant to be adaptive and flexible so each product has a product-specific technical assessment (FDA, 2012). Medicines are evaluated by the FDA on their risk profile but also on their predicted benefit (FDA, 2012). Emerging medicines using nanotechnology are subject to a premarket approval process and requirements where producers must submit data to prove “safety, effectiveness (where applicable), or regulatory status of the product” (FDA, 2012). The FDA Nanotechnology Task Force outlined that the FDA should recommend that manufacturers of nanotechnology contact the FDA when they are in the product development stage (FDA, 2012). Doing so allows for the FDA to provide feedback, review safety information, and assist in any necessary post-marketing safety oversight (FDA, 2012). The industry will be responsible for ensuring that the product meets all legal and safety standards, which is why the FDA encourages industry to work with them (FDA, 2012). To ensure safety, the FDA will monitor the marketplace for products that contain nanomaterials and will take necessary action if needed and works with international regulatory agencies to share information on nanotechnology products and intended uses (FDA, 2012). The FDA has begun to develop expertise in nanotechnology and encourage consideration of new information on nanotechnology and “evaluate current testing approaches to assess the safety, effectiveness, and quality of nanoscale materials” (FDA, 2012). The FDA’s method focuses on each product and its intended in its appropriate context to address health and safety risks (FDA, 2012).

Consumer Product Safety Commission (CPSC)

This agency was created to provide protections to the public from "unreasonable risks of serious injury or death from certain types of consumer products" (Sargent Jr., 2011). In order to do so, the Consumer Product Safety Commission (CPSC) utilizes the Consumer Product Safety Act and the Federal Hazardous Substances Act to address nanotechnology (Sargent Jr., 2011). These two acts do not evaluate a product's risk to humans until after it has been distributed into markets (Sargent Jr., 2011). As of 2011, the CPSC was unable to make any general statement about potential consumer exposure to nanotechnology along with health effects that could occur from exposure during consumer use or disposal (Sargent Jr., 2011). They do however recognize the regulatory challenges such as identification of specific nanotechnology; characterization of nanotechnology exposure; size assessment of nanotechnology; and toxicological data analysis for particle sizes (Sargent Jr., 2011).

Environmental Protection Agency (EPA)

The Environmental Protection Agency (EPA) is supporting research on the "toxicology, fate, transport, transformation, bioavailability, and exposure of humans… to nanomaterials to obtain information for us in risk assessment" (Sargent Jr., 2011). The International Organization for Standardization and the Organization for Economic Cooperation and Development are examples of international organizations that the EPA is continually engaged with in nanotechnology-related regulations (Sargent Jr., 2011). The EPA uses its authority to regulate nanotechnology materials and devices through the Clean Air Act (CAA), Clean Water Act (CWA), and Toxic Substances Control Act (TSCA) (Sargent Jr., 2011) (Shvedova, et al., 2009).

Up until 2010, it was not clear whether the Environmental Protection Agency (EPA) would treat CNTs as "new molecular entities" (NMEs) under the Toxic Substances Control Act (TSCA) (Shvedova, et al., 2009). As of 2010, the EPA issued significant new use rules (SNURs) under TSCA requiring producers of a "new" nanotechnology to provide the EPA with a premanufacture notification (PMN) (Sargent Jr., 2011) (EPA, 2010). The PMN requires any person that intends on manufacturing, importing, or processing CNTs to notify the EPA beforehand (EPA, 2010). The EPA then has 90 days to restrict the use, require more information from the manufacturers, or approve manufacturing (Sargent Jr., 2011). If a substance is on the approved inventory list, then any use is considered legitimate, although the EPA has the authority to assess the risk (Sargent Jr., 2011). Under these regulations, the EPA can assess the potential harm and would have the authority to prohibit or limit the manufacture before it may occur (EPA, 2010).

Occupational Safety and Health Administration (OSHA)

Based on the Occupational Safety and Health Administration (OSHA), carbon nanotube (CNT) exposure limits are classified the same as graphite exposure at 5mg/m^3 (Shvedova, et al., 2009). However, the United States National Institute for Occupational Safety and Health (NIOSH) has instead set a 0.5 (high aspect ratio, 0.01 fibers/cm^3) for soluble nanomaterials and fibrous manufactured nanomaterials (Lee, et al., 2010).

There are few occupational exposure limits that exist for nanomaterials so "existing occupational exposure limits for a substance may not provide adequate protection from nanoparticles of that substance" (OSHA, 2013). It is recommended by OSHA that "worker exposure to respirable carbon nanotubes and carbon nanofibers not exceed 1.0 micrograms per cubic meter (μg/m^3) as an 8-hour time-weighted average (OSHA, 2013). This is based on the National Institute for Occupational Safety and Health (NIOSH) proposed Recommended Exposure Limit (REL) (OSHA, 2013). It is also recommended that employers assess worker exposure to nanomaterials to determine if controls used will be effective in reducing or eliminating exposure (OSHA, 2013). If carbon nanotubes are not covered by these standards then OSHA has other regulatory mechanisms to attempt to protect workers safety. First, any inhalable nanoparticles such as carbon nanotubes will be covered under the $5 mg/m^3$ "particulates not otherwise regulated" or called "nuisance dust" (29 CFR 1910.1000) (Lustberg & Silbergeld, 2002). Second, the respiratory protection standard (CFR 1910.134) requires workers to use respirators and protective devices (Balbus, Florini, Denison, & Walsh, 2006). Third, the OSHA hazard communication standard (CFR 1910.1200) requires producers or importers to develop material safety data sheets (MSDS) which provide information on hazardous ingredients workers are using (Balbus, Florini, Denison, & Walsh, 2006). Fourth, the general duty clause [Section 5(a)(1)] is meant to protect workers from exposure on toxic effects that are not addressed by an OSHA standard, but it only applies to "recognized" hazards (Balbus, Florini, Denison, & Walsh, 2006).

III. Analyses Scope and Framework

Carbon nanotubes have seen a rise in production and function, and this growth is projected to continue to expand. Since carbon nanotubes are relatively new, research is still being done on the human health risks, and it can be complicated to elucidate how carbon nanotubes interact with the human body.

Before choosing a framework, a literature review was completed to review existing research on carbon nanotubes used in the pharmaceutical industry for pharmaceutical transportation. Examples of some of the search terms for this research included: carbon nanotubes (CNT), single walled CNT, multiwalled CNT, CNT functionalization, CNT health effects, CNT exposure, CNT toxicity, CNT research, CNT production, CNT use, CNT excretion, CNT release, CNT accidental release, CNT environment, CNT cancer, CNT pharmaceuticals, CNT biomedical, CNT medicine, CNT life cycle, CNT safety, CNT benefits, CNT growth, CNT government, CNT federal agency, CNT restrictions, and many more. These search terms were used in databases such as: Google Scholar, Medline, PubMed.gov, ScienceDirect (SciVerse), University of Minnesota Library Catalog, EBSCOhost, etc. Information pertaining to federal research and programs were pulled from governmental entities such as the Environmental Protection Agency (EPA), Government Accountability Office (GAO), National Nanotechnology Initiative (NNI), or the National Science Foundation (NSF). Peer-reviewed research from carbon nanotube researchers such as Dr. Nadine Wong Shi Kam, Dr. Lara Lacerda, Dr. Davide Pantarotto, Dr. Mihail C. Roco, Dr. Anna A. Shedova, and Dr. Ravi Singh were used to identify where information existed and what information was missing.

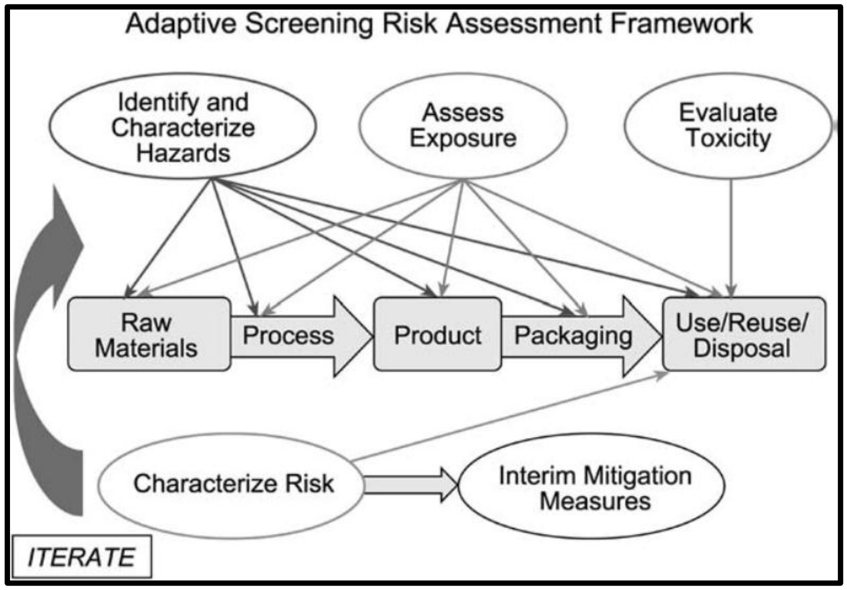
After reviewing existing research, it was found that there were information gaps. In order to understand where gaps occurred, it became important to analyze carbon nanotubes from cradle-to-grave. Existing life cycle assessments were identified and five were compared: CNT Characteristics and Potential Adverse Effects by Shatkin; Nanotechnology (NANO) Life Cycle Risk Assessment by Shatkin; Nanoproduct Life Cycle by Som, Berges, Chaudhry, Dusinska, Fernandes, Olsen, and Nowack; Life Cycle Assessment as a Tool to Enhance the Environmental

Performance of Carbon Nanotube Products: A Review by Upadhyayula; and Life Cycle Assessment by the Environmental Protection Agency. While all five were strong models, the Nanotechnology (NANO) Life Cycle Risk Assessment by Shatkin was the best choice for this assessment of carbon nanotubes because it best identifies where information is available, what health risks may exist, and where future research is needed. This framework was chosen over other models because it indicates where information gaps exist and it helps to evaluate the toxicity of nanomaterial. While various forms of life cycle analysis/assessment exist, modeling done by Shatkin helped to capture the cradle-to-grave life cycle of nanomaterials.

Shatkin defines life cycle assessments as "a compilation and evaluation of the inputs and outputs and the potential impacts of a product system through its life cycle" (Shatkin, 2009). Her model is called a Nanotechnology (NANO) Life Cycle Risk Assessment (LCRA) which is defined as a "screening tool to identify and prioritize key health … issues, which may be applied at a very early stage of nanomaterial development when little information is available for risk assessment" (Shatkin J. A., 2008). LCRA helps to identify the information that is needed for officials to make better decisions. When the information is compiled in a LCRA, it will allow researchers to address the information gaps and insert conclusions when new information is available (Shatkin J. A., 2008). To better understand how the NANO LCRA operates, see **Figure VI** below.

Risk is composed of level of exposure and severity of effects at different exposure levels and pathways. When addressing emerging technologies it is important to understand both exposure and effects (Shatkin J. A., 2008). In the lab, toxicological evaluations for different levels of exposure are measured, but without adequate exposure information in the real-world, it can be difficult to address risk (Shatkin J. A., 2008). LCRA focuses on exposure pathways at different parts of the life cycle of a nanomaterial.

FIGURE VI: Nanotechnology (NANO) Life Cycle Risk Assessment (LCRA)



The NANO LCRA begins the analysis at the raw material stage, and thus carbon nanotubes would start with metals such as iron and carbon as raw materials (Shatkin J. A., 2008). It is important to conduct a life cycle assessment since carbon nanotube exposure can exist through every stage such as synthesis, production, processing and assembly, product usage, recycling processes, and final disposal (Kohler, Som, Helland, & Gottschalk, 2008). This paper adapts Shatkin's previous work and makes it applicable to carbon nanotubes in biomedicine.

Shatkin's model is structured through a nanotechnology lens focused on environmental health, and allows for a continuation of the cycle through iterations, identifies hazards, assesses exposure, and evaluates toxicity. To make the model more applicable to carbon nanotubes in the pharmaceutical industry, it will require restructuring to focus on human health. The information analyzed in the five stages of raw materials, processing, product use, packaging and disposal, will stay primarily the same, but categories are restructured and additional analyses included. In order to better represent the changes that will be made, each stage has been color coded for quicker identification (**FIGURE VII**). This will assist in a comparison of Shatkin's NANO LCRA and the adapted Carbon Nanotube LCRA in this paper.

For the purpose of this study "raw materials" will now be titled "extraction" and their color is yellow. This stage will cover health effects and exposure that can occur during extraction and provide an of the potential health effects, from the raw materials. The stages, "process," "product," and "packing" have all been merged into "production" and their color is orange. These were merged together because Shatkin's definition for each could easily be combined into one stage that addresses the carbon nanotubes creation to its product disbursement. Shatkin's fifth stage, "use/reuse/disposal" was divided to account for pharmaceutical uses. For the purpose of this study, "use" will still be titled "pharmaceutical use" and will be colored blue. This stage is being separated because the exposure and health effects from clinical use could be very different than those from medical disposal. Since each stage is evaluated independently, it is important to differentiate main stages. For example, pharmaceutical use may have different exposure routes and health effects. The "disposal" portion will be relabeled as "accidental release/disposal" and will be colored red. The title "accidental release/disposal" addresses accidental releases from clinical setting and risk associated with this. Accidental release is important to include as this can occur at many stages, and deserves to be addressed as emergency response can be much different than standard operating procedures. The “reuse” portion will be renamed “excretion/environmental” and colored purple. This stage was renamed because after carbon nanotubes are used in the pharmaceutical industry, they enter the human body and are excreted through urine. The comparison between Shatkin’s model and the new model can be seen in **FIGURE VII**.

The reinterpretation of Shatkin’s work on Life Cycle Risk Assessment allows for a model that is specifically adapted to the use of carbon nanotubes in the pharmaceutical industry in the context of human health. For this Life Cycle Risk Assessment of Carbon Nanotubes, it is important to incorporate the routes of exposure to humans at each stage. The influence diagram illustrated in **Figure VIII** identifies routes of exposure (green). In this model, they are inhalation, dermal, ingestion, and intravenous, and their likelihood at each life cycle phase in **Figure VII** will be discussed below.

FIGURE VII: Reinterpreted Life Cycle Risk Assessment (LCRA)

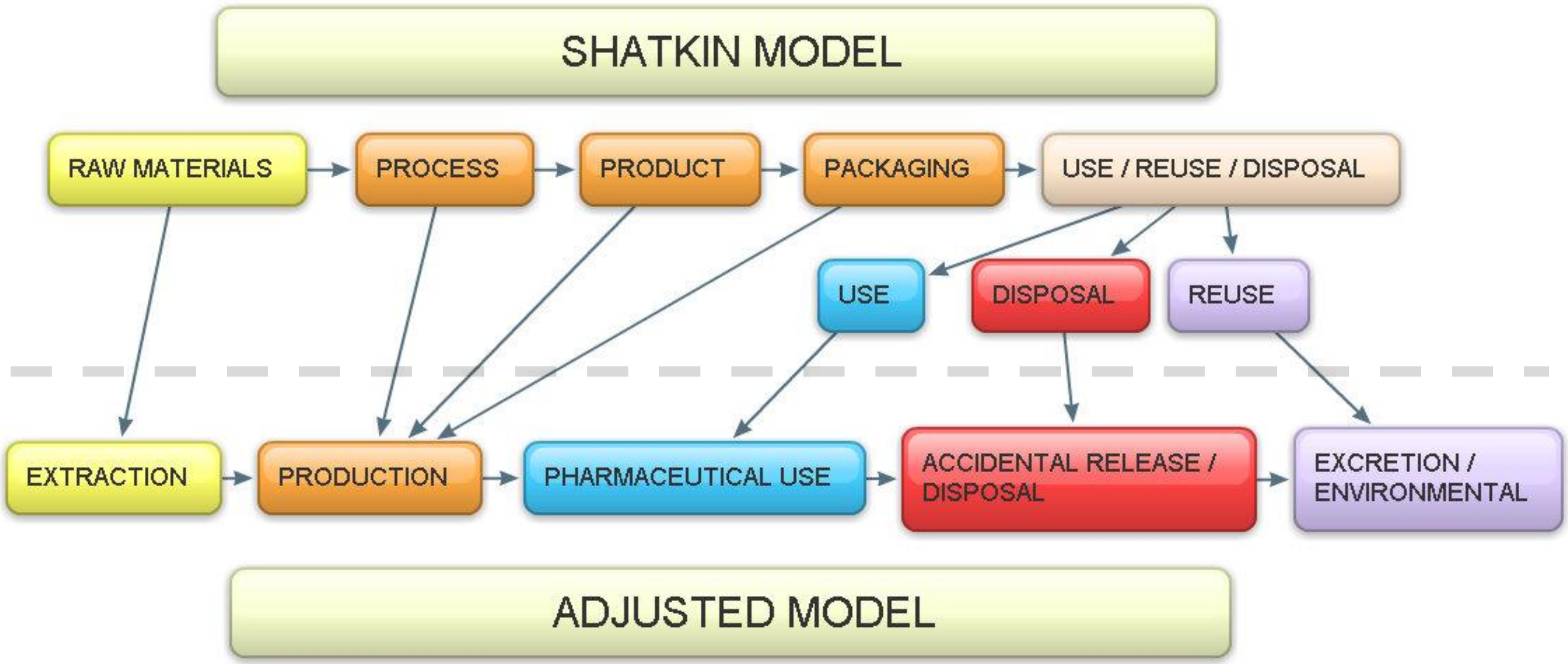
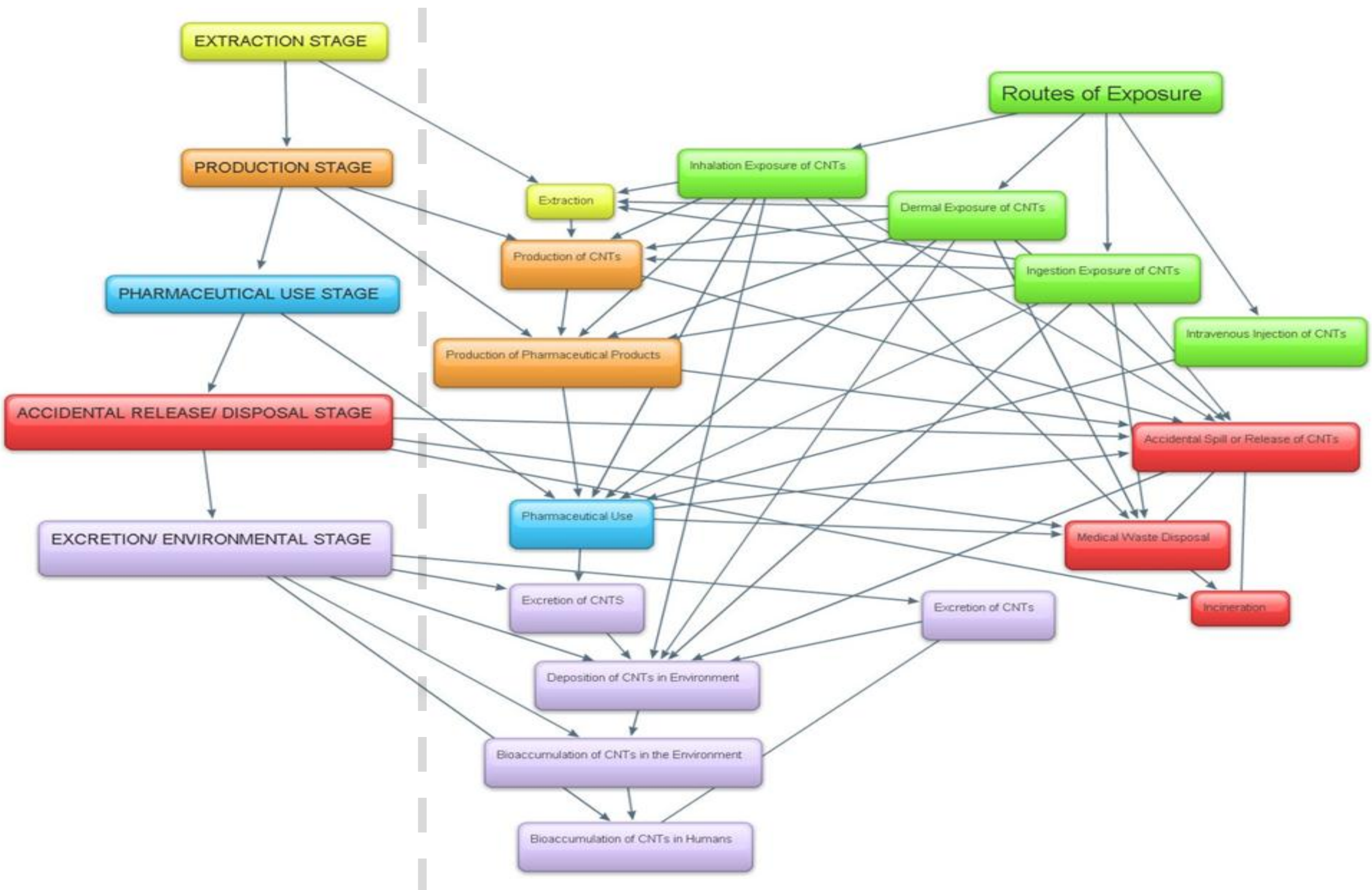


FIGURE VIII: Influence Diagram



IV. Life Cycle Risk Assessment

A. Routes of Exposure

i. Inhalation

Inhalation is the act of drawing air into the lungs through complex alterations in the diaphragm and muscles in the abdomen and thorax, which causes the chest cavity to expand (Merriam-Webster, 2013). An average adult lung moves roughly 2,641 gallons of air (BLF, 2013). Thus, concern for inhalation of carbon nanotubes (CNTs) is important because it can occur during all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental as seen in **FIGURE VII** and **TABLE III**. Caution is recommended when working with carbon nanotubes as they can be released during an aerosol release (Maynard, et al., 2004). Deposits on gloves have the propensity to become airborne which may lead to increased levels of exposure (Maynard, et al., 2004). Different CNT production methods can lead to different types of aerosol releases (Maynard, et al., 2004). Such a release could occur through "abrasion (e.g. wear and tear) "or "physiochemical ageing (e.g. due to corrosion or thermal influence)" (Kohler, Som, Helland, & Gottschalk, 2008). This could result in the agitation of unrefined single-walled carbon nanotubes which have the potential to release fine particulates into the air at $< 53 \mu g/m^3$ (Maynard, et al., 2004). Due to the fine size of the CNTs, there is a potential that they can penetrate deep into the lungs (Maynard, et al., 2004). Current restrictions under the National Ambient Air Quality Standards (NAAQS) are set for 24 hour exposure to be at $35 \mu g/m^3$ for $PM_{2.5}$[4] (EPA, 2013). This indicates that a onetime aerosol exposure of CNTs could be higher than a 24 hour regulated exposure for particulate matter.

[4] PM stands for particulate matter

TABLE III: Life Cycle Risk Assessment: Health Effects and Exposure at Each Stage

This table identifies the four routes of exposure (inhalation, dermal, ingestion, and intravenous) that are discussed in this paper along with how each exposure can occur and their health effects. A red box indicates that the type of exposure or health effect **does not** occur at that stage. For example, during inhalation, an individual could be exposed to a carbon nanotube via an aerosol release **not** during the Extraction phase. A green box indicates that the type of exposure or health effect **does** occur at that stage. For example, during inhalation, an individual could be exposed to a CNT via an aerosol release **during** the Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental stages.

Red indicates that it **does not** occur during this stage				Green indicates that it **does** occur during this stage				
			References	Extraction	Production	Pharmaceutical Use	Accidental Release/ Disposal	Excretion/ Environmental
Inhalation	Exposure	Aerosol release	(Han, et al., 2008); (Lee, et al., 2010); (Maynard, et al., 2004); (NIH, Graphite, 2012)					
		Abrasion	(Maynard, et al., 2004); (NIH, Graphite, 2012)					
		Physiochemical ageing (corrosion or thermal influence)	(Kohler, Som, Helland, & Gottschalk, 2008); (Maynard, et al., 2004); (NIH, Graphite, 2012)					
	Effects	May elicit inflammatory response	(Lam, James, McCluskey, & Hunter, 2004)					
		May elicit granulomatous response	(Lam, James, McCluskey, & Hunter, 2004)					
		May elicit fibrogenic response	(Lam, James, McCluskey, & Hunter, 2004)					
		Asbestos-like tendencies (concern for pathogenicity)	(Donaldson, et al., 2006); (Green & Vallyathan, 1998)					
		May cause lung toxicity	(Oberdorster, Oberdorster, & Oberdorster, 2005)					
		Increased risk of cancer in nose region (Iron Use)	(Feron, Arts, Kuper, Slootweg, & Woutersen, 2002)					
		Increased likelihood of Carboconioses (Graphite Mining)	(NIH, 2012)					
		Increased likelihood of Dust Bronchitis (Graphite Mining)	(NIH, 2012)					
		Mixed Dust Pneumococniosis (Graphite Mining)	(Domeh, et al., 2002); (Mazzucchelli, Radelfinger, & Kraft, 1996); (Uragoda, 1997)					
		Radiographic lesions found in 4.1% of miners (Graphite Mining)	(Uragoda, 1997)					
Dermal	Exposure	Epidermis, dermis, and hair follicles	(Han, et al., 2008); (Maynard, et al., 2004); (Tinkle, et al., 2003); (Tsuji, et al., 2006)					
		Gloves	(Maynard, et al., 2004)					
	Effects	Concern for accumulation in skin	(Tsuji, et al., 2006)					
		Potential to penetrate epidermal and dermal layers	(Tinkle, et al., 2003)					
		Cell viability decreased 50%	(Patlolla, Knighten, & Tchounwou, 2010)					
		Cell apoptosis	(Bottini, et al., 2006); (Patlolla, Knighten, & Tchounwou, 2010)					
		No irritation from patch test	(Huczko & Lange, 2001)					
		Increased likelihood of Fiber Dermatitis	(Shvedova, et al., 2003)					
		Increased likelihood of Hyperkeratosis	(Shvedova, et al., 2003)					
		Increased likelihood of Naevi	(Shvedova, et al., 2003)					
		Oxidative stress	(Shedova, et al., 2003)					

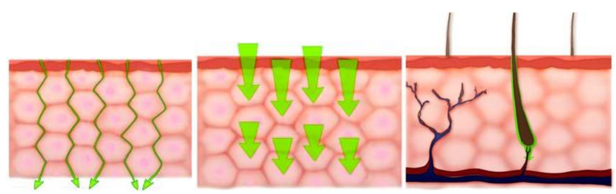
		Cellular toxicity	(Shvedova, et al., 2003)					
Ingestion	Exposure	Touching mouth or consumption after exposure	(Han, et al., 2008)					
	Effects	Demonstrated DNA damage	(Cicchetti, Divizia, Valentini, & Argentin, 2011)					
		Significant decline in cell proliferation	(Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005)					
		Decreased cell survival	(Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005)					
		Some apoptosis	(Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005)					
		No change in body weight	(Kolosnjaj-Tabi, et al., 2010)					
		No abnormalities	(Kolosnjaj-Tabi, et al., 2010)					
		Inflammation in peritoneum	(Poland, et al., 2008)					
		Increased concentration of metals	(Lam, James, McCluskey, & Hunter, 2004)					
		Highest dose in mice caused death in 5 out of 9 mice (possibly from nickel exposure	(Lam, James, McCluskey, & Hunter, 2004)					
		Highest dose lost weight	(Lam, James, McCluskey, & Hunter, 2004)					
		Acute toxicity	(Lam, James, McCluskey, & Hunter, 2004)					
		CNTs with iron elicited inflammation after 90 days	(Lam, James, McCluskey, & Hunter, 2004)					
		Decrease cellular adhesion	(Cui, Tian, Ozkan, Wang, & Gao, 2005)					
		Induced oxidative stress	(Cicchetti, Divizia, Valentini, & Argentin, 2011)					
		No death in mice	(Kolosnjaj-Tabi, et al., 2010)					
Intravenous	Exposure	Injection of medicine with CNT content	(Cherukuri, et al., 2006); (Deng, et al., 2007) (Kolosnjaj-Tabi, et al., 2010) (Lacerda, et al., 2008); (Lacerda, et al., 2008); (Schipper, et al., 2008); (Singh R. , et al., 2005); (Yang, et al., 2007); (Yang, et al., 2008); (Zhuang, et al., 2007)					
	Effects	Formed fiber-like structures (similar to asbestos)	(Kolosnjaj-Tabi, et al., 2010)					
		Higher doses induced granuloma formation	(Kolosnjaj-Tabi, et al., 2010)					
		No adverse health effects (mice)	(Cherukuri, et al., 2006); (Schipper, et al., 2008); (Singh R. , et al., 2005); (Yang, et al., 2007); (Yang, et al., 2008); (Zhuang, et al., 2007)					
		Persistence within liver and spleen for 4 months without toxicity	(Schipper, et al., 2008)					
		Persistence within organs up to 2 months	(Zhuang, et al., 2007)					
		CNTs only in liver after 24 hours	(Cherukuri, et al., 2006)					
		Liver accumulation	(Lacerda, et al., 2008); (Yang, et al., 2007)					
		No apoptosis	(Yang, et al., 2008)					
		Accumulation and persistence in liver, lung, and spleen for 3 months	(Yang, et al., 2008)					
		CNTs excreted through urine	(Deng, et al., 2007); (Lacerda, et al., 2008); (Lacerda, et al., 2008); (Singh R. , et al., 2005); (Yang, et al., 2008)					
		Low toxicity	(Deng, et al., 2007)					

ii. Dermal

Human skin is considered the largest organ, accounting for more than 10 percent of body mass (CDC, 2012). Thus, concern for dermal exposure of carbon nanotubes (CNTs) is important because it can occur during all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental as seen in **FIGURE VII** and **TABLE III**. Dermal absorption occurs when an agent is transported from the outer surface of the skin and enters into the skin and into the body (CDC, 2012). Dermal absorption often occurs without workers' knowledge (CDC, 2012).

IMAGE IV: Dermal Absorption

Images from left to right are as follows: Intercellular Lipid Pathway, Transcellular Permeation, and Through the Appendages (Hair Follicles, Glands) (CDC, 2012).



There are three ways that dermal absorption can occur: intercellular lipid pathway, transcellular permeation, and through appendages (CDC, 2012). All three exposure routes can be seen in **IMAGE IV**. The intercellular lipid pathway occurs when agents penetrate between cells where fats, oils, or waxes (known as lipids) exist (CDC, 2012). The transcellular permeation occurs when agents diffuse directly into the cells (CDC, 2012). The final route is through the appendages (hair follicles, glands, which are often the least significant because the surface area of the appendages is small, compared to the total area of skin (CDC, 2012).

The epidermis, dermis, and hair follicles all pose potential routes of exposure (Tsuji, et al., 2006). Due to their small nature, CNTs have the potential to penetrate the epidermal and dermal layers of the skin (Tinkle, et al., 2003). In order to best understand the exposure levels and limits, the skin needs to be analyzed before and after exposure (Tsuji, et al., 2006).

Deposits from handling single-walled carbon nanotubes (CNTs) on gloves were found to exist between 0.2 and 6 mg per hand (Maynard, et al., 2004). This indicates that deposits on gloves have the propensity to become airborne, may lead to additional negative health effects on dermal exposure or unprotected regions (Maynard, et al., 2004). Handling CNTs have shown to release particles into the work-place "such as CNT preparation, opening the CNT spray cover, wafer heating, opening the water bath, and ultrasonic dispersion" (Lee, et al., 2010).

iii. Ingestion

Concern for exposure through ingestion arises when an individual who has been handling or in the vicinity of carbon nanotubes (CNTs) either touches his or her mouth after exposure or consumes something that has been exposed to CNTs (Han, et al., 2008). Ingestion can also occur when a mucociliary clearance occurs after the inhalation of carbon nanotubes (Aschberger, et al., 2010). This indicates that while inhalation exposure occurs, there is a potential for ingestion exposure. Thus, concern for ingestion exposure of carbon nanotubes (CNTs) is important because it can occur during all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental as seen in **FIGURE VII** and **TABLE III**.

iv. Intravenous

Exposure via intravenous usually occurs when medications or fluids are transferred via a needle or tube into a vein which allows for immediate access to the blood supply (MedlinePlus, 2013). Thus, intravenous exposure of carbon nanotubes (CNTs) can occur during the Pharmaceutical Use stage as seen in **FIGURE VII** and **TABLE III**. Accidental exposure could occur during the Accidental Release/ Disposal stage, but that is outside the scope of this paper. Some research studies reviewed in this paper have used intravenous injections as a delivery mechanism for medicines (Kolosnjaj-Tabi, et al., 2010) (Deng, et al., 2007) (Cherukuri, et al., 2006) (Zhuang, et al., 2007) (Yang, et al., 2008) (Schipper, et al., 2008) (Yang, et al., 2007) (Lacerda, et al., 2008) (Lacerda, et al., 2008) (Singh R. , et al., 2005). These studies indicate that intravenous testing on animals has already exists and that when pharmaceuticals move into clinical trials, they will introduce the intravenous exposure route to possible CNT exposure routes. An example of a company moving into clinical trials is Cyto Wave Technologies Inc. is set to begin clinical trials in 2013 (Friedman, 2013). Gold-plated carbon nanotubes with a biological coating will be intravenously injected into patients in order to target cancer cells.

B. Health Effects

i. Inhalation

As shown in **FIGURE VII**, inhalation health effects of carbon nanotubes (CNTs) are applicable to all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental. The questions that need to be understood include: can carbon nanotubes be cleared from the lungs and what is their state; what are long-term tissue responses to persisting nanotubes; and how does their shape affect toxicity; and do metal impurities for nickel and iron affect toxicity (Tsuji, et al., 2006). The health effects discussed below are summarized in **TABLE III**. Catalyst metals are used in CNTs and studies conducted on rats indicate that there is an increased risk of cancers in the nose region and modeling indicates that similar results would occur in humans (Feron, Arts, Kuper, Slootweg, & Woutersen, 2002). These metals can be released during the production stage when catalyst preparation in chemical vapor deposition occurs (Lee, et al., 2010).

A study conducted on mice found that after seven and ninety days that carbon nanotubes inhaled into the lungs may elicit an inflammatory, granulomatous[5], and fibrogenic[6] response (Lam, James, McCluskey, & Hunter, 2004). A mouse study found that after a single inhalation exposure[7] of six hours, multi-walled carbon nanotubes (MWCNT) were found in the subpleural wall and within the subpleural macrophages leading to subpleural fibrosis (Ryman-Rasmussen, et al., 2009). But when the dose was lowered[8] , no effects were seen (Ryman-Rasmussen, et al., 2009). Subpleural fibrosis in mice indicates that MWCNT's may behave like asbestos, posing a potential health risk (Ryman-Rasmussen, et al., 2009). Ultrafine nanoparticles can cause lung toxicity when compared to larger particles of similar chemical composition based on studies conducted on mice and rats (Oberdorster, Oberdorster, & Oberdorster, 2005). Donaldson argues that carbon nanotubes have properties such as a needle-like appearance similar to asbestos (Donaldson, et al., 2006). Based on historical evidence, the graphitic nature of CNTs has been compared to asbestos exposure, in which graphite-containing dusts accumulate in lungs (Green

[5] Granulomatous is defined as relating to or characterized by granuloma. Granuloma is defined as a mass or nodule of chronically inflamed tissue with granulations that is usually associated with an ineffective process (NIH, Granuloma, 2013)

[6] Fibrogenic is defined as relating to fibrosis. Fibrosis is defined as any pathological condition where fibrous connective tissue invades any organ, usually as a consequence of inflammation or other injury (NIH, Fibrosis, 2013)

[7] Exposure of 30 mg m^{-3} (Ryman-Rasmussen, et al., 2009)

[8] Exposure of 1 mg m^{-3} (Ryman-Rasmussen, et al., 2009)

& Vallyathan, 1998). This research indicates that minimizing inhalation of MWCNTs and use of protective masks and clothing are important until further testing is completed (Ryman-Rasmussen, et al., 2009).

ii. Dermal

As shown in **FIGURE VII**, dermal health effects of carbon nanotubes (CNTs) are applicable to all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental. The health effects discussed below are summarized in **TABLE III**. Current concerns for dermal penetration include: skin or other organ cytotoxicity; accumulation in skin or other organs resulting in toxicity after long-term exposure; and metabolism may produce even smaller particles with potentially increased toxicity (Tsuji, et al., 2006). A study on mice found that nanoparticles could penetrate the epidermal and dermal layers of the skin through an *in vitro* flexed skin model and it demonstrated that it could penetrate human skin and "reach the epidermis and, occasionally, the dermis" (Tinkle, et al., 2003). More information and studies need to be conducted to discriminate between the epidermis, dermis, and hair follicles (Tsuji, et al., 2006).

A study produced by the National Institute of Health (NIH) that utilized *in vitro* normal human dermal fibroblast cells (NHDF) found that there was a dose-dependency decrease in cell viability with a 50% cell viability reduction occurred at the highest dose[9] of multi-walled carbon nanotubes (MWCNT) (Patlolla, Knighten, & Tchounwou, 2010). Early apoptosis[10] existed

[9] Dose existed at 400 µg/ml (Patlolla, Knighten, & Tchounwou, 2010)
[10] Apoptosis is defined as cell death.

when MWCNT were introduced to the dermal cells and a dose-dependency increase occurred (Patlolla, Knighten, & Tchounwou, 2010).

A patch test that exposed forty human participants to carbon nanotubes (CNTs) for ninety-six hours found no irritation (Huczko & Lange, 2001). Another test exposed four rabbits to CNTs for seventy-two hours and found no irritation (Huczko & Lange, 2001). The researchers in this test concluded that "no special precautions have to be taken while handling these carbon nanostructures" (Huczko & Lange, 2001); however another study showed exposure from carbon nanotubes has been associated with increased incidence of carbon fiber dermatitis, hyperkeratosis, and naevi in human keratinocyte cells (Shedova, et al., 2003). After eighteen hours of exposure to single-walled carbon nanotubes (SWCNTs), oxidative stress and cellular toxicity occurred (Shedova, et al., 2003).

iii. Ingestion

As shown in **FIGURE VII**, ingestion health effects of carbon nanotubes (CNTs) are applicable to all five stages: Extraction, Production, Pharmaceutical Use, Accidental Release/ Disposal, and Excretion/ Environmental. The health effects discussed below are summarized in **TABLE III.** The oral effects have been studied through the application of carbon nanotubes (CNT) on human gingival fibroblasts[11] (Cicchetti, Divizia, Valentini, & Argentin, 2011). Exposure of 24 hours found that exposure demonstrated DNA damage, significant decline in cell proliferation[12], decreased cell survival[13], and in some cases apoptosis[14] (Cicchetti, Divizia, Valentini, &

[11] Gingival fibroblasts are the main portion of gingival connective tissue in the mouth which have immunologic receptors (Ozawa, et al., 2003)
[12] Doses at 75, 100, 125, and 150 µg/ml (Cicchetti, Divizia, Valentini, & Argentin, 2011).
[13] Doses at 100, 125, and 150 µg/ml (Cicchetti, Divizia, Valentini, & Argentin, 2011).

Argentin, 2011). Mice were exposed to three types of single-walled carbon nanotubes (SWCNTs) in which no change in body weight was found and no abnormalities were found (Kolosnjaj-Tabi, et al., 2010). Inflammation at the surface of the peritoneum[15] has been found in mice when injected into the abdominal cavity (Poland, et al., 2008). A study conducted on HEK293 Human cells found that single-walled carbon nanotubes (SWCNT) can inhibit cell growth, induce cell apoptosis[16], and decrease cellular adhesion ability (Cui, Tian, Ozkan, Wang, & Gao, 2005).

A study sponsored by NASA on mice used single-walled carbon nanotubes found increased concentration of metals in three types of single-walled carbon nanotubes (SWCNT) (Lam, James, McCluskey, & Hunter, 2004). Raw SWCNT's contained 27% iron, 1% molybdenum, 0.8% nickel, and 0.4% copper; purified SWCNT's contained 2% iron; and SWCNT's contained 26% nickel, 5% yttrium, and 0.5% iron after 90 days of instillation (Lam, James, McCluskey, & Hunter, 2004). Since this test did not utilize natural inhalation delivery, its unnatural method may have exaggerated the effects on the lungs; it could have missed effects on upper airways, effects on the delivery, along with any properties of the particles (Lam, James, McCluskey, & Hunter, 2004). This study found that the highest dose level of SWCNT's caused deaths in five out of nine mice, which may be a result of a nickel release from the SWCNT during sonication, which would "not likely occur by aerosol inhalation" (Lam, James, McCluskey, & Hunter, 2004). The highest dose level of SWCNT also found that the group lost weight, while the raw SWCNT group had transient signs of acute toxicity (Lam, James, McCluskey, & Hunter, 2004).

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[14] Doses at 125 and 150 µg/ml (Cicchetti, Divizia, Valentini, & Argentin, 2011).
[15] Peritoneum is defined as "the tissue that lines the abdominal wall and covers most of the organs in the abdomen (NIH, Peritoneum Definition, 2013)
[16] Apoptosis is defined as cell death.

Carbon nanotubes that contained iron elicited inflammation that was present after 90 days (Lam, James, McCluskey, & Hunter, 2004)

iv. Intravenous

As shown in **FIGURE VII**, intravenous health effects of carbon nanotubes (CNTs) are applicable to one stage: Pharmaceutical Use. The health effects discussed below are summarized in **TABLE III.** Even though it is the least common route of exposure, intravenous exposure is considered a concern in the pharmaceutical industry. Injection of pharmaceuticals with carbon nanotubes is the main mode of exposure. Even though it is less likely to occur, in the medical industry it is possible to obtain an unintentional needle prick.

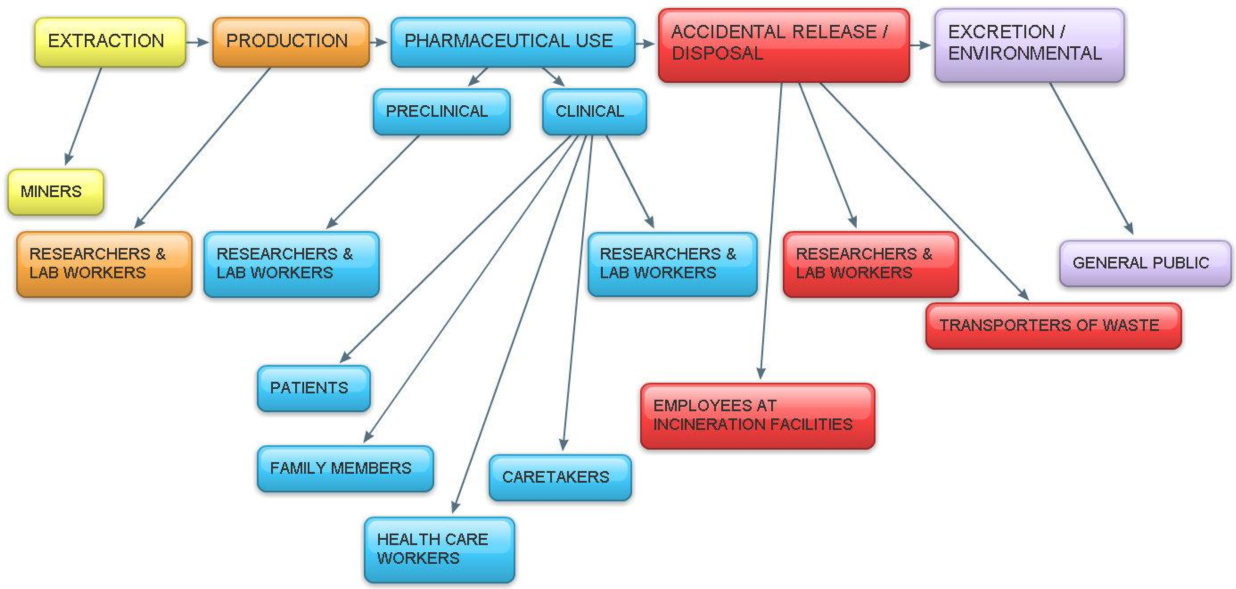
A study on mice has found that after intravenous injection, the carbon nanotubes (CNTs) formed fiber-like structures similar to asbestos fibers (Kolosnjaj-Tabi, et al., 2010). The study also found that at higher doses, granuloma formation was induced (Kolosnjaj-Tabi, et al., 2010). One study using mice found low toxicity of CNTs after intravenous injection (Deng, et al., 2007). Four studies found persistence of CNTs within the liver after 24 hours in rabbits and mice (Cherukuri, et al., 2006), within the organs up to two months in mice (Zhuang, et al., 2007), within the liver, lung, and spleen for three months in mice (Yang, et al., 2008), and within the liver and spleen for four months in mice (Schipper, et al., 2008). Along with persistence of CNTs in the liver, there was found to be accumulation of CNTs in mice (Lacerda, et al., 2008) (Yang, et al., 2007). A study found no cell apoptosis (cell death) (Yang, et al., 2007) and multiple studies have concluded that there are no adverse health effects from intravenous injection of CNTs in rabbits and mice (Cherukuri, et al., 2006) (Schipper, et al., 2008) (Singh R. , et al., 2005) (Yang, et al., 2007) (Yang, et al., 2008) (Zhuang, et al., 2007). Multiple studies have also indicated that CNTs are excreted through urine in mice, indicating that CNTs most likely would pass through human urine (Deng, et al., 2007) (Lacerda, et al., 2008) (Lacerda, et al., 2008) (Singh R. , et al., 2005) (Yang, et al., 2008).

C. Five Stages of the Life Cycle Risk Assessment

i. Extraction Stage

The first of the five phases is "extraction," as shown in yellow in **FIGURE VII** and **FIGURE VIII**. This phase has a single step which in the influence diagram is titled "Raw Materials & Extraction." During the extraction phase of the Life Cycle Risk Assessment, there are no obvious risks, as carbon nanotubes (CNTs) do not exist in nature. However indirect exposure and health effects do occur from mining and transportation of metals and graphite that are used in CNT formation. Components that must be extracted from the earth that are often vital components of CNTs include iron, cobalt, yttrium, and nickel. The health effects from the components of CNTs are shown in **TABLE III**. Graphite is one of the most common types of carbon and is most frequently used in the production of CNTs. Mining graphite can cause carboconioses and dust bronchitis which can lead to the development of lung carcinoma in humans (NIH, 2012). Mixed dust pneumoconiosis is caused by long-term occupational exposure to graphite dust and is considered a rare disease (Domeh, et al., 2002) (Mazzucchelli, Radelfinger, & Kraft, 1996). Radiographic lesions were found in graphite miners in 4.1% of workers in 1993 with 18 cases of graphite pneumoconiosis and seven cases of pulmonary tuberculosis (Uragoda, 1997). These studies all indicate that the route of exposure can be either inhalation, dermal, or ingestion, although inhalation is the most common and frequent route of exposure. Based on the studies reviewed, the group that has the highest likelihood of exposure would be those in the mining industry (**FIGURE IX**).

FIGURE IX: Groups Most Impacted by Exposure



ii. Production Stage

The second of the five stages is “production,” as highlighted in orange in **FIGURE VII** and **FIGURE VIII**. This phase has two steps which are shown in the influence diagram. The first step is “Production of CNTs.” In this step, the carbon nanotubes (CNTs) are created using raw materials. The second step is the “Production of Pharmaceutical Products.” In this step the CNTs are added to substances or devices in the biomedical industry.

Based on their desired needs, highly specialized laboratories and facilities utilize raw materials and manipulate them in a way that will showcase CNTs abilities. Several methods are used in the creation and synthesis of carbon nanotubes.

The first method is the arc-evaporation method involving two graphite electrodes with a current between them in an atmosphere of helium with metal catalysts (Co or Ni) (Awasthi, Srivastava, & Srivastava, 2005).

The second method is the chemical vapor deposition (CVD) technique that deposits materials using the chemical reaction of vapor on the surfaces of the sample (UC, 2010). This method is the most popular method to use because it allows for the greatest scalability and has the least residual metal catalysts involved (Awasthi, Srivastava, & Srivastava, 2005). Chemical vapor deposition can be produced either through the HiPco or CoMoCAT processes (Awasthi, Srivastava, & Srivastava, 2005). The HiPco process uses a gas-phase homogeneous process that forms CNTs through the disproportionate use of carbon monoxide over catalytic iron nanoparticles (Awasthi, Srivastava, & Srivastava, 2005). The CoMoCAT technology is a heterogeneous process that creates CNTs through catalytic decomposition (Awasthi, Srivastava, & Srivastava, 2005). This will create silica, residual cobalt, molybdenum particles, and amorphous carbon that are then removed in order to access the carbon nanotube (Awasthi, Srivastava, & Srivastava, 2005). This removal occurs using “silica leaching, froth flotation, acid treatment, and filtration purification processes (Awasthi, Srivastava, & Srivastava, 2005).”

The third form of creating CNTs is through laser ablation (Awasthi, Srivastava, & Srivastava, 2005). This method uses a laser to vaporize cobalt and nickel (Awasthi, Srivastava, & Srivastava, 2005). Based on the studies reviewed, the group that has the highest likelihood of exposure would be researchers and lab workers (**FIGURE IX**).

As outlined in **FIGURE VIII** and **TABLE III**, the production stage has three main exposure routes, which are inhalation, dermal, and ingestion. Employees working in a manufacturing facility that specializes in carbon nanotube (CNT) production or research should be aware of safety precautions and exposure limits. During chemical vapor deposition (CVD) production, exposure can occur when the chamber is opened for catalyst preparation[17], or during synthesis (Han, et al., 2008). In order to reduce exposure to workers, it is recommended that production facilities and labs use the CVD technique because the CNTs are in a vacuum, operated environment (UC, 2010).

iii. Pharmaceutical Use Stage

The third of the five stages is "Pharmaceutical Use," as shown in blue in **FIGURE VII** and **FIGURE VIII**. In the influence diagram shown in **FIGURE VIII**, this stage is identified as "Pharmaceutical Use."

Carbon nanotubes (CNTs) are used in cancer treatment because they can be attached to chemotherapy drugs which are then targeted to tumors (Liu, Tabakman, Welsher, & Dai, 2009). Two types of drug delivery systems have been used in testing. Passive targeting that is based on the enhanced permeability and retention (EPR) effect[18] of cancerous tumors (Liu, Tabakman, Welsher, & Dai, 2009). Due to cancerous tumors exhibiting poor lymphatic drainage and fenestrated vasculature, there are large gaps between adjacent endothelial cells which allows for

[17] 20 to 30 nm-sized particles
[18] Enhanced permeability and retention (EPR) effect is defined as, "The accumulation of particulate substances in angiogenic tumors due to enhanced permeability of particles across leaky tumor endothelium and retention of the particles inside the tumor due to its defective lymphatic drainage system" or as a passive targeting technique that "targets cancer cells" (Ontology, 2013).

passive targeting of drugs to the tumor site (Vlerken, Vyas, & Amiji, 2007). The poor lymphatic drainage leads to enhanced retention of the drugs which make for a more effective delivery mechanism (Vlerken, Vyas, & Amiji, 2007). The second delivery mechanism is active targeting which utilizes tumor targeting ligands (Liu, Tabakman, Welsher, & Dai, 2009).

If multi-walled carbon nanotubes (MWCNTs) are used as a drug carrier, it is recommended that measures be taken to reduce clearance of MWCNTs by the reticuloendothelial system (RES) (Deng, et al., 2007). Organs other than the lung need to block the RES by altering the surface of the MWCNTs (Deng, et al., 2007). Applying coatings of “hydrophilic molecules, polymers, surfactants, etc. can create a cloud of chains at the particle surface, will repel plasma proteins and hence evade the RES” (Deng, et al., 2007). Human subjects research (HSR) poses risks to groups other than just the subjects themselves (Ramachandran, Howard, Maynard, & Philbert, 2012). These “exposures can occur during the handling and administering of the pharmaceutical by the health care workers or the family members (if they are involved in drug administration)” or to researchers and lab workers, health care workers, family members, and caretakers (all of which would be labeled as “third-party” exposures (Ramachandran, Howard, Maynard, & Philbert, 2012). Based on the studies reviewed, pharmaceutical use stage should be analyzed in two groups: preclinical and clinical. The group that has the highest exposure in the preclinical would be researchers and lab workers. For the clinical stage, multiple groups could be at risk for exposure. Besides patients, the “third-party” exposures indicated by Ramachandran, Howard, Maynard and Philbert identify the following groups may be at risk for exposure during the clinical portion of the pharmaceutical use stage: family members, health care workers, caretakers, researchers, and lab workers (**FIGURE IX**).

iv. Accidental Release / Disposal Stage

The fourth stage is "Accidental Release / Disposal," as shown in red in **FIGURE VII** and **FIGURE VIII**. This stage has three components in the influence diagram. "Medical Waste Disposal" is included in this stage because containers holding medication with carbon nanotubes (CNTs) generally contain remnants of the medicine and must therefore be disposed of properly. Since these containers have the potential to break and release these remnants into the environment and impact individuals in the area, "Accidental Spill/ Release of CNTs" is the second component of this stage. While these amounts may be small, they still must be accounted for in the life cycle. The third component, "Incineration" is important because medical waste must be incinerated in order to destroy components of the medicine. Incineration of medical waste with CNTs present would most likely destroy them, the "uncontrolled incineration in open fires that occur frequently in developing countries will lead to emissions of CNTs as it is an incomplete combustion process" (Ramachandran, 2013). There is a potential for further accidental release of CNTs while it is being transported during the destruction process. A study conducted by the United States Geological Survey in 2008, mixed industrial waste varied with four concentrations[19] of multi-walled carbon nanotubes (MWCNTs) in distilled water (USGS, 2008). It found that MWCNTs could pose toxicity risk in a biological wastewater treatment facility with increasing toxicity when MWCNT concentration was increased (USGS, 2008). The study further concluded that current waste removal systems would not be able to destroy or eliminate CNTs from wastewater (USGS, 2008).

As outlined in **FIGURE VIII** and **TABLE III**, the "Accidental Release / Disposal" stage has three main exposure routes, which are inhalation, dermal, and ingestion. Individuals that work in

[19] 0.6, 1.44, 2.16, and 3.24 g/L

laboratories, production plants, health care, and in waste removal or abatement services may be more prone to exposure during this stage. Cleaning supplies that are used around carbon nanotube use or research are discarded directly into the hazardous waste stream (Helland, Wick, Koehler, Schmid, & Som, 2007). In order to address exposure during this stage facilities such as the University of California have set standard operating procedures specifically formed for carbon nanotubes (CNTs). Carbon nanotubes must be disposed of in a clearly labeled container and is treated as hazardous waste through California Environmental Health and Safety Guidelines (UC, 2010). Many company and university safety standards require that production chambers be cleaned with solvents or water with tissues, brushes, and sponges, all of which tend to be discarded (Helland, Wick, Koehler, Schmid, & Som, 2007). Based on the studies reviewed, the groups that have the highest likelihood of exposure would be researchers, lab workers, employees at incineration facilities, and employees involved in transporting waste that contains carbon nanotubes (**FIGURE IX**).

v. Excretion / Environmental Stage

The fifth stage is "Excretion/ Environmental," as shown in purple in **FIGURE VII** and **FIGURE VIII**. In the influence diagram shown in **FIGURE VIII**, this stage is identified by five components.

Once medicine with carbon nanotubes (CNTs) has been disbursed to patients, the first component occurs: "Excretion of CNTs." Studies have indicated that CNTs are excreted through urine (Deng, et al., 2007) (Lacerda, et al., 2008) (Singh R. , et al., 2005) (Yang, et al., 2008). Once the CNTs have been excreted through urine, they enter the environment during the component "Deposition of CNTs in Environment." After they have been deposited in the environment, the next component is "Bioaccumulation of CNTs in the Environment." A study conducted on earthworm populations grown in multi-walled carbon nanotubes-contaminated (MWCNTs) soil found that the bioaccumulation factor for was low at 0.015 (Li, Irin, Atore, Green, & Canas-Carrell, 2013). To determine if these earthworms could readily remove the MWCNTs from their system, they were transferred to uncontaminated MWCNT soil (Li, Irin, Atore, Green, & Canas-Carrell, 2013). They found that from their transfer from MWCNT-contaminated soil to uncontaminated soil, their concentration decreased significantly[20] five days after transfer (Li, Irin, Atore, Green, & Canas-Carrell, 2013). When carbon nanotubes (CNTs) are in pristine condition, they are completely insoluble in water (Lam, James, McCluskey, & Hunter, 2004). Another study conducted on sediment and/or food matrices to determine the bioaccumulation and bioavailability in marine environments using single-walled carbon nanotubes (SWCNTs) found that after 28 days of exposure, there was no indication of any bioaccumulation or toxicity (Parks, et al., 2013). While two other studies found that the degradation of CNTs is considered low, posing a concern for biopersistence and bioavailability (Warheit, et al., 2004) (Kohler, Som, Helland, & Gottschalk, 2008). Some manipulations of the surface on CNTs can increase hydrophilicity and improve dispersion in some liquids (Fubini, 1997). They are lipophilic, which means they can dissolve in fats, oils, lipids, and non-polar solvents (Wu, et al., 2006). Due to their insolubility and lipophilicity, CNTs have the potential to bioaccumulate in the environment. They are also expected to be biologically biopersistent due to their graphitic nature (Donaldson, et al., 2006). If CNTs can bioaccumulate in the environment, then there is a potential for human exposure during the component "Bioaccumulation of CNTs in Humans." If they bioaccumulate in humans, then there is potential for the last component "Excretion of CNTs," creating a feedback loop for this stage.

[20] 85 ± 13 μg/g to 21 ± 6 μg/g (Li, Irin, Atore, Green, & Canas-Carrell, 2013)

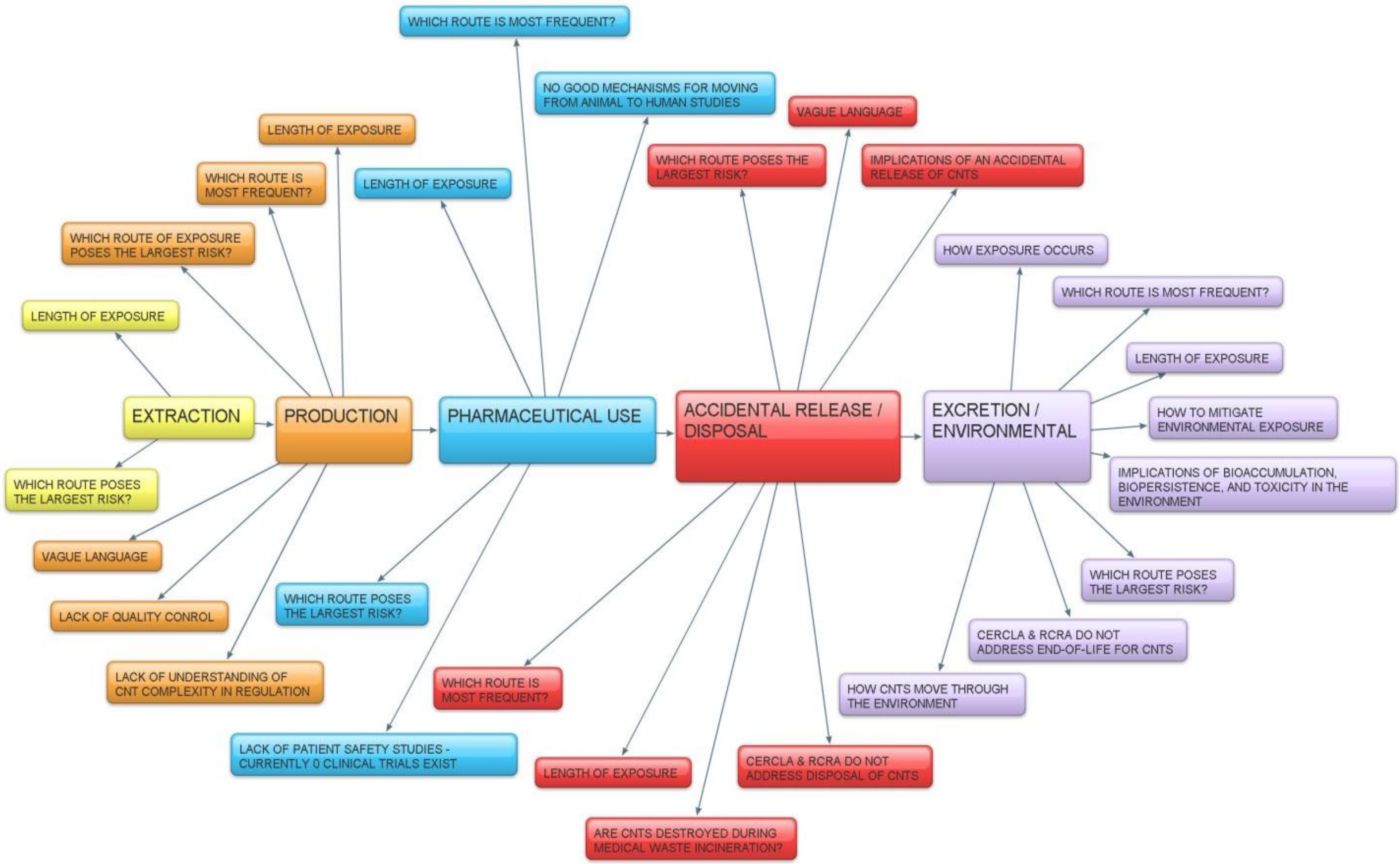
The information available for exposure of CNTs after they have been used in the pharmaceutical industry is next to none. Due to this lack of information, it is assumed that the general public would be the main group at risk (**FIGURE IX**).

V. Discussion

The information presented in this Life Cycle Risk Assessment has aimed to study carbon nanotubes (CNTs) throughout their entire life cycle. The physiochemical properties of CNTs were presented in order to better understand how they behave and to highlight qualities that are desired by researchers and producers of CNTs. Existing pharmaceutical uses were discussed in order to provide context for how CNTs are used in a medical setting. Cancer treatments were specifically highlighted as this area of research has seen numerous types of treatments and applications. The policy context was included in order to address how nanotechnology, specifically CNTs are regulated or not regulated in the United States. A Life Cycle Risk Assessment was then applied to CNTs to better understand the health effects and exposure routes of CNTs in the pharmaceutical industry.

Great attempts to better understand carbon nanotubes (CNTs) both scientifically and regulatory have been made. However, in spite of extensive research from the last twenty years, there are still areas that have shown gaps in knowledge of how to evaluate, produce, regulate, and distribute CNTs. The gaps that will be discussed are shown in **FIGURE X**.

FIGURE X: Carbon Nanotube Life Cycle Risk Assessment Gaps



A. Gaps in the Extraction Stage

The information presented in **TABLE III** indicates that there still is room for further research. There were no studies that were reviewed that stated that carbon nanotube (CNT) exposure and health effects were experienced during the "extraction" phase. Studies that were reviewed identified health exposure caused by mining and extraction of minerals and graphite. For the extraction stage, the routes of exposure that are possible are inhalation, dermal, and ingestion. Based on a literature review, miners have the highest exposure (**FIGURE IX**), most frequently via inhalation exposure (**TABLE III**). Further information should identify the threshold for length of exposure as well as which route of exposure poses the largest risk (**FIGURE X** and **TABLE IV**).

TABLE IV: Extraction Summary

Summary	
Studies Reviewed	(Domeh, et al., 2002); (Mazzucchelli, Radelfinger, & Kraft, 1996); (NIH, Graphite, 2012); (Uragoda, 1997)
Gaps in Literature	Length of Exposure
	Which Route of Exposure Poses the Largest Risk?

B. Gaps in the Production Stage

The information presented in **TABLE III** indicates room for further research. While the human health routes of exposure can be identified, there is a lack of research on how these routes of exposure occur during production and how they may be mitigated. Based on this review, only a few studies were found that identified the routes of exposure. A deeper understanding of how these routes of exposure can occur will provide researchers and producers of CNTs a clearer understanding of how to properly handle these materials. Researchers and lab workers have been identified as the group that has the highest likelihood of exposure (**FIGURE IX**) (Ramachandran, Howard, Maynard, & Philbert, 2012) via inhalation exposure (**TABLE III**).

Information gaps that have been identified are shown in **FIGURE X** and **TABLE V**. The literature reviewed does not identify which route of exposure could be most frequent or which poses the largest risk. But based on the literature reviewed in this article, I have ranked the likely route of exposure based on likelihood. In order, they are inhalation, dermal, and then ingestion. I excluded intravenous (IV) exposure due to its non-concern for the production stage because workers would not yet be exposed to IV needles.

The literature does not identify the length of exposure that is appropriate, if any. Some routes may need a larger amount of exposure or longer time of exposure to pose the same amount of risk. This analysis found one study that claimed that ingestion was a route of exposure (see **TABLE III**).

TABLE V: Production Summary

Summary	
Studies Reviewed	(Bottini, et al., 2006); (Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005); (Donaldson, et al., 2006); (Feron, Arts, Kuper, Slootweg, & Woutersen, 2002); (Green & Vallyathan, 1998); (Han, et al., 2008); (Huczko & Lange, 2001); (Kohler, Som, Helland, & Gottschalk, 2008); (Lam, James, McCluskey, & Hunter, 2004); (Lee, et al., 2010); (Maynard, et al., 2004); (Oberdorster, Oberdorster, & Oberdorster, 2005); (Patlolla, Knighten, & Tchounwou, 2010); (Poland, et al., 2008); (Shvedova, et al., 2003); (Tinkle, et al., 2003); (Tsuji, et al., 2006)
Gaps in Literature	Which Route Poses the Largest Risk?
	Which Route is Most Frequent?
	Length of Exposure
	Vague Language
	Lack of Quality Control
	Lack of Understanding CNT Complexity in Regulation

Although the likelihood of it occurring would be fairly low, a worker could be exposed if he or she touched an affected area on the worker's protective suit and then touch his or her mouth if they were not wearing gloves. In order to address these issues, there is a need for further research on these gaps in the literature.

The studies reviewed for health effects that occur during production indicate varying levels of health effects. Some of the studies presented use words such as “may” or “some” which indicate that there is not definitive evidence to say that the CNTs used in the studies conclusively cause a health effect. This indicates that further research is needed in order to determine whether the “may” means “yes, this causes …” or “no, this does not cause….” In regards to “some,” it would be useful to have further research conducted to see if the “some” is a normal occurrence. A greater understanding of the number and intensity of effects on health through additional research and studies will better define the implications of the CNT production stage.

Information presented in this study has shown that the gaps that exist in pharmaceutical manufacturing fall more on protections than from regulations from federal agencies. Many research and industry specialists have practices that meet or exceed current standards in order to reduce exposure and minimize health effects. One of the largest gaps in pharmaceutical manufacturing is producing carbon nanotubes (CNTs) that are the same every single time (Lacerda, Bianco, Prato, & Kostarelos, 2006) as seen in **TABLE IX**. In the production process, it can be difficult to produce the exact same carbon nanotube every single time, so high quality control and reducing impurities is a major challenge (Lacerda, Bianco, Prato, & Kostarelos, 2006). The complexities in CNT production can result in varying forms of production that can result in varying levels of exposure and health effects. Since CNTs can vary vastly, it is important that policies and regulations that address manufacturing address the complexities of CNTs. Research identifying health effects and exposure sometimes has vague language when describing the implications. Either the researchers are using a precautious approach or they implications may be unpredictable or vary.

C. Gaps in the Pharmaceutical Use Stage

The information presented in **TABLE III** indicates there is room for further research. All four routes of exposure (inhalation, dermal, ingestion, and intravenous) are possible during the "Pharmaceutical Use" stage. Based on this review, there is evidence for varying levels of concern for human health effects. In the preclinical trial portion of this stage, the researchers and lab workers have the highest potential for exposure (**FIGURE IX**) via inhalation exposure (**TABLE III**). In the clinical trials, "third-party" exposure can occur to family members, health care workers, caretakers, researchers and lab workers (**FIGURE IX**) (Ramachandran, Howard, Maynard, & Philbert, 2012). Conclusions on the gaps in this stage are shown in **FIGURE X** and **TABLE VI**. In order for researchers to move forward on pharmaceutical use, there will need to be further research on which carbon nanotubes (CNTs) pose different level of risks.

TABLE VI: Use in the Biomedical Industry Summary

Summary	
Studies Reviewed	(Bottini, et al., 2006); (Cherukuri, et al., 2006); (Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005); (Deng, et al., 2007); (Donaldson, et al., 2006); (Feron, Arts, Kuper, Slootweg, & Woutersen, 2002); (Han, et al., 2008); (Huczko & Lange, 2001); (Kohler, Som, Helland, & Gottschalk, 2008); (Kolosnjaj-Tabi, et al., 2010); (Lacerda, et al., 2008); (Lam, James, McCluskey, & Hunter, 2004); (Lee, et al., 2010); (Maynard, et al., 2004); (Oberdorster, Oberdorster, & Oberdorster, 2005); (Patlolla, Knighten, & Tchounwou, 2010); (Poland, et al., 2008); (Schipper, et al., 2008); (Shvedova, et al., 2003); (Singh R. , et al., 2005); (Tinkle, et al., 2003); (Tsuji, et al., 2006); (Yang, et al., 2008); (Yang, et al., 2007); (Zhuang, et al., 2007)
Gaps in Literature	Which Route is Most Frequent?
	Which Route Poses the Largest Risk?
	Length of Exposure
	Lack of Patient Safety Studies, Currently 0 Clinical Trials Exist
	No Good Mechanisms for Moving from Animal to Human Studies

Existing health effects studies found that if carbon nanotubes (CNTs) are inhaled in mice that they may elicit inflammatory, granulomatous, and fibrogenic responses (Lam, James, McCluskey, & Hunter, 2004) and that inhalation exposure on mice found that multi-walled carbon nanotubes were found in the subpleural wall and within the subpleural macrophages leading to subpleural fibrosis (Ryman-Rasmussen, et al., 2009). Catalyst metals and graphite used in CNT production have raised concerns for asbestos-like size and shape has raised concerns for potential pathogenicity and lung toxicity (Donaldson, et al., 2006) (Green & Vallyathan, 1998) (Oberdorster, Oberdorster, & Oberdorster, 2005) (Tsuji, et al., 2006).

Dermal exposure in mice found nanoparticles can penetrate epidermal and dermal layers of skin (Tinkle, et al., 2003) (Tsuji, et al., 2006) and that apoptosis existed on a dose-dependent increase (Patlolla, Knighten, & Tchounwou, 2010). One study on mice found increased incidence of carbon fiber dermatitis, hyperkeratosis, naevi, oxidative stress and cellular toxicity occurred (Shedova, et al., 2003), while another using mice found no irritation (Huczko & Lange, 2001).

Exposure via ingestion was found that DNA damage, significant decline in cell proliferation, decreased cell survival, and in some cases apoptosis occurred in a mice study (Cicchetti, Divizia, Valentini, & Argentin, 2011) while a study on human HEK293 cells found inhibited cell growth, induced cell apoptosis, and decreased cellular adhesion (Cui, Tian, Ozkan, Wang, & Gao, 2005). Another study found that no abnormalities or body weight change was shown in mice (Kolosnjaj-Tabi, et al., 2010). Inflammation in the peritoneum was found in mice (Poland, et al., 2008) and one study found five out of nine mice deaths at the highest dose level which may have been caused by metal exposure (Lam, James, McCluskey, & Hunter, 2004).

Intravenous exposure is the most likely route for pharmaceutical use, which a study on mice found that intravenous injection caused carbon nanotubes (CNTs) to form fiber-like structures similar to asbestos and granuloma formation at its highest doses (Kolosnjaj-Tabi, et al., 2010). Four studies found a persistence of CNTS in mice and rabbits in organs (Lacerda, et al., 2008) (Schipper, et al., 2008) (Yang, et al., 2008) (Zhuang, et al., 2007). Multiple studies in rabbits and mice have found no adverse health effects in rabbits and mice (Cherukuri, et al., 2006) (Schipper, et al., 2008) (Singh R. , et al., 2005) (Yang, et al., 2007) (Yang, et al., 2008) (Zhuang, et al., 2007). Finally, multiple studies have indicated that intravenous injections have resulted in urine excretion in mice indicating that CNTs are likely to pass through human urine (Deng, et al., 2007) (Lacerda, et al., 2008) (Lacerda, et al., 2008) (Singh R. , et al., 2005) (Yang, et al., 2008).

Based on these health effect conclusions, it is clear that there are varying types of potential health effects and these are important to note. The likelihood of exposure during this stage would be intravenous, ingestion, dermal, then inhalation. Intravenous is the most likely to occur because most drugs would be administered intravenously. These studies indicate that animal testing exists and as pharmaceuticals move into clinical trials. The company Cyto Wave Technologies Inc. is beginning clinical trials with gold-plated carbon nanotubes (CNTs) with a biological coating to be injected into patients in order to target cancer cells (Friedman, 2013). Discussions about clinical trials for CNTs in colorectal cancer treatment that use CNTs that are coated in B-vitamin folate so that the CNTs fit with folate receptors on cancer cells and the discussions are occurring between the Food and Drug Administration and the Center for Cancer Nanotechnology Excellence Focused on Therapy Response at Stanford University (NCI, 2013).

Medicines would then be most likely to be administered via ingestion. Based on this assumption, health effects and risk from CNT exposure are most important at this stage. The next highest physician and patient CNT exposure rate would be via dermal exposure while handling medication. If physicians wore gloves while administering the medication, the likelihood of exposure could be reduced. Finally inhalation is also a potential if the medicine becomes airborne. Although these conclusions are based on my literature review, research does not adequately identify which route of exposure is most likely. It also fails to identify which route of exposure may pose the greatest risk. The research is also needed to identify the length of exposure in which health effects become detrimental. Future research will need to address these issues and more.

Since most applications of carbon nanotubes (CNTs) have been in the pre-clinical trial phase, it is difficult to provide data that shows the health effects or indicates safety (**TABLE III)**. There is a gap in information about how animal studies translate into clinical settings with humans Similar to the gap in pharmaceutical manufacturing, there is also an issue with quality control and ensuring that every CNT will be identical and will behave identically (**TABLE IX**). Each new batch of CNTs may be different and quality control is an issue that is continually being addressed. In order to move forward, federal agencies are going to have to work with producers of CNTs in order to understand how their CNTs will behave and react in a human body. Most of these studies have conducted their research on mice and rats and are hoping to move into clinical trials once they pass through legal, regulatory, and funding hoops. Studies shown (**TABLE III)** indicate that functionalized CNTs may be safer to utilize in a clinical setting when compared to nonfunctionalized CNTs. Numerous studies have indicated that CNTs are passed via urine, which researchers believe is a good indicator that they could be used in clinical settings (Singh R. , et al., 2005).

Both single-walled carbon nanotubes and multi-walled carbon nanotubes are classified as fibrous, high aspect ratio nanoparticles (HARN) and their dimensions reflect similar structure as asbestos, which raises concerns (Shvedova, et al., 2009). Further research is needed to understand whether or not HARN, such as SWCNT and MWCNT actually behave like asbestos and whether or not these need to be classified likewise (Shvedova, et al., 2009).

Patient safety and drug approval is currently processed through the Food and Drug Administration (FDA) and must not conflict with any other regulations such as the Toxic Substances Control ACT (TSCA), Clean Water Act (CWA), or the Clean Air Act (CAA). The research and testing that has been completed has not provided conclusive evidence that there are no health effects involved in the use of carbon nanotubes (CNT). As Shatkin's Life Cycle Risk Assessment identifies, exposure and toxicity are both important in understanding the risk. While health effects may exist, they may only exist at extremely high doses which may not be used in pharmaceutical applications. The FDA approaches each new medicine on an individual basis and evaluates the risk accordingly. A major gap is lack of patient safety research studies as seen in **FIGURE X** and **TABLE IX**.

D. Gaps in the Accidental Release / Disposal Stage

The information that is presented in **TABLE III** indicates that there is further need for research for "Accidental Release/ Disposal" of carbon nanotubes (CNTs). While multiple studies identify health effects from inhalation, dermal, and ingestion exposure, there is a lack of information on the potential exposure from accidental release during disposal. During this stage, employees at incineration facilities, researchers and lab workers, and transporters of waste have been shown to have the highest likelihood of exposure (**FIGURE IX**). While research on CNTs has been generally on the front end of the CNT life cycle, but it is important to address the end of the CNT life cycle. Conclusions from this stage are shown in **FIGURE X** and **TABLE VII**. In order for researchers to understand the life cycle of CNTs, there will need to be further research on how CNTs may expose other subgroups and what health effects may pose the most risk (potentially via "third-party" exposures) (Ramachandran, Howard, Maynard, & Philbert, 2012). Like the other stages, the literature fails to identify how much exposure is appropriate, which route would be most frequent during this stage, and what is an acceptable length of exposure. While a single study was found on the effects of CNTs in

TABLE VII: Accidental Release/ Disposal Summary

Summary	
Studies Reviewed	(Bottini, et al., 2006); (Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005); (Donaldson, et al., 2006); (Feron, Arts, Kuper, Slootweg, & Woutersen, 2002); (Han, et al., 2008); (Huczko & Lange, 2001); (Kohler, Som, Helland, & Gottschalk, 2008); (Lam, James, McCluskey, & Hunter, 2004); (Lee, et al., 2010); (Maynard, et al., 2004); (Oberdorster, Oberdorster, & Oberdorster, 2005); (Patlolla, Knighten, & Tchounwou, 2010); (Poland, et al., 2008); (Shvedova, et al., 2003); (Tinkle, et al., 2003); (Tsuji, et al., 2006); (USGS, 2008)
Gaps in Literature	Length of Exposure
	Which Route is Most Frequent?
	Which Route Poses the Largest Risk?
	Vague Language
	CERCLA & RCRA do not Address Disposal of CNTs
	Implications of an Accidental Release of CNTs
	Are CNTs Destroyed During Medical Waste Incineration?

wastewater, there needs to be further research on accidental releases of CNTs. The literature analyzed in this paper did not identify whether CNTs are fully destroyed during incineration. If researchers know the temperature at which CNTs are broken down and destroyed, this information should be published.

Currently the Environmental Protection Agency operates under two Federal statutes that handle waste. The first is the Resource Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as the Superfund law) (Dolinar & Fearn-Desrosiers, 2009). These regulations do not directly address the issues surrounding the disposal of carbon nanotubes (Dolinar & Fearn-Desrosiers, 2009). To address this issue, both CERCLA and RCRA will need to draft new insurance policies, interpret existing policies, plan for potential liabilities, and take into account the potential environmental risks posed by the release or disposal of carbon nanotubes (Dolinar & Fearn-Desrosiers, 2009).

E. Gaps in the Excretion / Environmental Stage

The information presented in **TABLE III** indicates that there is a further need for research for the stage "Excretion / Environmental." While information identifies different modes of exposure, they do not adequately address environmental exposure. From the studies identified in this paper, few address environmental exposure to humans. Due to this lack of information, it is assumed that the general public would be the group potentially impacted by exposure (**FIGURE IX**). Since there is a lack of information on exposure routes from the environment to humans, it is uncertain which route (inhalation, dermal, or ingestion) would pose the most risk or be most likely occurring. Most research on carbon nanotubes (CNTs) has focused on the production stage of exposure and health effects, but little has addressed environmental exposure as well as bioaccumulation, biopersistence, and toxicity in the environment. Conclusions from this stage are shown in **FIGURE X** and **TABLE VIII**. In order for researchers to understand the life cycle of CNTs, they will need to address how exposure to humans could occur in the environment, which exposure route would be most frequent, which route may pose the greatest risk, what length of exposure is tolerable, and how this lack of information could be addressed. As CNTs are attached to particles in the environment, they could move throughout the ecosystem affecting health of plants, animals, and potentially affecting human health. Since production of CNTs is expected to increase, it is likely that an increase of CNTs in the environment will occur. As a result, it is critical to address this lack of research information.

TABLE VIII: Excretion/ Environmental Summary

Summary	
Studies Reviewed	(Bottini, et al., 2006); (Cicchetti, Divizia, Valentini, & Argentin, 2011); (Cui, Tian, Ozkan, Wang, & Gao, 2005); (Donaldson, et al., 2006); (Feron, Arts, Kuper, Slootweg, & Woutersen, 2002); (Han, et al., 2008); (Huczko & Lange, 2001); (Kohler, Som, Helland, & Gottschalk, 2008); (Lam, James, McCluskey, & Hunter, 2004); (Lee, et al., 2010); (Maynard, et al., 2004); (Oberdorster, Oberdorster, & Oberdorster, 2005); (Patlolla, Knighten, & Tchounwou, 2010); (Poland, et al., 2008); (Shvedova, et al., 2003); (Tinkle, et al., 2003); (Tsuji, et al., 2006); (USGS, 2008)
Gaps in Literature	How Exposure Occurs
	Which Route is Most Frequent?
	Which Route Poses the Largest Risk?
	Length of Exposure
	How CNTs Move Through the Environment
	CERCLA & RCRA do not Address End-of-Life for CNTs
	Implications of Bioaccumulation, Biopersistence, and Toxicity in the Environment
	How to Mitigate Environmental Exposure

The RCRA and CERCLA regulations do not directly address the issues surrounding the end-of-life of carbon nanotubes (Dolinar & Fearn-Desrosiers, 2009). Further research is needed on CNTs biopersistence and bioavailability in order to determine exposure and health effects. Research needs to focus on understanding the exposure routes and health effects of human exposure of CNTs in the environment.

Conclusions

Based on the Life Cycle Risk Assessment and policy analysis in this paper, it is clear that there are health safety gaps that exist in the life cycle of carbon nanotubes (CNTs). Due to the complexities of CNTs extensive research and development is going to be necessary before researchers and policymakers truly understand how best to classify CNTs. While funding levels for nanotechnology may be increasing, it may not be enough in order to address all emerging technologies including CNTs. The United States federal government has taken long strides towards a stronger regulatory approach that attempts to address nanotechnology as a whole, but constant re-evaluation will be necessary in order to assess health effects and exposure routes for the emerging and evolving technologies for carbon nanotubes production and use.

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