EXHIBIT 4

I, Lieutenant Colonel Theresa Long, MD, MPH, FS, declare under the penalty of perjury of the laws of the United States of America, and state upon personal knowledge that:

I am an adult of sound mind, 47 years old, and declare that the information herein is true, correct and complete and that I have voluntarily affirmed this affidavit based upon my own personal knowledge, education, and experience, and under the penalty of perjury of the laws of the United States of America.

SUBSCRIBED AND SWORN TO BEFORE ME on the 22nd day of September 2021, to certify which witness my hand and official seal.

/S/ Nicholas S. Babel
Notary Public for the Judge Advocates General, Alabama

UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF COLORADO

DANIEL ROBERT	*	
SSGT, U.S. ARMY	*	
	*	
HOLLI MULVIHILL	*	
SSGT, USMC	*	
	*	
Plaintiffs,	*	
	*	
v.	*	
	*	**Civil Action No. 1:21-cv-002228**
LLOYD AUSTIN	*	
Secretary of Defense,	*	
U.S. DEPARTMENT OF DEFENSE	*	
Washington, D.C. 20301	*	
	*	
and	*	
	*	
XAVIER BECERRA	*	
Secretary of the U.S. Department of	*	
Health and Human Services	*	
U.S. DEPARTMENT OF HEALTH	*	
AND HUMAN SERVICES	*	
	*	
and	*	
	*	
JANET WOODCOCK, Acting	*	
Commissioner of the Food & Drug	*	

Administration	*
U.S. FOOD AND	*
DRUG ADMINISTRATION	*
	*
UNITED STATES OF AMERICA	*
	*
Defendants.	*

AFFIDAVIT OF LTC. THERESA LONG M.D. IN SUPPORT OF A MOTION FOR A PRELIMINARY INJUNCTION ORDER

I, Lieutenant Colonel **Theresa Long**, MD, MPH, FS being duly sworn, depose and state as follows:

1. I make this affidavit, as a whistle blower under the Military Whistleblower Protection Act, Title 10 U.S.C. § 1034, in support of the above referenced MOTION as expert testimony in support thereof.

2. The expert opinions expressed here are my own and arrived at from my persons, professional and educational experiences taken in context, where appropriate, by scientific data, publications, treatises, opinions, documents, reports and other information relevant to the subject matter and are not necessarily those of the Army or Department of Defense.

Experience & Credentials

3. I am competent to testify to the facts and matters set forth herein. A true and accurate copy of my *curriculum vitae* is attached hereto as **Exhibit A**.

4. After receiving a bachelor's degree from the University of Texas Austin, completed my medical degree from the University of Texas Health Science Center at Houston Medical School in 2008. I served as a Field Surgeon for ten years and went on to complete a residency in Aerospace and Occupational Medicine at the United States Army School of Aviation Medicine, Fort Rucker, AL. I hold a Master's in Public Health, and I have been trained by the Combat Readiness Center at Ft. Rucker as an Aviation Safety Officer. Additionally, I have trained in the Medical Management of Chemical and Biological Causalities at Fort Detrick and USAMIIRD.

5. I am board certified in flight Aerospace Medicine and board eligible in Occupational Medicine.

6. I am currently serving as the Brigade Surgeon for the 1st Aviation Brigade Ft. Rucker, Alabama and am responsible for certifying the health, mental and physical ability, and readiness for all nearly 4,000 individuals on flight status on this post.

7. My appended *curriculum vitae* further demonstrates my academic and scientific achievements by me over the past thirteen years.

8. Prior to the outset of the pandemic, I received specialized military training from Infectious Disease doctors from the Army, Navy and Air Force on emerging infectious disease threats, FEMA training, Emergency preparedness training, Medical effects of Ionizing Radiation, OSHA, Aerospace Toxicology, Epidemiology, Biostatistics, medical research and disaster

planning. More recently I have functioned as a medical and scientific advisor to an Aviation training Brigade seeking to identify risk mitigation strategies, and bio statistical analysis of SARS-Cov-2 ("Covid 19") infections in both vaccinated and unvaccinated Soldiers. In so doing, I have identified, diagnosed and treated Covid 19 pathogenic infections. I have observed vaccine adverse events following the administration of EUA vaccines, and followed the success of Soldiers who obtained various Covid 19 therapies outside the military. The majority of the service members within the DOD population are young and in good physical condition. Military aviators are a subset of the military population that has to meet the most stringent medical standards to be on flight status. The population of student pilots I take care of are primarily in their 20s-30s, males and in excellent physical condition. The risk of serious illness or death in this population from SARs-CoV-2 is minimal, with a survival rate of 99.997%.

9. In observing, studying and analyzing all the available data, information, samples, experiences, histories and results of these treatments and inoculations provided, I have formulated a professional opinion, which requires me to report those findings to superiors in the chain of command and colleagues in the military. I have done so with mixed results in terms of acceptance, rejection and threats of punishment for so sharing.

10. The application of risk management is critical to the safety and success in both medicine and aviation. Aerospace Medicine is a specialty devoted to safety of flight by the aeromedical dispositioning and treatment of flight crew members, as accomplished by the consistent and careful application of risk mitigation and management strategies. ATP 5-19, 1-3. Risk Management (RM)[1] outlines a disciplined approach to express a risk level in terms readily understood at all echelons.

[1] adminpubs.tradoc.army.mil/regulations/TR385-2withChange1.docx

11. 1-6. States, "A risk decision is a commander, leader, or individual's determination to accept or not accept. The risk(s) associated with an action he or she will take or will direct others to take. RM is only effective when specific information about hazards and risks is passed to the appropriate level of command for a risk decision. Subordinates must pass specific risk information up the chain of command."

12. "When the specific information about hazards and risks is passed to the appropriate level of command for a risk decision. Subordinates must pass specific risk information up the chain of command. Conversely, the higher command must provide subordinates making risk decisions or implementing controls with the established risk tolerance—the level of risk the responsible commander is willing to accept. RM application must be inclusive; those executing an operation and those directing it participate in an integrated process".

13. 1-7. States, "In the context of RM, a control is an action taken to eliminate a hazard or to reduce its risk. Commanders establish local policies and regulations if appropriate".

14. The five steps of Risk management include; 1. Identify the hazards, 2. Assess the hazards, 3. Develop controls and make risk decisions, 4. Implement controls, 5. Supervise and evaluate.

15. It is therefore my responsibility and that of every leaders to apply the steps of risk management to the current pandemic and countermeasures used. **The CDC and the FDA are civilian agencies that do not have the mission of National Defense that the DOD has.** Guidance and recommendations made by these civilian agencies must be filtered through strategic perspective of national defense and the potential risks recommendations may have on the health

of the entire fighting force. Ensuring that the health of the fighting force is not compromised is a strategic imperative, for which **every** military physician is responsible to ensure.

16. **Step 1: Identify the hazards:** As defined by FM 1-02.1 Operational Terms, pg. 1-48, hazard is a condition with the potential to cause injury, illness, or death of personnel; damage to or loss of equipment or property; or mission degradation.

17. **Step 2: Assess the Hazards:** There are numerous therapeutic agents that have been proven to significantly reduce infection and therefore provide protection from the harmful effects of SARs-CoV-2.

18. Literature has demonstrated that natural immunity is durable, completed, and superior to vaccination immunity to SARs-CoV-2. mRNA vaccines produced by Pfizer and Moderna both have been linked to myocarditis, especially in young males between 16-24 years old,[2] The majority of young new Army aviators are in their early twenties. We know there is a risk of myocarditis with **each** mRNA vaccination. We additionally now know that vaccination does not necessarily prevent infection or transmission of SARs-CoV-2Therefore individuals fully vaccinated with mRNA vaccines have at least two independent risk factors for myocarditis after vaccination. Additional boaster shots add more risk. It is impossible to perform a risk/benefit analysis on the use of mRNA as counter measures to SARs-CoV-2 without further data... Use of mRNA vaccines in our fighting force, presents a risk of undetermined magnitude, in a population in which **less than 20 active-duty personnel out of 1.4 million, died of the underlying SARs-CoV-2.**

19. Aircrew Training Program (ATP) 5-19, 1-8. **Accept No Unnecessary Risk**, states, "An unnecessary risk is any risk that, if taken, **will not contribute meaningfully to mission**

[2] https://www.fda.gov/media/151733/download

accomplishment or will needlessly endanger lives or resources. Army leaders accept only a level of risk in which the potential benefit outweighs the potential loss.

20. Research shows that most individuals with myocarditis do not have any symptoms. Complications of myocarditis include dilated cardiomyopathy, arrhythmias, sudden cardiac death and carries a mortality rate of 20% at one year and 50% at 5 years. According to the National Center for Biotechnology Information, U.S. National Library of Medicine, "despite optimal medical management, overall mortality has not changed in the last 30 years".

21. **Step 3: Develop controls and make risk decisions:** Because vaccination with mRNA increase the risk of myocarditis, a comprehensive screening program should be implemented immediately to identify individuals who have been affected and attempt to mitigate immediate risks and long-term disability.

22. **Step 4: Implement Controls:** Send out clear guidance to all DOD healthcare professionals on risks of-vaccination myocarditis. Compulsory SARs-CoV-2 mRNA vaccination program should be immediately suspended until research can be done to determine the true magnitude of risk of myocarditis in individuals who have been vaccinated. We must evaluate and immediately implement alternatives to mRNA vaccines, to include Ivermectin (FDA approved 1996), Remdesivir (FDA approved 2020), Hydroxychloroquine (FDA approved 1955), Regeneron (FDA EU approved 2020). Review VAERS data for deaths from COVID for age-matched data and data from active duty COVID deaths within the DOD to perform a risk/benefit analysis.

23. **Step 5: Supervise and evaluate:** We must establish a screening program to identify those at increased risk of myocarditis, i.e. those that have, received mRNA vaccinations with Comirnaty, BioNTech or Moderna, or have any of the following symptoms chest pain, shortness of breath or palpitations They should have screening tested performed in accordance

with the CDC recommendations prior to return to flight duties. Per the CDC guidelines the initial evaluation of individuals identified according to the above criteria include; ECG, troponion level, inflammatory markers such as the C-reactive protein and erythrocyte sedimentation rate. It should be noted that the gold standard for diagnosis of myocarditis is end myocardial biopsy (EMB).

24. Given that the labels for Comirnaty and BioNtech clearly state that the vaccination should not be given to individuals that are allergic to ingredients. I have noted that one of the primary ingredients of the Lipid Nanoparticle delivery system is "ALC 1035" (two attachments, parts highlighted) in the Pfizer shots. The forth attachment is the toxicity report on ALC-1035, which comprises between 30-50% of the total ingredients.[3] The Safety Data Sheet, (attached as Exhibit B) for this primary ingredient states that it is Category 2 under the OSHA HCS regulations (21 CFR 1910) and includes several concerning warnings, including but not limited to:

a. Seek medical attention if it comes into contact with your skin;

b. If inhaled and If breathing is difficult, give cardiopulmonary resuscitation

c. Evacuate if there is an environmental spill

d. the chemical, physical, and toxicological properties have not been completely investigated

e. Caution: Product has not been fully validated for medical applications. For research use only

25. Other journals and scientific papers also denote that this particular ingredient has never been used in humans before.[4] To be abundantly clear, one of the listed primary

[3] https://thetattyjournal.org/2021/07/17/expert-evidence-regarding-comirnaty-pfizer-covid-19-mrna-vaccine-for-children/

[4] https://www.verywellhealth.com/peg-compound-in-covid-19-vaccine-5119161#citation-2

ingredients of these injectables is Polyethylene glycol ("PEG") which is a derivative of ethylene oxide. Polyethylene Glycol is the active ingredient in antifreeze. While it is hard to believe this is a key ingredient in these vaccines, it would explain the increased cardiovascular risk to users of the BioNTech or Comirnaty shots. I cannot discern what form of alchemy Pfizer and the FDA have discovered that would make antifreeze into a healthful cure to the human body. Others seem to agree my point per recent scientific studies that caused a group of 57 doctors and scientists to call for an immediate halt to the vaccination program.[5] In short, this antifreeze ingredient is being studied for the first time in human injectables. According to the VAERS data, which admittedly underreports by as much as 100 times the actual SAE's, there are well more than 600,000 documented Serious Adverse Events (ones requiring medical attention) alone and more than 13,000 fatalities directly linked to this particular vaccine. I cannot understand how this vaccine remains on the list of available options to treat Covid, when there are so many other non-deadly or injurious options available.

26. As such, I believe it is reasonable to conclude that many humans are allergic to these dangerous and deadly toxins and therefore should not take vaccinations with either Comirnaty or BioNtech. Again, I have identified an agent that possess a significant hazard to Soldiers, which would fall under DA Pam 385-61 Toxic Safety Standards cited in 2-11.

27. My assessment is that ALC 0315 is a known toxin with little study, specifically restricted to "research only" and effectively has no prior use history, with the SDS

[5] https://en-volve.com/2021/05/08/57-top-scientists-and-doctors-release-shocking-study-on-covid-vaccines-and-demand-immediate-stop-to-all-vaccinations/"

designation of (GHS02), listed as H315 and H319, in other words, hazardous if inhaled, ingested or in contact with skin and a health hazard with the designation (P313). A review of the SDS outlines that it is not for human or veterinary use,

28. I have not taken significant time to delineate the risks of other Covid 19 Vaccines other than the Safety Data Sheet of Moderna's key ingredient, SM-102 (attached as Exhibit C). Suffice it to say that SM-102 is significantly more dangerous than the Pfizer ALC 3015 and it appears that the DOD is not actively acquiring or distributing this IND/EUA. If the DOD were to undertake use of the Moderna vaccine, one can expect a much higher Serious Adverse Event and fatality rate given that SM-102 carries an express warning "Skull and Crossbones" characterized under the GHS06 and GHS08. In other words, this Moderna ingredient is deadly.

29. Given that these Covid 19 Vaccines were both Investigational New Drugs and Emergency Use Authorization vaccines, I have taken considerable time to understand potential risks, hazards and dangers these and any new drug or Investigational New Drug will may have on the health, safety and operational readiness or ability of pilots under my care and at this post. I have sought to research military records and track systems for recording events and Serious Adverse Events and fatalities associated with vaccines, new vaccines and Emergency Use, investigational vaccines in computer data systems recommended by the General Accounting Office in 2002 and ordered to be developed and implemented by the Secretary of Defense in 2003.

30. A weekly MEDSITREP report fails to report the CDC data from VAERS or internal data regarding vaccine adverse events. Despite recommendation made by the Government Accountability Office in the GAO's survey of Guard and Reserve Pilots and

Aircrew GAO-02-445, published Sep 20,2002, in which it was recommended that the Secretary of Defense should direct the establishment of an active surveillance program (unlike the passive VAERS) to identify and monitor adverse events, was not implemented. I have been unable to locate, access or asses any data, data base or internal system to track, store, evaluate or research the effects of vaccines on our military members or pilots.

31. I have also reviewed scientific data and peer reviewed studies that discuss, analyze results and conclude that natural immunity is at least as good if not far superior to any Covid Vaccine available at this time. I have also reviewed Dr. Peter McCullough's sworn affidavit in support of and in relation to the Complaint filed in this case and have reviewed its supporting data. An additional peer-reviewed study not referenced in Dr. McCullough's materials also supports the same conclusions drawn and reports that natural immunity provides a 13 fold better protection against Covid 19 infections than any currently available Covid 19 Vaccine[6]. More recently, in a meeting of the FDA Advisory Committee on September 17 of this year, fourteen of seventeen members voted against the authorization of any Covid booster vaccines in the juvenile age group having noted that the vaccine program has breached the defining test under the EUA statute as to whether the experimental treatment benefits outweigh the risks; in fact, they found the shots are far more dangerous than helpful in this age group and some voiced concerns that this would apply generally to all age groups.[7]

[6] https://www.sciencemag.org/news/2021/08/having-sars-cov-2-once-confers-much-greater-immunity-vaccine-no-infection-parties

[7] https://www.thegatewaypundit.com/2021/09/fda-hearing-doctors-experts-testify-government-data-demonstrates-covid-shots-dangerous-may-kill-save-video/ & https://www.youtube.com/watch?v=WFph7-6t34M

32. I am also aware of the Secretary of Defense Austin's order in relation to Covid Vaccine mandates made this week. In an information paper, it was stated that, "Unit personnel should use only as much force as necessary to assist medical personnel with immunizations." The use of force to administer a medical treatment or therapy against the will of a mentally competent individual constitutes medical battery and universally violates medical ethics. Currently, I am not aware of the Comirnaty available within the DOD. Emergency Use Authorized vaccines, despite the attempt to characterize some of them as approved despite such approved versions not being available and regardless of a military member's prior immunity to Covid 19; even where it may be demonstrated with a recent antibody test.

33. Finally, I have reviewed a recent study *entitled "US COVID-19 Vaccines Proven to Cause More Harm than Good Based on Pivotal Clinical Trial Data Analyzed Using the Proper Scientific Endpoint, All Cause Severe Morbidity," by J. Bart Classen, MD and published in Trends in Internal Medicine; August 25, 2021.* Attached as Exhibit D.

34. I have also seen policies, memoranda and guidance as it relates to exemptions for vaccinations as fully detailed in Army Regulation 40-562, which purport to eliminate any exemption for prior immunity by our military personnel.

Opinion

35. I have reviewed the Motion for a Preliminary Injunction which discusses the issue of prior immunity benefits outweighing the risks of using experimental Covid 19 Vaccines, together with proposed exhibits and materials cited therein. In opinion on this

subject matter, I am also drawing my own conclusions that will be put into practice in my current role as an Army flight surgeon knowing full well the horrific repercussions this decision may befall me in terms of my career, my relationships and life as an Army doctor.

36. I personally observed the most physically fit female Soldier I have seen in over 20 years in the Army, go from Colligate level athlete training for Ranger School, to being physically debilitated with cardiac problems, newly diagnosed pituitary brain tumor, thyroid dysfunction within weeks of getting vaccinated. Several military physicians have shared with me their firsthand experience with a significant increase in the number of young Soldiers with migraines, menstrual irregularities, cancer, suspected myocarditis and reporting cardiac symptoms after vaccination. Numerous Soldiers and DOD civilians have told me of how they were sick, bed-ridden, debilitated, and unable to work for days to weeks after vaccination. I have also recently reviewed three flight crew members' medical records, all of which presented with both significant and aggressive systemic health issues. Today I received word of one fatality and two ICU cases on Fort Hood; the deceased was an Army pilot who could have been flying at the time. All three pulmonary embolism events happened within 48 hours of their vaccination. I cannot attribute this result to anything other than the Covid 19 vaccines as the source of these events. Each person was in top physical condition before the inoculation and each suffered the event within 2 days post vaccination. Correlation by itself does not equal causation, however, significant causal patterns do exist that raise correlation into a probable cause; and the burden to prove otherwise falls on the authorities such as the

CDC, FDA, and pharmaceutical manufacturers. I find the illnesses, injuries and fatalities observed to be the proximate and causal effect of the Covid 19 vaccinations.

38. I can report of knowing over fifteen military physicians and healthcare providers who have shared experiences of having their safety concerns ignored and being ostracized for expressing or reporting safety concerns as they relate to COVID vaccinations. The politicization of SARs-CoV-2, treatments and vaccination strategies have completely compromised long-standing safety mechanisms, open and honest dialogue, and the trust of our service members in their health system and healthcare providers.

39. The subject matter of this Motion for a Preliminary Injunction and its devastating effects on members of the military compel me to conclude and conduct accordingly as follows:

a) None of the ordered Emergency Use Covid 19 vaccines can or will provide better immunity than an infection-recovered person;

b) All three of the EUA Covid 19 vaccines (Comirnaty is not available), in the age group and fitness level of my patients, are more risky, harmful and dangerous than having no vaccine at all, whether a person is Covid recovered or facing a Covid 19 infection;

c) Direct evidence exists and suggests that all persons who have received a Covid 19 Vaccine are damaged in their cardiovascular system in an irreparable and irrevocable manner;

d) Due to the Spike protein production that is engineered into the user's genome, each such recipient of the Covid 19 Vaccines already has micro clots in their cardiovascular system that present a danger to their health and safety;

e) That such micro clots over time will become bigger clots by the very nature of the shape and composition of the Spike proteins being produced and said proteins are found throughout the user's body, including the brain;

f) That at the initial stage of this damage the micro clots can only be discovered by a biopsy or Magnetic Resonance Image ("MRI") scan;

g) That due to the fact that there is no functional myocardial screening currently being conducted, it is my professional opinion that substantial foreseen risks currently exist, which require proper screening of all flight crews.

h) That, by virtue of their occupations, said flight crews present extraordinary risks to themselves and others given the equipment they operate, munitions carried thereon and areas of operation in close proximity to populated areas.

i) That, without any current screening procedures in place, including any Aero Message (flight surgeon notice) relating to this demonstrable and identifiable risk, I must and will therefore ground all active flight personnel who received the vaccinations until such time as the causation of these serious systemic health risks can be more fully and adequately assessed.

j) That, based on the DOD's own protocols and studies, the only two valuable methodologies to adequately assess this risk are through MRI imaging or cardio biopsy which must be carried-out.

k) That, in accordance with the foregoing, I hereby recommend to the Secretary of Defense that all pilots, crew and flight personnel in the military service who required hospitalization from injection or received any Covid 19 vaccination be grounded similarly for further dispositive assessment.

l) That this Court should grant an immediate injunction to stop the further harm to all military personnel to protect the health and safety of our active duty, reservists and National Guard troops.

40. I am competent to opine on the medical and flight readiness aspects of these allegations based upon my above-referenced education and professional medical, aviation and military experience and the basis of my opinions are formed as a result of my education, practice, training and experience.

41 As an Aerospace Medicine Specialist, and flight surgeon responsible for the lives of our Army pilots, I confirm and attest to the accuracy and truthfulness of my foregoing statements, analysis and attachments or references hereto:

_____/S/_____

LTC Theresa Long, MD, MPH, FS

State of Alabama §

§

County of ____Dale_____ §

The undersigned, being duly sworn, deposes and says:

I, Lieutenant Colonel Theresa Long, MD, MPH, FS, declare under the penalty of perjury of the laws of the United States of America, and state upon personal knowledge that:

I am an adult of sound mind, 47 years old, and declare that the information herein is true, correct and complete and that I have voluntarily affirmed this affidavit based upon my own personal knowledge, education, and experience, and under the penalty of perjury of the laws of the United States of America.

SUBSCRIBED AND SWORN TO BEFORE ME on the 22nd day of September 2021, to certify which witness my hand and official seal.

/S/ Nicholas S. Babel
Notary Public for the Judge Advocates General, Alabama

EXHIBIT 4
A - Long CV

THERESA MARIE LONG, MD, MPH, FS
LTC, MEDICAL CORPS, U.S. Army
Mobile Phone: 512-554-xxxx
theresa.m.long.mil@mail.mil

Medical Education
United States Army School of Aviation Medicine
Aerospace/Occupational Medicine Residency
University of West Florida
Graduate Student -MPH
06/2019-6/2021

Carl R. Darnall Army Medical Center, Fort Hood, Texas
Family Medicine Internship
06/2008-11/2010
Unrestricted Medical License, IN

09/2003 - 06/2008
University of Texas Medical School at Houston, Houston, Texas
06/2008 M.D.

08/2001 - 08/2004
Undergraduate - University of Texas at Austin, Austin, TX
05/2004 B.S. Neurobiology

Research Experience
08/2018 – 5/2020
School of Aviation Medicine
University of West Florida MPH program
https://tml526.wixsite.com/website
Performed a cross-sectional study on Intervertebral Disc Disease Among Army Aviators and Air Crew

08/2002 - 05/2003
University of Texas at Austin, Texas
Research Assistant, Dr. Dee Silverthorn
Performed academic research in effort to update medical facts and the latest research information for the publication of the fourth edition of Human Physiology

09/2000 - 11/2000
Neuropharmacology Research, Texas
Lab Tech, Dr. Silverthorn
Acquisition of rat cerebellums for research in gene sequencing. The focus of the project was to determine the DNA sequence of the receptor in the developing fetal brain that binds to ethanol and induces apoptosis leading to fetal alcohol syndrome.

Publications/Presentations/Poster Sessions
Presentations/Posters

Poster: Intervertebral Disc Disease Among Army Aviators and Air Crew, presented during the 2021 American Occupational Healthcare Conference.
Long, Theresa M., Sorensen, Christian, Victoria Zumberge. (2003, May). Sodium dependent transport of Chlorophenol red uptake by Malpighian tubules of acheta domesticus. Poster presented at: University of Texas at Houston; Austin, TX.

Volunteer Experience
08/ 2005 - 09/2005
University of Texas - Houston, Health Science Ctr, Texas
Medical Student -Provided medical aid and support for Acute Care and triage of Hurricane Katrina evacuees.

Work Experience

06/2021- Present
1st Aviation Brigade TOMS Surgeon
Serve as the Medical Advisor to the 1st Aviation Brigade Commander regarding health and fitness of over 3600 officers, warrant officers and Soldiers. The Brigade is comprised of three aviation training battalions, responsible for initial entry rotary wing/ fixed wing flight training, advanced aircraft training. as well as Specific duties include ensuring safety of flight in Army Aviation operations by functioning as Flight Surgeon, while ensuring the health and fitness of military police, firefighters and military working dogs that support Ft. Rucker. Tasked with conducting epidemiological and biostatistical analysis of injuries and illnesses (SARs CoV-2) and medical trends that occur during training and identify and implement strategies to mitigate delays or lost training time.
05/2018-06/2021
Aerospace and Occupational Medicine Resident

Graduate Medical Education training in Aerospace and Occupational Medicine while obtaining a Master's in Public Health. Specialty training included the Flight surgeon course, The Instructor/Trainer course, Space Cadre Course, Medical Effects of Ionizing Radiation, Medical Management of Chemical and Biological Casualties course at USAMIIRD, Ft. Detrick, NASA, 7th Special Forces, Aviation Safety Officer Course, Global Medicine Symposium, OSHA, Dept of Transportation, Textron Bell Helicopters, Brigade Healthcare Course, Preventative Medicine Senior Leaders Course, Joint Enroute Critical Care Course, Army Aeromedical Activity, research on Intervertebral Disc Disease.

05/2015-05/2018
Department of Rehabilitation Services
General Medical Officer
Assigned to Carl R. Darnall Army Medical Center Physical Medicine clinic with special duties Function as General Medical Officer, to mitigate the number of high risk patients get referred off-post to Pain management and PM&R clinics. Functioned as the Performance Improvement officer for PM&R, the Chiropractic Clinic OIC, and the MEB/IDES Subject Matter Expert to IPMC multi-disciplinary team. Significantly increased access to care to the Physical Medicine clinic. Was instrumental in leading the hospital transition for the Chiropractic clinic, contributing to the subsequent successful Joint Commission inspection. Increased access to care in the Chiropractic clinic by 500%.

9/2013- 5/2015
Department of Pediatrics/ Department of Deployment & Operational Medicine
General Medical Officer
Assigned to the Carl R. Darnall Army Medical center Pediatric Clinic with special duties within the Department of Deployment & Operational Medicine. Provided acute and routine medical care for newborn to age 18 and collaborated with Lactation Team Leader to develop research matrix to ensure effective use of resources to meet Perinatal Core Measures PC-05 for Joint Commission Accreditation. Demonstrated initiative by providing emergency medical care to one of the victims of the April 2, 2014 FT Hood shooting.

10/2012-9/2013
Department of Deployment Medicine/ Emergency Medicine
General Medical Officer
Assigned to the Department of Deployment & Operational Medicine at Carl R Darnall Army Medical Center (CRDAMC) with specific duties directed by the CRDAMC DCCS. Supported soldier deployment/redeployment from combat, while also performing clinical rotations within the Emergency and Internal Medicine Departments to increase access to care for acutely ill patients. Improved productivity of the SMRC by conducting ETS, Chapter, Special Forces, Airborne, Ranger, SERE, and OCS/WOCS physicals. Ensured DODM success with 90% CRDAMC staff compliance of their annual PHA's. Selected to become an ACLS instructor.

06/2012-10/01/2012
Department of the Army Inspector General Agency
Disability Medicine Subject Matter Expert (SME) - Temporary Dept of the Army Inspector General
Assistant Inspector General on Medical Disability (Subject Matter Expert)
Selected above my peers, from across the Army AMEDD as one of three medical NARSUM Subject Matter Experts to function as a temporary assistant Inspector General, in a SECARMY directed inspection of the MEB/IDES system. Planed, coordinated, and conducted inspections of agencies/commands and to gather required data and perspectives relevant to the inspection topic. Developed inspection concepts, objectives, methodologies while coordinating inspection site requirements with major Army Commands ASCC, DRUs, Installations and Components. Identified trends, analyzed root causes to systemic problems and proposed solutions to the IG, Army Chief of Staff and Secretary of the Army for service-wide implementation.

06/2011-06/2012
Carl R. Darnall Army Medical Center
Integrated Disability Evaluation System
Increased patient access to care by conducting 203 acute care appointments in four months. Increased productivity by 25% by completing 202 NARSUMs, 12 TDRLs, 42 Psychiatric addendums in nine months with only a single case returned from the PEB. Performed duties of MEB chief and QA physician in their absence by performing QA on seven NARSUMS, and reviewing 13 cases for initial intake. Functioned as IDES Physician Training officer, applying PDA training to develop a comprehensive training program for new MEB/IDES NARSUM physicians.

11/2010-05/2011
Carl R. Darnall Army Medical Center, Hospital Operations, Clinical Plans and Medical Operations Officer
Served as Clinical Plans and Medical Operations Officer for Hospital Operation (HOD), responsible for the synchronization of external and internal MEDCEN operations supporting over 3,000 MEDCEN employee as well as the DoD's largest military installation and surrounding civilian population; assisted in development and execution of medical plans supporting Installation, Garrison, MEDCEN and Civilian AT/FP and MASCAL events

06/2005 - 07/2005
United States Army, Texas, Officer Basic Course - Class 1st Sergeant
Supervised 306 medical, dental, and veterinarian HPSP scholarship recipients for Officer Basic training.

10/2002 - 08/2003
United States Army - **Texas National Guard, Texas Flight Medic -**EMT/BCLS Instructor Training

10/2001 - 10/2002
United States Army Reserve, Texas, Instructor/Trainer

Instructor/ Trainer of the Total Army Instructor Trainer Course and Instructor Candidate for NCO leadership development courses.

EXHIBIT 4
B

MCE® MedChemExpress

Safety Data Sheet

Revision Date: Mar.-23-2021
Print Date: Sep.-9-2021

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifier

Product name : ALC-0315

Catalog No. : HY-138170

CAS No. : 2036272-55-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, manufacture of substances.

1.3 Details of the supplier of the safety data sheet

Company: MedChemExpress USA

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: sales@medchemexpress.com

1.4 Emergency telephone number

Emergency Phone #: 609-228-6898

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Skin corrosion/irritation (Category 2),H315

Serious eye damage/eye irritation (Category 2A),H319

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Warning

Hazard statement(s)

H315 Causes skin irritation

H319 Causes serious eye irritation

Precautionary statement(s)

P264 Wash hands thoroughly after handling

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 IF ON SKIN: Wash with plenty of soap and water.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P313 Get medical advice/attention.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

P362 Take off contaminated clothing and wash before reuse.

2.3 Other hazards

None.

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula:	$C_{48}H_{95}NO_5$
Molecular Weight:	766.27
CAS No. :	2036272-55-4

4. FIRST AID MEASURES

4.1 Description of first aid measures

Eye contact

Remove any contact lenses, locate eye-wash station, and flush eyes immediately with large amounts of water. Separate eyelids with fingers to ensure adequate flushing. Promptly call a physician.

Skin contact

Rinse skin thoroughly with large amounts of water. Remove contaminated clothing and shoes and call a physician.

Inhalation

Immediately relocate self or casualty to fresh air. If breathing is difficult, give cardiopulmonary resuscitation (CPR). Avoid mouth-to-mouth resuscitation.

Ingestion

Wash out mouth with water; Do NOT induce vomiting; call a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2).

4.3 Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

5. FIRE FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, dry chemical, foam, and carbon dioxide fire extinguisher.

5.2 Special hazards arising from the substance or mixture

During combustion, may emit irritant fumes.

5.3 Advice for firefighters

Wear self-contained breathing apparatus and protective clothing.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use full personal protective equipment. Avoid breathing vapors, mist, dust or gas. Ensure adequate ventilation. Evacuate

personnel to safe areas.

Refer to protective measures listed in sections 8.

6.2 Environmental precautions

Try to prevent further leakage or spillage. Keep the product away from drains or water courses.

6.3 Methods and materials for containment and cleaning up

Absorb solutions with finely-powdered liquid-binding material (diatomite, universal binders); Decontaminate surfaces and equipment by scrubbing with alcohol; Dispose of contaminated material according to Section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Avoid inhalation, contact with eyes and skin. Avoid dust and aerosol formation. Use only in areas with appropriate exhaust ventilation.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly sealed in cool, well-ventilated area. Keep away from direct sunlight and sources of ignition.

Recommended storage temperature: 4°C, protect from light

* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)

Shipping at room temperature if less than 2 weeks.

7.3 Specific end use(s)

No data available.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Engineering controls

Ensure adequate ventilation. Provide accessible safety shower and eye wash station.

Personal protective equipment

Eye protection	Safety goggles with side-shields.
Hand protection	Protective gloves.
Skin and body protection	Impervious clothing.
Respiratory protection	Suitable respirator.
Environmental exposure controls	Keep the product away from drains, water courses or the soil. Clean spillages in a safe way as soon as possible.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Viscous liquid
Odor	No data available
Odor threshold	No data available

pH	No data available
Melting/freezing point	No data available
Boiling point/range	No data available
Flash point	No data available
Evaporation rate	No data available
Flammability (solid, gas)	No data available
Upper/lower flammability or explosive limits	No data available
Vapor pressure	No data available
Vapor density	No data available
Relative density	No data available
Water Solubility	No data available
Partition coefficient	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidizing properties	No data available

9.2 Other safety information

No data available.

10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available.

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to avoid

No data available.

10.5 Incompatible materials

Strong acids/alkalis, strong oxidising/reducing agents.

10.6 Hazardous decomposition products

Under fire conditions, may decompose and emit toxic fumes.

Other decomposition products - no data available.

11.TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

Classified based on available data. For more details, see section 2

Skin corrosion/irritation

Classified based on available data. For more details, see section 2

Serious eye damage/irritation

Classified based on available data. For more details, see section 2

Respiratory or skin sensitization

Classified based on available data. For more details, see section 2

Germ cell mutagenicity

Classified based on available data. For more details, see section 2

Carcinogenicity

IARC: No component of this product present at a level equal to or greater than 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by ACGIH.

NTP: No component of this product present at a level equal to or greater than 0.1% is identified as a anticipated or confirmed carcinogen by NTP.

OSHA: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by OSHA.

Reproductive toxicity

Classified based on available data. For more details, see section 2

Specific target organ toxicity - single exposure

Classified based on available data. For more details, see section 2

Specific target organ toxicity - repeated exposure

Classified based on available data. For more details, see section 2

Aspiration hazard

Classified based on available data. For more details, see section 2

Additional information

This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity

No data available.

12.2 Persistence and degradability

No data available.

12.3 Bioaccumlative potential

No data available.

12.4 Mobility in soil

No data available.

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment unavailable as chemical safety assessment not required or not conducted.

12.6 Other adverse effects

No data available.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Dispose substance in accordance with prevailing country, federal, state and local regulations.

Contaminated packaging

Conduct recycling or disposal in accordance with prevailing country, federal, state and local regulations.

14. TRANSPORT INFORMATION

DOT (US)

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IMDG

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IATA

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

15. REGULATORY INFORMATION

SARA 302 Components:

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components:

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards:

No SARA Hazards.

Massachusetts Right To Know Components:

No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components:

No components are subject to the Pennsylvania Right to Know Act.

New Jersey Right To Know Components:

No components are subject to the New Jersey Right to Know Act.

California Prop. 65 Components:

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or anyother reproductive harm.

16. OTHER INFORMATION

 The above information is correct to the best of our present knowledge but does not purport to be all inclusive and should be used only as a guide. The product is for research use only and for experienced personnel. It must only be handled by suitably qualified experienced scientists in appropriately equipped and authorized facilities. The burden of safe use of this material rests entirely with the user. MedChemExpress disclaims all liability for any damage resulting from handling or from contact with this product.

EXHIBIT 4
C

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/11/2021 Revision date 04/11/2021

1 Identification

· **Product identifier**

· **Trade name: SM-102**
· **Synonym** 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester

· **Article number:** 33474
· **Application of the substance / the mixture** For research use only, not for human or veterinary use.

· **Details of the supplier of the safety data sheet**
· **Manufacturer/Supplier:**
Cayman Chemical Co.
1180 E. Ellsworth Rd.
Ann Arbor, MI 48108
USA

· **Information department:** Product safety department
· **Emergency telephone number:**
During normal opening times: +1 (734) 971-3335
US/CANADA: 800-424-9300
Outside US/CANADA: 703-741-5970

2 Hazard(s) identification

· **Classification of the substance or mixture**


GHS02 Flame

Flam. Liq. 2 H225 Highly flammable liquid and vapor.


GHS06 Skull and crossbones

Acute Tox. 2 H310 Fatal in contact with skin.


GHS08 Health hazard

Carc. 2 H351 Suspected of causing cancer.
Repr. 2 H361 Suspected of damaging fertility or the unborn child.
STOT RE 1 H372 Causes damage to the central nervous system, the kidneys, the liver and the respiratory system through prolonged or repeated exposure.


GHS09 Environment

Aquatic Chronic 1 H410 Very toxic to aquatic life with long lasting effects.


GHS07

(Contd. on page 2)

EXHIBIT 4
D

Research Article

Trends in Internal Medicine

US COVID-19 Vaccines Proven to Cause More Harm than Good Based on Pivotal Clinical Trial Data Analyzed Using the Proper Scientific Endpoint, "All Cause Severe Morbidity"

J. Bart Classen, MD*

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Received: 24 July 2021; **Accepted:** 25 August 2021

Citation: Classen B. US COVID-19 Vaccines Proven to Cause More Harm than Good Based on Pivotal Clinical Trial Data Analyzed Using the Proper Scientific Endpoint, "All Cause Severe Morbidity". Trends Int Med. 2021; 1(1): 1-6.

ABSTRACT

Three COVID-19 vaccines in the US have been released for sale by the FDA under Emergency Use Authorization (EUA) based on a clinical trial design employing a surrogate primary endpoint for health, severe infections with COVID-19. This clinical trial design has been proven dangerously misleading. Many fields of medicine, oncology for example, have abandoned the use of disease specific endpoints for the primary endpoint of pivotal clinical trials (cancer deaths for example) and have adopted "all cause mortality or morbidity" as the proper scientific endpoint of a clinical trial. Pivotal clinical trial data from the 3 marketed COVID-19 vaccines was reanalyzed using "all cause severe morbidity", a scientific measure of health, as the primary endpoint. "All cause severe morbidity" in the treatment group and control group was calculated by adding all severe events reported in the clinical trials. Severe events included both severe infections with COVID-19 and all other severe adverse events in the treatment arm and control arm respectively. This analysis gives reduction in severe COVID-19 infections the same weight as adverse events of equivalent severity. Results prove that none of the vaccines provide a health benefit and all pivotal trials show a statically significant increase in "all cause severe morbidity" in the vaccinated group compared to the placebo group. The Moderna immunized group suffered 3,042 more severe events than the control group (p=0.00001). The Pfizer data was grossly incomplete but data provided showed the vaccination group suffered 90 more severe events than the control group (p=0.000014), when only including "unsolicited" adverse events. The Janssen immunized group suffered 264 more severe events than the control group (p=0.00001). These findings contrast the manufacturers' inappropriate surrogate endpoints: Janssen claims that their vaccine prevents 6 cases of severe COVD-19 requiring medical attention out of 19,630 immunized; Pfizer claims their vaccine prevents 8 cases of severe COVID-19 out of 21,720 immunized; Moderna claims its vaccine prevents 30 cases of severe COVID-19 out of 15,210 immunized. Based on this data it is all but a certainty that mass COVID-19 immunization is hurting the health of the population in general. Scientific principles dictate that the mass immunization with COVID-19 vaccines must be halted immediately because we face a looming vaccine induced public health catastrophe.

Keywords

Clinical trial, Vaccines, COVID-19.

Introduction

For decades, true scientists have warned that pivotal clinical trial designs for vaccines are dangerously flawed and outdated [1]. Vaccines have been promoted and widely utilized under the false claim they have been shown to improve health. However, this claim is only a philosophical argument and not science based. In a true scientific fashion to show a health benefit one would need to show fewer overall deaths during an extended period in the vaccinated group compared to a control group. Less stringent

indicators of a health benefit would include fewer severe events of all kinds, fewer days hospitalized for any reason, lower heath care expenses of all types, fewer missed days from work for any health reason. No pivotal clinical trial for a vaccine preventing an infectious disease has ever demonstrated an improvement in health using these scientific measurements of health as a primary endpoint. Instead, vaccine clinical trials have relied on misleading surrogate endpoints of health such as infection rates with a specific infectious agent. Manufactures and government agents have made the scientifically disproved and dangerous philosophical argument that these surrogate endpoints equate to a health benefit.

True medical scientists, outside the vaccine fields, have embraced the use of true health measurements as the proven proper scientific endpoint of clinical trials. Decades ago, a pharmaceutical manufacturer would only need to show that a chemotherapeutic agent shrank a tumor or reduce cancer deaths to obtain FDA approval. Manufacturers would market their products under the fraudulent philosophical argument that shrinking tumors or reducing cancer deaths equates to improved survival. However, many of the toxic chemotherapeutic agents would destroy vital organs and actually reduce survival while decreasing cancer deaths at the same time. The FDA and comparable agencies around the world switched to “all cause mortality” as the primary endpoint for pivotal cancer drug trails. The gold standard for marketing approval is to show that those receiving a cancer drug actually live longer than those who do not. Typically, new “miracle” anticancer drugs only prolong survival about 2 months but this added time may be spent severely ill suffering from adverse events caused by the chemotherapy. Application of true scientific principles often severely deflates the hype promoting pharmaceutical products.

All previous vaccine trials have suffered not only from lacking a proper primary clinical endpoint put also from insufficient perspective follow up of adverse events. The trials have failed to account for the well-established toxicity data and epidemiology data that vaccines are associated with chronic immune mediated disorders that may not develop for years after immunization. These adverse events, for example type 1 diabetes, are quite common, develop 3 or more years after immunization, and can exceed the reduction in infectious complications induced by the vaccine as was shown with a hemophilus vaccine [1]. Pivotal trials for the recombinant hepatitis B vaccine prospectively recorded adverse events for about 7 days after immunization and newer vaccines typically prospectively follow patients 6 months for adverse events.

Use of “all cause morbidity or mortality” as the primary endpoint is warranted in vaccine trials for several reasons. First, the recipients are generally healthy (relative to patients with terminal cancer for example) and the risk of severe morbidity from the target infection is low so even rare adverse events can result in an unfavorable risk benefit. Second, stimulating the immune system with a vaccine can lead to almost any type of adverse event including increasing the incidence or severity of diseases already present in the population. One needs a trial design with a primary endpoint that captures both a decline in infectious complications as well as small rises in hundreds of different immune modified disorders of similar or worse severity as the infectious complications.

Three COVID-19 vaccines are approved by the US FDA under Emergency Use Authorization (EUA). These vaccines have been developed by Pfizer-BioNTech, Moderna, and Janssen. Since marketing has begun multiple reports of potential, adverse events have been recorded. These reports include prion disease [2,3] , clotting disorders [4], myocarditis, reproductive issues, death and many more. A clear difference in frequency of adverse events between different COVID-19 vaccines has been published [3]. The clinical trial designs of the pivotal trials and the resulting data was evaluated to determine if scientifically the results support mass immunization with the vaccines for COVID-19. The published data from the manufacturers’ own clinical trials was re analyzed using the proper scientific endpoint “all cause severe morbidity”.

Method

Data from all three US COVID-19 vaccines was published in the New England Journal of Medicine [4-6]. Data from these three publications and the accompanying published appendixes provided the bulk of the information analyzed. On rare occasions supplemental data was found on the FDA’s website (https://www.fda.gov/advisory-committees/advisory-committee-calendar) in briefing documents pertaining to FDA advisory panel committees for COVID-19 vaccines from Pfizer-BioNTech, Moderna, and Janssen. The scientific primary endpoint, “all severe events", in the treatment group and controls was calculated by adding all severe or life threatening events reported in the clinical trials by the manufacturers. Severe events included both severe cases of COVID-19 and all other severe events in the treatment arm and control arm respectively.

A Chi square analysis using a 2x2 table was used to calculate statistical p values. An online statistical chi square calculator (***https://www.socscistatistics.com/tests/chisquare***) was used. Statistical calculations ignored small differences in total subject number between efficacy and adverse event populations. The randomized number, shown in Table 1, was used as the study population for statistical calculations. In general, the population for adverse events was slightly higher than that for efficacy. Given the statistical significant p, values generated (see Table 1), these small differences do not appear to be material.

The FDA document entitled Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007, provided the following definitions for adverse events.

Grades 3, Severe: Prevents daily activity and requires medical intervention.
Grades 4, Potentially life threatening: ER visit or hospitalization.

Results

Moderna

The Moderna pivotal Phase III trial results and protocol are published in the New England Journal of Medicine (NEJM) [5]. The primary endpoint was COVID-19 illness starting 14 days after the second dose of vaccine however the trial had a secondary endpoint

which was patients developing severe COVID-19 symptoms. This later endpoint allowed for a direct comparison to severe adverse events. The study randomized 30,420 individuals, 15,210 were randomized to receive injections with Moderna's mRNA-1273 vaccine and 15,210 were randomized to receive injections with placebo. Two shots were administered 28 days apart. "Solicited" adverse events were collected 7 days after immunization and "unsolicited" adverse events were reported up to 28 days after administration of each vaccine or approximately 56 days after the first dose according to protocol. Because of dropouts, adverse events were recorded on 15,185 vaccinated patients and 15,166 placebo patients (reference 5, appendix table S8). The treatment group had 11 cases of symptomatic COVID-19 infections and 0 cases severe COVID-19 infections (reference 5, appendix table S13). There were 234 cases of severe "unsolicited" adverse events in the treatment group (reference 5, appendix table S8), and an additional 3,751 "solicited" severe or life threatening (Grade 3 or Grade 4) adverse events (reference 5, appendix table S3 and S4). By contrast, the control group had 185 cases of symptomatic COVID-19 infections and 30 cases of severe COVID-19 infections. However, only one of these case of COVID-19 out of 15,166 controls required admission to an intensive care unit (see reference 5, appendix table S13). There were 202 cases of severe "unsolicited" adverse events in the placebo group and an additional 711 "solicited" severe or life threatening (Grade 3 or Grade 4) adverse events. There were 3 deaths in the placebo group and 2 in the vaccinated group (reference 5, appendix table S8).

Pfizer-BioNTech

The Pfizer-BioNTech (Pfizer) pivotal Phase III trial results are published in the New England Journal of Medicine [6]. The Pfizer trial was classified as a Phase 1/2/3 trial. Two shots were administered 21 days apart. The primary endpoint was confirmed COVID-19 infections 7 days after the second dose. A post hoc analysis of severe COVID-19 infections was included in the appendix published by the NEJM. The study randomized 43,548 individuals of which 100 did not receive injections, 21,720 received injections with the vaccine and 21,728 received injections with placebo. "Solicited" adverse events were collected 7 days after immunization and "unsolicited" severe adverse events were reported up to 14 weeks after administration of the second dose. However, median safety follow up for "unsolicited" events was only approximately 2 months after the second dose at the time of publication in the NEJM. In the treatment arm there was 1 case of severe Covid-19 (reference 6, appendix table S5), 240 "unsolicited" severe adverse events and 21 "unsolicited" life threatening adverse events (reference 6, appendix table S3). In the placebo arm, there were 9 cases of severe COVID-19, 139 "unsolicited" severe adverse events and 24 "unsolicited" life threatening adverse events. Pfizer used a safety subset of approximately 8,183 (both vaccinated and unvaccinated) to record "solicited" adverse events at 7 days. These data that are not shown in Table 1 in part because the data was depicted graphically in the NEJM manuscript. However, graphical data in the NEJM strongly

Table 1: All Cause Severe Morbidity

	Moderna		**Control**		**Difference**	**P value**
Randomized	15,210		15,210			
Days of Safety Follow Up	56		56			
# Severe COVID-19 Cases	0		30			
# Unsolicited Severe Adverse Events	234		202			
# Solicited Grade 3 AE, Shot 1	848		361			
# Solicited Grade 4 AE, Shot 1	5		6			
# Solicited Grade 3 AE, Shot 2	2884		341			
# Solicited Grade 4 AE, Shot 2	14		3			
# Total Severe Events	3985		943		3042	p=0.00001
#Deaths	2		3			
	Pfizer		**Control**		**Difference**	**P value**
Randomized	21,720		21,728			
Days of Safety Follow Up	81		81			
# Severe COVID-19 Cases	1		9			
# Unsolicited Severe Adverse Events	240		139			
# Unsolicited Life Threatening Adverse Events	21		24			
# Total Severe Events	262		172		90	p=0.000014
#Deaths	2		4			
	Jansen	**Jansen**	**Control**	**Control**	**Difference**	**P value**
Randomized	19,630		19,691			
Safety Subset		3,356		3,386		
Days of Safety Follow Up	28		28			
# Severe COVID-19 Cases	21		78			
# Solicited Grade 3 Adverse Events						
Local (**extrapolated**)	**135**	23	**35**	6		
Systemic (**extrapolated**)	**357**	61	**122**	21		
# Unsolicited Grade 3-4 Adverse Events	83		96			
# Total Severe Events	595		331		264	p=0.00001
# Deaths	3		16			

indicates the vaccinated group has more "solicited" adverse events of all grade levels than the control group.

Janssen

The Janssen pivotal Phase III trial design and trial results are published in the New England Journal of Medicine [4]. The primary endpoint was prevention of molecularly confirmed, moderate to severe–critical COVID-19 14 days post vaccination however a secondary endpoint was prevention of molecularly confirmed, severe–critical COVID-19 14 days post vaccination. This later endpoint allowed for a direct comparison to severe adverse events. The study randomized 19,630 to receive a single injection with Janssen's adenovirus COVID-19 vaccine and randomized 19,691 to receive a single injection with placebo. "Solicited" adverse events were collected 7 days after immunization and "unsolicited" adverse events were reported up to 28 days after administration of the single dose of vaccine. The treatment group had 21 cases of severe or critical COVID-19 infections while the placebo control group had 78 (reference 4, appendix table S9). Further analysis shows that only 2 of 19,514 immunized patients needed medical intervention for COVID-19 infections starting 14 days after immunization, while only 8 of 19,544 controls needed medical intervention for COVID-19 infections starting 14 days after placebo injection where the COVID-19 infection was confirmed by a central lab (reference 4, appendix table S10). There were 83 "unsolicited" and approximately 492 "solicited" serious adverse events in the vaccinated group compared to 96 "unsolicited" and approximately 157 "solicited" serious adverse events in the control group (reference 4, appendix table S7). There were 3 deaths in the treatment group and 16 in the control group (reference 4, appendix table S7).

Janssen did not collect "solicited" adverse events from the whole group at day 7 but instead collected these adverse events from a safety group comprising 3,356 vaccinated and 3,380 control patients. FDA briefing document Table 23, page 39 [7] provided the number of "solicited" Grade 3 adverse events in each group. These figures as well as the number of patients randomized were used to extrapolate the number of solicited severe adverse events in the full vaccinated and placebo group as recorded in Table 1.

Discussion

Scientific analysis of the data from pivotal clinical trials for US COVID-19 vaccines indicates the vaccines fail to show any health benefit and in fact, all the vaccines cause a decline in health in the immunized groups. Health is the sum of all medical events or lack there of. COVID-19 vaccines are promoted as improving health while in fact there is no evidence that these vaccines actual improve health in the individual or population as a whole. The current analysis used the proper scientific endpoint of "all cause severe morbidity", a true measure of health. By contrast, manufactures and government officials promote the vaccines using a surrogate measure of health, severe infections with COVID-19, and the disproved philosophical argument that this surrogate endpoint equates to health. This substitution of philosophy for science is extremely dangerous and is certainly leading to a catastrophic public health event.

Review of data from the three COVID-19 vaccines marketed in the US shows complete lack of a health benefit and even an increase in severe events among vaccine recipients. The proper scientific clinical trial endpoint, "all cause severe morbidity" was created by combing all severe and or life threatening events, both infectious and non-infectious, occurring in the vaccinated and placebo control groups respectively. The data (Table 1) shows there are clearly more severe events in the vaccinated groups. The results are highly statistically significant. The use of a true scientific measure of health as an endpoint for a vaccine trial gives a contrasting result compared to the use of a non-scientific surrogate endpoint of heath, severe infections with COVID-19.

Clinical trial data show there were actually few very "severe" cases of COVID-19 in either the vaccinated or the placebo group. Moderna data shows that only one of 15,166 unvaccinated patients required admission to an intensive care unit for COVID-19. Data provided by Janssen shows that only a few of the "severe" COVID-19 infections required medical intervention. Table S10 in the appendix published in the New England Journal of Medicine [4] , shows only 2 of 19,514 patients immunized with the Janssen vaccine needed medical intervention for severe COVID-19 infections starting 14 days after immunization, while only 8 of 19,544 controls needed medical intervention for severe COVID-19 infections starting 14 days after placebo, where the infection was confirmed by a central lab. This benefit, reduction in 6 case of COVID-19 requiring medical intervention, in 19,630 vaccinated patients is simply statistically insignificant in a population that has a hundred fold more severe events of any cause. The Janssen vaccinated group had 595 severe Grade 3 or 4 events in the first 28 days post immunization. Science thus does not support a health benefit with COVID-19 vaccines. All arguments for immunization are purely philosophical and based on false, discredited, assumptions.

Reductions in infection rates, hospitalization rates and even death with COVID-19 are poor surrogate markers for health and are not proper primary endpoints for a vaccine clinical trial. As discussed earlier with cancer treatments, a trial endpoint showing reduced cancer deaths is not equivalent to enhanced survival. One could apply enough radiation (or cytotoxic chemotherapy) to cancer patients to kill all their cancer cells and prevent cancer deaths but these cancer patients would die of radiation sickness (or chemotherapy induced organ failure) faster than if they died naturally of cancer. In the same manner, reducing severe COVID-19 infections does not equate to enhanced survival especially when the vaccine can cause clotting, heart disease and many other severe adverse events. Potential vaccine recipients need to know if the vaccine improves their survival in order for them to make an informed consent to be immunized. Unfortunately, the current studies with COVID-19 vaccines in fact show they cause a decline in health.

The actual health decline caused by the vaccines is probably much worse than what is depicted in Table 1 for many reasons. First manufactures took a haphazardly approach to recording adverse events in contrast to recording a reduction in COVID-19 events. At

the time of publication, patients were only followed prospectively for approximately 7 days after immunization for "solicited" adverse events, and then relied on "unsolicited" reports of adverse events for approximately 30-60 days after immunization. Serious non-infectious events occurring after this 30-60 day period were not part of the published data. By contrast, infections with COVID-19 were followed indefinitely since the time of immunization. Both Janssen and Pfizer were specifically lax recording adverse events and only recorded "solicited" adverse events at day 7 in a safety cohort representing less than 20% of the study population. Given that some of the vaccine clinical trials recruited patients in the third world, patients with low education, and potentially even elderly with dementia the patients can not be expected to understand when they may be having an serious event that needs reporting or how to report it. For these and others reason only 5% of adverse events are generally ever reported [8].

COVID-19 vaccines were released for marketing under a EUA. Use of such a protocol should be reserved for outbreaks of life threatening epidemics. If this were, actually the case with COVID-19 then reduction in "all cause mortality" should be the primary outcome for the vaccine trials and "all cause severe morbidity" should be the secondary endpoint. However, the manufacturers show no evidence of a survival benefit. Deaths in the trials were extremely rare and of 30 deaths, out of roughly 110,000 trial participants, only about 6 deaths were confirmed to have COVID-19 at the time of death. Regrettably, the vaccines did not reduce morbidity but caused an increase in severe events. Worse, the pivotal clinical trials were never designed to show a benefit in "all-cause mortality" or reduction "in all cause severe morbidity". The fact that the trials were never designed to show these health benefits is an admission that those developing the vaccines never expected the vaccines to result in measurable health benefits. Regrettably some manufacturers have published the false claim [6] that the vaccine have been proven to be "effective" and that its now "unethical" to withhold immunization from the control group. They advocate abolishing the control group by immunizing them. This unscientific act only further proves the pharmaceutical industry is unaccountable to any one and does not feel the need to adhere to principles of science, ethics, or public health.

The COVID-19 vaccine pivotal clinical trials were of very short duration and the question exists whether longer-term follow up will reverse the vaccine induced health decline and show a health benefit. The question is purely philosophical. Some manufactures have already threatened to destroy the randomization by immunizing the control group, as stated above, making further scientific study impossible. While it is possible that the vaccines will continue to prevent severe infectious disease long after the immunization, the reality is that immunity wanes with time and vaccine resistant variants keep developing. Another issue is that severe adverse events will continue to occur over time. Given evidence of prion genic activity by both established pathophysiology [2], animal toxicity data [9] and epidemiology data [3] one can expect an increase in adverse events in the vaccinated group for decades.

Yearly booster are unlikely to improve the health outcome with COVID-19 vaccines. A booster may provide a small incremental benefit in preventing severe COVID-19 infections however, the boosters are likely to cause many more severe adverse events. Looking at the data on secondary injections with the Moderna vaccine (Table 1) there are approximately 3 times as many Grade 3 or 4 adverse events after the second dose than after the first dose. However, this is not the case following the second dose of placebo in the Moderna placebo group. The net is that adding a booster shot is highly unlikely to induce a favorable health benefit that was missing with the first series of immunization.

Government officials are promoting COVID-19 vaccines as a way to stop the epidemic. There is however no scientific data that the COVID-19 vaccines can improve the health of the population. In fact, the data from the clinical trials seems to point in the opposite direction. Given that the population is the sum of the individuals, and the vaccines cause a decline in health in the individuals, then mass immunization is likely to erode the health of the general population, not improve it. Immunization may even cause a selection bias for new variants. Finally, if the COVID-19 outbreak is the result of a bioweapons attack and vaccine resistant variants represent the release of different prototypes then immunization is almost certain to fail [10].

There is an old saying, fool me once shame on you, fool me twice shame on me. This saying can be applied to the COVID-19 mass immunization program. The US anthrax attack of 2001, which originated at US army is Fort Detrick, has demonstrated that there are people in the US government who desire to attack US citizens with bioweapons [10]. According to the chief FBI agent leading the investigation of the US anthrax attack, conspirators were likely not apprehended in part because the investigation was prematurely ended and prior to stopping the investigation, people at the top of the FBI deliberately tried to sabotage the investigation [11]. In the US anthrax attack of 2001, people high in the US government publicly anticipated the anthrax attack as early as 1999 [10]. Similarly with the COVID-19 attack, people high in government anticipated the COVID-19 attack [12,13] several years before the attack took place [10]. There is even data that an effort was made in 2018 to protect certain populations against COVID-19 by immunizing them with MMR vaccine [14].

In such a hostile government environment, the citizens need to individually evaluate the science of immunization with COVID-19 vaccines and not rely on philosophical arguments propagated by government officials. In this case there is no scientific evidence that the COVID-19 vaccines improve the health of the individual, much less of the population as a whole. Mass immunization with COVID-19 vaccines is certainly leading to a catastrophic public health event.

References

1. Classen JB, Classen DC. Clustering of cases of insulin dependent diabetes (IDDM) occurring three years after

Hemophilus influenza B (HiB) immunization support causal relationship between immunization and IDDM. Autoimmunity. 2002; 35: 247-253.

2. Classen JB. COVID-19 RNA based vaccines and the risk of prion disease. Microbiol Infect Dis. 2021; 5: 1-3.
3. Classen JB. COVID-19 Vaccine associated Parkinson's disease, a prion disease signal in the UK Yellow Card adverse event database. J Med - Clin Res & Rev. 2021; 5: 1-6.
4. Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med. 2021; 384: 2187-2201.
5. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021; 384: 403-416.
6. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020; 383: 2603-2615.
7. FDA Briefing Document, Janssen Ad26.COV2.S vaccine for the prevention of COVID-19. Vaccines and Related Biological Products Advisory Committee Meeting February 26, 2021.
8. Hazell L, Shakir SAW. Under reporting of adverse drug reactions: a systematic review. Drug Saf. 2006; 29: 385-396.
9. Philippens IHCHM, Böszörményi KP, Wubben JA, et al. SARS-CoV-2 causes brain inflammation and induces Lewy body formation in macaques. bioRxiv preprint. 2021.
10. Classen JB. Review of COVID-19 vaccines and the risk of chronic adverse events including neurological degeneration. J Med - Clin Res & Rev. 2021; 5: 1-7.
11. Richard L. Lambert versus Attorney General Eric Holder, Robert Muller III and others. Eastern District of Tennessee. Case 3:15-cv-00147-PLR-HBG. Filed April 2, 2015.
12. Schoch-Spana M, Brunson E, Chandler H, et al. Recommendations on how to manage anticipated communication dilemmas involving medical countermeasures in an emergency. Public health reports. 2018; 133: 366-378.
13. https://www.usatoday.com/story/news/factcheck/2020/07/29/fact-check-2017-anthony-fauci-warned-potential-outbreak/5494601002/
14. Classen JB. COVID-19, MMR vaccine, and bioweapons. Diabetes Complications. 2020; 4: 1-8.

EXHIBIT 5
Sigaloff Affidavit

UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF COLORADO

DANIEL ROBERT	*	
SSGT, U.S. ARMY	*	
	*	
HOLLI MULVIHILL	*	
SSGT, USMC	*	
	*	
Plaintiffs,	*	
	*	
v.	*	
	*	Civil Action No. 21-02228
LLOYD AUSTIN	*	
Secretary of Defense,	*	
U.S. DEPARTMENT OF DEFENSE	*	
Washington, D.C. 20301	*	
	*	
and	*	
	*	
XAVIER BECERRA	*	
Secretary of the U.S. Department of	*	
Health and Human Services	*	
U.S. DEPARTMENT OF HEALTH	*	
AND HUMAN SERVICES	*	
	*	
and	*	
	*	

JANET WOODCOCK, Acting *
Commissioner of the Food & Drug *
Administration *
U.S. FOOD AND *
DRUG ADMINISTRATION *
*
UNITED STATES OF AMERICA *
*
Defendants. *

* * * * * * * * * * * * *

MOTION FOR PRELIMINARY INJUNCTION

AFFIDAVIT OF DR. SAMUEL SIGOLOFF IN SUPPORT OF PRELIMINARY INJUNCTION MOTION

I, Samuel N. Sigoloff, Doctor of Osteopathy, being duly sworn, depose and state as follows:

1. I make this affidavit in support of the above referenced MOTION as expert testimony in support thereof.

2. The expert opinions expressed here are my own and arrived at from my persons, professional and educational experiences taken in context, where appropriate, by scientific data, publications, treatises, opinions, documents, reports and other information relevant to the subject matter.

Experience & Credentials

3. I am competent to testify to the facts and matters set forth herein. A true and accurate copy of my *curriculum vitae* is attached hereto as **Exhibit A**.

4. After receiving a bachelor's degree from Saint Mary's University in San Antonio Texas in 2007, I completed a medical degree from Heritage College of Osteopathic Medicine in Athens, Ohio in 2012. I went on to complete a Family Medicine Residency at Martin Army Community Hospital at Fort Benning, Georgia in June 2015.

5. I have been board certified in Family Medicine since July, 2015.

6. I am currently serving as the Medical Director for Raymond W. Bliss Army Health Clinic at Fort Huachuca, Arizona. I am responsible for supervising Physician's Assistant and Nurse Practitioners and Physicians. I have held this position since August of 2021. I have held this similar position previously at Fort Sill, Oklahoma from 2016-2017 and at Camp Buehring Kuwait from 2017-2018.

7. Since before the declaration of this pandemic, which was declared by the WHO on March 11, 2020, I have been watching and studying any and all resources available so that I would be useful to my unit/community at Fort Wainwright, AK. Due to my initial concerns with SARS-CoV2 (COVID-19) and significant amount of reading that I completed about pandemics (historic references of what was done in 1918, SARS and MERS), and because I felt I had the most optimum health when compared to my peers, I felt it a duty to volunteer to work the 'covid clinic' at the hospital. I also wanted to reduce possible exposure by limiting the number of clinicians that would work in the 'covid clinic.' I was the only physician that volunteered for this role and there were no other clinicians requesting to share the work load.

8. As the clinician in charge of the 'covid clinic' I helped the nursing staff establish and improve procedures for safe handling of patient samples, which at that time it was thought to be a definite death sentence if contracted by anyone. Other hardships during this time included below 0 temperatures in the mornings (Ft Wainwright, AK). I was given very strict and narrow guidelines on when to test patients for SARS-CoV2. I believed it important to be liberal with testing prior to a clear outbreak in our community that way we can determine as early as possible when it has entered our community. My superiors did support my ideas and I was very liberal with testing. After 1-1/2 months I was moved back into the hospital, during that time not a single positive test had been resulted. This information is for a more clear understanding of how vested I am in this topic.

9. Given that these SARS-CoV2 preventative therapies were both Investigational New Drugs and

Emergency Use Authorization for biologics. I have spent a considerable amount of time to understand potential risks, hazards and dangers that these Investigational New Drug and biologics may have on the health, safety and operational readiness of patients and service members in my care.

10. As part of my investigation to determine if the only FDA approved biologic, Comirnaty, would be safe for service members and other patients, one must look at the ingredient list which is provided by the package insert (Exhibit B). The first 3 ingredients of Comirnaty are:

1. ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (alternative names ALC-0315, CAS No. 2036272-55-4)

2. 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide (Alternative names ALC-0159, CAS No. 1849616-42-7)

3. 1,2-distearoyl-sn-glycero-3-phosphocholine (Alternative name DSPC)

11. On August 24, 2021 the Secretary of Defense, Lloyd J. Austin III, issued guidance for mandatory Coronavirus Disease 2019 Vaccination of Department of Defense Service Members (Exhibit C).

12. The above listed chemicals are lipid-nanoparticles and the DoDI 6050.05 (DoD Hazard Communication Program) will apply for safe handling. The DoDI 6050.05 (Exhibit D) states:

1. Paragraph 3.2.2 – an inventory of all engineered nanomaterials in the work place in accordance with paragraph 3.2.c

2. Paragraph 3.2.c – All DoD workplaces, or DoD-manufactured materials where engineered nanomaterials are used, should include engineered nanomaterials that are not incorporated into articles or otherwise excluded from Part 1910.1200 of Title 29, CFR into their written HAZCOM plans when there is knowledge of the presence of such engineered nanomaterials.

3. Paragraph 3.4.b.1 – Copies of the appropriate SDS will be: (1) Readily accessible before hazardous chemicals are used and accessible at all times thereafter.

4. Paragraph 3.4.d.2 – Rejects incomplete hazardous material information that does not comply with the requirements of Part 1910.1200 of Title 29, CFR. Laboratory verification of technical elements is not required. DoD Components will return incomplete or inadequate SDSs and labels to the supplier for correction. The contracting officer or buyer must consult with the manufacturer or distributer for resolution of SDS discrepancies.

5. Paragraph G.2 DEFINITIONS: engineered nanomaterials. Discrete materials having structures with at least one dimension between 1 and 100 nanometers that are intentionally created, as opposed to those that are naturally or incidentally formed. They do not include larger materials that may have nanoscale features (e.g., etched silicon wafers), biomolecules (e.g., proteins, nucleic acids, carbohydrates), and materials with occupational exposure limits that address nanoparticles for that substance.

13. DoDI 6050.05 (DoD Hazard Communication Program) for safe handling references "Approaches to Safe Nanotechnology, Managing the Health and Safety Concerns Associated with Engineered Nanomaterials." March 2009 (Exhibit E).

Opinion

14. I have reviewed the Motion for Temporary Restraining Order which discusses the issue of prior immunity benefits outweighing the risks of using experimental genetic therapy Covid 19 Vaccines, together with proposed exhibits and materials cited therein. My opinion on this subject matter, I am drawing my own conclusions as an Army Physician and Medical Director of a Troop Medical Facility. I understand that I am willingly taking on enormous risk to my personal carrier as a physician, however it is balanced with the risk of potentially not stopping the intentional poisoning of our entire fighting force, as directed by the current Secretary of Defense Lloyd J. Austin III.

15. The first ingredient in the FDA approved Comirnaty biologic: ((4 hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (alternative names ALC-0315, CAS No. 2036272-55-4)

1. Two separate material safety data sheets (MSDS) both say that is for research use only and not for human use. (See Exhibit F and G)
2. SDS by ChemScene dated 23MAR2021 states that:

-Acute toxicity - Classified based on available data

-Skin corrosion/irritation – Classified based on available data

-Serious eye damage/irritation – Classified based on available data

-Respiratory or skin sensitization – Classified based on available data

-Germ cell mutagenicity – Classified based on available data

-Reproductive toxicity – Classified based on available data

-Specific target organ toxicity - single exposure – Classified based on available data

-Specific target organ toxicity - repeated exposure – Classified based on available data

-Aspiration hazard – Classified based on available data

-Additional information – This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

-The product is for research use only and for experienced personnel. It must only be handled by suitably qualified experienced scientists in appropriately equipped and authorized facilities. The burden of safe use of this material rests entirely with the user.

-Caution: Product has not been fully validated for medical applications. For research use only.

16. The second ingredient in the FDA approved Comirnaty biologic: 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide (Alternative names ALC-0159, CAS No. 1849616-42-7)

1. Two spate material safety data sheets (MSDS) both say that is for research use only and not for human use. (See Exhibit H and I)

2. SDS by MCE MedChemExpress dated 31JUL2021 states that:

-Acute toxicity - Classified based on available data

-Skin corrosion/irritation – Classified based on available data

-Serious eye damage/irritation – Classified based on available data

-Respiratory or skin sensitization – Classified based on available data

-Germ cell mutagenicity – Classified based on available data

-Reproductive toxicity – Classified based on available data

-Specific target organ toxicity - single exposure – Classified based on available data

-Specific target organ toxicity - repeated exposure – Classified based on available data

-Aspiration hazard – Classified based on available data

-Additional information – This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

-The product is for research use only and for experienced personnel. It must only be handled by suitably qualified experienced scientists in appropriately equipped and authorized facilities. The burden of safe use of this material rests entirely with the user. MedChemExpress disclaims all liability for any damage resulting from handling or from contact with this product.

-Caution: Product has not been fully validated for medical applications. For research use only.

17. The third ingredient in the FDA approved Comirnaty biologic: 1,2-distearoyl-sn-glycero-3-phosphocholine (Alternative name DSPC, see Exhibit J)

1. The material safety data sheet (MSDS) states:

Relevant identified uses: For research use only, not for human or veterinary use.

18. It is my opinion that the above stated chemicals listed on the package insert for Comirnaty render this product NOT safe to inject into service members nor any humans nor animals.

19. The Secretary of Defense, Lloyd J. Austin III, is in volition of DoDI 6050.05 (DoD Hazard Communication Program) and is choosing to willfully expose the entire United States Department of Defense to chemicals that are not approved for medical use and to a chemical that is not even approved for veterinary use, which may put the ability to defend this country from or foreign or domestic adversaries, in great peril.

20. I am competent to opine on the medical readiness aspects of these allegations based upon my above-referenced education and professional medical, and military experience and the basis of my opinions are formed as a result of my education, practice, training and experience.

21. As a Doctor of Osteopathy and Board Certified Family Medicine physician I am committed 'To Conserve Fighting Strength,' and as a Commissioned Officer in the US Army, I confirm and attest to the accuracy and truthfulness of my foregoing statements, analysis and attachments or references hereto:

_____/S/_____

MAJ Samuel N. Sigoloff, DO FM

State of Arizona §
§
County of Cochise §

The undersigned, being duly sworn, deposes and says:
I, Major Samuel N Sigoloff, DO, declare under the penalty of perjury of the laws of the United States of America, and state upon personal knowledge that:
I am an adult of sound mind, 36 years old, and declare that the information herein is true, correct and complete and that I have voluntarily affirmed this affidavit based upon my own personal knowledge, education, and experience, and under the penalty of perjury of the laws of the United States of America.

SUBSCRIBED AND SWORN TO BEFORE ME on the 21st day of _September 22, 2021,
to certify which witness my hand and official seal.
Notary Public for the State of Arizona

My Commission Expires:
___Jonathan Smith_____ Expires: June 10, 2024
Notary Public

EXHIBIT 5
A Sigoloff CV

Samuel N. Sigoloff, DO
4290 S. Silva, Sierra Vista, AZ 85650
210-872-1357, Samuel.Sigoloff@1791.com

Employment

Raymond W. Bliss Army Health Clinic, Ft Huachuca	2021-presnt
Bassett Army Community Hospital, Ft Wainwright, AK	2018-2021
Adjunct professor for Basic EMT course with Central Texas College, At Camp Buehring, Kuwait	2017
Reynolds Army Health Clinic, Ft Sill, OK	2015-2018

Education

Family Medicine Residency at Martin Army Community Hospital, Ft Benning, GA	2012-2015
Ohio University Heritage College of Osteopathic Medicine	2007-2012
Saint Mary's University - San Antonio, Texas Bachelor of Arts Degree in Biology and Minor in Military Science	2003-2007

Licensure and Certification

American Board of Family Medicine, Diplomate ABFM ID: 164257	2015-present
Medical License, Nebraska License number 1149	2013-2021
Medical License, Texas License number S3747	2019-present

Publication

Adams, J.R & Layton, M.C. & Sigoloff, S.N.. (2015). "Folliculitis". In F. Domino (Ed.), *The 5-Minute Clinical Consult Standard 2016.* Philadelphia, PA: Wolters Kulwer.

Leadership

Medical Director, Raymond W. Bliss Army Health Clinic, Ft Huachuca	2021-present
Medical Director, Camp Buehring, Kuwait	2017-2018
Chief of Patient Centered Medical Home, Ft Sill OK	2016-2017
Clinical Pharm D OIC, Ft Sill OK	2015-2016
Association of Military Osteopathic Physicians and Surgeons (AMOPS) Ohio University Heritage College of Medicine Chapter President	2008-2010
Medical Students for Life OMSI Liaison	2007-2008
College Cadet Battalion Training Officer for ROTC	2007
College Cadet Battalion Commander for Reserve Officer Training Corps ROTC Saint Mary's University	2006
College Cadet Sergeant Major for ROTC	2005
College Ranger Challenge Team Captain	2005
Eagle Scout Project	2002

Community Service

Men's Health Fair, Camp Buehring, Kuwait	2017
TCCC training of swat team in La Grange GA	2015
Shaw High School physical, Columbus GA	2015
Health Fair, Ft Benning GA	2014
EFMP Olympics, Ft Benning GA	2014
Shaw High school physical, Columbus GA	2013
No smoking campaign at Faith Middle School, Ft Benning GA	2012

Military Training

Joint Forces Combat Trauma Management Course	2017
Basic Airborne Course, Class 21-14	2014
Combat Casualty Care Course	2012
Medical Corps Officer Basic Course	2008

EXHIBIT 5
B Comirnaty
Pkg. Insert

appendix 1

Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use COMIRNATY safely and effectively. See full prescribing information for COMIRNATY.

COMIRNATY® (COVID-19 Vaccine, mRNA) suspension for injection, for intramuscular use
Initial U.S. Approval: 2021

---------- INDICATIONS AND USAGE----------
COMIRNATY is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 16 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------
- For intramuscular injection only. (2.2)
- COMIRNATY is administered intramuscularly as a series of 2 doses (0.3 mL each) 3 weeks apart. (2.3)

---------- DOSAGE FORMS AND STRENGTHS----------
Suspension for injection. After preparation, a single dose is 0.3 mL. (3)

---------- CONTRAINDICATIONS ----------
Known history of a severe allergic reaction (e.g., anaphylaxis) to any component of COMIRNATY. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. (5.2)
- Syncope (fainting) may occur in association with administration of injectable vaccines, including COMIRNATY. Procedures should be in place to avoid injury from fainting. (5.4)

---------- ADVERSE REACTIONS ----------
- In clinical studies of participants 16 through 55 years of age, the most commonly reported adverse reactions (≥10%) were pain at the injection site (88.6%), fatigue (70.1%), headache (64.9%), muscle pain (45.5%), chills (41.5%), joint pain (27.5%), fever (17.8%), and injection site swelling (10.6%). (6.1)
- In clinical studies of participants 56 years of age and older, the most commonly reported adverse reactions (≥10%) were pain at the injection site (78.2%), fatigue (56.9%), headache, (45.9%), muscle pain (32.5%), chills (24.8%), joint pain (21.5%), injection site swelling (11.8%), fever (11.5%), and injection site redness (10.4%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc. at 1-800-438-1985 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 8/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

COMIRNATY is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 16 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Preparation for Administration

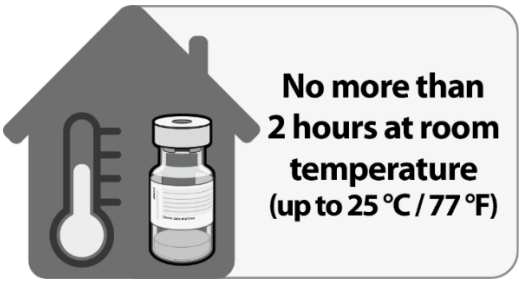
Prior to Dilution

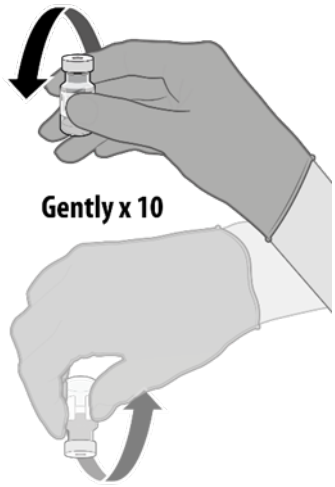
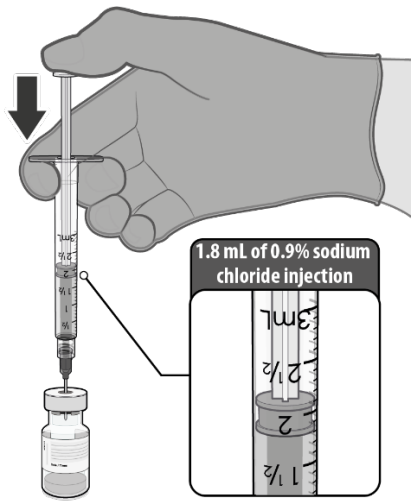
- COMIRNATY Multiple Dose Vial contains a volume of 0.45 mL, supplied as a frozen suspension that does not contain preservative. Each vial must be thawed and diluted prior to administration.
- Vials may be thawed in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)] or at room temperature [up to 25ºC (77ºF)] *[see How Supplied/Storage and Handling (16)]*.
- Refer to thawing instructions in the panels below.

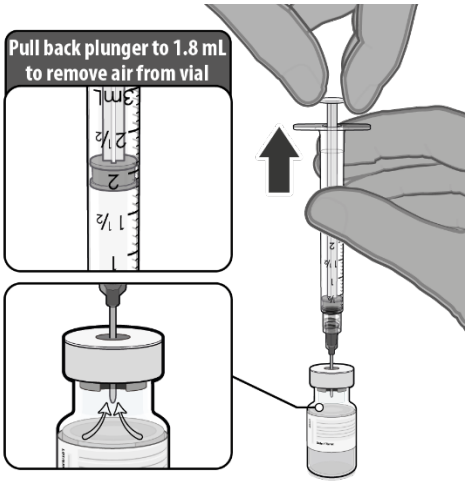
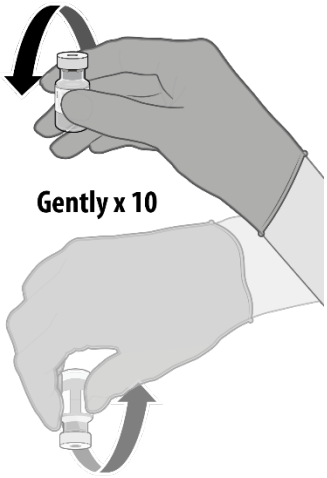
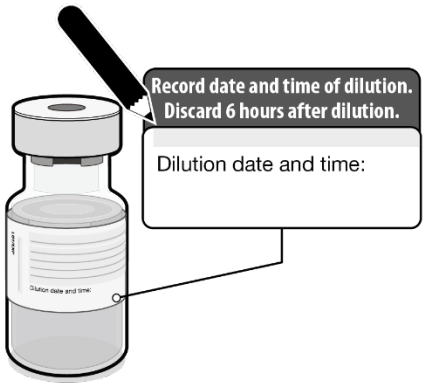
Dilution

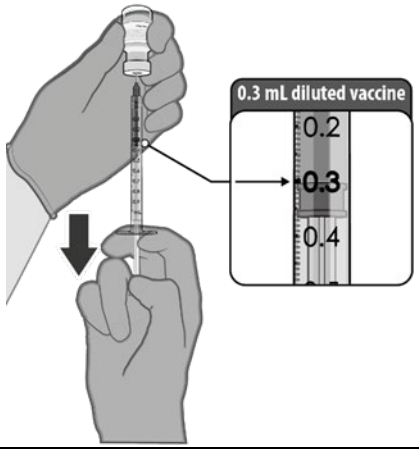
- Dilute the vial contents using 1.8 mL of sterile 0.9% Sodium Chloride Injection, USP to form COMIRNATY. Do not add more than 1.8 mL of diluent.
- ONLY use sterile 0.9% Sodium Chloride Injection, USP as the diluent. Do not use bacteriostatic 0.9% Sodium Chloride Injection or any other diluent.
- Vials of sterile 0.9% Sodium Chloride Injection, USP are provided but shipped separately. Use the provided diluent or another sterile 0.9% Sodium Chloride Injection, USP as the diluent.
    - Provided diluent vials are single-use only; discard after 1.8 mL is withdrawn.
    - If another sterile 0.9% Sodium Chloride Injection, USP is used as the diluent, discard after 1.8 mL is withdrawn.
    - Do not dilute more than 1 vial of COMIRNATY using the same diluent vial.
- After dilution, 1 vial of COMIRNATY contains 6 doses of 0.3 mL each.
- Refer to dilution and dose preparation instructions in the panels below.

THAWING PRIOR TO DILUTION	

	• Thaw vial(s) of COMIRNATY before dilution either by:
	  ○ Allowing vial(s) to thaw in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)]. A carton of vials may take up to 3 hours to thaw, and thawed vials can be stored in the refrigerator for up to 1 month.
	  ○ Allowing vial(s) to sit at room temperature [up to 25ºC (77ºF)] for 30 minutes.
	• Using either thawing method, vials must reach room temperature before dilution and must be diluted within 2 hours.

	• Before dilution invert vaccine vial gently 10 times. • <u>Do not shake.</u> • Inspect the liquid in the vaccine vial prior to dilution. The liquid is a white to off-white suspension and may contain white to off-white opaque amorphous particles. • Do not use if liquid is discolored or if other particles are observed.
DILUTION	
	• ONLY use sterile 0.9% Sodium Chloride Injection, USP as the diluent. • Withdraw 1.8 mL of diluent into a transfer syringe (21-gauge or narrower needle). • Add 1.8 mL of sterile 0.9% Sodium Chloride Injection, USP into the vaccine vial.

	• Equalize vial pressure before removing the needle from the vaccine vial by withdrawing 1.8 mL air into the empty diluent syringe.
	• Gently invert the vial containing COMIRNATY 10 times to mix. • Do not shake. • Inspect the vaccine in the vial. • The vaccine will be an off-white suspension. Do not use if vaccine is discolored or contains particulate matter.
	• Record the date and time of dilution on the COMIRNATY vial label. • Store between 2°C to 25°C (35°F to 77°F). • Discard any unused vaccine 6 hours after dilution.

PREPARATION OF INDIVIDUAL 0.3 mL DOSES OF COMIRNATY	
	• Withdraw 0.3 mL of COMIRNATY preferentially using low dead-volume syringes and/or needles. • Each dose must contain 0.3 mL of vaccine. • If the amount of vaccine remaining in a single vial cannot provide a full dose of 0.3 mL, discard the vial and any excess volume. • Administer immediately.

After dilution, vials of COMIRNATY contain 6 doses of 0.3 mL of vaccine. Low dead-volume syringes and/or needles can be used to extract 6 doses from a single vial. If standard syringes and needles are used, there may not be sufficient volume to extract a sixth dose from a single vial. Irrespective of the type of syringe and needle,

- each dose must contain 0.3 mL of vaccine.
- if the amount of vaccine remaining in the vial cannot provide a full dose of 0.3 mL, discard the vial and any excess volume.
- do not pool excess vaccine from multiple vials.

2.2 Administration Information

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The vaccine will be an off-white suspension. Do not administer if vaccine is discolored or contains particulate matter.

Administer a single 0.3 mL dose of COMIRNATY intramuscularly.

2.3 Vaccination Schedule

COMIRNATY is administered intramuscularly as a series of 2 doses (0.3 mL each) 3 weeks apart.

There are no data available on the interchangeability of COMIRNATY with other COVID-19 vaccines to complete the vaccination series. Individuals who have received 1 dose of COMIRNATY should receive a second dose of COMIRNATY to complete the vaccination series.

3 DOSAGE FORMS AND STRENGTHS

COMIRNATY is a suspension for injection. After preparation, a single dose is 0.3 mL.

4 CONTRAINDICATIONS

Do not administer COMIRNATY to individuals with known history of a severe allergic reaction (e.g., anaphylaxis) to any component of the COMIRNATY *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment used to manage immediate allergic reactions must be immediately available in the event an acute anaphylactic reaction occurs following administration of COMIRNATY.

5.2 Myocarditis and Pericarditis

Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. The observed risk is higher among males under 40 years of age than among females and older males. The observed risk is highest in males 12 through 17 years of age. Although some cases required intensive care support, available data from short-term follow-up suggest that most individuals have had resolution of symptoms with conservative management. Information is not yet available about potential long-term sequelae. The CDC has published considerations related to myocarditis and pericarditis after vaccination, including for vaccination of individuals with a history of myocarditis or pericarditis (https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html).

5.3 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines, including COMIRNATY. Procedures should be in place to avoid injury from fainting.

5.4 Altered Immunocompetence

Immunocompromised persons, including individuals receiving immunosuppressant therapy, may have a diminished immune response to the COMIRNATY.

5.5 Limitation of Effectiveness

COMIRNATY may not protect all vaccine recipients.

6 ADVERSE REACTIONS

In clinical studies, the most commonly reported (≥10%) adverse reactions in participants 16 through 55 years of age following any dose were pain at the injection site (88.6%), fatigue (70.1%), headache (64.9%), muscle pain (45.5%), chills (41.5%), joint pain (27.5%), fever (17.8%), and injection site swelling (10.6%).

In clinical studies, the most commonly reported (≥10%) adverse reactions in participants 56 years of age and older following any dose were pain at the injection site (78.2%), fatigue (56.9%), headache, (45.9%), muscle pain (32.5%), chills (24.8%), joint pain (21.5%), injection site swelling (11.8%), fever (11.5%), and injection site redness (10.4%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of COMIRNATY was evaluated in participants 16 years of age and older in 2 clinical studies conducted in Germany (Study 1), United States, Argentina, Brazil, Turkey, South Africa, and Germany (Study 2). Study BNT162-01 (Study 1) was a Phase 2-part, dose-escalation trial that enrolled 60 participants, 18 through 55 years of age and 36 participants, 56 through 85 years of age. Study C4591001 (Study 2) is a Phase 1/2/3 multicenter, multinational, randomized, saline placebo-controlled, double-blinded (Phase 2/3), dose-finding, vaccine candidate-selection and efficacy study that has enrolled approximately 44,047 participants (22,026 COMIRNATY; 22,021 placebo) 16 years of age or older (including 378 and 376 participants 16 through 17 years of age in the vaccine and placebo groups, respectively). Upon issuance of the Emergency Use Authorization (December 11, 2020) for COMIRNATY, participants were unblinded to offer placebo participants COMIRNATY. Participants were unblinded in a phased manner over a period of months to offer placebo participants COMIRNATY. Study 2 also included 200 participants with confirmed stable human immunodeficiency virus (HIV) infection; HIV-positive participants are included in safety population disposition but are summarized separately in safety analyses. Confirmed stable HIV infection was defined as documented viral load <50 copies/mL and CD4 count >200 cells/mm^3 within 6 months before enrollment, and on stable antiretroviral therapy for at least 6 months.

At the time of the analysis of the ongoing Study 2 with a data cut-off of March 13, 2021, there were 25,651 (58.2%) participants (13,031 COMIRNATY and 12,620 placebo) 16 years of age and older followed for ≥4 months after the second dose.

Participants 16 years and older in the reactogenicity subset were monitored for solicited local and systemic reactions and use of antipyretic medication after each vaccination in an electronic diary. Participants are being monitored for unsolicited adverse events, including serious adverse events, throughout the study [from Dose 1 through 1 month (all unsolicited adverse events) or 6 months (serious adverse events) after the last vaccination].

Demographic characteristics in Study 2 were generally similar with regard to age, gender, race, and ethnicity among participants who received COMIRNATY and those who received placebo. Overall, among the total participants who received either COMIRNATY or placebo, 50.9% were male, 49.1% were female, 79.3% were 16 through 64 years of age, 20.7% were 65 years of age and older, 82.0% were White, 9.6% were Black or African American, 25.9% were Hispanic/Latino, 4.3% were Asian, and 1.0% were American Indian or Alaska Native.

Local and Systemic Adverse Reactions Solicited in the Study 2

Table 1 and Table 2 present the frequency and severity of reported solicited local and systemic reactions, respectively, within 7 days following each dose of COMIRNATY and placebo in the subset of participants 16 through 55 years of age included in the safety population who were monitored for reactogenicity with an electronic diary.

Table 3 and Table 4 present the frequency and severity of reported solicited local and systemic reactions, respectively, within 7 days of each dose of COMIRNATY and placebo for participants 56 years of age and older.

In participants 16 through 55 years of age after receiving Dose 2, the mean duration of pain at the injection site was 2.5 days (range 1 to 70 days), for redness 2.2 days (range 1 to 9 days), and for swelling 2.1 days (range 1 to 8 days) for participants in the COMIRNATY group. In participants 56 years of age and older after receiving Dose 2, the mean duration of pain at the injection site was 2.4 days (range 1 to 36 days), for redness 3.0 days (range 1 to 34 days), and for swelling 2.6 days (range 1 to 34 days) for participants in the COMIRNATY group.

Table 1: Study 2 – Frequency and Percentages of Participants with Solicited Local Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 16 Through 55 Years of Age – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	Placebo Dose 1 N[a]=2908 n[b] (%)	COMIRNATY Dose 2 N[a]=2682 n[b] (%)	Placebo Dose 2 N[a]=2684 n[b] (%)
Redness[c]				
Any (>2.0 cm)	156 (5.4)	28 (1.0)	151 (5.6)	18 (0.7)
Mild	113 (3.9)	19 (0.7)	90 (3.4)	12 (0.4)
Moderate	36 (1.2)	6 (0.2)	50 (1.9)	6 (0.2)
Severe	7 (0.2)	3 (0.1)	11 (0.4)	0
Swelling[c]				
Any (>2.0 cm)	184 (6.3)	16 (0.6)	183 (6.8)	5 (0.2)
Mild	124 (4.3)	6 (0.2)	110 (4.1)	3 (0.1)
Moderate	54 (1.9)	8 (0.3)	66 (2.5)	2 (0.1)
Severe	6 (0.2)	2 (0.1)	7 (0.3)	0
Pain at the injection site[d]				
Any	2426 (83.7)	414 (14.2)	2101 (78.3)	312 (11.6)
Mild	1464 (50.5)	391 (13.4)	1274 (47.5)	284 (10.6)
Moderate	923 (31.8)	20 (0.7)	788 (29.4)	28 (1.0)
Severe	39 (1.3)	3 (0.1)	39 (1.5)	0

Notes: Reactions were collected in the electronic diary (e-diary) from Day 1 to Day 7 after vaccination.
No Grade 4 solicited local reactions were reported in participants 16 through 55 years of age.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction was the same, therefore, this information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: >2.0 to ≤5.0 cm; Moderate: >5.0 to ≤10.0 cm; Severe: >10.0 cm.

d. Mild: does not interfere with activity; Moderate: interferes with activity; Severe: prevents daily activity.

Table 2: Study 2 – Frequency and Percentages of Participants with Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 16 Through 55 Years of Age – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	Placebo Dose 1 N[a]=2908 n[b] (%)	COMIRNATY Dose 2 N[a]=2682 n[b] (%)	Placebo Dose 2 N[a]=2684 n[b] (%)
Fever				
≥38.0°C	119 (4.1)	25 (0.9)	440 (16.4)	11 (0.4)
≥38.0°C to 38.4°C	86 (3.0)	16 (0.6)	254 (9.5)	5 (0.2)
>38.4°C to 38.9°C	25 (0.9)	5 (0.2)	146 (5.4)	4 (0.1)
>38.9°C to 40.0°C	8 (0.3)	4 (0.1)	39 (1.5)	2 (0.1)
>40.0°C	0	0	1 (0.0)	0
Fatigue[c]				
Any	1431 (49.4)	960 (33.0)	1649 (61.5)	614 (22.9)
Mild	760 (26.2)	570 (19.6)	558 (20.8)	317 (11.8)
Moderate	630 (21.7)	372 (12.8)	949 (35.4)	283 (10.5)
Severe	41 (1.4)	18 (0.6)	142 (5.3)	14 (0.5)

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	Placebo Dose 1 N[a]=2908 n[b] (%)	COMIRNATY Dose 2 N[a]=2682 n[b] (%)	Placebo Dose 2 N[a]=2684 n[b] (%)
Headache[c]				
Any	1262 (43.5)	975 (33.5)	1448 (54.0)	652 (24.3)
Mild	785 (27.1)	633 (21.8)	699 (26.1)	404 (15.1)
Moderate	444 (15.3)	318 (10.9)	658 (24.5)	230 (8.6)
Severe	33 (1.1)	24 (0.8)	91 (3.4)	18 (0.7)
Chills[c]				
Any	479 (16.5)	199 (6.8)	1015 (37.8)	114 (4.2)
Mild	338 (11.7)	148 (5.1)	477 (17.8)	89 (3.3)
Moderate	126 (4.3)	49 (1.7)	469 (17.5)	23 (0.9)
Severe	15 (0.5)	2 (0.1)	69 (2.6)	2 (0.1)
Vomiting[d]				
Any	34 (1.2)	36 (1.2)	58 (2.2)	30 (1.1)
Mild	29 (1.0)	30 (1.0)	42 (1.6)	20 (0.7)
Moderate	5 (0.2)	5 (0.2)	12 (0.4)	10 (0.4)
Severe	0	1 (0.0)	4 (0.1)	0
Diarrhea[e]				
Any	309 (10.7)	323 (11.1)	269 (10.0)	205 (7.6)
Mild	251 (8.7)	264 (9.1)	219 (8.2)	169 (6.3)
Moderate	55 (1.9)	58 (2.0)	44 (1.6)	35 (1.3)
Severe	3 (0.1)	1 (0.0)	6 (0.2)	1 (0.0)
New or worsened muscle pain[c]				
Any	664 (22.9)	329 (11.3)	1055 (39.3)	237 (8.8)
Mild	353 (12.2)	231 (7.9)	441 (16.4)	150 (5.6)
Moderate	296 (10.2)	96 (3.3)	552 (20.6)	84 (3.1)
Severe	15 (0.5)	2 (0.1)	62 (2.3)	3 (0.1)
New or worsened joint pain[c]				
Any	342 (11.8)	168 (5.8)	638 (23.8)	147 (5.5)
Mild	200 (6.9)	112 (3.9)	291 (10.9)	82 (3.1)
Moderate	137 (4.7)	55 (1.9)	320 (11.9)	61 (2.3)
Severe	5 (0.2)	1 (0.0)	27 (1.0)	4 (0.1)
Use of antipyretic or pain medication[f]	805 (27.8)	398 (13.7)	1213 (45.2)	320 (11.9)

Notes: Reactions and use of antipyretic or pain medication were collected in the electronic diary (e-diary) from Day 1 to Day 7 after each dose.

No Grade 4 solicited systemic reactions were reported in participants 16 through 55 years of age.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction or use of antipyretic or pain medication was the same, therefore, this information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: does not interfere with activity; Moderate: some interference with activity; Severe: prevents daily activity.

d. Mild: 1 to 2 times in 24 hours; Moderate: >2 times in 24 hours; Severe: requires intravenous hydration.

e. Mild: 2 to 3 loose stools in 24 hours; Moderate: 4 to 5 loose stools in 24 hours; Severe: 6 or more loose stools in 24 hours.

f. Severity was not collected for use of antipyretic or pain medication.

Table 3: Study 2 – Frequency and Percentages of Participants with Solicited Local Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 56 Years of Age and Older – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
Redness[c]				
Any (>2.0 cm)	106 (5.3)	20 (1.0)	133 (7.2)	14 (0.8)
Mild	71 (3.5)	13 (0.7)	65 (3.5)	10 (0.5)
Moderate	30 (1.5)	5 (0.3)	58 (3.1)	3 (0.2)
Severe	5 (0.2)	2 (0.1)	10 (0.5)	1 (0.1)
Swelling[c]				
Any (>2.0 cm)	141 (7.0)	23 (1.2)	145 (7.8)	13 (0.7)
Mild	87 (4.3)	11 (0.6)	80 (4.3)	5 (0.3)
Moderate	52 (2.6)	12 (0.6)	61 (3.3)	7 (0.4)
Severe	2 (0.1)	0	4 (0.2)	1 (0.1)
Pain at the injection site[d]				
Any (>2.0 cm)	1408 (70.1)	185 (9.3)	1230 (66.1)	143 (7.8)
Mild	1108 (55.2)	177 (8.9)	873 (46.9)	138 (7.5)
Moderate	296 (14.7)	8 (0.4)	347 (18.7)	5 (0.3)
Severe	4 (0.2)	0	10 (0.5)	0

Notes: Reactions were collected in the electronic diary (e-diary) from Day 1 to Day 7 after vaccination.
No Grade 4 solicited local reactions were reported in participants 56 years of age and older.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction was the same, therefore, the information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: >2.0 to ≤5.0 cm; Moderate: >5.0 to ≤10.0 cm; Severe: >10.0 cm.

d. Mild: does not interfere with activity; Moderate: interferes with activity; Severe: prevents daily activity.

Table 4: Study 2 – Frequency and Percentages of Participants with Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 56 Years of Age and Older – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
Fever				
≥38.0°C	26 (1.3)	8 (0.4)	219 (11.8)	4 (0.2)
≥38.0°C to 38.4°C	23 (1.1)	3 (0.2)	158 (8.5)	2 (0.1)
>38.4°C to 38.9°C	2 (0.1)	3 (0.2)	54 (2.9)	1 (0.1)
>38.9°C to 40.0°C	1 (0.0)	2 (0.1)	7 (0.4)	1 (0.1)
>40.0°C	0	0	0	0

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
Fatigue[c]				
Any	677 (33.7)	447 (22.5)	949 (51.0)	306 (16.7)
Mild	415 (20.7)	281 (14.1)	391 (21.0)	183 (10.0)
Moderate	259 (12.9)	163 (8.2)	497 (26.7)	121 (6.6)
Severe	3 (0.1)	3 (0.2)	60 (3.2)	2 (0.1)
Grade 4	0	0	1 (0.1)	0
Headache[c]				
Any	503 (25.0)	363 (18.3)	733 (39.4)	259 (14.1)
Mild	381 (19.0)	267 (13.4)	464 (24.9)	189 (10.3)
Moderate	120 (6.0)	93 (4.7)	256 (13.8)	65 (3.5)
Severe	2 (0.1)	3 (0.2)	13 (0.7)	5 (0.3)
Chills[c]				
Any	130 (6.5)	69 (3.5)	435 (23.4)	57 (3.1)
Mild	102 (5.1)	49 (2.5)	229 (12.3)	45 (2.5)
Moderate	28 (1.4)	19 (1.0)	185 (9.9)	12 (0.7)
Severe	0	1 (0.1)	21 (1.1)	0
Vomiting[d]				
Any	10 (0.5)	9 (0.5)	13 (0.7)	5 (0.3)
Mild	9 (0.4)	9 (0.5)	10 (0.5)	5 (0.3)
Moderate	1 (0.0)	0	1 (0.1)	0
Severe	0	0	2 (0.1)	0
Diarrhea[e]				
Any	168 (8.4)	130 (6.5)	152 (8.2)	102 (5.6)
Mild	137 (6.8)	109 (5.5)	125 (6.7)	76 (4.1)
Moderate	27 (1.3)	20 (1.0)	25 (1.3)	22 (1.2)
Severe	4 (0.2)	1 (0.1)	2 (0.1)	4 (0.2)
New or worsened muscle pain[c]				
Any	274 (13.6)	165 (8.3)	537 (28.9)	99 (5.4)
Mild	183 (9.1)	111 (5.6)	229 (12.3)	65 (3.5)
Moderate	90 (4.5)	51 (2.6)	288 (15.5)	33 (1.8)
Severe	1 (0.0)	3 (0.2)	20 (1.1)	1 (0.1)
New or worsened joint pain[c]				
Any	175 (8.7)	124 (6.2)	353 (19.0)	72 (3.9)
Mild	119 (5.9)	78 (3.9)	183 (9.8)	44 (2.4)
Moderate	53 (2.6)	45 (2.3)	161 (8.7)	27 (1.5)
Severe	3 (0.1)	1 (0.1)	9 (0.5)	1 (0.1)
Use of antipyretic or pain medication[f]	382 (19.0)	224 (11.3)	688 (37.0)	170 (9.3)

Notes: Reactions and use of antipyretic or pain medication were collected in the electronic diary (e-diary) from Day 1 to Day 7 after each dose.

The only Grade 4 solicited systemic reaction reported in participants 56 years of age and older was fatigue.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. N for each reaction or use of antipyretic or pain medication was the same, therefore was included in the column header.

b. n = Number of participants with the specified reaction.

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)

c. Mild: does not interfere with activity; Moderate: some interference with activity; Severe: prevents daily activity; Grade 4 reactions were defined in the clinical study protocol as emergency room visit or hospitalization for severe fatigue, severe headache, severe chills, severe muscle pain, or severe joint pain.
d. Mild: 1 to 2 times in 24 hours; Moderate: >2 times in 24 hours; Severe: requires intravenous hydration; Grade 4 emergency visit or hospitalization for severe vomiting.
e. Mild: 2 to 3 loose stools in 24 hours; Moderate: 4 to 5 loose stools in 24 hours; Severe: 6 or more loose stools in 24 hours; Grade 4: emergency room or hospitalization for severe diarrhea.
f. Severity was not collected for use of antipyretic or pain medication.

In participants with chronic, stable HIV infection the frequencies of solicited local and systemic adverse reactions were similar to or lower than those observed for all participants 16 years of age and older.

Unsolicited Adverse Events

Overall, 11,253 (51.1%) participants in the COMIRNATY group and 11,316 (51.4%) participants in the placebo group had follow-up time between ≥4 months to <6 months after Dose 2 in the blinded placebo-controlled follow-up period with an additional 1,778 (8.1%) and 1,304 (5.9%) with ≥6 months of blinded follow-up time in the COMIRNATY and placebo groups, respectively.

A total of 12,006 (54.5%) participants originally randomized to COMIRNATY had ≥6 months total (blinded and unblinded) follow-up after Dose 2.

In an analysis of all unsolicited adverse events reported following any dose, through 1 month after Dose 2, in participants 16 years of age and older (N=43,847; 21,926 COMIRNATY group vs. 21,921 placebo group), those assessed as adverse reactions not already captured by solicited local and systemic reactions were nausea (274 vs. 87), malaise (130 vs. 22), lymphadenopathy (83 vs. 7), asthenia (76 vs. 25), decreased appetite (39 vs. 9), hyperhidrosis (31 vs. 9), lethargy (25 vs. 6), and night sweats (17 vs. 3).

In analyses of all unsolicited adverse events in Study 2 from Dose 1 up to the participant unblinding date, 58.2% of study participants had at least 4 months of follow-up after Dose 2. Among participants 16 through 55 years of age who received at least one dose of study vaccine, 12,995 of whom received COMIRNATY and 13,026 of whom received placebo, unsolicited adverse events were reported by 4,396 (33.8%) participants in the COMIRNATY group and 2,136 (16.4%) participants in the placebo group. In a similar analysis in participants 56 years of age and older that included 8,931 COMIRNATY recipients and 8,895 placebo recipients, unsolicited adverse events were reported by 2,551 (28.6%) participants in the COMIRNATY group and 1,432 (16.1%) participants in the placebo group. Among participants with confirmed stable HIV infection that included 100 COMIRNATY recipients and 100 placebo recipients, unsolicited adverse events were reported by 29 (29%) participants in the COMIRNATY group and 15 (15%) participants in the placebo group. The higher frequency of reported unsolicited adverse events among COMIRNATY recipients compared to placebo recipients was primarily attributed to events that are consistent with adverse reactions solicited among participants in the reactogenicity subset (Table 3 and Table 4).

Throughout the placebo-controlled safety follow-up period, Bell's palsy (facial paralysis) was reported by 4 participants in the COMIRNATY group and 2 participants in the placebo group. Onset of facial paralysis was Day 37 after Dose 1 (participant did not receive Dose 2) and Days 3, 9, and 48 after Dose 2. In the placebo group the onset of facial paralysis was Day 32 and Day 102. Currently available information is insufficient to determine a causal relationship with the vaccine. In the analysis of blinded, placebo-controlled follow-up, there

were no other notable patterns or numerical imbalances between treatment groups for specific categories of non-serious adverse events (including other neurologic or neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of non-serious adverse events that would suggest a causal relationship to COMIRNATY.

Serious Adverse Events

In Study 2, among participants 16 through 55 years of age who had received at least 1 dose of vaccine or placebo (COMIRNATY =12,995; placebo = 13,026), serious adverse events from Dose 1 up to the participant unblinding date in ongoing follow-up were reported by 103 (0.8%) COMIRNATY recipients and 117 (0.9%) placebo recipients. In a similar analysis, in participants 56 years of age and older (COMIRNATY = 8,931; placebo = 8,895), serious adverse events were reported by 165 (1.8%) COMIRNATY recipients and 151 (1.7%) placebo recipients who received at least 1 dose of COMIRNATY or placebo, respectively. In these analyses, 58.2% of study participants had at least 4 months of follow-up after Dose 2. Among participants with confirmed stable HIV infection serious adverse events from Dose 1 up to the participant unblinding date in ongoing follow-up were reported by 2 (2%) COMIRNATY recipients and 2 (2%) placebo recipients.

In the analysis of blinded, placebo-controlled follow-up, there were no notable patterns between treatment groups for specific categories of serious adverse events (including neurologic, neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of serious adverse events that would suggest a causal relationship to COMIRNATY.

6.2 Postmarketing Experience

The following adverse reactions have been identified during postmarketing use of COMIRNATY, including under Emergency Use Authorization. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Cardiac Disorders: myocarditis, pericarditis
Gastrointestinal Disorders: diarrhea, vomiting
Immune System Disorders: severe allergic reactions, including anaphylaxis, and other hypersensitivity reactions (e.g., rash, pruritus, urticaria, angioedema)
Musculoskeletal and Connective Tissue Disorders: pain in extremity (arm)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to COMIRNATY during pregnancy. Women who are vaccinated with COMIRNATY during pregnancy are encouraged to enroll in the registry by visiting https://mothertobaby.org/ongoing-study/covid19-vaccines/.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to

4% and 15% to 20%, respectively. Available data on COMIRNATY administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study has been performed in female rats administered the equivalent of a single human dose of COMIRNATY on 4 occasions; twice prior to mating and twice during gestation. These studies revealed no evidence of harm to the fetus due to the vaccine *(see Animal Data)*.

Data

Animal Data

In a developmental toxicity study, 0.06 mL of a vaccine formulation containing the same quantity of nucleoside-modified messenger ribonucleic acid (mRNA) (30 mcg) and other ingredients included in a single human dose of COMIRNATY was administered to female rats by the intramuscular route on 4 occasions: 21 and 14 days prior to mating, and on gestation days 9 and 20. No vaccine-related adverse effects on female fertility, fetal development, or postnatal development were reported in the study.

8.2 Lactation

Risk Summary

It is not known whether COMIRNATY is excreted in human milk. Data are not available to assess the effects of COMIRNATY on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for COMIRNATY and any potential adverse effects on the breastfed child from COMIRNATY or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of COMIRNATY in individuals 16 through 17 years of age is based on safety and effectiveness data in this age group and in adults *[see Adverse Reactions (6) and Clinical Studies (14.1)]*.

The safety and effectiveness of COMIRNATY in individuals younger than 16 years of age have not been established.

8.5 Geriatric Use

Of the total number of COMIRNATY recipients in Study 2 as of March 13, 2021 (N = 22,026), 20.7% (n = 4,552) were 65 years of age and older and 4.2% (n = 925) were 75 years of age and older *[see Clinical Studies (14.1)]*. No overall differences in safety or effectiveness were observed between these recipients and younger recipients.

11 DESCRIPTION

COMIRNATY (COVID-19 Vaccine, mRNA) is a sterile suspension for injection for intramuscular use. COMIRNATY is supplied as a frozen suspension in multiple dose vials; each vial must be diluted with 1.8 mL of sterile 0.9% Sodium Chloride Injection, USP prior to use to form the vaccine. Each dose of COMIRNATY contains 30 mcg of a nucleoside-modified messenger RNA (mRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2.

Each 0.3 mL dose of the COMIRNATY also includes the following ingredients: lipids (0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 0.05 mg 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.2 mg cholesterol), 0.01 mg potassium chloride, 0.01 mg monobasic potassium phosphate, 0.36 mg sodium chloride, 0.07 mg dibasic sodium phosphate dihydrate, and 6 mg sucrose. The diluent (0.9% Sodium Chloride Injection, USP) contributes an additional 2.16 mg sodium chloride per dose.

COMIRNATY does not contain preservative.

The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The nucleoside-modified mRNA in COMIRNATY is formulated in lipid particles, which enable delivery of the mRNA into host cells to allow expression of the SARS-CoV-2 S antigen. The vaccine elicits an immune response to the S antigen, which protects against COVID-19.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

COMIRNATY has not been evaluated for the potential to cause carcinogenicity, genotoxicity, or impairment of male fertility. In a developmental toxicity study in rats with COMIRNATY there were no vaccine-related effects on female fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

Efficacy in Participants 16 Years of Age and Older

Study 2 is an ongoing, multicenter, multinational, randomized, placebo-controlled, observer-blind, dose-finding, vaccine candidate–selection, and efficacy study in participants 12 years of age and older. Randomization was stratified by age: 12 through 15 years of age, 16 through 55 years of age, or 56 years of age and older, with a minimum of 40% of participants in the ≥56-year stratum. The study excluded participants who were immunocompromised and those who had previous clinical or microbiological diagnosis of COVID-19. Participants with preexisting stable disease, defined as disease not requiring significant change in therapy or hospitalization for worsening disease during the 6 weeks before enrollment, were included as were participants with known stable infection with HIV, hepatitis C virus (HCV), or hepatitis B virus (HBV).

In Study 2, based on data accrued through March 13, 2021, approximately 44,000 participants 16 years of age and older were randomized equally and received 2 doses of COMIRNATY or placebo. Participants are planned to be followed for up to 24 months, for assessments of safety and efficacy against COVID-19.

Overall, among the total participants who received COMIRNATY or placebo, 51.4% or 50.3% were male and 48.6% or 49.7% were female, 79.1% or 79.2% were 16 through 64 years of age, 20.9% or 20.8% were 65 years of age and older, 81.9% or 82.1% were White, 9.5% or 9.6% were Black or African American, 1.0% or 0.9% were American Indian or Alaska Native, 4.4% or 4.3% were Asian, 0.3% or 0.2% Native Hawaiian or other Pacific Islander, 25.6% or 25.4% were Hispanic/Latino, 73.9% or 74.1% were non-Hispanic/Latino, 0.5% or 0.5% did not report ethnicity, 46.0% or 45.7% had comorbidities [participants who have 1 or more

comorbidities that increase the risk of severe COVID-19 disease: defined as subjects who had at least one of the Charlson comorbidity index category or body mass index (BMI) ≥30 kg/m^2], respectively. The mean age at vaccination was 49.8 or 49.7 years and median age was 51.0 or 51.0 in participants who received COMIRNATY or placebo, respectively.

Efficacy Against COVID-19

The population for the analysis of the protocol pre-specified primary efficacy endpoint included 36,621 participants 12 years of age and older (18,242 in the COMIRNATY group and 18,379 in the placebo group) who did not have evidence of prior infection with SARS-CoV-2 through 7 days after the second dose. The population in the protocol pre-specified primary efficacy analysis included all participants 12 years of age and older who had been enrolled from July 27, 2020, and followed for the development of COVID-19 through November 14, 2020. Participants 18 through 55 years of age and 56 years of age and older began enrollment from July 27, 2020, 16 through 17 years of age began enrollment from September 16, 2020, and 12 through 15 years of age began enrollment from October 15, 2020.

For participants without evidence of SARS-CoV-2 infection prior to 7 days after Dose 2, vaccine efficacy against confirmed COVID-19 occurring at least 7 days after Dose 2 was 95.0% (95% credible interval: 90.3, 97.6), which met the pre-specified success criterion. The case split was 8 COVID-19 cases in the COMIRNATY group compared to 162 COVID-19 cases in the placebo group.

The population for the updated vaccine efficacy analysis included participants 16 years of age and older who had been enrolled from July 27, 2020, and followed for the development of COVID-19 during blinded placebo-controlled follow-up through March 13, 2021, representing up to 6 months of follow-up after Dose 2. There were 12,796 (60.8%) participants in the COMIRNATY group and 12,449 (58.7%) in the placebo group followed for ≥4 months after Dose 2 in the blinded placebo-controlled follow-up period.

SARS-CoV-2 variants of concern identified from COVID-19 cases in this study include B.1.1.7 (Alpha) and B.1.351 (Beta). Representation of identified variants among cases in vaccine versus placebo recipients did not suggest decreased vaccine effectiveness against these variants.

The updated vaccine efficacy information is presented in Table 5.

Table 5: Vaccine Efficacy – First COVID-19 Occurrence From 7 Days After Dose 2, by Age Subgroup – Participants 16 Years of Age and Older Without Evidence of Infection and Participants With or Without Evidence of Infection Prior to 7 Days After Dose 2 – Evaluable Efficacy (7 Days) Population During the Placebo-Controlled Follow-up Period

First COVID-19 occurrence from 7 days after Dose 2 in participants without evidence of prior SARS-CoV-2 infection*			
Subgroup	**COMIRNATY N[a]=19,993 Cases n1[b] Surveillance Time[c] (n2[d])**	**Placebo N[a]=20,118 Cases n1[b] Surveillance Time[c] (n2[d])**	**Vaccine Efficacy % (95% CI[e])**
All participants[f]	77 6.092 (19,711)	833 5.857 (19,741)	91.1 (88.8, 93.1)
16 through 64 years	70 4.859 (15,519)	709 4.654 (15,515)	90.5 (87.9, 92.7)
65 years and older	7 1.233 (4192)	124 1.202 (4226)	94.5 (88.3, 97.8)

First COVID-19 occurrence from 7 days after Dose 2 in participants with or without* evidence of prior SARS-CoV-2 infection			
Subgroup	**COMIRNATY N[a]=21,047 Cases n1[b] Surveillance Time[c] (n2[d])**	**Placebo N[a]=21,210 Cases n1[b] Surveillance Time[c] (n2[d])**	**Vaccine Efficacy % (95% CI[e])**
All participants	81 6.340 (20,533)	854 6.110 (20,595)	90.9 (88.5, 92.8)
16 through 64 years	74 5.073 (16,218)	726 4.879 (16,269)	90.2 (87.5, 92.4)
65 years and older	7 1.267 (4315)	128 1.232 (4326)	94.7 (88.7, 97.9)

Note: Confirmed cases were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and at least 1 symptom consistent with COVID-19 (symptoms included: fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; vomiting).

* Participants who had no evidence of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit prior to 7 days after Dose 2 were included in the analysis.

a. N = Number of participants in the specified group.
b. n1 = Number of participants meeting the endpoint definition.
c. Total surveillance time in 1000 person-years for the given endpoint across all participants within each group at risk for the endpoint. Time period for COVID-19 case accrual is from 7 days after Dose 2 to the end of the surveillance period.
d. n2 = Number of participants at risk for the endpoint.
e. Two-sided confidence interval (CI) for vaccine efficacy is derived based on the Clopper and Pearson method adjusted to the surveillance time.

Subgroup analyses of vaccine efficacy (although limited by small numbers of cases in some subgroups) did not suggest meaningful differences in efficacy across genders, ethnic groups, geographies, or for participants with obesity or medical comorbidities associated with high risk of severe COVID-19.

Efficacy Against Severe COVID-19

Efficacy analyses of secondary efficacy endpoints supported benefit of COMIRNATY in preventing severe COVID-19. Vaccine efficacy against severe COVID-19 is presented only for participants with or without prior SARS-CoV-2 infection (Table 6) as the COVID-19 case counts in participants without prior SARS-CoV-2 infection were the same as those in participants with or without prior SARS-CoV-2 infection in both the COMIRNATY and placebo groups.

Table 6: Vaccine Efficacy – First Severe COVID-19 Occurrence in Participants 16 Years of Age and Older With or Without* Prior SARS-CoV-2 Infection Based on Protocol† or Centers for Disease Control and Prevention (CDC)‡ Definition From 7 Days After Dose 2 – Evaluable Efficacy (7 Days) Population During the Placebo-Controlled Follow-up

Vaccine Efficacy – First Severe COVID-19 Occurrence			
	COMIRNATY Cases n1[a] Surveillance Time[b] (n2[c])	**Placebo Cases n1[a] Surveillance Time[b] (n2[c])**	**Vaccine Efficacy % (95% CI[d])**
7 days after Dose 2[d]	1 6.353 (20,540)	21 6.237 (20,629)	95.3 (70.9, 99.9)
Vaccine Efficacy – First Severe COVID-19 Occurrence Based on CDC Definition			
	COMIRNATY Cases n1[a] Surveillance Time[b] (n2[c])	**Placebo Cases n1[a] Surveillance Time[b] (n2[c])**	**Vaccine Efficacy % (95% CI[d])**
7 days after Dose 2[d]	0 6.345 (20,513)	31 6.225 (20,593)	100 (87.6, 100.0)

Note: Confirmed cases were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and at least 1 symptom consistent with COVID-19 (symptoms included: fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; vomiting).

* Participants who had no evidence of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit prior to 7 days after Dose 2 were included in the analysis.

† Severe illness from COVID-19 is defined in the protocol as confirmed COVID-19 and presence of at least 1 of the following:

- Clinical signs at rest indicative of severe systemic illness (respiratory rate ≥30 breaths per minute, heart rate ≥125 beats per minute, saturation of oxygen ≤93% on room air at sea level, or ratio of arterial oxygen partial pressure to fractional inspired oxygen <300 mm Hg);
- Respiratory failure [defined as needing high-flow oxygen, noninvasive ventilation, mechanical ventilation or extracorporeal membrane oxygenation (ECMO)];
- Evidence of shock (systolic blood pressure <90 mm Hg, diastolic blood pressure <60 mm Hg, or requiring vasopressors);
- Significant acute renal, hepatic, or neurologic dysfunction;
- Admission to an Intensive Care Unit;
- Death.

‡ Severe illness from COVID-19 as defined by CDC is confirmed COVID-19 and presence of at least 1 of the following:

- Hospitalization;
- Admission to the Intensive Care Unit;
- Intubation or mechanical ventilation;
- Death.

a. n1 = Number of participants meeting the endpoint definition.
b. Total surveillance time in 1000 person-years for the given endpoint across all participants within each group at risk for the endpoint. Time period for COVID-19 case accrual is from 7 days after Dose 2 to the end of the surveillance period.
c. n2 = Number of participants at risk for the endpoint.
d. Two-side confidence interval (CI) for vaccine efficacy is derived based on the Clopper and Pearson method adjusted to the surveillance time.

16 HOW SUPPLIED/STORAGE AND HANDLING

COMIRNATY Suspension for Intramuscular Injection, Multiple Dose Vials are supplied in a carton containing 25 multiple dose vials (NDC 0069-1000-03) or 195 multiple dose vials (NDC 0069-1000-02). A 0.9% Sodium Chloride Injection, USP diluent is provided but shipped separately, and should be stored at controlled room temperature 20°C to 25°C (68°F to 77°F) [see USP Controlled Room Temperature]. The provided 0.9% Sodium Chloride Injection, USP diluent will be supplied either as cartons of 10 mL single-use vials manufactured by

Hospira, Inc (NDC 0409-4888-10), or 2 mL single-use vials manufactured by Fresenius Kabi USA, LLC (NDC 63323-186-02).

After dilution, 1 vial contains 6 doses of 0.3 mL.

During storage, minimize exposure to room light, and avoid exposure to direct sunlight and ultraviolet light.

Do not refreeze thawed vials.

Frozen Vials Prior to Use

Cartons of COMIRNATY Multiple Dose Vials arrive in thermal containers with dry ice. Once received, remove the vial cartons immediately from the thermal container and preferably store in an ultra-low temperature freezer between -90ºC to -60ºC (-130ºF to -76ºF) until the expiry date printed on the label. Alternatively, vials may be stored at -25°C to -15°C (-13°F to 5°F) for up to 2 weeks. Vials must be kept frozen and protected from light, in the original cartons, until ready to use. Vials stored at -25°C to -15°C (-13°F to 5°F) for up to 2 weeks may be returned 1 time to the recommended storage condition of -90ºC to -60ºC (-130ºF to -76ºF). Total cumulative time the vials are stored at -25°C to -15°C (-13°F to 5°F) should be tracked and should not exceed 2 weeks.

If an ultra-low temperature freezer is not available, the thermal container in which COMIRNATY arrives may be used as temporary storage when consistently re-filled to the top of the container with dry ice. Refer to the re-icing guidelines packed in the original thermal container for instructions regarding the use of the thermal container for temporary storage. The thermal container maintains a temperature range of -90ºC to -60ºC (-130ºF to -76ºF). Storage of the vials between -96°C to -60°C (-141°F to -76°F) is not considered an excursion from the recommended storage condition.

Transportation of Frozen Vials

If local redistribution is needed and full cartons containing vials cannot be transported at -90°C to -60°C (-130°F to -76°F), vials may be transported at -25°C to -15°C (-13°F to 5°F). Any hours used for transport at -25°C to -15°C (-13°F to 5°F) count against the 2-week limit for storage at -25°C to -15°C (-13°F to 5°F). Frozen vials transported at -25°C to -15°C (-13°F to 5°F) may be returned 1 time to the recommended storage condition of -90ºC to -60ºC (-130ºF to -76ºF).

Thawed Vials Before Dilution

Thawed Under Refrigeration

Thaw and then store undiluted vials in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)] for up to 1 month. A carton of 25 vials or 195 vials may take up to 2 or 3 hours, respectively, to thaw in the refrigerator, whereas a fewer number of vials will thaw in less time.

Thawed at Room Temperature

For immediate use, thaw undiluted vials at room temperature [up to 25ºC (77ºF)] for 30 minutes. Thawed vials can be handled in room light conditions.

Vials must reach room temperature before dilution.

Undiluted vials may be stored at room temperature for no more than 2 hours.

Transportation of Thawed Vials

Available data support transportation of 1 or more thawed vials at 2°C to 8°C (35°F to 46°F) for up to 12 hours.

Vials After Dilution

After dilution, store vials between 2°C to 25°C (35°F to 77°F) and use within 6 hours from the time of dilution. During storage, minimize exposure to room light, and avoid exposure to direct sunlight and ultraviolet light. Any vaccine remaining in vials must be discarded after 6 hours. Do not refreeze.

17 PATIENT COUNSELING INFORMATION

Inform vaccine recipient of the potential benefits and risks of vaccination with COMIRNATY.

Inform vaccine recipient of the importance of completing the two dose vaccination series.

There is a pregnancy exposure registry for COMIRNATY. Encourage individuals exposed to COMIRNATY around the time of conception or during pregnancy to register by visiting https://mothertobaby.org/ongoing-study/covid19-vaccines/.

Advise vaccine recipient to report any adverse events to their healthcare provider or to the Vaccine Adverse Event Reporting System at 1-800-822-7967 and www.vaers.hhs.gov.

This product's labeling may have been updated. For the most recent prescribing information, please visit https://dailymed.nlm.nih.gov/dailymed/.

BIONTECH
Manufactured for
BioNTech Manufacturing GmbH
An der Goldgrube 12
55131 Mainz, Germany

Pfizer
Manufactured by
Pfizer Inc., New York, NY 10017

LAB-1448-0.9

US Govt. License No. x

EXHIBIT 5
C SecDef Memo

SECRETARY OF DEFENSE
1000 DEFENSE PENTAGON
WASHINGTON, DC 20301-1000

AUG 24 2021

MEMORANDUM FOR SENIOR PENTAGON LEADERSHIP
COMMANDERS OF THE COMBATANT COMMANDS
DEFENSE AGENCY AND DOD FIELD ACTIVITY DIRECTORS

SUBJECT: Mandatory Coronavirus Disease 2019 Vaccination of Department of Defense Service Members

To defend this Nation, we need a healthy and ready force. After careful consultation with medical experts and military leadership, and with the support of the President, I have determined that mandatory vaccination against coronavirus disease 2019 (COVID-19) is necessary to protect the Force and defend the American people.

Mandatory vaccinations are familiar to all of our Service members, and mission-critical inoculation is almost as old as the U.S. military itself. Our administration of safe, effective COVID-19 vaccines has produced admirable results to date, and I know the Department of Defense will come together to finish the job, with urgency, professionalism, and compassion.

I therefore direct the Secretaries of the Military Departments to immediately begin full vaccination of all members of the Armed Forces under DoD authority on active duty or in the Ready Reserve, including the National Guard, who are not fully vaccinated against COVID-19.

Service members are considered fully vaccinated two weeks after completing the second dose of a two-dose COVID-19 vaccine or two weeks after receiving a single dose of a one-dose vaccine. Those with previous COVID-19 infection are not considered fully vaccinated.

Mandatory vaccination against COVID-19 will only use COVID-19 vaccines that receive full licensure from the Food and Drug Administration (FDA), in accordance with FDA-approved labeling and guidance. Service members voluntarily immunized with a COVID-19 vaccine under FDA Emergency Use Authorization or World Health Organization Emergency Use Listing in accordance with applicable dose requirements prior to, or after, the establishment of this policy are considered fully vaccinated. Service members who are actively participating in COVID-19 clinical trials are exempted from mandatory vaccination against COVID-19 until the trial is complete in order to avoid invalidating such clinical trial results.

Mandatory vaccination requirements will be implemented consistent with DoD Instruction 6205.02, "DoD Immunization Program," July 23, 2019. The Military Departments should use existing policies and procedures to manage mandatory vaccination of Service members to the extent practicable. Mandatory vaccination of Service members will be subject to any identified contraindications and any administrative or other exemptions established in Military Department policy. The Military Departments may promulgate appropriate guidance to carry out the requirements set out above. The Under Secretary of Defense for Personnel and

Readiness may provide additional guidance to implement and comply with FDA requirements or Centers for Disease Control and Prevention recommendations.

The Secretaries of the Military Departments should impose ambitious timelines for implementation. Military Departments will report regularly on vaccination completion using established systems for other mandatory vaccine reporting.

Our vaccination of the Force will save lives. Thank you for your focus on this critical mission.

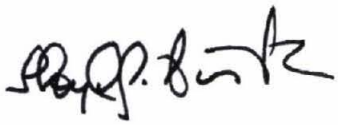


EXHIBIT 5
D DOD Hazcom program

DoD Instruction 6050.05

DoD Hazard Communication (HAZCOM) Program

Originating Component:	Office of the Under Secretary of Defense for Personnel and Readiness
Effective:	February 26, 2019
Change 1 Effective:	June 10, 2019
Releasability:	Cleared for public release. Available on the Directives Division Website at https://www.esd.whs.mil/DD/.
Reissues and Cancels:	DoD Instruction 6050.05, "DoD Hazard Communication (HAZCOM) Program," August 15, 2006, as amended
Approved by:	Ellen M. Lord, Under Secretary of Defense for Acquisition and Sustainment
Change 1 Approved by:	James N. Stewart, Assistant Secretary of Defense for Manpower and Reserve Affairs, Performing the Duties of the Under Secretary of Defense for Personnel and Readiness

Purpose: In accordance with the authority in DoD Directive (DoDD) 5124.01, DoD Instruction (DoDI) 6055.01, and the April 10, 2019 Office of the Deputy Secretary of Defense Memorandum, and the guidance in DoDI 6055.01, this issuance:

- Establishes policy, assigns responsibilities, and provides procedures for the DoD HAZCOM Program, which protects Service members and DoD civilian employees (referred to collectively in this issuance as "employee") who use or produce hazardous chemicals.
- Implements regulatory requirements of Parts 1910.120, 1910.1200, 1910.1450, 1915.1200, and 1926.59 of Title 29, Code of Federal Regulations (CFR).

TABLE OF CONTENTS

SECTION 1: GENERAL ISSUANCE INFORMATION

1.1. APPLICABILITY. This issuance applies to OSD, the Military Departments, the Office of the Chairman of the Joint Chiefs of Staff and the Joint Staff, the Combatant Commands, the Office of the Inspector General of the Department of Defense, the Defense Agencies, the DoD Field Activities, and all other organizational entities within the DoD (referred to collectively in this issuance as the "DoD Components").

1.2. POLICY. The DoD:

a. Protects DoD personnel from accidental death, injury, or occupational illness in accordance with DoDI 6055.01.

b. Manages hazardous materials to minimize health and environmental risks and operational costs.

c. Oversees establishment of HAZCOM programs at locations outside of the United States, where feasible, subject to the limitations detailed in DoDI 6055.01.

d. Applies HAZCOM procedures for all military personnel and civilian employees in non-uniquely military operations within the DoD and workplaces in accordance with this issuance and DoDI 6055.01.

e. Provides known hazard information to military personnel and civilian employees using hazardous chemicals, including engineered nanomaterials.

1.3. INFORMATION COLLECTIONS. The Enterprise Data Repository, referred to throughout this issuance, has been assigned report control symbol DD-A&S-1486 in accordance with the procedures in Volume 1 of DoD Manual 8910.01. The expiration date of this information collection is listed in the DoD Information Collections System at https://apps.sp.pentagon.mil/sites/dodiic/Pages/default.aspx.

1.4. SUMMARY OF CHANGE 1. This change reassigns the office of primary responsibility for this issuance to the Under Secretary of Defense for Personnel and Readiness (USD(P&R)) in accordance with the April 10, 2019 Office of the Deputy Secretary of Defense Memorandum and updates authoritative references accordingly.

SECTION 2: RESPONSIBILITIES

2.1. USD(P&R). The USD(P&R):

a. Establishes policy for the operation of the DoD HAZCOM Program.

b. Oversees the implementation of this issuance.

2.2. ASSISTANT SECRETARY OF DEFENSE FOR READINESS. Under the authority, direction, and control of the USD(P&R), the Assistant Secretary of Defense for Readiness:

a. Advises the USD(P&R) on implementation of this issuance.

b. Develops policy and conducts advocacy and oversight of the DoD HAZCOM Program.

c. Conducts annual management reviews of the DoD Components' HAZCOM programs in accordance with DoDI 6055.01.

d. Establishes and administers a configuration control process:

(1) To support the HAZCOM requirements described in this issuance.

(2) In accordance with the DoD Business Enterprise Architecture.

(3) Pursuant to Section 2222 of Title 10, United States Code (U.S.C.).

e. Provides guidance and oversight for hazardous material management in the systems acquisition process to help program managers implement the requirements of Section 16 of Enclosure 3 of DoDI 5000.02.

2.3. DIRECTOR, DEFENSE LOGISTICS AGENCY (DLA). In addition to the responsibilities in Paragraph 2.4., and under the authority, direction, and control of the Under Secretary of Defense for Acquisition and Sustainment, the Director, DLA, as the lead DoD Component and administrator for enterprise data management:

a. Establishes and operates the Enterprise Data Repository for the storage and retrieval of data in accordance with Paragraph 3.7.b.

b. Implements and sustains the capability to store, use, and export regulatory reference data and enterprise product hazard data to DoD HAZCOM officials.

c. Receives and processes compliant hazardous materials information received from the DoD Component HAZCOM officials, General Services Administration officials, and other federal agency officials.

d. Makes available product hazard data, which is accessible to military personnel and civilian employees who use or are at risk of exposure to hazardous materials, immediately after completing quality control and records release.

e. Negotiates agreements with other federal agency offices of primary responsibility for interaction with the Enterprise Data Repository.

2.4. DOD COMPONENT HEADS. The DoD Component heads:

a. Establish and maintain a HAZCOM program and develop HAZCOM implementing guidance that conforms to the requirements of this issuance and is consistent with Parts 1910.1200, 1910.1450, 1915.1200, and 1926.59 of Title 29, CFR.

b. Designate a HAZCOM office of primary responsibility to oversee and implement policy and guidance, and report changes to the Hazardous Materials Information Systems Manager at Headquarters, DLA.

c. Designate a DoD Component HAZCOM official to:

(1) Obtain, evaluate, enter, and provide compliant hazardous material information to the Enterprise Data Repository.

(2) Represent the DoD Component in the configuration control process.

d. Assess their component's HAZCOM program during annual workplace visits in accordance with DoDI 6055.01.

e. Require contracts that purchase hazardous materials include a requirement for the contractor to provide compliant hazardous material information to the office of the contracting activity before contract award, as required by Federal Standard FED-STD-313E. The contracting activity will then forward this information to the DoD Component HAZCOM official.

f. Address multi-employer workplaces pursuant to the requirements of Part 1910.1200(e)(2) of Title 29, CFR, in their HAZCOM programs.

g. Make available appropriate occupational and environmental health, environmental, and safety personnel (including explosives safety, as appropriate) to provide installation and workplace HAZCOM support in areas such as training, safety data sheet (SDS) generation, hazard classification, and HAZCOM labeling.

SECTION 3: PROCEDURES

3.1. GENERAL. The DoD HAZCOM Program provides the framework to communicate hazards consistent with:

a. The requirements of Parts 1910.1200, 1915.1200, and 1926.59 of Title 29, CFR for hazardous chemicals, also known and referred to in this issuance as the "Occupational Safety and Health Administration (OSHA) HAZCOM Standard."

b. The requirements of Part 1910.1450 of Title 29, CFR for hazardous chemicals, also known and referred to in this issuance as the "OSHA HAZCOM Standard for Laboratories."

c. The requirements of Part 1910.120 of Title 29, CFR, also known and referred to in this issuance as the "OSHA Hazardous Waste Operations and Emergency Response (HAZWOPER) Standard," for hazardous substance cleanup operations including:

(1) Emergency response operations in areas used primarily for hazardous waste treatment, storage, or disposal.

(2) Emergency response to hazardous substances, also known and referred to in this issuance as "HAZWOPER operations."

d. Host nation (HN) HAZCOM requirements at overseas locations when a SOFA or final governing standard (FGS) requires adoptions of HN HAZCOM requirements.

e. Paragraphs 3.2.a.(2), 3.2.c., 3.4.c., and 3.6.d., for the known presence of engineered nanomaterials that are not incorporated into an article.

3.2. WRITTEN HAZCOM PLANS.

a. All DoD workplaces using or producing hazardous chemicals must have a written HAZCOM plan that includes:

(1) A list of hazardous chemicals present in each workplace.

(2) An inventory of all engineered nanomaterials in the workplace in accordance with Paragraph 3.2.c.

(3) Hazard classification procedures in accordance with Paragraph 3.3.

(4) Container labeling procedures and requirements in accordance with Paragraph 3.5.

(5) Employee training in the safe use of hazardous materials and SDS accessibility to employees and other affected personnel in accordance with Paragraph 3.6.

(6) Procedures for preserving inventories of employee exposure records consistent with Part 1910.1020 of Title 29, CFR and pursuant to DoDI 6055.05.

(7) Procedures for informing employees regarding hazards of non-routine tasks and the hazards associated with chemicals contained in unlabeled pipes in the workplace.

(8) Requirements for contractors bringing hazardous materials onto DoD installations. These requirements will include providing hazardous material and label information compliant with Part 1910.1200 of Title 29, CFR, to the contracting officers in accordance with Subpart 223.3, Defense Federal Acquisition Regulation Supplement (DFARS). The contracting officers will then forward the information to the proper environmental, safety (including explosives safety, as appropriate), and health officials.

b. All DoD workplaces with laboratories must develop a written chemical hygiene plan in accordance with the OSHA HAZCOM Standard for Laboratories. These written chemical hygiene plans must:

(1) Be readily available to all affected personnel and include any installation-unique procedures about the local purchase of hazardous chemicals.

(2) Address engineered nanomaterials, not included in an article, used within the laboratory.

c. All DoD workplaces, or DoD-manufactured materials where engineered nanomaterials are used, should include engineered nanomaterials that are not incorporated into articles or otherwise excluded from Part 1910.1200 of Title 29, CFR into their written HAZCOM plans when there is knowledge of the presence of such engineered nanomaterials.

d. DoD Components stationed outside the United States must take measures to include HN requirements in HAZCOM plans if required to do so by SOFAs and FGSs.

e. All DoD workplaces conducting HAZWOPER operations must have a written HAZCOM plan that includes a list of hazardous wastes managed or hazardous substances that military personnel and civilian employees may encounter during emergency response or cleanup operations in accordance with Part 1910.120(b) of Title 29, CFR.

3.3. HAZARD CLASSIFICATION.

a. The DoD Components will obtain and use hazard information based on the hazard classification and any additional information provided on the SDSs. If an occupational or environmental health risk assessment or health hazard assessment is conducted in accordance with DoDI 6055.05, this information will supplement the manufacturer's information.

b. For DoD-manufactured or imported materials, the DoD activity controlling the formulation, or the DoD activity manufacturing the chemical, performs the hazard classification and produces the SDS and HAZCOM label with the required information following the guidelines specified in Part 1910.1200 of Title 29, CFR.

(1) The DoD activity producing the material will include hazard classification procedures in their written program, and train their military personnel and civilian employees on the hazards and handling of hazardous material and the prevention and handling of spillage incidents.

(2) If the DoD activity producing the material transfers the material to other organizations, they will provide the SDS and HAZCOM label to the receiving organization and the DoD Component HAZCOM official.

c. When engineered nanomaterials are present (but not incorporated into articles), regardless of quantity, the DoD activity using the material or controlling the formulation will refer to the National Institute for Occupational Safety and Health Publication Number 2009-125 (or most current report on nanomaterial toxicity and risk management) for what is currently known about the nanoparticle toxicity, process emissions, exposure assessment, engineering controls, and personal protective equipment.

d. The DoD activity will follow the guidelines in Technical Bulletin 700-2/Naval Sea Systems Command Instruction 8020.8C/Technical Order 11A-1-47 for classifying the hazards of DoD ammunition and explosives. This publication establishes procedures for classifying the physical hazards of ammunition and explosives in accordance with Department of Transportation regulations. This classification is used primarily for transporting and storing ammunition and explosives.

e. The DoD activity will identify risks at HAZWOPER operations consistent with Part 1910.120(b)(7) of Title 29, CFR.

3.4. HAZARDOUS MATERIAL INFORMATION.

a. The DoD Components will make SDSs compliant with Parts 1910.1200 and 1910.1450 of Title 29, CFR. SDSs will be readily accessible to employees at all times when they are in their work area, required to use hazardous chemicals, or at risk of exposure to hazardous chemicals.

b. Copies of the appropriate SDS will be:

(1) Readily accessible before hazardous chemicals are used and accessible at all times thereafter.

(2) Submitted for inclusion in the Enterprise Data Repository as soon as practical in accordance with Paragraph 3.7.

(3) Available to safety (including explosives safety, as appropriate), environmental, and fire officials in case of an accident.

c. DoD Components will make available their occupational and environmental health, environmental, and safety (including explosives safety, as appropriate) military personnel and civilian employees, upon request, to assess and explain SDSs and labels to supervisors and affected employees and assist in HAZCOM training.

d. Consistent with Part 1910.1200 of Title 29, CFR, the controlling DoD Component procurement activity:

(1) Electronically provides the most current, compliant SDSs and HAZCOM labels for users and the DoD Component HAZCOM official to include in the Enterprise Data Repository, as specified by the Business Enterprise Architecture; Subpart 223.3, DFARS; and Clause 52.223-3 of the Federal Acquisition Regulation Supplement.

(2) Rejects incomplete hazardous material information that does not comply with the requirements of Part 1910.1200 of Title 29, CFR. Laboratory verification of technical elements is not required. DoD Components will return incomplete or inadequate SDSs and labels to the supplier for correction. The contracting officer or buyer must consult with the manufacturer or distributer for resolution of SDS discrepancies.

e. Purchase requests for applicable supply items must include:

(1) A requirement for contracting activities to obtain manufacturer, importer, or supplier SDSs.

(2) The requirement for warning labels compliant with Part 1910.1200 of Title 29, CFR for U.S. locations or the Globally Harmonized System of Classification and Labelling of Chemicals for non-U.S. locations, in accordance with Military Standard MIL-STD-129R, Federal Standard FED-STD-313E, and Subpart 223.3, DFARS.

f. DoD Components will protect and use proprietary formulas or trade secret information in an SDS only as a management tool for exposure and mishap prevention, hazardous chemicals education, and medical diagnosis and treatment of exposed military personnel and civilian employees consistent with Part 1910.1200 of Title 29, CFR, and Volume 4 of DoD Manual 5200.01.

g. For nationally stock-listed and locally purchased nonstandard stock hazardous chemicals, the responsible contracting officer must contractually require and obtain compliant electronic SDSs and HAZCOM labels.

(1) For locally purchased chemicals, the purchaser or contracting officer confirms before the contract award or purchase:

(a) The adequate completion of an environmental, safety, and health assessment of the SDSs and HAZCOM labels.

(b) The correct SDSs and labels, as required in Part 1910.1200 of Title 29, CFR.

(2) The installation point of contact electronically forwards the SDSs and HAZCOM labels to the DoD Component HAZCOM official for processing.

h. For foreign manufactured products used outside the United States, the contracting office and purchaser will obtain SDSs and HAZCOM labels that are available in English.

(1) The SDSs and HAZCOM labels must contain all information required in Part 1910.1200 of Title 29, CFR.

(2) The contracting office and purchaser must electronically forward the SDSs, translated by other than the chemical manufacturer, to installation SDS focal points for entry into the Enterprise Data Repository with markings showing the SDS has translated hazardous material information.

i. The lead DoD Component managing the first non-U.S. entry point must establish procedures to make the appropriate SDS information available to all users of supplied hazardous chemical supply items.

(1) Unless the governing SOFA specifically mandates the use of HN SDS data and formats, SDSs will conform to Parts 1910.1200 and 1910.1450 of Title 29, CFR and Paragraph 3.4.h.

(2) Special procedures may be necessary for certain workplaces outside the United States with foreign national employees, including multi-employer sites similar to Part 1910.1200(e)(2) of Title 29, CFR. Hazardous material information and SDSs will reflect the predominant language spoken in addition to English. If required by the governing SOFA, FGS, or other binding agreement, the SDSs available in workplaces with foreign nationals may need to account for HN variations in SDS format or data. The lead DoD Component should establish those procedures using the guidance in this issuance, the relevant SOFA, the FGS for the location, or DoD 4715.05-G.

3.5. LABELING.

a. Hazardous chemicals used by the DoD Components:

(1) Must be appropriately classified and labeled consistent with Parts 1910.1200 and 1910.1450 of Title 29, CFR.

(2) That are specifically identified in Parts 1910.1001 through 1910.1052 in Subpart Z of Title 29, CFR, must be classified and labeled following additional substance-specific standards.

b. Commercial suppliers are required to label all hazardous materials with HAZCOM Standard-compliant labels consistent with Part 1910.1200 of Title 29, CFR.

c. The DoD Components will use the commercial or manufacturer's HAZCOM label for marking hazardous chemicals, including laboratory chemicals, and this label must not be removed from the products or defaced. If a DoD Component generates a hazardous chemical label, it must comply with Part 1910.1200 of Title 29, CFR.

d. If suppliers of hazardous materials have not properly labeled containers in accordance with Parts 1910.1200 and 1910.1450 of Title 29, CFR, the DoD Component must properly label containers. Hazardous material cannot be issued to downstream customers or used until compliance is met.

e. The label information must contain:

(1) Product identifier.

(2) Signal word.

(3) Hazard statements.

(4) Pictograms.

(5) Precautionary statements.

(6) Chemical manufacturer, importer, or other responsible party's name, address, and telephone number.

f. Navy ships may use alternate HAZCOM Standard-compliant labeling (e.g., tags or markings) for repackaging, breakdown containers, or unlabeled containers aboard ship, consistent with the exclusion for uniquely military equipment, systems, operations, or workplaces in Executive Order 12196. The Navy must label all hazardous chemicals in accordance with the provisions of this issuance before being off-loaded or transferred to a shore facility.

g. Hazardous chemicals excluded from HAZCOM labeling requirements are described in Part 1910.1200(b)(5) of Title 29, CFR. Outside of the United States, hazardous chemicals must be labeled in accordance with applicable HAZCOM regulations as specified in the SOFA, FGSs, or other HN agreement. Many of these chemicals, though excluded from HAZCOM, have alternative labeling requirements such as chemicals regulated by:

(1) Section 2015 of Title 15, U.S.C., also known as the "Consumer Product Safety Act."

(2) Section 136 of Title 7, U.S.C., also known as the "Federal Insecticide, Fungicide, and Rodenticide Act."

(3) Section 201 of Title 27, U.S.C., also known as the "Federal Alcohol Administration Act."

(4) Section 2601 of Title 15, U.S.C., also known as the "Toxic Substances Control Act."

3.6. EMPLOYEE INFORMATION AND TRAINING. The DoD Components will:

a. Provide HAZCOM information and training and document training to employees who may become exposed to:

(1) Hazardous chemicals while carrying out their duties, in accordance with Parts 1910.1200 and 1910.1450 of Title 29, CFR.

(2) Hazardous materials during HAZWOPER operations, consistent with the OSHA HAZWOPER Standard.

b. Inform contractors and subcontractors at DoD workplaces of site emergency response procedures consistent with Part 1910.120(b)(1)(iv) of Title 29, CFR.

c. Consider HN regulations for personnel information and training for the local national workforce if authorized or required to do so by SOFAs or FGSs.

d. Provide known hazard information of engineered nanomaterials used at DoD workplaces.

3.7. ENTERPRISE DATA REPOSITORY.

a. The DoD Components will implement procedures to provide hazardous materials information to the Enterprise Data Repository consistent with Part 1910.1200 of Title 29, CFR. They will submit the hazardous materials information through the media (hard or electronic copy) appropriate to the technological capabilities or availability suitable for the DoD Component's system.

b. The DLA operates the Enterprise Data Repository for the storage and retrieval of data and:

(1) Makes SDS image and associated ingredient, transportation, disposal, and label information accessible by national item identification number; local item identification number, if applicable; trade name and part number; SDS serial number; hazard characteristic code; hazardous ingredient(s); contract number; and manufacturer, importer, or distributor (or other responsible party) Commercial and Government Entity Codes. For local purchases of hazardous chemicals made without a formal contract number assigned, or from companies who do not have a Commercial and Government Entity Code, this information may not be available for entering into the Enterprise Data Repository. All other SDS information will be available.

(2) Allows for expansion as required by future safety, health, environmental, or transportation legislation or regulation.

(3) Permanently retains SDS information electronically.

(4) Provides SDS and corresponding product hazard data for all hazardous material inventory items for use as a reference throughout the procurement and the supply chain distribution process.

(5) Establishes a capability for the management of HN SDS information and images for hazardous materials used by the DoD outside the United States.

GLOSSARY

G.1. ACRONYMS.

CFR	Code of Federal Regulations
DFARS	Defense Federal Acquisition Regulation Supplement
DLA	Defense Logistics Agency
DoDD	DoD directive
DoDI	DoD instruction
FGS	final governing standard
HAZCOM	hazard communication
HAZWOPER	hazardous waste operations and emergency response
HN	host nation
OSHA	Occupational Safety and Health Administration
SDS	safety data sheet
SOFA	status-of-forces agreement
U.S.C.	United States Code
USD(P&R)	Under Secretary of Defense for Personnel and Readiness

G.2. DEFINITIONS. Unless otherwise noted, these terms and their definitions are for the purpose of this issuance.

article. Defined in Part 1910.1200(c) of Title 29, CFR.

DoD workplaces with laboratories. Defined in the OSHA HAZCOM Standard for Laboratories.

engineered nanomaterials. Discrete materials having structures with at least one dimension between 1 and 100 nanometers that are intentionally created, as opposed to those that are naturally or incidentally formed. They do not include larger materials that may have nanoscale features (e.g., etched silicon wafers), biomolecules (e.g., proteins, nucleic acids, carbohydrates), and materials with occupational exposure limits that address nanoparticles for that substance.

hazard classification. Defined in Part 1910.1200(c) of Title 29, CFR.

hazardous chemical. Defined in Part 1910.1200(c) of Title 29, CFR.

hazardous material. Hazardous chemicals, hazardous substances, hazardous wastes, or engineered nanomaterials, where applicable.

hazardous substance. Defined in Part 1910.120(c) of Title 29, CFR.

hazardous waste. Defined in Part 261.3 of Title 40, CFR and Part 171.8 of Title 49, CFR, in accordance with the OSHA HAZWOPER Standard.

HAZWOPER. Defined in Part 120(a)(1) of Title 29, CFR.

product hazard data. The comprehensive set of material, chemical, and regulatory data necessary to develop and implement ESOH controls for mission activities involving hazardous materials.

uniquely military equipment, systems, and operations. Defined in Part 1960.2(i) of Title 29, CFR.

REFERENCES

Code of Federal Regulations, Title 29

Code of Federal Regulations, Title 40, Part 261.3

Code of Federal Regulations, Title 49, Part 171.8

Defense Federal Acquisition Regulation Supplement, Subpart 223.3

DoD 4715.05-G, "Overseas Environmental Baseline Guidance Document," May 1, 2007

DoD Directive 5124.01, "Under Secretary of Defense for Personnel and Readiness (USD(P&R))," June 23, 2008

DoD Instruction 5000.02, "Operation of the Defense Acquisition System," January 7, 2015, as amended

DoD Instruction 6055.01, "DoD Safety and Occupational Health (SOH) Program," October 14, 2014

DoD Instruction 6055.05, "Occupational and Environmental Health (OEH)," November 11, 2008, as amended

DoD Manual 5200.01, Volume 4, "DoD Information Security Program: Controlled Unclassified Information (CUI)," February 24, 2018, as amended

DoD Manual 8910.01, Volume 1, "DoD Information Collections Manual: Procedures for DoD Internal Information Collections," June 30, 2014, as amended

Executive Order 12196, "Occupational Safety and Health Programs for Federal Employees," February 26, 1980, as amended

Federal Acquisition Regulation Supplement, Clause 52.223-3

Federal Standard FED-STD-313E, "Material Safety Data, Transportation Data, and Disposal Data for Hazardous Materials Furnished to Government Activities," July 1, 2014

Military Standard MIL-STD-129R, "Department of Defense Standard Practice: Military Marking for Shipment and Storage," February 18, 2014

National Institute for Occupational Safety and Health Publication Number 2009-125, "Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials," March 2009

Office of the Deputy Chief Management Officer, "Business Enterprise Architecture (BEA)," current edition[1]

Office of the Deputy Secretary of Defense Memorandum, "Safety and Occupational Health Policy and Oversight Functions," April 10, 2019

TB 700-2/NAVSEAINST 8020.8C/TO 11A-1-47, "Department of Defense Ammunition and Explosives Hazard Classification Procedures," July 30, 2012[2]

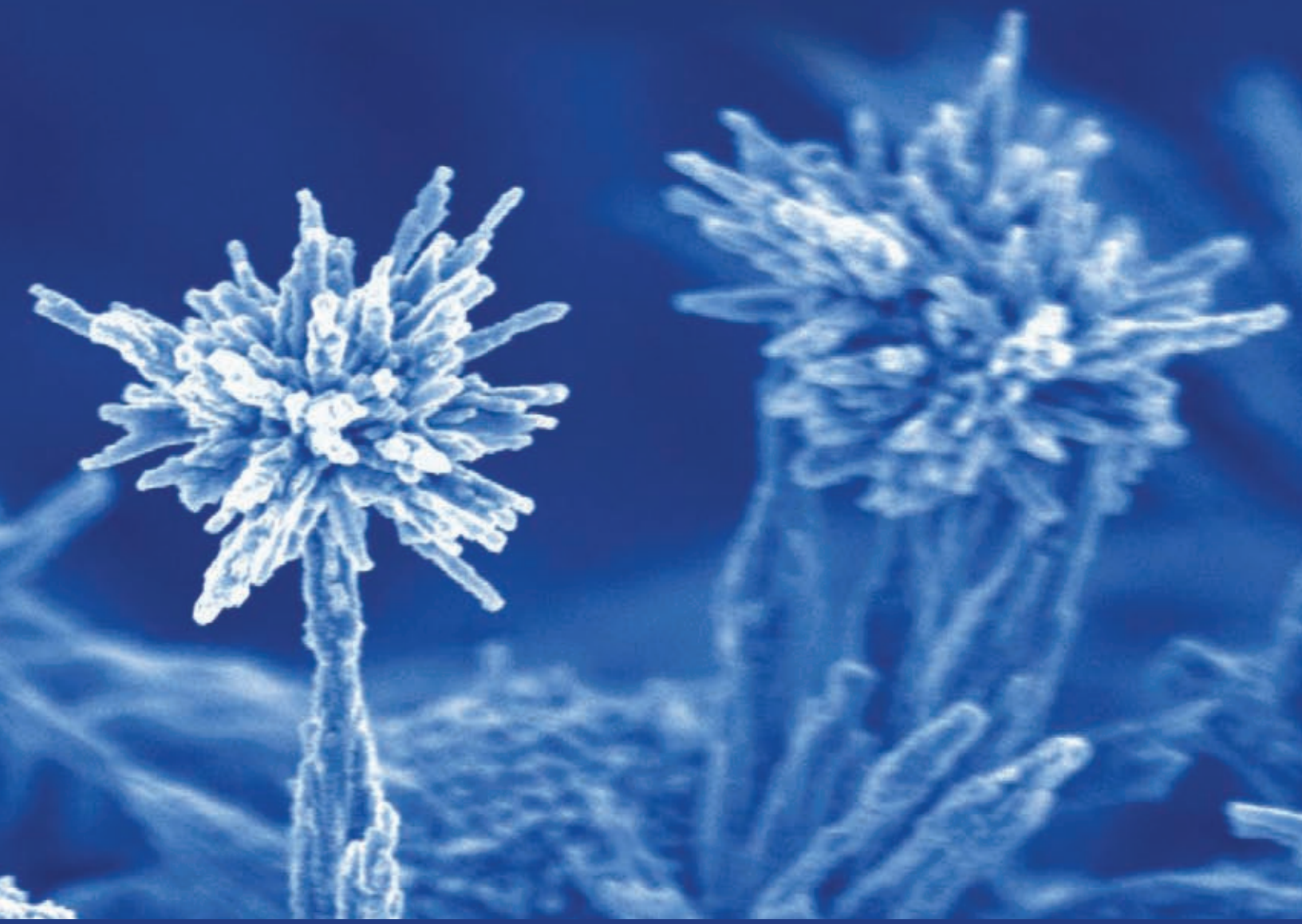
United Nations, "Globally Harmonized System of Classification and Labelling of Chemicals (GHS)," Revision 4, New York and Geneva, 2011[3]

[1] Available at http://cmo.defense.gov/Products-and-Services/Business-Enterprise-Architecture/
[2] Available at https://www.ddesb.pentagon.mil/docs/TB700-2.pdf
[3] Available at http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

EXHIBIT 5
E Safe
Nanotech

Approaches to Safe Nanotechnology

Managing the Health and Safety Concerns Associated with Engineered Nanomaterials



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

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Approaches to Safe Nanotechnology

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DHHS (NIOSH) Publication No. 2009–125

March 2009

SAFER • HEALTHIER • PEOPLE™

Foreword

Nanotechnology—the manipulation of matter on a near-atomic scale to produce new structures, materials, and devices—offers the promise of unprecedented scientific advancement for many sectors, such as medicine, consumer products, energy, materials, and manufacturing. Nanotechnology has the power not only to improve existing technologies, but to dramatically enhance the effectiveness of new applications.

Research on the potential applications of nanotechnology continues to expand rapidly worldwide. New nanotechnology consumer products emerge at a rate of three to four per week. Over the course of the next decade, nanotechnology could have a $1 trillion impact on the global economy and employ two million workers—half of them residing in the U.S.

While nanomaterials present seemingly limitless possibilities, they bring with them new challenges to understanding, predicting, and managing potential safety and health risks to workers. The National Institute for Occupational Safety and Health (NIOSH) remains committed to protecting workers now and in the future, as nanotechnology applications and uses expand.

As part of these efforts, in October 2005, NIOSH released for public comment the draft document, Approaches to Safe Nanotechnology: An Information Exchange with NIOSH. Based on feedback received, NIOSH revised and updated the document in July 2006 and sought further public comment. This draft report has been widely cited, and the final version of the report should serve as a vital resource for stakeholders (including occupational safety and health professionals, researchers, policy makers, risk assessors, and workers in the industry) who wish to understand more about the safety and health implications of nanotechnology in the workplace.

With the publication of the Approaches to Safe Nanotechnology document, NIOSH hopes to: raise awareness of the occupational safety and health issues involved with nanotechnology; make recommendations on occupational safety and health best practices in the production and use of nanomaterials; facilitate dialogue between NIOSH and its external partners in industry, labor and academia; respond to requests for authoritative safety and health guidelines; and, identify information gaps and areas for future study and research.

As our knowledge of nanoscience increases, so too will our efforts to provide valuable guidance on the safe handling of nanoparticles and for protecting the lives and livelihoods of nanotechnology workers.

Christine M. Branche, Ph.D.
Acting Director, National Institute
for Occupational Safety and Health
Centers for Disease Control and Prevention

Executive Summary

Nanotechnology has the potential to dramatically improve the effectiveness of a number of existing consumer and industrial products and could have a substantial impact on the development of new products in all sectors, ranging from disease diagnosis and treatment to environmental remediation. Because of the broad range of possible nanotechnology applications, continued evaluation of the potential health risks associated with exposure to nanomaterials is essential to ensure their safe handling. Engineered nanoparticles are materials purposefully produced with at least one dimension between 1 and 100 nanometers. Nanoparticles* often exhibit unique physical and chemical properties that impart specific characteristics essential in making engineered materials, but little is known about what effect these properties may have on human health. Research has shown that the physicochemical characteristics of particles can influence their effects in biological systems. These characteristics include particle size, shape, surface area, charge, chemical properties, solubility, oxidant generation potential, and degree of agglomeration. Until the results from research studies can fully elucidate the characteristics of nanoparticles that may pose a health risk, precautionary measures are warranted.

NIOSH has developed this document to provide an overview of what is known about the potential hazards of engineered nanoparticles and measures that can be taken to minimize workplace exposures. Following is a summary of findings and key recommendations.

*In an attempt at standardization of terminology, the International Organization for Standardization-Technical Committee 229 has used the term nanomaterial to describe engineered nanoparticles.

Potential Health Concerns

- The potential for nanomaterials to enter the body is among several factors that scientists examine in determining whether such materials may pose an occupational health hazard. Nanomaterials have the greatest potential to enter the body through the respiratory system if they are airborne and in the form of respirable-sized particles (nanoparticles). They may also come into contact with the skin or be ingested.
- Based on results from human and animal studies, airborne nanoparticles can be inhaled and deposit in the respiratory tract; and based on animal studies, nanoparticles can enter the blood stream, and translocate to other organs.
- Experimental studies in rats have shown that equivalent mass doses of insoluble incidental nanoparticles are more potent than large particles of similar composition in causing pulmonary inflammation and lung tumors. Results from in vitro cell culture studies with similar materials are generally supportive of the biological responses observed in animals.
- Experimental studies in animals, cell cultures, and cell-free systems have shown that changes in the chemical

composition, crystal structure, and size of particles can influence their oxidant generation properties and cytotoxicity.

- Studies in workers exposed to aerosols of some manufactured or incidental microscopic (fine) and nanoscale (ultrafine) particles have reported adverse lung effects including lung function decrements and obstructive and fibrotic lung diseases. The implications of these studies to engineered nanoparticles, which may have different particle properties, are uncertain.
- Research is needed to determine the key physical and chemical characteristics of nanoparticles that determine their hazard potential.

Potential Safety Concerns

- Although insufficient information exists to predict the fire and explosion risk associated with powders of nanomaterials, nanoscale combustible material could present a higher risk than coarser material with a similar mass concentration given its increased particle surface area and potentially unique properties due to the nanoscale.
- Some nanomaterials may initiate catalytic reactions depending on their composition and structure that would not otherwise be anticipated based on their chemical composition.

Working with Engineered Nanomaterials

- Nanomaterial-enabled products such as nanocomposites, surface-coated materials, and materials comprised of nanostructures, such as integrated circuits, are unlikely to pose a risk of exposure during their handling and use as materials of non-inhalable size. However, some of the processes used in their production (e.g., formulating and applying nanoscale coatings) may lead to exposure to nanomaterials, and the cutting or grinding of such products could release respirable-sized nanoparticles.
- Maintenance on production systems (including cleaning and disposal of materials from dust collection systems) is likely to result in exposure to nanoparticles if deposited nanomaterials are disturbed.
- The following workplace tasks can increase the risk of exposure to nanoparticles:
  - Working with nanomaterials in liquid media without adequate protection (e.g., gloves)
  - Working with nanomaterials in liquid during pouring or mixing operations, or where a high degree of agitation is involved
  - Generating nanoparticles in non-enclosed systems
  - Handling (e.g., weighing, blending, spraying) powders of nanomaterials
  - Maintenance on equipment and processes used to produce or fabricate nanomaterials and the cleaning-up of spills and waste material containing nanomaterials
  - Cleaning of dust collection systems used to capture nanoparticles
  - Machining, sanding, drilling, or other mechanical disruptions of materials containing nanoparticles

Exposure Assessment and Characterization

- Until more information becomes available on the mechanisms underlying nanomaterial toxicity, it is uncertain what measurement technique should be used to monitor exposures in the workplace. Current research indicates that mass and bulk chemistry may be less important than particle size and shape, surface area, and surface chemistry (or activity) for some nanostructured materials.
- Many of the sampling techniques that are available for measuring airborne nanoaerosols vary in complexity but can provide useful information for evaluating occupational exposures with respect to particle size, mass, surface area, number concentration, and composition. Unfortunately, relatively few of these techniques are readily applicable to routine exposure monitoring. NIOSH has initiated exposure assessment studies in workplaces that manufacture or use engineered nanoparticles (see Appendix *Nanoparticle Emission Assessment Technique for Identification of Sources and Releases of Engineered Nanomaterials*).
- Regardless of the metric or measurement method used for evaluating nanoaerosol exposures, it is critical that background nanoscale particle measurements be conducted before the production, processing, or handling of nanomaterials.
- When feasible, personal sampling is preferred to ensure an accurate representation of the worker's exposure, whereas area sampling (e.g., size-fractionated aerosol samples) and real-time (direct reading) exposure measurements may be more useful for evaluating the need for improvement of engineering controls and work practices.

Precautionary Measures

- Given the limited amount of information about health risks that may be associated with nanomaterials, taking measures to minimize worker exposures is prudent.
- For most processes and job tasks, the control of airborne exposure to nanoaerosols can be accomplished using a variety of engineering control techniques similar to those used in reducing exposure to general aerosols.
- The implementation of a risk management program in workplaces where exposure to nanomaterials exists can help to minimize the potential for exposure to nanoparticles. Elements of such a program should include the following:
  - Evaluating the hazard posed by the nanomaterial based on available physical and chemical property data, toxicology, or health-effects data
  - Assessing the worker's job task to determine the potential for exposure
  - Educating and training workers in the proper handling of nanomaterials (e.g., good work practices)
  - Establishing criteria and procedures for installing and evaluating engineering controls (e.g., exhaust ventilation) at locations where exposure to nanomaterials might occur

  - Developing procedures for determining the need for and selecting proper personal protective equipment (e.g., clothing, gloves, respirators)
  - Systematically evaluating exposures to ensure that control measures are working properly and that workers are being provided the appropriate personal protective equipment

- Engineering control techniques such as source enclosure (i.e., isolating the generation source from the worker) and local exhaust ventilation systems should be effective for capturing airborne nanoparticles. Current knowledge indicates that a well-designed exhaust ventilation system with a high-efficiency particulate air (HEPA) filter should effectively remove nanomaterials.
- The use of good work practices can help to minimize worker exposures to nanomaterials. Examples of good practices include cleaning of work areas using HEPA vacuum pickup and wet wiping methods, preventing the consumption of food or beverages in workplaces where nanomaterials are handled, providing hand-washing facilities, and providing facilities for showering and changing clothes.
- No guidelines are currently available on the selection of clothing or other apparel (e.g., gloves) for the prevention of dermal exposure to nanoaerosols. However, some clothing standards incorporate testing with nanometer-sized particles and therefore provide some indication of the effectiveness of protective clothing.
- Respirators may be necessary when engineering and administrative controls do not adequately prevent exposures. Currently, there are no specific limits for airborne exposures to engineered nanoparticles although occupational exposure limits exist for some larger particles of similar chemical composition. It should be recognized that exposure limits recommended for nonnanoscale particles may not be health protective for nanoparticle exposures (e.g., the OSHA Permissible Exposure Limit [PEL] for graphite may not be a safe exposure limit for carbon nanotubes). The decision to use respiratory protection should be based on professional judgment that takes into account toxicity information, exposure measurement data, and the frequency and likelihood of the worker's exposure. While research is continuing, preliminary evidence indicates that NIOSH-certified respirators will be useful for protecting workers from nanoparticle inhalation when properly selected and fit tested as part of a complete respiratory protection program.

Occupational Health Surveillance

Occupational health surveillance is an essential component of an effective occupational safety and health program. The unique physical and chemical properties of nanomaterials, the increasing growth of nanotechnology in the workplace, and information suggesting that exposure to some engineered nanomaterials can cause adverse health effects in laboratory animals all support consideration of an occupational health surveillance program for workers potentially exposed to engineered

nanomaterials. Continued evaluation of toxicologic research and workers potentially exposed to engineered nanomaterials is needed to inform NIOSH and other groups regarding the appropriate components of occupational health surveillance for nanotechnology workers. NIOSH has formulated interim guidance relevant to medical screening (one component of an occupational health surveillance program) for nanotechnology workers (see NIOSH *Current Intelligence Bulletin Interim Guidance for Medical Screening and Hazard Surveillance for Workers Potentially Exposed to Engineered Nanoparticles* at www.cdc.gov/niosh/review/public/115/). In this document NIOSH concluded that insufficient scientific and medical evidence now exist to recommend the specific medical screening of workers potentially exposed to engineered nanoparticles. However, NIOSH did recommend that hazard surveillance be conducted as the basis for implementing control measures.

Contents

Acknowledgments

This report was developed by the scientists and staff of the National Institute for Occupational Safety and Health (NIOSH) who participate in the NIOSH Nanotechnology Research Center (NTRC). Paul Schulte is the manager and Charles Geraci, coordinator of the NIOSH NORA nanotechnology cross-sector program. Special thanks go to Ralph Zumwalde and Laura Hodson for writing and organizing the report and to Mark Methner for the development of the *Appendix: Nanoparticle Emission Assessment Technique for Identification of Sources and Releases of Engineered Nanomaterials*.

Others who contributed substantially to the writing and research described here include: Eileen Birch, Fred Blosser, Vincent Castranova, Brian Curwin, Douglas Evans, Pengfei Gao, Donna Heidel, Mark Hoover, John Howard, Vijia Karra, Bon-Ki Ku, Eileen Kuempel, Robert Mercer, Arthur Miller, Vladimir Murashov, Terri Pearce, Appavoo Rengasamy, Ronald Shaffer, Anna Shvedova, Petia Simeonova, Aleksandr Stefaniak, Douglas Trout, and Leonid Turkevich.

The NIOSH NTRC also acknowledges the contributions of Vanessa Becks and Gino Fazio for desktop publishing and graphic design, and Elizabeth Fryer for editing the report.

1 Introduction

Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials, and devices. This technology has the ability to transform many industries and can be applied in many ways to areas ranging from medicine to manufacturing. Research in nanoscale technologies is growing rapidly worldwide. Lux Research [2007] projects that new emerging nanotechnology applications will affect nearly every type of manufactured product through the middle of the next decade, becoming incorporated into 15% of global manufacturing output, totaling $2.6 trillion in 2014.

Nanomaterials present new challenges to understanding, predicting, and managing potential health risks to workers. As with any material being developed, scientific data on the health effects in exposed workers are largely unavailable. **In the case of nanomaterials, the uncertainties are great because the characteristics of nanoparticles may be different from those of larger particles with the same chemical composition.** Safety and health practitioners recognize the critical lack of specific guidance on the safe handling of nanomaterials—especially now, when the degree of risk to exposed workers is unknown. In the meantime, the extensive scientific literature on airborne particles—including toxicology and epidemiological studies, measurement techniques, and engineering controls—provides the best available data from which to develop interim approaches for working safely with nanomaterials and to develop hypotheses for studies of new nanomaterials.

The National Institute for Occupational Safety and Health (NIOSH) is working in parallel with the development and implementation of commercial nanotechnology through (*1*) conducting strategic planning and research, (*2*) partnering with public- and private-sector colleagues from the United States and abroad, and (*3*), making information widely available. The NIOSH goal is to provide national and world leadership for incorporating research findings about the implications and applications of nanotechnology into good occupational safety and health practice for the benefit of all nanotechnology workers. NIOSH has developed a strategic plan for coordinating nanotechnology research and for use as a guide for enhancing the development of new research efforts (www.cdc.gov/niosh/topics/nanotech/strat_plan.html).

2 Purpose

With the publication of this *Approaches to Safe Nanotechnology* document, NIOSH hopes to do the following:

- **Raise awareness** of the occupational safety and health issues being identified in the rapidly moving and changing science involving implications and applications of nanotechnology.
- **Use the best information available to make recommendations** on occupational safety and health practices in the production and use of nanomaterials (These recommendations will be updated as appropriate to reflect new information. They will address key components of occupational safety and health, including exposure monitoring, engineering controls, personal protective equipment, and administrative controls. They will draw from the ongoing NIOSH assessment of current best practices, technical knowledge, and professional judgment. Throughout the development of these guidelines, the utility of a hazard-based approach to risk assessment and control was evaluated and, where appropriate, recommendations are provided.)
- **Facilitate an exchange of information** between NIOSH and its external partners from ongoing research, including success stories, applications, and case studies.
- **Respond to requests** from industry, labor, academia, and other partners who are seeking science-based, authoritative guidelines.
- **Identify information gaps** where few or no data exist and where research is needed.

3 Scope

This document has been developed to provide a resource for stakeholders who wish to understand more about the safety and health implications and applications of nanotechnology in the workplace. The information and guidelines presented here are intended to aid in evaluating the potential hazard of exposure to engineered nanomaterials and to set the stage for the development of more comprehensive guidelines for reducing potential workplace exposures in the wide range of tasks and processes that use nanomaterials. The information in this document will be of specific interest to the following:

- Occupational safety and health professionals who must (*1*) understand how nanotechnology may affect occupational health and (*2*) devise strategies for working safely with nanomaterials
- Researchers working with or planning to work with engineered nanomaterials and studying the potential occupational safety and health impacts of nanomaterials
- Policy and decision makers in government agencies and industry
- Risk evaluation professionals
- People working with or potentially exposed to engineered nanomaterials in the workplace

Established safe work practices are generally based on an understanding of the hazards associated with the chemical and physical properties of a material. Engineered nanomaterials may exhibit unique properties that are related to their physical size, shape, structure, and chemical composition. Considerable uncertainty still exists as to whether these unique properties present occupational health risks. Current information about the potential adverse health effects of engineered nanomaterials, exposure assessment, and exposure control is limited. However, the large body of scientific literature that exists on exposures to and responses of animals and humans to ultrafine and other airborne particles may be useful in making preliminary assessments as to the health risks posed by engineered nanomaterials. **Until further information is available, interim safe working practices should be used based on the best available information.** The information and recommendations in this document are intended to aid in assessment of the potential hazard of engineered nanomaterials and to set the stage for the development of more comprehensive guidelines for reducing potential workplace exposures.

4 Descriptions and Definitions

Nanotechnology involves the manipulation of matter at nanometer[†] scales to produce new materials, structures, and devices. The U.S. National Nanotechnology Initiative (see http://nano.gov/html/facts/whatIsNano.html) defines a technology as nanotechnology only if it involves all of the following:

- Research and technology development involving structures with at least one dimension in the range of 1–100 nanometers (nm), frequently with atomic/molecular precision
- Creating and using structures, devices, and systems that have unique properties and functions because of their nanoscale dimensions
- The ability to control or manipulate on the atomic scale

Nanotechnology is an enabling technology that offers the potential for unprecedented advances in many diverse fields. The ability to manipulate matter at the atomic or molecular scale makes it possible to form new materials, structures, and devices that exploit the unique physical and chemical properties associated with nanoscale structures. The promise of nanotechnology goes far beyond extending the use of current materials. New materials and devices with intricate and closely engineered structures will allow for (*1*) new directions in optics, electronics, and optoelectronics, (*2*) development of new medical imaging and treatment technologies, and (*3*) production of advanced materials with unique properties and high-efficiency energy storage and generation.

Although nanotechnology-based products are generally thought to be at the precompetitive stage, an increasing number of products and materials are becoming commercially available. These include nanoscale powders, solutions, and suspensions of nanoscale materials as well as composite materials and devices having a nanostructure. Nanoscale products and materials are increasingly used in optoelectronic, electronic, magnetic, medical imaging, drug delivery, cosmetic, catalytic, and materials applications. New nanotechnology consumer products are coming on the market at the rate of three to four per week, a finding based on the latest update to the nanotechnology consumer product inventory maintained by the Project on Emerging Nanotechnologies (PEN)[‡] (www.nanotechproject.org/inventories/consumer). The number of consumer products using nanotechnology has grown from 212 to 609 since PEN launched the world's first online inventory of manufacturer-identified nanotech goods in March 2006.

According to Lux Research [2007], in 2006, governments, corporations, and venture capitalists worldwide spent $11.8 billion on nanotechnology research and development (R&D), which was up 13% from 2005. By 2014, Lux estimates $2.6 trillion in manufactured goods

[†]1 nanometer (nm) = 1 billionth of a meter (10^{-9}).

[‡]The Project on Emerging Nanotechnologies was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts.

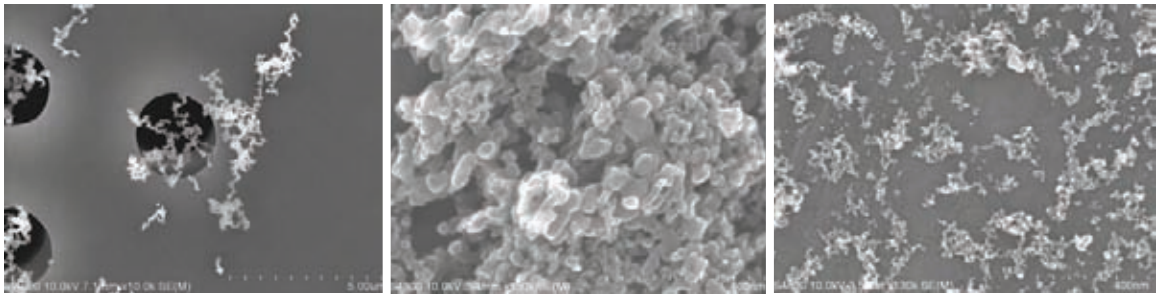

Figure 4–1. Photomicrographs of airborne exposure to ultrafine (nanoscale) particles of welding fumes, diesel exhaust, and cerium oxide

will incorporate nanotechnology—or about 15% of total global output.

4.1 Nano-objects

The International Organization for Standardization Technical Committee 229 (Nanotechnologies) is developing globally recognized nomenclature and terminology for nanomaterials. According to ISO/TS 27687:2008, *nano-object* is defined as material with one, two, or three external dimensions in the size range from approximately 1–100 nm. Subcategories of nano-object are (*1*) *nanoplate*, a nano-object with one external dimension at the nanoscale; (*2*) *nanofiber*, a nano-object with two external dimensions at the nanoscale with a nanotube defined as a hollow nanofiber and a nanorod as a solid nanofiber; and (*3*) *nanoparticle*, a nano-object with all three external dimensions at the nanoscale. Nano-objects are commonly incorporated in a larger matrix or substrate referred to as a *nanomaterial*. Nano-objects may be suspended in a gas (as a nanoaerosol), suspended in a liquid (as a colloid or nanohydrosol), or embedded in a matrix (as a nanocomposite).

The precise definition of particle diameter depends on particle shape as well as how the diameter is measured. Particle morphologies may vary widely at the nanoscale. For instance, carbon fullerenes represent nano-objects with identical dimensions in all directions (i.e., spherical), whereas single-walled carbon nanotubes (SWCNTs) typically form convoluted, fiber-like nano-objects. Many regular but nonspherical particle morphologies can be engineered at the nanoscale, including flower- and belt-like structures. Please see www.nanoscience.gatech.edu/zlwang/research.html for examples of some nanoscale structures.

4.2 Ultrafine Particles

The term *ultrafine particle* has traditionally been used by the aerosol research and occupational and environmental health communities to describe airborne particles smaller than 100 nm in diameter. *Ultrafine* is frequently used in the context of nanometer-diameter particles that have not been intentionally produced but are the incidental products of processes involving combustion, welding, or diesel engines (see Figure 4–1). The term *nanoparticle* is frequently used with respect to particles demonstrating size-dependent physicochemical properties, particularly from a materials science perspective. **The two terms are sometimes used to differentiate between engineered**

(*nanoparticle*) and incidental (*ultrafine*) nanoscale particles.

It is currently unclear whether the use of source-based definitions of nanoparticles and ultrafine particles is justified from a safety and health perspective. This is particularly the case where data on non-engineered, nanometer-diameter particles are of direct relevance to the impact of engineered particles. An attempt has been made in this document to follow the general convention of preferentially using *nanoparticle* in the context of intentionally produced or engineered particles and *ultrafine* in the context of incidentally produced particles (e.g., combustion products). However, this does not necessarily imply specific differences in the properties of these particles as related to hazard assessment, measurement, or control of exposures, and this remains an active area of research. *Nanoparticle* and *ultrafine particle* are not rigid definitions. For example, since the term *ultrafine* has been in existence longer, some intentionally produced particles with primary particle sizes in the nanosize range (e.g., TiO_2) are often called *ultrafine* in the literature.

4.3 Engineered Nanoparticles

Engineered nanoparticles are intentionally produced, whereas *ultrafine particles* (often referred to as *incidental nanoparticles)* are typically byproducts of processes such as combustion and vaporization. Engineered nanoparticles are designed with very specific properties or compositions (e.g., shape, size, surface properties, and chemistry). Incidental nanoparticles are generated in a relatively uncontrolled manner and are usually physically and chemically heterogeneous compared with engineered nanoparticles.

4.4 Nanoaerosol

A *nanoaerosol* is a collection of nanoparticles suspended in a gas. The particles may be present as discrete nano-objects, or as aggregates or agglomerates of nano-objects. These agglomerates may have diameters larger than 100 nm. In the case of an aerosol consisting of micrometer-diameter particles formed as agglomerates of nano-objects, the definition of nanoaerosol is open to interpretation. It is generally accepted that if the nanostructure associated with the nano-object is accessible (through physical, chemical, or biological interactions), then the aerosol may be considered a nanoaerosol. However, if the nanostructure within individual micrometer-diameter particles does not directly influence particle behavior (for instance, if the nanoparticles were inaccessibly embedded in a solid matrix), the aerosol would not be described as a nanoaerosol.

4.5 Agglomerate

An *agglomerate* is a group of nanoparticles held together by relatively weak forces, including van der Waals forces, electrostatic forces, and surface tension [ISO 2006].

4.6 Aggregate

An *aggregate* is a heterogeneous particle in which the various components are held together by relatively strong forces, and thus not easily broken apart [ISO 2006]. Aggregated nanoparticles would be an example of a nanostructured material.

5 Potential Health Concerns

Nanotechnology is an emerging field. As such, there are many uncertainties as to whether the unique properties of engineered nanomaterials (which underpin their commercial and scientific potential) also pose occupational health risks. These uncertainties arise because of gaps in knowledge about the factors that are essential for predicting health risks—factors such as routes of exposure, translocation of materials once they enter the body, and interaction of the materials with the body's biological systems. The potential health risk following exposure to a substance is generally associated with the magnitude and duration of the exposure, the persistence of the material in the body, the inherent toxicity of the material, and the susceptibility or health status of the person exposed. More data are needed on the health risks associated with exposure to engineered nanomaterials. Results of existing studies in animals and humans on exposure and response to ultrafine or other respirable particles provide a basis for preliminary estimates of the possible adverse health effects from exposures to similar engineered materials on a nanoscale. Experimental studies in rodents and cell cultures have shown that the toxicity of ultrafine or nanoparticles is greater than that of the same mass of larger particles of similar chemical composition [Oberdörster et al. 1992, 1994a, b; Lison et al. 1997; Tran et al. 1999, 2000; Brown et al. 2001; Barlow et al. 2005; Duffin et al. 2007]. In addition to particle surface area, other particle characteristics may influence toxicity, including surface functionalization or coatings, solubility, shape, and the ability to generate oxidant species and to adsorb biological proteins or bind to receptors [Duffin et al. 2002; Oberdörster et al. 2005a; Maynard and Kuempel 2005; Donaldson et al. 2006]. More research is needed on the influence of particle properties on interactions with biological systems and the potential for adverse effects. International research strategies for evaluating the safety of nanomaterials are actively being developed through cooperative efforts [Thomas et al. 2006].

Existing toxicity information about a given material of larger particle size can provide a baseline for anticipating the possible adverse health effects that may occur from exposure to a nanoscale material that has some of the same physicochemical properties (e.g., chemistry, density). However, predicting the toxicity of an engineered nanomaterial based on its physicochemical properties may not provide an adequate level of protection.

5.1 Exposure Routes

Inhalation is the most common route of exposure to airborne particles in the workplace. The deposition of discrete nano-objects in the respiratory tract is determined by the particle's aerodynamic or thermodynamic diameter (i.e., the particle shape and size). Agglomerates of nano-objects will deposit according to the diameter of the agglomerate, not constituent nano-objects. Research is ongoing to determine the physical factors that contribute to the agglomeration and de-agglomeration of nano-objects in air, suspended in aqueous media, or once in contact with lung lining fluid and/or biological proteins. Evidence indicates

that the degree of agglomeration can affect the toxicity of inhaled nano-objects [Shvedova et al. 2007].

Discrete nanoparticles are deposited in the lungs to a greater extent than larger respirable particles [ICRP 1994], and deposition increases with exercise due to increase in breathing rate and change from nasal to mouth breathing [Jaques and Kim 2000; Daigle et al. 2003] and among persons with existing lung diseases or conditions (e.g., asthma, emphysema) [Brown et al. 2002]. Based on animal studies, discrete nanoparticles may enter the bloodstream from the lungs and translocate to other organs [Takenaka et al. 2001; Nemmar et al. 2002; Oberdörster et al. 2002].

Discrete nanoparticles (35–37-nm median diameter) that deposit in the nasal region may be able to enter the brain by translocation along the olfactory nerve, as was observed in rats [Oberdörster et al. 2004; Oberdörster et al. 2005a; Elder et al. 2006]. The transport of insoluble particles from 20–500 nm-diameter to the brain via sensory nerves (including olfactory and trigeminus) was reported in earlier studies in several animal models [De Lorenzo 1970; Adams and Bray 1983; Hunter and Dey 1998]. This exposure route for nanoparticles and to nanoscale biological agents has not been studied in humans.

Some studies suggest that nanomaterials could potentially enter the body through the skin during occupational exposure. Tinkle et al. [2003] have shown that particles smaller than 1 μm in diameter may penetrate into mechanically flexed skin samples. A more recent study reported that nanoparticles with varying physicochemical properties were able to penetrate the intact skin of pigs [Ryman-Rasmussen et al. 2006]. These nanoparticles were quantum dots of different size, shape, and surface coatings. They were reported to penetrate the stratum corenum barrier by passive diffusion and localize within the epidermal and dermal layers within 8–24 hours. The dosing solutions were 2- to 4-fold dilutions of quantum dots as commercially supplied and thus represent occupationally relevant doses.

At this time, it is not fully known whether skin penetration of nanoparticles would result in adverse effects in animal models. However, topical application of raw SWCNT to nude mice has been shown to cause dermal irritation [Murray et al. 2007]. Studies conducted in vitro using primary or cultured human skin cells have shown that both SWCNT and multi-walled carbon nanotubes (MWCNT) can enter cells and cause release of pro-inflammatory cytokines, oxidative stress, and decreased viability [Monteiro-Riviere et al. 2005; Shvedova et al. 2003]. It remains unclear, however, how these findings may be extrapolated to a potential occupational risk, given that additional data are not yet available for comparing the cell model studies with actual conditions of occupational exposure. Research on the dermal exposure of nanomaterials is ongoing (www.uni-leipzig.de/~nanoderm/).

Ingestion can occur from unintentional hand to mouth transfer of materials; this has been found to happen with traditional materials, and it is scientifically reasonable to assume that it also could happen during handling of nanomaterials. Ingestion may also accompany inhalation exposure because particles that are cleared from the respiratory tract via the mucociliary escalator may be swallowed [ICRP 1994]. Little is known about possible adverse effects from the ingestion of nanomaterials.

5.2 Effects Seen in Animal Studies

Experimental studies in rats have shown that at equivalent mass doses, insoluble ultrafine particles are more potent than larger particles of similar composition in causing pulmonary inflammation, tissue damage, and lung tumors [Lee et al. 1985; Oberdörster and Yu 1990; Oberdörster et al. 1992, 1994a,b; Heinrich et al. 1995; Driscoll 1996; Lison et al. 1997; Tran et al. 1999, 2000; Brown et al. 2001; Duffin et al. 2002; Renwick et al. 2004; Barlow et al. 2005]. These studies have shown that for poorly-soluble low toxicity (PSLT) particles, the dose-response relationships are consistent across particle sizes when dose is expressed as particle surface area. In addition to particle size and surface area, studies have shown that other particle characteristics can influence toxicity. For example, although the relationship between particle surface area dose and pulmonary inflammation is consistent among PSLT particles, crystalline silica is much more inflammogenic than PSLT particles at a given surface area dose [Duffin et al. 2007].

Reactive oxidant generation on the particle surface is an important factor influencing lung response to particles, which can be related to crystal structure. A recent study of the lung effects of rats dosed with either ultrafine *anatase* titanium dioxide ($TiO_{2)}$ or ultrafine *rutile* TiO_2 showed that the *anatase* TiO_2 had more reactive surfaces and caused greater pulmonary inflammation and cell proliferation in the lungs of rats [Warheit et al. 2007]. In a cell-free assay designed to investigate the role of surface area and crystal structure on particle reactive oxygen species (ROS)-generation, Jiang et al. [2008] observed that size, surface area, and crystal structure all contribute to ROS generation.

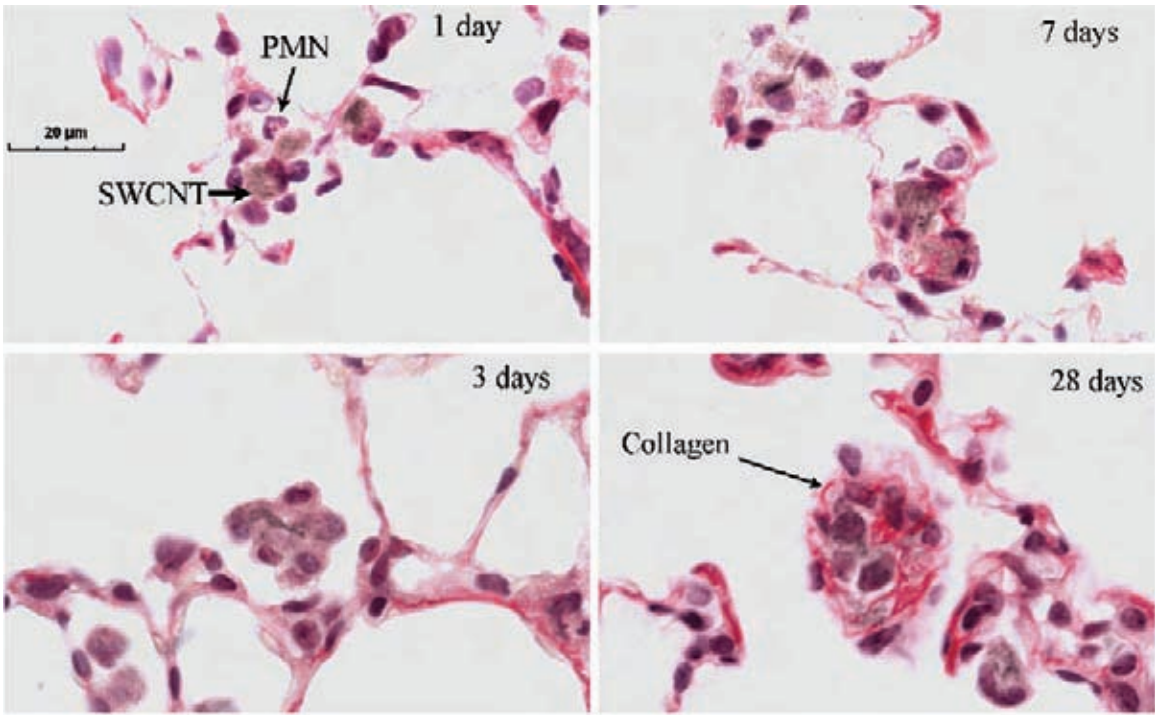

Figure 5–1. Formation of collagen following deposition of SWCNTs in the lungs of mice

Oxidant generation was apparently associated with the number of defective sites per surface area, which varied in nanoparticles in some size ranges [Jiang et al. 2008].

These studies indicate that for nanoparticles with similar properties (e.g., PSLT), the toxicity of a given mass dose will increase with decreasing particle size due to the increasing surface area. However, the dose-response relationship may differ for particles with different chemical composition and other properties. Consistent with these findings, a recent pulmonary instillation study with rats dosed with either fine or ultrafine TiO_2 reported no significant difference in lung responses when compared to controls, while crystalline silica caused more severe lung responses at the same dose [Warheit et al. 2006]. However, Warheit et al. [2006] were unable to adequately test the hypotheses about the relationship between particle surface area dose and toxicity because the diameters of the fine and ultrafine TiO_2-instilled particles did not significantly differ due to particle agglomeration, both being in excess of 2 μm. When efforts were made to more effectively disperse fine and ultrafine particles, the effect of surface area on the pulmonary response in rats after intratracheal instillation was verified [Sager et al. 2008].

5.2.1 Polytetrafluoroethylene fume

Among ultrafine particles, freshly generated polytetrafluoroethylene (PTFE) fume (generated at temperatures of more than 425°C) is known to be highly toxic to the lungs. Freshly generated PTFE fume caused hemorrhagic pulmonary edema and death in rats exposed to less than 60 $\mu g/m^3$ [Oberdörster et al. 1995]. In contrast, aged PTFE fume was much less toxic and did not result in mortality. This low toxicity was attributed to the increase in particle size from accumulation and to changes in surface chemistry [Johnston et al. 2000; Oberdörster et al. 2005a]. Human case studies have reported pulmonary edema in workers exposed to PTFE fume and an accidental death in a worker when an equipment malfunction caused overheating of the PTFE resin and release of the PTFE pyrolysis products in the workplace [Goldstein et al. 1987; Lee et al. 1997]. While PTFE fume differs from engineered nanoparticles, these studies illustrate properties of ultrafine particles that have been associated with an acute toxic hazard. Enclosed processes and other engineering controls appear to have been effective at eliminating worker exposures to PTFE fume in normal operations, and thus may provide examples of control systems that may be implemented to prevent exposure to nanoparticles that may have similar properties.

5.2.2 Carbon nanotubes

Carbon nanotubes (CNT) are specialized forms or structures of engineered nanomaterials that have had increasing production and use [Donaldson et al. 2006]. Consequently, a number of toxicologic studies of CNT have been performed in recent years. These studies have shown that the toxicity of CNT may differ from that of other nanomaterials of similar chemical composition. For example, single-walled CNTs (SWCNT) have been shown to produce adverse effects including granulomas in the lungs of mice and rats at mass doses at which ultrafine carbon black did not produce these adverse effects [Shvedova et al. 2005; Lam et al. 2004]. While both SWCNTs and carbon black are carbon-based, SWCNTs have a unique, convoluted, fibrous structure and specific surface chemistry that offers excellent electrical conductive properties. How these characteristics may influence

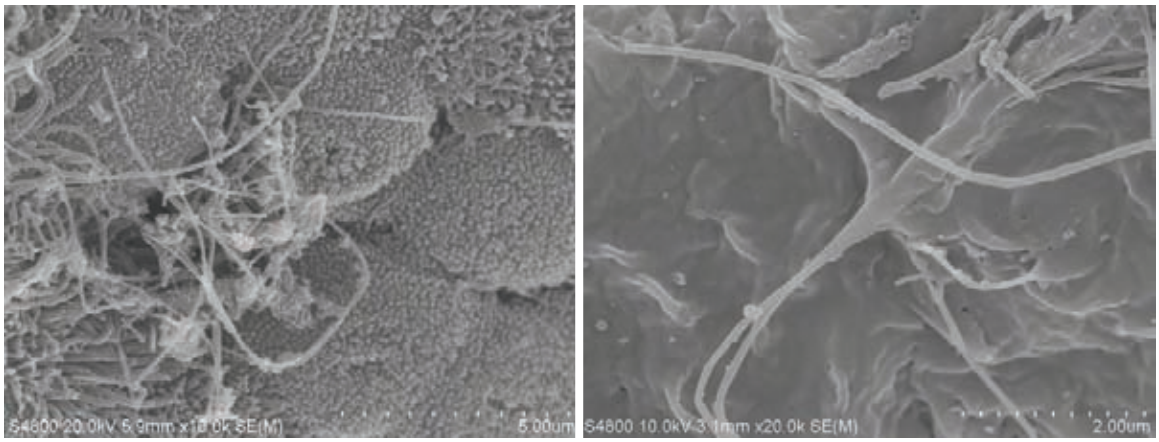

Figure 5–2. Deposition and clearance of MWCNTs from the conducting airways of mice following inhalation exposure

toxicity is not known. Carbon nanotubes may contain metal catalysts as byproducts of their production, which could contribute to their toxicity, or the CNTs may provide a structure that promotes fibroblast cell growth [Wang et al. 2008].

In a study of SWCNTs instilled into the lungs of rats, multi-focal granulomas (without transient inflammation or persistent lesions) were observed at doses of 1 or 5 mg/kg body weight [Warheit et al. 2004]. In a study of mice instilled with one of several types of SWCNTs (i.e., raw, purified, iron-containing, and nickel-containing) at doses of 0.1 or 0.5 mg/mouse (approximately 3 or 16 mg/kg body weight), dose-dependent epithelioid granulomas were observed at 7 days, which persisted at 90 days [Lam et al. 2004, 2006]. Both the raw and purified forms produced interstitial inflammation, while mortality (5/9 mice) was observed in the high dose group of the Ni-containing SWCNT.

NIOSH researchers recently reported adverse lung effects following pharyngeal aspiration of SWCNTs in mice using doses between 10–40 μg/mouse (approximately 0.5–2 mg/kg body weight) [Shvedova et al. 2005]. The findings showed that exposure to SWCNTs in mice lead to transient pulmonary inflammation, oxidative stress, decrease in pulmonary function, decrease in bacterial clearance, and early onset of interstitial fibrosis. Deposition of agglomerates resulted in development of granulomas, while deposition of dispersed nanotube structures in the aspirated suspension resulted in the rapid development of interstitial fibrosis (within 7 days), which progressed over a 30–60 day post-exposure period [Shvedova et al. 2005; Mercer et al. 2008]. Evidence indicates that when efforts were made to more fully disperse the SWCNT and obtain smaller structures in the aspiration suspension, fewer granulomas occurred but a 4-fold more potent interstitial fibrotic response was observed [Mercer et al. 2008].

Exposure to SWCNT has been observed to be more fibrogenic than an equal mass of either ultrafine carbon black or fine quartz [Shvedova et al. 2005; Lam et al. 2004]. Based on their findings in mice, Shvedova et al. [2005] estimated that workers may be at risk of developing lung lesions if they were exposed to SWCNT over a period of 20 days at the current

OSHA PEL for graphite (5 mg/m^3). Lam et al. [2004, 2006] provided similar estimates and suggested that the graphite PEL should not be used (e.g., on MSDS) as a safe concentration for workers exposed to CNTs. Compared to instillation, the pharyngeal aspiration technique may approximate more closely the particle deposition that occurs during inhalation. Inhalation studies of CNTs may provide more definitive information about their potential toxicity in humans [Donaldson et al. 2006]. Recently, NIOSH scientists designed a system to generate an aerosol of SWCNT for a rat inhalation study [Baron et al. 2008]. Results of the inhalation exposure to SWCNT [Shvedova et al. 2008] were qualitatively similar to those of the aspiration study [Shvedova et al. 2004] with a 4-fold more potent interstitial fibrotic response similar to that reported by Mercer et al. [2008]. Another NIOSH study found markers of inflammation in the lung, aorta, and heart tissues of ApoE-/- mice after a single intra-pharyngeal instillation dose of SWCNT (10 and 40 μg/mouse) and accelerated plaque formation after repeated doses (20 μg/mouse once every other week for 8 weeks in mice fed an atherogenic diet) [Li et al. 2007].

MWCNTs were recently studied by intratracheal instillation in Sprague-Dawley rats receiving 0.5, 2, or 5 mg (approximately 2, 9, or 22 mg/kg body weight) of either ground MWCNT or unground MWCNT [Muller et al. 2005]. Both forms produced pulmonary inflammation and fibrosis. Rats that received ground MWCNT showed greater dispersion in the lungs, and fibrotic lesions were observed in the deep lungs (alveolar region). In rats treated with MWCNT (not ground) fibrosis showed mainly in their airways rather than in their lungs. The biopersistence of the unground MWCNT was greater than that of the ground MWCNT, with 81% vs. 36%, respectively, remaining in the lungs at day 60. At an equal mass dose, ground MWCNT produced a similar inflammatory and fibrogenic response as chrysotile asbestos and a greater response than ultrafine carbon black [Muller et al. 2005]. Effects from the vehicle (1% Tween 80) used for administering ground and unground MWCNT to rats were not reported; the control group used in the study was exposed to only saline. NIOSH scientists have exposed mice by aspiration to MWCNT suspended in a simulated alveolar lining fluid rather than Tween 80. Control studies show that this suspension medium was not inflammatory and did not mask the biological activity of the particle surface. Data indicate that aspiration of dispersed MWCNT produced pulmonary inflammation, which peaked 7 days post exposure. The inflammatory response to MWCNT was greater than the inflammatory response to SWCNT [Sriram et al. 2007].

Two recent studies investigated the hypotheses that CNTs can behave like asbestos. In the first study, Takagi et al. [2008] administered to p53 (+/-) mice MWCNT, fullerene, or crocidolite asbestos by intraperitoneal injection at doses of 3 mg/mouse. The average width of the MWCNT was approximately 100 nm, and approximately 28% of the particles were longer than 5 μm. The particle number concentrations of MWCNT and crocidolite were 1×10^9 and 1×10^{10} (in 1-ml suspensions), respectively, although the MWCNT sample was also reported to contain mainly large aggregates, indicating that the number of MWCNT fibers was vastly underestimated and much larger than for the asbestos exposure. At the termination of the study (25 weeks), mesothelial responses in the MWCNT-treated mice included moderate to severe fibrous peritoneal adhesion and peritoneal tumors. The asbestos-treated mice had similar responses but to a lesser extent, while the

fullerene-treated group did not show these responses. Mesothelioma was considered by the authors as the primary cause of death, and constriction of the ileus due to severe peritoneal adhesion was considered to be the second major cause of death, suggesting that 3 mg/mouse exceeded the maximum tolerated dose of MWCNT. Whether mesotheliomia was a primary cause of death is somewhat speculative.

In a second study, Poland et al. [2008] administered to mice either MWCNT (two short and two long CNT samples), nanoscale carbon black, or amosite (short or long) at doses of 50 μg/mouse by intraperitoneal injection. The short CNTs were 10 nm or 15 nm in width, with no fibers larger than 15 μm in length detected; the long CNTs were 85 nm or 165 nm in width, and 24% or 84%, respectively, were larger than 15 μm in length (the percentage of fibers longer than 5 μm was not reported). After either 24 hours or 7 days, the long MWCNT caused inflammation and granulomatous lesions that were qualitatively and quantitatively similar to that caused by the long asbestos. The short, low-aspect-ratio, tangled aggregates of MWCNT did not produce these responses at the doses used in this study. Additional studies are needed to determine if this inflammatory response to MWCNT would be persistent and result in tumors of the abdominal wall. Additionally, the potential for migration of MWCNT through the lungs to the mesothelium after inhalation requires investigation. Long-term studies are also needed to determine whether CNTs can cause cancer such as mesothelioma in laboratory animals, including exposures by typical routes in humans (i.e., inhalation, dermal penetration, and ingestion) and at doses that include those equivalent to potential workplace exposures.

These studies indicate the need for more data on exposures of workers to CNTs. Maynard et al. [2004] reported relatively low short-term (approximately 30 min) airborne mass concentrations of SWCNT (0.007–0.053 mg/m^3) in a laboratory production facility. A recent study by Han et al. [2008] reported total airborne mass concentrations of MWCNT from 0.21–0.43 mg/m^3 (4–6-hr sampling) in a laboratory research facility prior to use of engineering control measures; after implementing controls, the concentration decreased to nondetectable. Workers could also be exposed to ground CNTs used in polymer composites and other matrices or during cutting, grinding, or polishing of these materials. Given that exposure to SWCNT and MWCNT causes interstitial fibrosis and pulmonary inflammation, respectively, in rodent lungs at relatively low mass doses, it is prudent to minimize worker exposure to airborne CNTs (see Chapter 8 *Guidelines for Working with Engineered Nanomaterials*).

5.3 Observations from Epidemiological Studies Involving Fine and Ultrafine Particles

Epidemiological studies in workers exposed to aerosols including fine and ultrafine particles have reported lung function decrements, adverse respiratory symptoms, chronic obstructive pulmonary disease, and fibrosis [Kreiss et al. 1997; Gardiner et al. 2001; Antonini 2003]. In addition, some studies have found lung disease including elevated lung cancer and neurological effects among workers exposed to certain ultrafine particles (i.e., diesel exhaust particulate) [Steenland et al. 1998; Garshick et al. 2004, 2006; Hart et al. 2006] or welding fumes [Antonini 2003; Park

et al. 2006; Ambroise et al. 2007; Bowler et al. 2007]. The implications of these studies to engineered nanomaterials, which may have different particle properties, are uncertain. Studies of airborne particles and fibers in the workplace do provide relevant background information about the particle-related lung diseases and mechanisms, and some limited quantitative estimates of exposures and risk of adverse health effects. As such, these studies provide a point of reference, including baseline information and estimates regarding possible health risks of exposure to other nanoscale particles depending on the extent to which the exposure conditions and particle-biological interactions may be similar.

Epidemiological studies in the general population have also shown associations between particulate air pollution and increased morbidity and mortality from respiratory and cardiovascular diseases [Dockery et al. 1993; HEI 2000; Pope et al. 2002, 2004]. Some epidemiological studies have shown adverse health effects associated with exposure to the ultrafine particulate fraction of air pollution [Peters et al. 1997, 2004; Penttinen et al. 2001; Ibald-Mulli et al. 2002; Timonen et al. 2004; Ruckerl et al. 2006] although uncertainty exists about the role of ultrafine particles relative to other air pollutants in causing the observed adverse health effects. The associations in these studies have been based on measurements of the particle number or mass concentrations of particles within certain size fractions (e.g., particulate matter with diameter of 2.5 μm and smaller [$PM_{2.5}$]). In an experimental study of healthy and asthmatic subjects inhaling ultrafine carbon particles, changes were observed in the expression of adhesion molecules by blood leukocyte, which may relate to possible cardiovascular effects of ultrafine particle exposure [Frampton et al. 2006]. Short-term diesel exhaust exposure (0.3 mg/m^3 for 1 hr) in healthy volunteers was associated with mild systemic inflammation and impaired endothelial-dependent vasodilation [Törnqvist et al. 2007].

5.4 Hypotheses from Animal and Epidemiological Studies

The existing literature on particles and fibers provides a scientific basis from which to evaluate the potential hazards of engineered nanomaterials. While the properties of engineered nanomaterials can vary widely, the basic physicochemical and toxicokinetic principles learned from the existing studies are relevant to understanding the potential toxicity of nanomaterials. For example, it is known from studies in humans that a greater proportion of inhaled nanoparticles will deposit in the respiratory tract (both at rest and with exercise) compared to larger particles [ICRP 1994; Jaques and Kim 2000; Daigle et al. 2003; Kim and Jaques 2004]. It is also known from studies in animals that nanoparticles in the lungs can be translocated to other organs in the body; how the chemical and physical properties of the nanoparticles influence this translocation is not completely known [Takenaka et al. 2001; Kreyling et al. 2002; Oberdörster et al. 2002, 2004; Semmler et al. 2004; Geiser et al. 2005]. Due to their small size, nanoparticles can cross cell membranes and interact with subcellular structures such as mitochondria, where they have been shown to cause oxidative damage and to impair function of cells in culture [Möller et al. 2002, 2005; Li et al. 2003; Geiser et al. 2005]. Nanoparticles have also been observed inside cell nuclei [Porter et al. 2007a, b]. Animal studies have shown that nanoparticles are more biologically

active due to their greater surface area per mass compared with larger-sized particles of the same chemistry [Oberdörster et al. 1992; 1994a,b; 2005a; Driscoll 1996; Lison et al. 1997; Brown et al. 2001; Duffin et al. 2002; Renwick et al. 2004; Barlow et al. 2005; Sager et al. 2008]. While this increased biological activity is a fundamental component to the utility of nanoparticles for industrial, commercial, and medical applications, the consequences of unintentional exposures of workers to nanoparticles are uncertain.

Research reported from laboratory animal studies and from epidemiological studies have lead to hypotheses regarding the potential adverse health effects of engineered nanomaterials. These hypotheses are based on the scientific literature of particle exposures in animals and humans. This literature has been recently reviewed [Donaldson et al. 2005; Maynard and Kuempel 2005; Oberdörster et al. 2005a, Donaldson et al. 2006; Kreyling et al. 2006]. In general, the particles used in past studies have not been characterized to the extent recommended for new studies in order to more fully understand the physicochemical properties of the particles that influence toxicity [Oberdörster et al. 2005b; Thomas et al. 2006]. As this research continues, more data will become available to support or refute the following hypotheses for engineered nanoparticles.

Hypothesis 1: Exposure to engineered nanoparticles is likely to cause adverse health effects similar to ultrafine particles that have similar physical and chemical characteristics.

Studies in rodents and humans support the hypothesis that exposure to ultrafine particles poses a greater respiratory hazard than exposure to the same mass of larger particles with a similar chemical composition. Studies of existing particles have shown adverse health effects in workers exposed to ultrafine particles (e.g., diesel exhaust particulate, welding fumes), and animal studies have shown that ultrafine particles are more inflammogenic and tumorigenic in the lungs of rats than an equal mass of larger particles of similar composition [Oberdörster and Yu 1990; Driscoll 1996; Tran et al. 1999, 2000]. **If engineered nanoparticles have the same physicochemical characteristics that are associated with reported effects from ultrafine particles, they may pose the same health concerns.**

Although the physicochemical characteristics of ultrafine particles and engineered nanoparticles can differ, the toxicologic and dosimetric principles derived from available studies may be relevant to postulating the health concerns for newly engineered particles. The biological mechanisms of particle-related lung diseases (i.e., oxidative stress, inflammation, and production of cytokines, chemokines, and cell growth factors) [Mossman and Churg 1998; Castranova 2000; Donaldson and Tran 2002] appear to be a consistent lung response for respirable particles including ultrafine or engineered nanoparticles [Donaldson et al. 1998; Donaldson and Stone 2003; Oberdörster et al. 2005a]. Toxicological studies have shown that the chemical and physical properties that influence the fate and toxicity of ultrafine particles may also be relevant to mechanisms influencing biological exposure and response to other nanoscale particles [Duffin et al. 2002; Kreyling et al. 2002; Oberdörster et al. 2002; Semmler et al. 2004; Nel et al. 2006].

Hypothesis 2: Surface area and activity and particle number may be better predictors of potential hazard than mass.

The greater potential hazard may relate to the greater number or surface area of nanoparticles compared with that for the same mass

concentration of larger particles [Oberdörster et al. 1992, 1994a,b; Driscoll et al. 1996; Tran et al. 2000; Brown et al. 2001; Peters et al. 1997; Moshammer and Neuberger 2003; Sager et al. 2008]. This hypothesis is based primarily on the pulmonary effects observed in studies of rodents exposed to various types of ultrafine or fine particles (i.e., TiO_2, carbon black, barium sulfate, carbon black, diesel soot, coal fly ash, toner) and in humans exposed to aerosols, including nanoscale particles (e.g., diesel exhaust, welding fumes). These studies indicate that for a given mass of particles, relatively insoluble nanoparticles are more toxic than larger particles of similar chemical composition and surface properties. Studies of fine and ultrafine particles have shown that particles with less reactive surfaces are less toxic [Tran et al. 1999; Duffin et al. 2002]. However, even particles with low inherent toxicity (e.g., TiO_2) have been shown to cause pulmonary inflammation, tissue damage, and fibrosis at sufficiently high particle surface area doses [Oberdörster et al. 1992, 1994a,b; Tran et al. 1999, 2000].

Through engineering, the properties of nanomaterials can be modified. For example, a recent study has shown that the cytotoxicity of water-soluble fullerenes can be reduced by several orders of magnitude by modifying the structure of the fullerene molecules (e.g., by hydroxylation) [Sayes et al. 2004]. These structural modifications were shown to reduce the cytotoxicity by reducing the generation of oxygen radicals—which is a probable mechanism by which cell membrane damage and death occurred in these cell cultures. Increasing the sidewall functionalization of SWCNT also rendered these nanomaterials less cytotoxic to cells in culture [Sayes et al. 2005]. Cytotoxicity studies with quantum dots have shown that the type of surface coating can have a significant effect on cell motility and viability [Hoshino et al. 2004; Shiohara et al. 2004; Lovric et al. 2005]. Differences in the phase composition of nanocrystalline structures can influence their cytotoxicity; in a recent study comparing two types of TiO_2 nanoparticles exposed to UV radiation, anatase TiO_2 was more cytotoxic and produced more reactive species than did rutile TiO_2 with similar specific surface area (153 m^2g and 123 m^2g of TiO_2, respectively) [Sayes et al. 2006]. Reactive oxygen species were also associated with the cytotoxicity of TiO_2 nanoparticles to mouse microglia (brain cells) grown in culture [Long et al. 2006]. In contrast, in vitro generation of oxidant species is relatively low in purified SWCNT (contaminating metals removed), yet this material caused progressive interstitial fibrosis in vivo [Shvedova et al. 2004; 2005]. However, recent in vitro studies indicate that purified SWCNTs enhance proliferation and collagen production in fibroblasts [Wang et al. 2008]. Therefore, oxidant generation may not be the only mechanism driving the biological activity of nanomaterials.

The studies of ultrafine particles may provide useful data to develop preliminary hazard or risk assessments and to generate hypotheses for further testing. The studies in cell cultures provide information about the cytotoxic properties of nanomaterials that can guide further research and toxicity testing in whole organisms. More research is needed of the specific particle properties and other factors that influence the toxicity and disease development, including those characteristics that may be most predictive of the potential safety or toxicity of newly engineered nanomaterials.

6 Potential Safety Hazards

Very little is known about the safety risks that engineered nanomaterials might pose, beyond some data indicating that they possess certain properties associated with safety hazards in traditional materials. Based upon currently available information, the potential safety concerns most likely would involve catalytic effects or fire and explosion hazards if nanomaterials are found to behave similarly to traditional materials.

6.1 Fire and Explosion Risk

Although insufficient information exists to predict the fire and explosion risk associated with nanoscale powders, **nanoscale combustible material could present a higher risk than a similar quantity of coarser material, given its unique properties** [HSE 2004]. Decreasing the particle size of combustible materials can increase combustion potential and combustion rate, leading to the possibility of relatively inert materials becoming highly reactive in the nanometer size range. Dispersions of combustible nanomaterial in air may present a greater safety risk than dispersions of non-nanomaterials with similar compositions. Some nanomaterials are designed to generate heat through the progression of reactions at the nanoscale. Such materials may present a fire hazard that is unique to engineered nanomaterials. In the case of some metals, explosion risk can increase significantly as particle size decreases.

The greater activity of nanoscale materials forms a basis for research into nanoenergetics. For instance, nanoscale Al/MoO_3 thermites ignite more than 300 times faster than corresponding micrometer-scale material [Granier and Pantoya 2004].

6.2 Risks of Catalytic Reactions

Nanoscale particles and nanostructured porous materials have been used as effective catalysts for increasing the rate of reactions or decreasing the necessary temperature for reactions to occur in liquids and gases. **Depending on their composition and structure, some nanomaterials may initiate catalytic reactions that, based on their chemical composition, would not otherwise be anticipated [Pritchard 2004].**

7 Exposure Assessment and Characterization

There are currently no national or international consensus standards on measurement techniques for nanomaterials in the workplace. If the qualitative assessment of a process has identified potential exposure points and leads to the decision to measure nanomaterials, several factors must be kept in mind. Current research indicates that mass and bulk chemistry may be less important than particle size, surface area, and surface chemistry (or activity) for nanostructured materials [Oberdörster et al. 1992, 1994a,b; Duffin et al. 2002]. Research is ongoing into the relative importance of these different exposure metrics, and how to best characterize exposures to nanomaterials in the workplace. In addition, the unique shape and properties of some nanomaterials may pose additional challenges. For example, the techniques used to measure fiber concentrations in the workplace (e.g., phase contrast microscopy) would not be able to detect individual carbon nanotubes with diameters less than 100 nm nor bundles of carbon nanotubes with diameters less than 250 nm [Donaldson et al. 2006]. NIOSH and the National Institute of Standards and Technology (NIST) are collaborating on efforts to develop nanoscale reference materials for exposure assessment. Initial effort is focused on development of TiO_2 reference material.

7.1 Workplace Exposures

While research continues to address questions of nanomaterial toxicity, a number of exposure assessment approaches can be used to help determine worker exposures to airborne nanomaterials. These assessments can be performed using traditional industrial hygiene sampling methods including samplers placed at static locations (area sampling), samples collected in the breathing zone of the worker (personal sampling), or real-time devices or methods that can be personal or static. In general, personal sampling is preferred to ensure an accurate representation of the worker's exposure, whereas area samples (e.g., size-fractionated aerosol samples) and real-time (direct-reading) exposure measurements may be more useful for evaluating the need for improvement of engineering controls and work practices.

Many of the sampling techniques that are available for measuring nanoaerosols vary in complexity but can provide useful information for evaluating occupational exposures with respect to particle size, mass, surface area, number concentration, composition, and surface chemistry. Unfortunately, relatively few of these techniques are readily applicable to routine exposure monitoring. Research is ongoing into developing an analytical strategy for determining both TiO_2 surface area and titanium mass from 37-mm cassette filter samplers. Current measurement techniques are described below along with their applicability for monitoring nanometer aerosols.

For each measurement technique used, it is vital that the key parameters associated with the technique and sampling methodology be recorded when measuring exposure to nanoaerosols. This should include the response range of the instrumentation,

whether personal or static measurements are made, and the location of all potential aerosol sources including background aerosols. Comprehensive documentation will facilitate comparison of exposure measurements using different instruments or different exposure metrics and will aid the re-interpretation of historic data as further information is developed on health-appropriate exposure metrics. **Regardless of the metric and method selected for exposure monitoring, it is critical that measurements be taken before production or processing of a nanomaterial to obtain background nanoparticle exposure data.** Measurements made during production or processing can then be evaluated to determine if there has been an increase in particle number concentrations in relation to background measurements and whether that change represents worker exposure to the nanomaterial. Table 7–1 gives a listing of instruments and measurement methods that can be used in the evaluation of engineered nanoparticle exposures.

7.1.1 Size-fractionated aerosol sampling

Studies indicate that particle size plays an important role in determining the potential adverse effects of nanomaterials in the respiratory system: by influencing the physical, chemical, and biological nature of the material; by affecting the surface-area dose of deposited particles; and by enabling deposited particles to more readily translocate to other parts of the body. Animal studies indicate that the toxicity of inhaled nanoparticles is more closely associated with the particle surface area and particle number than with the particle mass concentration when comparing aerosols with different particle size distributions. However, mass concentration measurements may be applicable for evaluating occupational exposure to nanometer aerosols where a good correlation between the surface area of the aerosol and mass concentration can be determined or if toxicity data based on mass dose are available for a specific nanoscale particle associated

Figure 7–1. Examples of different sampling instruments used to measure occupational exposures to nanoparticles including the determination of real-time particle number concentrations and size-fractionated mass concentrations

with a known process (e.g., diesel exhaust particulate).

Aerosol samples can be collected using inhalable, thoracic, or respirable samplers, depending on the region of the respiratory system most susceptible to the inhaled particles. Since prevailing **information suggests that a large fraction of inhaled nanoparticles will deposit in the gas-exchange region of the lungs [ICRP 1994], respirable samplers would be appropriate.** Respirable samplers will also collect a nominal amount of nanoscale particles that can deposit in the upper airways and ultimately be cleared or transported to other parts of the body.

Table 7–1. Summary of instruments and measurement methods used in the evaluation of nanomaterial exposures*

Metric	Instrument or method	Remarks
Mass-Direct (total and/or elemental)	Size Selective Static Sampler	The only instruments offering a cut point around 100 nm are cascade impactors (Berner-type low pressure impactors, or Micro orifice impactors). Allows gravimetric and chemical analysis of samples on stages below 100 nm.
	TEOM® (Tapered Element Oscillating Microbalance)	Sensitive real-time monitors such as the TEOM may be useable to measure nanoaerosol mass concentration on-line with a suitable size selective inlet.
	Filter collection and elemental analysis	Filters may be collected with size selective pre-samplers or open face. Elemental analysis (e.g., carbon, metals) for mass determination.
Mass-Indirect (calculation)	ELPI™ (Electrical Low Pressure Impactor)	Real time size-selective (aerodynamic diameter) detection of active surface area concentration giving aerosol size distribution. Mass concentration of aerosols can be calculated when particle charge and density are known or assumed.
	MOUDI (Micro-Orfice Uniform Deposit Impactor)	Real time size-selective (aerodynamic diameter) by cascade impaction.
	DMAS (Differential Mobility Analyzing System)	Real time size-selective (mobility diameter) detection of number concentration, giving aerosol size distribution. Mass concentration of aerosols can be calculated when particle shape and density are known or assumed.

(continued)

See footnotes at end of table.

Table 7–1 (Continued). Summary of instruments and measurement methods used in the evaluation of nanomaterial exposures*

Metric	Instrument or method	Remarks
Number-Direct	CPC (Condensation Particle Counter)	CPCs provide real time number concentration measurements between their particle diameter detection limits. Without a nanoparticle pre-separator they are not specific to the nanometer size range. Some models have diffusion screen to limit top size to 1 μm.
	OPC (Optical Particle Counter)	OPCs provide real time number concentration measurements between their particle diameter detection limits. Particle size diameters begin at 300 nm and may go up to 10,000 nm.
	DMAS and SMPS (Scanning Mobility Particle Sizer)	Real time size-selective (mobility diameter) detection of number concentration giving number-based size distribution.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on size-specific aerosol number concentration.
Number-Indirect	ELPI™ and MOUDI	Real time size-selective (aerodynamic diameter) detection of active surface-area concentration giving aerosol size distribution. Data may be interpreted in terms of number concentration. Size-selective samples may be further analyzed off-line.
Surface Area-Direct	Diffusion Charger	Real-time measurement of aerosol active surface-area. Active surface-area does not scale directly with geometric surface-area above 100 nm. Note that not all commercially available diffusion chargers have a response that scales with particle active surface-area below 100 nm. Diffusion chargers are only specific to nanoparticles if used with appropriate inlet pre-separator.
	ELPI™ and MOUDI	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration. Active surface-area does not scale directly with geometric surface-area above 100 nm.

(continued)

See footnotes at end of table.

Table 7–1 (Continued). Summary of instruments and measurement methods used in the evaluation of nanomaterial exposures*

Metric	Instrument or method	Remarks
Surface Area-Direct (continued)	Electron Microscopy	Off-line analysis of electron microscope samples (previously collected on filters or other media) can provide information on particle surface-area with respect to size. TEM analysis provides direct information on the projected area of collected particles which may be related to geometric area for some particles shapes.
Surface Area-Indirect (calculation)	DMAS and SMPS	Real time size-selective (mobility diameter) detection of number concentration. Data may be interpreted in terms of aerosol surface-area under certain circumstances. For instance, the mobility diameter of open agglomerates has been shown to correlate with projected surface area.
	DMAS and ELPI™ used in parallel	Differences in measured aerodynamic and mobility can be used to infer particle fractal dimension which can be further used to estimate surface-area.

*Adapted from ISO/TR 12885

Note: Inherent to all air sampling instruments in this table is the fact that they cannot discriminate the nanoaerosol of interest from other airborne particles. Also, there is a general lack of validation regarding the response of these air sampling instruments to the full spectrum of nanoparticles that may be found in the workplace, including varieties of primary particles, agglomerates or aggregates, and other physical and chemical forms. A suite of nanoparticle reference materials are required to perform the needed validations.

Respirable samplers allow mass-based exposure measurements to be made using gravimetric and/or chemical analysis [NIOSH 1994]. However, they do not provide information on aerosol number, size, or surface-area concentration, unless the relationship between different exposure metrics for the aerosol (e.g., density, particle shape) has been previously characterized. Currently, no commercially available personal samplers are designed to measure the particle number, surface area, or mass concentration of nanoaerosols. However, several methods are available that can be used to estimate surface area, number, or mass concentration for particles smaller than 100 nm.

The use of conventional impactor samplers to assess nanoparticle exposure is limited since the impaction collection efficiencies are 200–300 nm. Low-pressure cascade impactors that can measure particles to 50 nm and larger may be used for static sampling since their size and complexity preclude their use as personal samplers [Marple et al. 2001; Hinds 1999]. A personal cascade impactor is available with a lower aerosol cut point of

250 nm [Misra et al. 2002], allowing an approximation of nanoscale particle mass concentration in the worker's breathing zone. For each method, the detection limits are on the order of a few micrograms of material on a filter or collection substrate [Vaughan et al. 1989]. Cascade impactor exposure data gathered from worksites where nanomaterials are being processed or handled can be used to make assessments as to the efficacy of exposure control measures.

7.1.2 Real-time aerosol sampling

The real-time (direct-reading) measurement of nanometer aerosol concentrations is limited by the sensitivity of the instrument to detect small particles. Many real-time aerosol mass monitors used in the workplace rely on light scattering from groups of particles (photometers). This methodology is generally insensitive to particles smaller than 100 nm [Hinds 1999]. Optical instruments that size individual particles and convert the measured distribution to a mass concentration are similarly limited to particles larger than 100 nm. Quantitative information gained by optical particle counters may also be limited by relatively poor counting efficiencies at smaller particle diameters (i.e., less than 500 nm). These instruments are capable of operating within certain concentration ranges that, when exceeded, affect the count or mass determination efficiencies due to coincidence errors at the detector. Similarly, the response of optical particle counters may be material-dependent according to the refractive index of the particle. The Scanning Mobility Particle Sizer (SMPS) is widely used as a research tool for characterizing nanoscale aerosols although its applicability for use in the workplace may be limited because of its size, cost, and the inclusion of a radioactive source. Additionally, the SMPS may take 2–3 minutes to scan an entire size distribution; thus, it may be of limited use in workplaces with highly variable aerosol size distributions, such as those close to a strong particle source. Fast (less than 1 second), mobility-based, particle-sizing instruments are now available commercially; however, having fewer channels, they lack the finer sizing resolution of the SMPS. The Electrical Low Pressure Impactor (ELPI) is an alternative instrument that combines diffusion charging and a cascade impactor with real-time (less than 1 second) aerosol charge measurements providing aerosol size distributions by aerodynamic diameter [Keskinen et al. 1992].

7.1.3 Surface-area measurements

Relatively few techniques exist to monitor exposures with respect to aerosol surface area. Particle surface is composed of internal surface area attributable to pores (cavities more deep than wide), external surface area due to roughness (cavities more wide than deep), and total surface area (the accessible area of all real particle surfaces). A standard gas adsorption technique (i.e., BET) is used to measure the total surface area of powders and can be adapted to measure the specific surface area (surface area per unit mass) of engineered nanomaterials [Brunauer et al. 1938]. However, surface-area analysis by gas adsorption requires relatively large quantities of material, is not element specific, and must be performed in a laboratory.

The first instrument designed to measure aerosol surface area was the epiphaniometer [Baltensperger et al. 1988]. This device measures the Fuchs, or active surface area, of the aerosols by measuring the attachment rate of

radioactive ions. For aerosols less than approximately 100 nm in size, measurement of the Fuchs surface area is probably a good indicator of external surface area (or geometric surface area). However, for aerosols greater than approximately 1 μm, the relationship with geometric particle surface area is lost [Fuchs 1964]. Measurements of active surface area are generally insensitive to particle porosity. The epiphaniometer is not well suited to widespread use in the workplace because of the inclusion of a radioactive source and the lack of effective temporal resolution.

This same measurement principle can be applied with the use of a portable aerosol diffusion charger. Studies have shown that these devices provide a good estimate of aerosol external surface area when airborne particles are smaller than 100 nm in diameter. For larger particles, diffusion chargers underestimate aerosol surface area. However, further research is needed to evaluate the degree of underestimation. Extensive field evaluations of commercial instruments are yet to be reported. However, laboratory evaluations with monodisperse silver particles have shown that two commercially available diffusion chargers can provide good measurement data on aerosol external surface area for particles smaller than 100 nm in diameter but underestimate the aerosol surface area for particles larger than 100 nm in diameter [Ku and Maynard 2005, 2006].

7.1.4 Particle number concentration measurement

Particle number concentration has been associated with adverse responses to air pollution in some human studies [Timonen et al. 2004; Ruckerl et al. 2005], while in toxicologic studies, particle surface area has generally been shown to be a better predictor than either particle number, mass, or volume concentration alone [Oberdörster and Yu 1990; Tran et al. 1999; Duffin et al. 2002]. A two-variable dose metric of particle size and volume has been shown to be the best predictor of lung cancer in rats from various types of particles [Borm et al. 2004; Pott and Roller 2005]. This illustrates some of the complexity of interpreting existing data on particle dose metric and response. While adverse health effects appear to be more closely related with particle surface area, the number of particles depositing in the respiratory tract or other organ systems may also play an important role.

Aerosol particle number concentration can be measured relatively easily using Condensation Particle Counters (CPCs). These are available as hand-held static instruments, and they are generally sensitive to particles greater than 10–20 nm in diameter. Condensation Particle Counters designed for the workplace do not have discrete size-selective inputs, and so they are typically sensitive to particles less than 1 μm in diameter. Commercial size-selective inlets are not available to restrict CPCs to the nanoparticle size range; however, the technology exists to construct size-selective inlets based on particle mobility or possibly on inertial pre-separation. An alternative approach to estimating nanoparticle number concentrations using a CPC is to use the instrument in parallel with an optical particle counter (OPC). The difference in particle count between the instruments will provide an indication of particle number concentration between the lower CPC detectable particle diameter and the lower OPC particle diameter (typically 300–500 nm).

A critical issue when characterizing exposure using particle number concentration

is selectivity. **Nanoscale particles are ubiquitous in many workplaces**, from sources such as combustion, vehicle emissions, and infiltration of outside air. Particle counters are generally insensitive to particle source or composition **making it difficult to differentiate between incidental and process-related nanoparticles using number concentration alone.** In a study of aerosol exposures during a carbon black bagging process, Kuhlbusch et al. [2004] found that peaks in number concentration measurements were associated with emissions from fork lift trucks and gas burners in the vicinity, rather than with the process itself. In a similar manner, during an ultrafine particle mapping exercise in an automotive facility, Peters et al. [2006] found that direct gas-fired heating systems systematically produced high particle number concentrations throughout the facility when the heating system was in operation. Through follow up measurements, Heitbrink et al. [2007] found a high proportion of ultrafine particles produced from these burners, yet little if any mass was associated with their emissions. Other non-process ultrafine sources were identified in an adjacent foundry [Evans et al. 2008]. Together with roof mounted gas-fired heating units, additional sources included cigarette-smoking and the exhaust from a propane fueled sweeper vehicle, with the latter contributing a large fraction of the ultrafine particles. Although these issues are not unique to particle number concentration measurements, orders of magnitude difference can exist in particle number concentrations depending on concomitant sources of particle emissions.

Although using nanoparticle number concentration as an exposure measurement may not be consistent with exposure metrics being used in animal toxicity studies, **such measurements may be useful for identifying nanoscale particle emissions and determining the efficacy of control measures.** Portable CPCs are capable of measuring localized particle concentrations allowing the assessment of particle releases occurring at various processes and job tasks [Brouwer et al. 2004].

7.1.5 Surface-area estimation

Information about the relationship between different measurement metrics can be used for approximating particle surface area. If the size distribution of an aerosol remains consistent, the relationship between particle number, surface area, and mass metrics will be constant. In particular, mass concentration measurements can be used for deriving surface-area concentrations, assuming the constant of proportionality is known. This constant is the specific surface area (surface to mass ratio).

Size distribution measurements may be obtained through the collection of filter samples and analysis by transmission electron microscopy to estimate particle surface area. If the measurements are weighted by particle number, information about particle geometry will be needed to estimate the surface area of particles with a given diameter. If the measurements are weighted by mass, additional information about particle density will be required. Estimates of particle-specific surface area from geometric relation with external particle dimensions depends upon the morphology regime of the material of interest and is only appropriate for smooth, regularly shaped, compact particles [Stefaniak et al. 2003; Weibel et al. 2005]. For example, Weibel et al. [2005] report that estimates of ultrafine TiO_2 surface area determined using a geometric relationship with the physical particle size

(using TEM) were 50% lower than measured using nitrogen gas adsorption.

If the airborne aerosol has a lognormal size distribution, particle surface-area concentration can be derived using three independent measurements. An approach has been proposed using three simultaneous measurements of the aerosol that included mass concentration, number concentration, and charge [Woo et al. 2001]. With knowledge of the response function of each instrument, minimization techniques can be used to estimate the parameters of the lognormal distribution leading to the three measurements used in estimating the particle surface area.

An alternative approach has been proposed whereby independent measurements of particle number and mass concentration are made, and the surface area is estimated by assuming the geometric standard deviation of the (assumed) lognormal distribution [Maynard 2003]. This method has the advantage of simplicity by relying on portable instruments that can be used in the workplace. Theoretical calculations have shown that estimates may be up to a factor of 10 different from the actual particle surface area, particularly when the aerosol has a bimodal distribution. Field measurements indicate that estimates are within a factor of 3 of the active surface area, particularly at higher concentrations. In workplace environments, particle surface-area concentrations can be expected to span up to 5 orders of magnitude; thus, surface-area estimates may be suited for initial or preliminary appraisals of occupational exposure concentrations.

Although such estimation methods are unlikely to become a long-term alternative to more accurate methods, they may provide a viable interim approach to estimating the surface area of nanoscale particles in the absence of precise measurement data. Additional research is needed on comparing methods used for estimating particle surface area with a more accurate particle surface-area-measurement method. NIOSH is conducting research in this area and will communicate results as they become available.

7.1.6 Particle number concentration mapping

To better understand particle sources and contaminant migration, some investigators have adopted an aerosol mapping technique, which integrated measurements of respirable mass, ultrafine particle number, and active surface-area concentrations in automotive manufacturing facilities [Peters et al. 2006; Heitbrink et al. 2007, 2008; Evans et al. 2008]. The process relies on portable aerosol sampling instrumentation for simultaneous measurements at predetermined positions throughout a facility. The technique is somewhat measurement-intensive but is useful for locating contaminant sources and determining the extent of contaminant migration. Leaks and other less obvious particle sources have been identified in this way and the procedure provides a powerful tool for facility staff to target their contaminant control approaches most effectively. This technique relies on successive measurements at various locations, making facilities with continuous processes or those likely to achieve steady state particle number concentrations most appropriate for this approach. The approach is less successful for facilities with batch processes or those likely to experience rapid concentration changes as, depending on where in the measurement cycle the release occurs, it may be overlooked. A high degree of variability between mapping events is expected in

facilities where sporadic or batch processing occurs.

7.2 Sampling Strategy

Currently, there is not one sampling method that can be used to characterize exposure to nanoscale aerosols. Therefore, any attempt to characterize workplace exposure to nanomaterials must involve a multifaceted approach incorporating many of the sampling techniques mentioned above. Brouwer et al. [2004] recommend that all relevant characteristics of nanomaterial exposure be measured, and a sampling strategy similar to theirs would provide a reasonable approach to characterizing workplace exposure. NIOSH has developed the Nanoparticle Emission Assessment Technique (NEAT) to qualitatively determine the release of engineered nanomaterials in the workplace (see Appendix). This approach may be helpful to others for the initial evaluation of workplaces where engineered nanomaterials are manufactured or used. If material release is found and if resources allow, then a more comprehensive and quantitative approach may be adopted [Methner et al. 2007].

The first step to characterizing workplace exposures would involve identifying the source of nanomaterial emissions. A CPC used in parallel with an OPC provides acceptable capability for this purpose. **It is critical to determine ambient or background particle counts before measuring particle counts during the manufacturing, processing, or handling of engineered nanomaterials.** However, investigators need to be aware that background nanoscale particle counts can vary both spatially and temporally depending on the unique conditions of the workplace. Subtraction of background nanoscale particle counts will be most challenging in these situations. In cases where nanomaterial handling or processing operations contribute only small elevations in particle counts, it may not be possible to adequately characterize these increases, particularly if the background particle count is relatively high.

If nanomaterials are detected in the process area at elevated concentrations relative to background particle number concentrations, then a pair of filter-based, area air samples should be collected for particle analysis via transmission electron microscopy (TEM) and for determining mass concentration. Transmission electron microscopy can provide an estimate of the particle size distribution and, if equipped with an energy dispersive X-ray analyzer (EDS), a determination of elemental composition

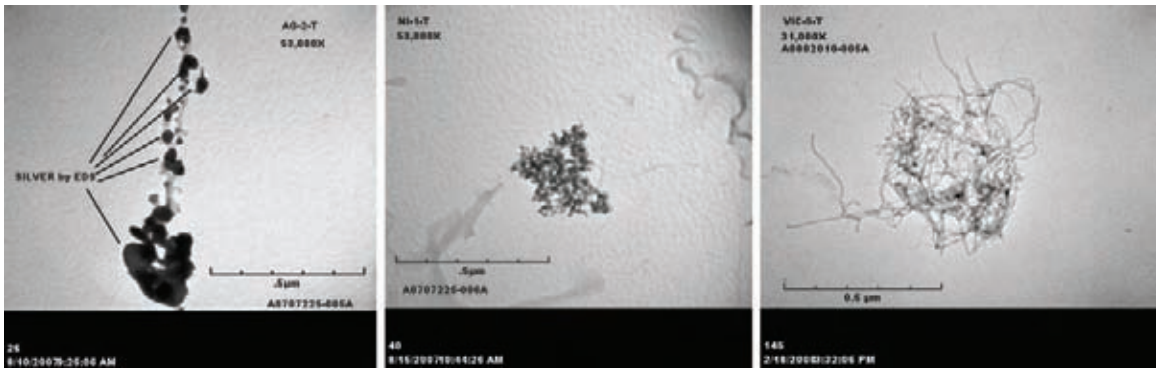

Figure 7–2. Photomicrographs of airborne engineered nanomaterials (airborne samples of engineered nanoparticles of silver, nickel, and MWCNT analyzed by TEM and EDS)

can be made to identify the nanomaterial (see Figure 7–2).

Analysis of filters for mass determination of air contaminants of interest can help identify the source of the particles. Standard analytical chemical methodologies (e.g., NMAM 5040 for carbon, NMAM 7303 for metals) should be employed [NIOSH 1994].

The combination of particle counters and samples for chemical analysis allows for an assessment of worker exposure to nanomaterials (see Figure 7–3) and the characterization of the important aerosol metrics. However, since this approach relies primarily on static or area sampling, some uncertainty will exist in estimating worker exposures.

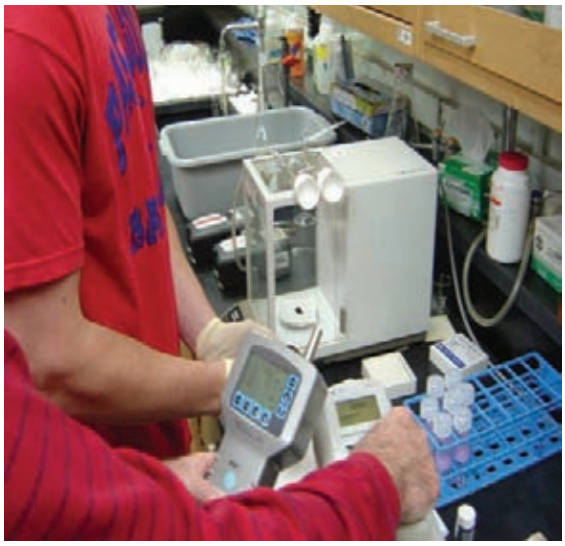

Figure 7–3. Combined use of the OPC, CPC, and two filter samples to determine the presence of nanomaterials

8 Guidelines for Working with Engineered Nanomaterials

Engineered nanomaterials are diverse in their physical, chemical, and biological nature. The processes used in research, material development, production, and use or introduction of nanomaterials have the potential to vary greatly. **Until further information on the possible health risks and extent of occupational exposure to nanomaterials becomes available, interim protective measures should be developed and implemented.** These measures should focus on the development of engineering controls and safe working practices tailored to the specific processes and materials where workers might be exposed. Hazard information that is available about common materials being manufactured in the nanoscale range (e.g., TiO_2, beryllium) should be considered as a starting point in developing appropriate controls and work practices.

The following recommendations are designed to aid in the assessment and control of workplace exposures to engineered nanomaterials. Using a hazard-based approach to evaluate exposures and for developing precautionary measures is consistent with good occupational safety and health practices [The Royal Society and The Royal Academy of Engineering 2004; Schulte et al. 2008].

8.1 Potential for Occupational Exposure

Few workplace measurement data exist on airborne exposure to nanomaterials that are purposely produced and not incidental to an industrial process. In general, it is likely that processes generating nanomaterials in the gas phase (after removal of the nanomaterial from an enclosed generation system), or using or producing nanomaterials as powders or slurries/suspensions/solutions (i.e., in liquid media), pose the greatest risk for releasing nanoparticles. In addition, **maintenance on production systems (including cleaning and disposing of materials from dust collection systems) is likely to result in exposure to nanoparticles if deposited nanomaterials are disturbed.** Exposures associated with waste streams containing nanomaterials may also occur.

The magnitude of exposure to nanomaterials when working with nanopowders depends on the likelihood of particles being released from the powders during handling. NIOSH is actively conducting research to quantitatively determine how various nanomaterials are dispersed in the workplace. Studies on exposure to SWCNTs and MWCNTs have indicated that the raw material may release visible particles into the air when handled, that the particle size of the agglomerate can be a few millimeters in diameter, and that the release rate of inhalable and respirable particles is relatively low (on a mass or number basis) compared with other nanopowders. Maynard et al. [2004] reported concentrations of respirable dust from 0.007 to 0.053 mg/m^3 when energy was applied (vortexing) to bulk SWCNT for approximately 30 minutes. Similar findings were reported by Han et al. [2008] at a laboratory producing MWCNTs in which exposure concentrations as high as 0.4 mg/m^3 were observed prior to the implementation of exposure controls. In a health hazard evaluation conducted by NIOSH at a

university-based research laboratory the potential release of airborne carbon nanotubes (CNFs) was observed at various processes [Methner et al. 2007]. General area exposure measurements indicated slight increases in airborne particle number and mass concentrations relative to background measurements during the transfer of CNFs prior to weighing and mixing, and during wet saw cutting of a composite material. Since data are lacking on the generation of inhalable/respirable particles during the production and use of engineered nanomaterials, further research is required to determine exposures under various conditions. NIOSH researchers are conducting both laboratory and field-based evaluations in order to address some of these knowledge gaps.

Devices comprised of nanostructures, such as integrated circuits, pose a minimal risk of exposure to nanomaterials during handling. However, some of the processes used in their production may lead to exposure to nanomaterials (e.g., exposure to commercial polishing compounds that contain nanoscale particles, exposure to nanoscale particles that are inadvertently dispersed or created during the manufacturing and handling processes). Likewise, large-scale components formed from nanocomposites will most likely not present significant exposure potential. However, if such materials are used or handled in such a manner that can generate nanoparticles (e.g., cutting, grinding) or undergo degradation processes that lead to the release of nanostructured material, then exposure may occur by the inhalation, ingestion, and/or dermal penetration of these particles.

8.2 Factors Affecting Exposure to Nanomaterials

Factors affecting exposure to engineered nanomaterials include the amount of material being used and whether the material can be easily dispersed (in the case of a powder) or form airborne sprays or droplets (in the case of suspensions). The degree of containment and duration of use will also influence exposure. In the case of airborne material, particle or droplet size will determine whether the material can enter the respiratory tract and where it is most likely to deposit. Respirable particles are those capable of depositing in the alveolar (gas exchange) region of the lungs, which includes particles smaller than approximately 10 μm in diameter [Lippmann 1977; ICRP 1994; ISO 1995]. The proportion of inhaled nanoparticles likely to deposit in any region of the human respiratory tract is approximately 30%–90% depending on factors such as breathing rate and particle size. Up to 50% of nanoparticles in the 10–100 nm size range may deposit in the alveolar region, while nanoparticles smaller than 10 nm are more likely to deposit in the head and thoracic regions [ICRP 1994]. **The mass deposition fraction of inhaled nanoparticles in the gas-exchange region of the lungs is greater than that for larger respirable particles.**

At present there is insufficient information to predict all of the situations and workplace scenarios that are likely to lead to exposure to nanomaterials. However, there are some workplace factors that can increase the potential for exposure:

- working with nanomaterials in liquid media without adequate protection (e.g., gloves)
- working with nanomaterials in liquid during pouring or mixing operations

or where a high degree of agitation is involved

- generating nanomaterials in the gas phase in non-enclosed systems
- handling (e.g., weighing, blending, spraying) powders of nanostructured materials
- maintenance on equipment and processes used to produce or fabricate nanomaterials
- cleaning up spills or waste material
- cleaning dust collection systems used to capture nanoparticles
- machining, sanding, drilling of nanomaterials, or other mechanical disruptions of nanomaterials can potentially lead to the aerosolization of nanoparticles.

8.3 Elements of a Risk Management Program

Given the limited information about the health risks associated with occupational exposure to engineered nanomaterials, appropriate steps should be taken to minimize the risk of worker exposure through the implementation of a risk management program [Schulte et al. 2008]. Risk management programs for nanomaterials should be seen as an integral part of an overall occupational safety and health program for any company or workplace producing or using nanomaterials or nanoenabled products. A critical element of the program should be the capability to anticipate new and emerging risks (hazard determination) and whether they are linked to changes in the manufacturing process, equipment, or the introduction of new materials. This will require an ongoing assessment of the potential risks to workers (risk evaluation) through the systematic collection of job and product information so that determinations can be made regarding scenarios (e.g., laboratory research, production and manufacture, nanoenabled product use) that place the worker in contact with nanomaterials (see Figure 8–1). This assessment should be an ongoing cyclic process that provides feedback on potential sources of exposure and solutions taken to correct those problems. For example, operations and job tasks that have the potential to aerosolize nanomaterials (e.g., handling dry powders, spray applications) deserve more attention and more stringent controls than those where the nanomaterials are imbedded in solid or liquid matrices. Elements of the risk management program should include guidelines for installing and evaluating engineering controls (e.g., exhaust ventilation, dust collection systems), the education and training of workers in the proper handling of nanomaterials (e.g., good work practices), and the selection and use of personal protective equipment (e.g., clothing, gloves, respirators).

When controlling potential exposures within a workplace, NIOSH has recommended a hierarchical approach to reduce worker exposures (see Table 8–1) [NIOSH 1990]. The philosophical basis for the hierarchy of controls is to eliminate the hazard when possible (i.e., substitute with a less hazardous material) or, if not feasible, control the hazard at or as close to the source as possible.

8.3.1 Engineering Controls

If the potential hazard can not be eliminated or substituted with a less hazardous or non-hazardous substance, then engineering controls should be installed and tailored to the process or job task. The type of engineering control used should take into

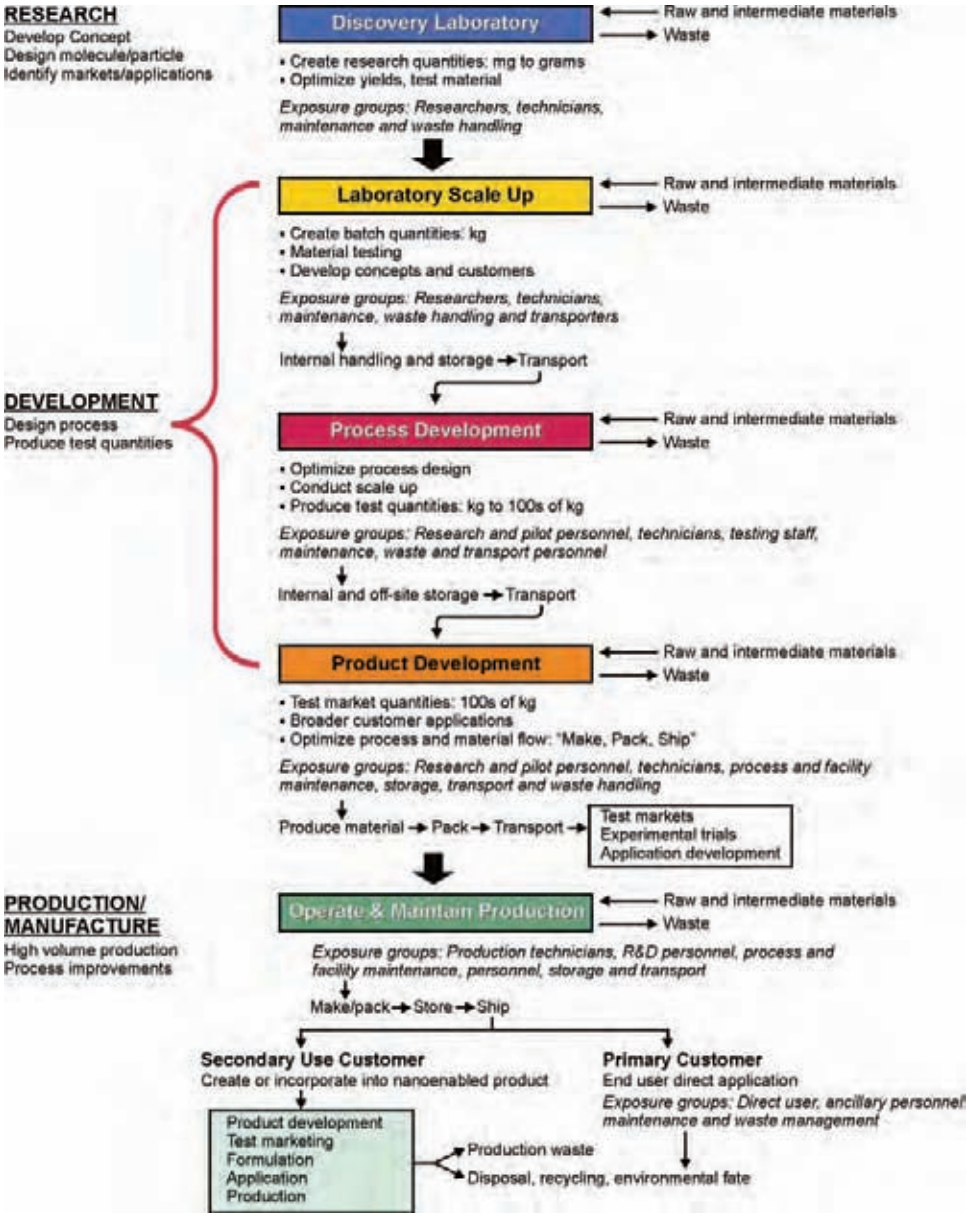

Figure 8–1. Workplaces with potential for occupational exposure to engineered nanomaterials. The figure illustrates the life cycle of nanomaterials from laboratory research development through product development, use, and disposal. Each step of the life cycle represents opportunities for potential worker exposure to nanomaterials. Adapted from Schulte et al. 2008a.

Table 8–1. Hierarchy of exposure controls*

Control method	Process, equipment, or job task
1. Elimination	Change design to eliminate hazard
2. Substitution	Replace a high hazard for a low hazard
3. Engineering	Isolation/enclosure, ventilation (local, general)
4. Administrative	Procedures, policies, shift design
5. Personal protective equipment	Respirators, clothing, gloves, goggles, ear plugs

*Control methods are typically implemented in this order to limit worker exposures to an acceptable concentration (e.g., occupational exposure limit or other pre-established limit).

Sources: Plog et al. 2002; NIOSH 1990.

account information on the potential hazardous properties of the precursor materials and intermediates as well as those of the resulting nanomaterial. In light of current scientific knowledge about the generation, transport, and capture of aerosols [Seinfeld and Pandis 1998; Hinds 1999], airborne exposure to nanomaterials can most likely be controlled at most processes and job tasks using a wide variety of engineering control techniques similar to those used in reducing exposures to general aerosols [Ratherman 1996; Burton 1997].

Engineering control techniques such as source enclosure (i.e., isolating the generation source from the worker) and local exhaust ventilation systems should be effective for capturing airborne nanomaterials, based on what is known of nanomaterial motion and behavior in air (see Figure 8–2). The quantity of the bulk nanomaterial that is synthesized or handled in the manufacture of a product will significantly influence the selection of the exposure controls.

Other factors that influence selection of engineering controls include the physical form of the nanomaterial and task duration and frequency. For instance, working with nanomaterial in the slurry form in low quantities would require a less rigorous control system than those that would be required for large quantities of nanomaterials in a free or fine powder form (see Figure 8–3). Unless cutting or grinding occurs, nanomaterials that are not in free form (encapsulated in a solid, nanocomposites, and surface coated materials) typically wouldn't require engineering controls.

Handling research quantities typically occurs in laboratories with ventilation controls. Since quantities are small, local containment and control can be applied, such as low-flow vented work stations and small glove box chambers. However, as quantities are increased, care must be taken to reduce the amount of nanomaterial that is released from the process equipment and to prevent the migration of nanomaterials into adjacent rooms or areas. For example, the installation of local exhaust ventilation at a

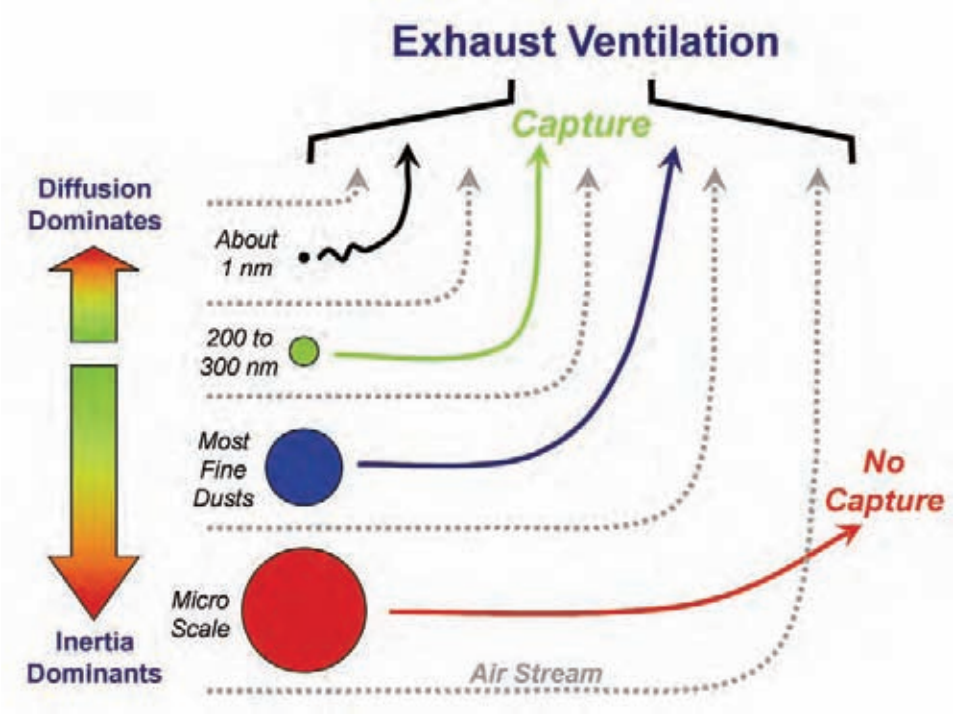

Figure 8–2. Exposure control of particles (illustration of how particle diameter-related diffusion and inertia influence particle capture efficiency in a ventilation system). Particles with a diameter of 200–300 nm have minimal diffusion and inertial properties and are easily transported by moving air and captured. Particle motion by diffusion increasingly dominates as particle diameter decreases below 200 nm. The inertial behavior of larger particles, especially those ejected from energetic processes such as grinding, increases significantly with particle diameter, enabling them to cross the streamlines of moving air and avoid capture. Adapted from Schulte et al. 2008a.

reactor used to make nanoscale engineered metal oxides and metals was found to reduce nanoparticle exposures by 96% (mean particle number concentration) [Methner 2008]. The use of exhaust ventilation systems should be designed, tested, and maintained using approaches recommended by the American Conference of Governmental Industrial Hygienists [ACGIH 2001].

A secondary but nonetheless important issue concerning the control of nanoparticle emissions is that of product loss. The properties of nanomaterials, along with the unique methods that may be employed for producing them, may mean that traditional exhaust ventilation may be more energetic than necessary for removing incidentally released nanoscale particles. For this reason, engineering controls need to be applied judiciously to ensure protection of workers without compromising production.

8.3.2 Dust collection efficiency of filters

Current knowledge indicates that a well-designed exhaust ventilation system with a

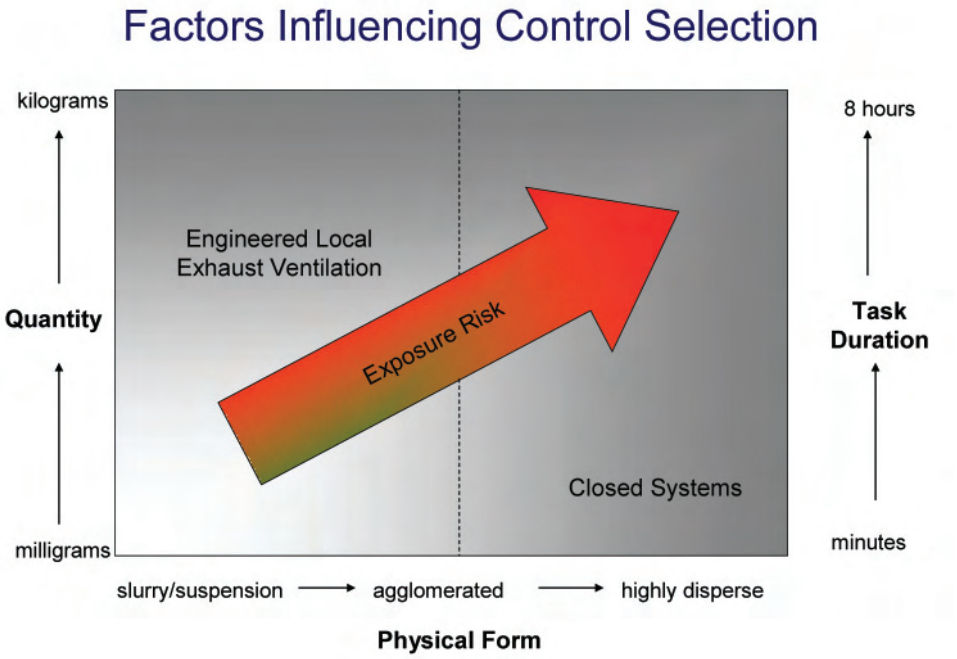

Figure 8–3. Factors influencing control selection. Several factors influence the selection of exposure controls for nanomaterials including quantity of nanomaterial handled or produced, physical form, and task duration. As each one of theses variables increase, exposure risk becomes greater as does the need for more efficient exposure control measures.

HEPA filter should effectively remove nanoparticles [Hinds 1999]. Limited studies have reported the efficacy of filter media typically found in control systems (including respirators) in capturing nanoparticles. The dearth of data on filtration performance against nanoparticles (in particular nanoparticles smaller than 20 nm) is primarily due to the challenges in generating and quantifying particles in those size ranges. Despite these limitations, the results of some studies [Van Osdell et al. 1990] using different filter media challenged with monodispersed aerosols (silver 4–10 nm and dioctylphthalate 32–420 nm) were in agreement with classical single-fiber theory showing an increase in filtration efficacy for smaller size particles. No evidence for particle thermal rebound was observed. Similar results have been recently reported by Kim et al. [2007] using different filter media challenged with particles ranging in size from 2.5–20 nm, indicating that other filter medias—including those used in air purifying respirators—would behave similarly.

If HEPA filters are used in the dust collection system, they should be coupled with well-designed filter housings. If the filter is improperly seated within the housing, nanoparticles have the potential to bypass the filter, leading to filter efficiencies much less than predicted [NIOSH 2003].

8.3.3 Work practices

An integral step in establishing good work practices is having knowledge of the potential

hazards in the workplace and developing formal procedures that describe actions to be taken to ensure the protection of workers. Incorporated in these procedures should be guidelines for good work practices intended to minimize worker exposure to nanomaterials and other potentially hazardous chemicals. Management should systemically review and update these procedures. Actions taken to resolve and/or improve workplace conditions should be routinely conveyed by management to workers.

Good practices for management

- Educating workers on the safe handling of engineered nano-objects or nano-object-containing materials to minimize the likelihood of inhalation exposure and skin contact.
- Providing information, as needed, on the hazardous properties of the precursor materials and those of the resulting nanomaterials product with instruction on measures to prevent exposure.
- Encouraging workers to use handwashing facilities before eating, smoking, or leaving the worksite.
- Providing additional control measures (e.g., use of a buffer area, decontamination facilities for workers if warranted by the hazard) to ensure that engineered nanomaterials are not transported outside the work area [US DOE 2007].
- Providing facilities for showering and changing clothes to prevent the inadvertent contamination of other areas (including take-home) caused by the transfer of nanomaterials on clothing and skin.

Good practices for workers

- Avoiding handling nanomaterials in the open air in a "*free particle*" state.
- Storing dispersible nanomaterials, whether suspended in liquids or in a dry particle form in closed (tightly sealed) containers whenever possible.
- Cleaning work areas at the end of each work shift, at a minimum, using either a HEPA-filtered vacuum cleaner or wet wiping methods. Dry sweeping or air hoses should not be used to clean work areas. Cleanup should be conducted in a manner that prevents worker contact with wastes. Disposal of all waste material should comply with all applicable Federal, State, and local regulations.
- Avoiding storing and consuming food or beverages in workplaces where nanomaterials are handled.

8.3.4 Personal protective clothing

Currently, there are no generally acceptable guidelines available based on scientific data for the selection of protective clothing or other apparel against exposure to nanomaterials. This is due in part to minimal data being available on the efficacy of existing protective clothing, including gloves. In any case, although nanoparticles may penetrate the epidermis, there has been little evidence to suggest that penetration leads to disease; and no dermal exposure standards have been proposed. However, based on a recent survey of nanotechnology workplaces [ICON 2006], 84% of employers recommended personal protective equipment and clothing for employees working with nanomaterials. These recommendations were generally based on conventional occupational hygiene practices

but also varied with the size of the company, the type of nanomaterials being handled, and the commercial sector. While some guidelines on the use of protective clothing and gloves have been developed by organizations for use in their own laboratories (e.g., US DOE 2007) or countries (e.g., British Standards Institute BSI 2008) or by consensus standards development organizations (e.g., ASTM, 2007), these are generally based upon good industrial hygiene practices rather than scientific data specific to nanomaterials.

A challenge to making appropriate recommendations for dermal protection against nanoparticles is the need to strike a balance between comfort and protection. Garments that provide the highest level of protection (e.g., an impermeable Level A suit) are also the least comfortable to wear for long periods of time, while garments that are probably the least protective (e.g., thin cotton lab coat) are the most breathable and comfortable for employees to wear. The two primary routes of exposure to particulates for workers using protective clothing are direct penetration through the materials and leakage through gaps, seams, defects, and interface and closure areas [Schneider et al. 1999, 2000]. The relative contributions from these two inward leakage sources are not well-understood. NIOSH has an active research program designed to assess the efficacy of barrier materials and ensembles for protection against particulate hazards, including nanoparticles.

The lack of available data is further complicated by the limitations and difficulties of current test methods, which fall into two basic categories: penetration tests on material swatches to determine barrier efficiency and system-level aerosol testing to determine product ensemble integrity. The former are usually bench-scale testing methods, while the latter require an exposure chamber that is large enough for at least one human test subject or mannequin. Chamber design requirements for system level aerosol testing have been reviewed by Gao et al. [2007]. Little scientific data exists, but some systems level test methods are available. ISO standard method 13982 [ISO 2004a] and EN standard method 943 [CEN 2002] specify the use of sodium chloride (NaCl) with a mass median aerodynamic diameter (MMAD) of 0.6 μm to determine the barrier efficiency of protective clothing against aerosols of dry, fine dusts. The standard method issued by National Fire Protection Association [2007] is a method that is not dependent on filtration-based approaches. Penetration of fluorophore-impregnated silica particles with a MMAD of 2.5 μm and a geometric standard deviation of 2.6 are qualitatively visualized by black light that causes the fluorescent glow of the challenge aerosol particles. Note that the polydisperse particle challenges used in these methods include a large number of nanoscale particles when measured by count rather than by mass.

Particle penetration test methods can be further categorized into those that are analogous to the process used in respirator filter testing and those that are not dependent on filtration-based approaches. Test methods that involve measuring aerosol concentrations using a sampling flow rate do not mimic in-situ situations because the skin does not "breathe." Standardized methodology is needed that is not dependent on filtration-based approaches for examining the overall barrier-effectiveness of the full protective clothing ensemble for different materials to particulate hazards. In this respect, NIOSH has presented preliminary results [Wang and Gao 2007] on development of a magnetic passive aerosol sampler for more accurate determination of particle penetration

through protective clothing. NIOSH is conducting research in this area and will communicate results as they become available.

The bulk of the penetration data available on clothing has been done with filtration based testing. One study found that penetration levels of 30–2,000-nm-sized potassium chloride particles through an unidentified military garment ranged from about 20%–60%, with the maximum penetration occurring in the range of 100–400 nm [Hofacre 2006]. Another group of researchers studied the barrier efficiency of 10 unidentified fabric samples (woven, non-woven, and laminated fabrics) using 477-nm-sized latex spheres at a flow rate of 1.8 cm/second [Shavlev et al. 2000]. Particle penetration measurements ranged from 0%–54%, with three of the fabrics exhibiting a measurable pressure drop and having penetration levels less than 1%. In general, these findings suggest that increased external air pressure (e.g., from wind) results in increased particle penetrations. Thus, only impermeable barrier materials are likely to provide complete barrier protection against aerosol penetration. Body movement (i.e., bellows effect) can also impact penetration [Bergman et al. 1989]. NIOSH will theoretically and empirically investigate wind-driven nanoparticle penetration through protective clothing in an attempt to obtain a predictive model based upon single-fiber theory. Results will be communicated as they become available.

Another widely used test method incorporates testing with nanoscale particles in solution, and therefore also provides some indication of the effectiveness of protective clothing to nanoparticles. ASTM standard F1671–03 [ASTM 2003] and ISO standard 16604 [ISO 2004b] specify the use of a 27-nm bacteriophage to evaluate the resistance of materials used in protective clothing from the penetration of blood-borne pathogens. One study [Edlich et al. 1999] evaluated the integrity of powder-free examination gloves and found that no bacteriophage penetration was detected for powder-free nitrile gloves, powder-free latex gloves, nor polyvinyl chloride synthetic gloves.

Based upon the uncertainty of the health effects of dermal exposure to nanoparticles, it is prudent to consider using protective equipment, clothing, and gloves to minimize dermal exposure, with particular attention given to preventing exposure of nanomaterials to abraded or lacerated skin. Until scientific data exist specific to the performance of protective clothing and gloves against nanomaterials, current industrial hygiene best practices should be followed.

8.3.5 Respirators

The use of respirators is often required when engineering and administrative controls do not adequately keep worker exposures to an airborne contaminant below a regulatory limit or an internal control target. Currently, there are no specific exposure limits in the United States for airborne exposures to engineered nanomaterials although occupational exposure limits and guidelines exist for airborne particles of similar chemical composition regardless of particle size. Current scientific evidence indicates that nanoparticles may be more biologically reactive than larger particles of similar chemical composition and thus may pose a greater health risk when inhaled. In determining the need for respirators, it would therefore be prudent to consider current exposure limits or guidelines (e.g., OSHA PELs, NIOSH RELs, ACGIH TLVs) for larger particles of similar composition, existing toxicologic

data on the specific nanoparticle, and the likelihood of worker exposure (e.g., airborne concentration, time exposed, job task).

The decision to institute respiratory protection should be based on a combination of professional judgment and the results of the hazard assessment and risk management practices recommended in this document. The effectiveness of administrative, work-practice, and engineering controls can be evaluated using the measurement techniques described in Chapter 7 Exposure Assessments and Characterization. If worker exposure to airborne nanomaterials remains a concern after instituting control measures, the use of respirators can provide further worker protection. Several classes of respirators exist that can provide different levels of protection when properly fit tested on the worker. Table 8–2 lists various types of particulate respirators that can be used; information is also provided on the level of exposure reduction that can be expected along with the advantages and disadvantages of each respirator type. To assist respirator users, NIOSH has published the document *NIOSH Respirator Selection Logic (RSL)* that provides a process that respirator program administrators can use to select appropriate respirators [NIOSH 2004] (see www.cdc.gov/niosh/docs/2005-100/default.html). As new toxicity data for individual nanomaterials become available, NIOSH will review the data and make recommendations for respirator protection.

When respirators are required for use in the workplace, the Occupational Safety and Health Administration (OSHA) respiratory protection standard [29 CFR 1910.134] requires that a respiratory program be established that includes the following program elements: (*1*) an evaluation of the worker's ability to perform the work while wearing a respirator, (*2*) regular training of personnel, (*3*) periodic environmental monitoring, (*4*) respirator fit testing, and (*5*) respirator maintenance, inspection, cleaning, and storage. The standard also requires that the selection of respirators be made by a person knowledgeable about the workplace and the limitations associated with each type of respirator. OSHA has also issued guidelines for employers who choose to establish the voluntary use of respirators [29 CFR 1910.134 Appendix D].

Table 8–2 lists the NIOSH assigned protection factors (APF) for various classes of respirators. The APF is defined as the minimum anticipated protection provided by a properly functioning respirator or class of respirators to a given percentage of properly fitted and trained users. The APF values developed by NIOSH are based in part on laboratory studies and take into consideration a variety of factors including the inward leakage caused by penetration through the filter and leakage around the respirator face seal. The relative contributions of these two sources of inward leakage are critical because for many applications the predominant source of exposure to the respirator wearer results from leakage around the face seal (due to a poor fit) and not penetration directly through the filter media. In 2006, OSHA published updated APF values that supersede the NIOSH APF values [Federal Register 2006]. In general there is good agreement between the NIOSH and OSHA APF values, but management should consult the OSHA standard prior to using the values in Table 8–2 directly.

NIOSH is not aware of any data specific to respirator face seal leakage of nanoparticles. However, numerous studies have

been conducted on larger particles and on gases/vapors with one total inward leakage (TIL) study that used nanoparticles. For example, work done by researchers at the U.S. Army RDECOM on a head-form showed that mask leakage (i.e., simulated respirator fit factor) measured using submicron aerosol challenges (0.72 μm polystyrene latex spheres) was representative of vapor challenges such as sulfur hexafluoride (SF_6) and isoamyl acetate (IAA) [Gardner et al. 2004]. Other studies using particles larger than 100 nm have shown that face seal leakage can be affected by particle size, however, the impact of this is still the subject of some debate. A recently completed laboratory study to measure TIL protection factors of four NIOSH certified N95 filtering facepiece respirator models donned by human test subjects exposed to 40–1,300 nm particles found that the minimal protection factors were observed for particles between 80–200 nm [Lee 2008]. The geometric mean of the protection factors for all four models across all particle sizes tested was 21.5; but wide model-to-model variation was observed. NIOSH is conducting a laboratory study to determine whether nanoparticle face seal leakage is consistent with the leakage observed for larger particles and gases/vapors. Results will be communicated as they become available.

NIOSH certifies respirators in accordance with 42 CFR Part 84. As noted earlier, the NIOSH RSL contains a process for selecting respirators for protection against particular hazards. The two respirator classes (air purifying respirators and powered air purifying respirators) most commonly used for protection against particulates use filter media to collect/trap particles before they reach the user's breathing zone. Among the various test methods and criteria NIOSH uses as part of the certification process, respirator filter performance testing is the one most affected by the particle size. Since respirator users are exposed to a variety of hazards in different scenarios, respirator certification filtration testing was designed to use worst-case test conditions (e.g., different particle sizes and flow rates), so that filter performance in the workplace would not be worse. The NIOSH certification test for N-designated respirators uses a polydisperse distribution of NaCl particles with a count median diameter (CMD) of 0.075 +/-0.020 μm and a geometric standard deviation (GSD) of less than 1.86 [NIOSH 2005a]. NIOSH tests R- and P-designated respirators using a polydispersal of dioctyl phthalate (DOP) particles with a CMD of 0.185 +/-0.020 μm and a GSD of less than 1.60 [NIOSH 2005b]. For the lognormal distribution of NaCl aerosols used in the N series certification test, a broad range of particle sizes (e.g., 95% of the particles lie in the range of 22–259 nm) with a MMD of about 240 nm is used to determine whether the respirator filter performance is at least 95, 99, or 99.97% efficient. Most of the particles penetrating through the filter are measured simultaneously using a forward light scattering photometer. However, as noted in a recent review, the instrumentation used in the NIOSH certification test is not capable of measuring the light scattering of all particles less than 100 nm [Eninger et al. 2008a].

Particles larger than 0.3 μm are collected most efficiently by impaction, interception, and gravitational settling, while particles smaller than 0.3 μm are collected most efficiently by diffusion or electrostatic attraction [Hinds 1999]. In the development of the test method used for respirator certification, penetration by particles with an approximate 0.3 μm diameter was considered

to be the worst case because these particles were considered to be in the range of the most penetrating particle size [Stevens and Moyer 1989; TSI 2005; NIOSH 1996]. However, in practice, the most penetrating particle size range (MPPS) for a given respirator can vary based on the type of filter media employed and the condition of the respirator. For example, the most penetrating particle size for N95 air purifying respirators containing electrostatically charged filter media can range from 50–100 nm [Martin and Moyer 2000; Richardson et al. 2005] to 30–70 nm [Balazy et al. 2006; Eninger et al. 2008b]. These test results were recently confirmed by NIOSH [Rengasamy et al. 2007] in which five different models of respirators with N95 filters were challenged with 11 different monodisperse NaCl particles ranging in size from 20–400 nm. The monodisperse aerosol penetrations showed that the MPPS was in the 40-nm range for all respirator models tested. Under the aggressive laboratory test conditions employed in the study, mean penetration levels for 40-nm particles ranged from 1.4%–5.2%, which suggested that the respirators would be effective at capturing nanoparticles in the workplace. The NIOSH study also investigated whether there was a correlation between filtration performance using the existing NIOSH certification protocol for N series air purifying respirators and the filtration performance against monodisperse particles at the MPPS. A good correlation ($r = 0.95$) was found (e.g., respirators that performed better using the NIOSH certification test also had higher filter efficiencies against monodisperse 40-nm nanoparticles), which is not surprising given that changes in filtration performance follow a consistent trend as a function of particle size.

According to single fiber filtration theory, below the most penetrating particle size, filtration efficiency will increase as particle size decreases. This trend will continue until the particles are so small that they behave like vapor molecules. As particles approach molecular size, they may be subject to thermal rebound effects, in which particles literally bounce through a filter. As a result, particle penetration will increase. The exact size at which thermal rebound will occur is unclear. However, a study by Heim et al. [2005] found that there was no discernable deviation from classical single-fiber theory for particles as small as 2.5-nm diameter. Subsequently, a NIOSH-funded contract with the University of Minnesota [Kim et al. 2007; Pui et al. 2006] and another study [Kim et al. 2006] showed that the penetration of nanoparticles through fibrous filter media decreased down to 2.5 nm as expected by the single fiber filtration theory. Thermal rebound phenomena were observed for nanoparticles below 2 nm diameter [Kim et al. 2006]. Recent studies provide additional data on nanoparticle penetration for NIOSH certified N95 and P100 filtering face-piece respirators [Rengasamy et al. 2008a], NIOSH certified N95 and European Certified FFP1 respirators [Huang et al. 2007], and FFP3 filter media [Golanski et al. 2008] using particles greater than 4 nm.

Based on these preliminary findings, NIOSH-certified respirators should provide the expected levels of protection if properly selected and fit tested as part of a complete respiratory protection program. However, as noted elsewhere [Rengsamy et al. 2007], in the unlikely event that the workplace exposure consists of a large percentage of particles in the most penetrating particle size range, management should take this information into account during the respirator

selection process, perhaps by choosing a respirator with higher levels of filtration performance (e.g., changing from an N95 to a P100, even though the APF will remain the same) as suggested by OSHA [Federal Register 2006] or by selecting a respirator with a higher APF (e.g., full face-piece respirator or powered air purifying respirator). Dust masks, commercially available at hardware/home improvement stores, are often confused with NIOSH approved N95 filtering facepiece respirators because of their similar appearance. However, dust masks are not respirators and are not approved by NIOSH for respiratory protection. One study found that penetration of 40-nm NaCl nanoparticles range from 4.3%–81.6% for the seven dust mask models studied [Rengasamy et al. 2008b]. **Dust masks should not be used in place of NIOSH-approved respirators for protection against nanoparticles**.

NIOSH is continuing to study the protection afforded by NIOSH-certified respirators against emerging hazards such as engineered nanomaterials—including workplace-protection-factor studies—to ensure they provide expected levels of protection. NIOSH is also committed to updating 42 CFR Part 84—the regulatory language that provides NIOSH the authority to certify the performance of respirators in the United States—using a modular approach to rulemaking. Recently, NIOSH proposed the use of a TIL test as part of the respirator certification process for half-mask air purifying particulate respirators, including those having elastomeric and filtering face-pieces. The test protocol used to obtain benchmark TIL data for 101 half-face piece respirator models used 40–60 nm size ambient nanoparticles [NIOSH 2007]. Once implemented as part of the NIOSH certification process, the TIL tests should result in half-mask respirators with increased fitting performance. Future rulemaking activities may also include revisions to the filtration test to reflect changes in filtration performance resulting from use of new technologies (e.g., electret filter media). Results will be communicated as they become available.

8.3.6 Cleanup and disposal of nanomaterials

No specific guidance is currently available on cleaning up nanomaterial spills or contamination on surfaces; however, recommendations developed in the pharmaceutical industry for the handling and cleanup of pharmaceutical compounds might be applicable to worksites where engineered nanomaterials are manufactured or used [Wood 2001]. Until relevant information is available, it would be prudent to base strategies for dealing with spills and contaminated surfaces on current good practices, together with available information on exposure risks including the relative importance of different exposure routes. Standard approaches for cleaning powder spills include using HEPA-filtered vacuum cleaners, or wiping up the powder using damp cloths or wetting the powder prior to dry wiping. Liquid spills are typically cleaned by applying absorbent materials/liquid traps.

Damp cleaning methods with soaps or cleaning oils are preferred. Cleaning cloths should be properly disposed. Use of commercially available wet or electrostatic microfiber cleaning cloths may also be effective in removing particles from surfaces with minimal dispersion into the air. Drying and reusing contaminated cloths can result in re-dispersion of particles.

Energetic cleaning methods such as dry sweeping or the using of compressed air

should be avoided or only used with precautions that assure that particles suspended by the cleaning action are trapped by HEPA filters. If vacuum cleaning is employed, care should be taken that HEPA filters are installed properly and bags and filters changed according to manufacturer's recommendations.

While vacuum cleaning may prove to be effective for many applications, the following issues should be considered. Forces of attraction may make it difficult to entrain particles off surfaces with a vacuum cleaner. The electrostatic charge on particles will cause them to be attracted to oppositely charged surfaces and repelled by similarly charged surfaces. A similarly charged vacuum brush or tool may repel particles, making it difficult to capture the aerosol or even causing it to be further dispersed. Vigorous scrubbing with a vacuum brush or tool or even the friction from high flow rates of material or air on the vacuum hose can generate a charge. The vacuum cleaners recommended for cleaning copier and printer toners have electrostatic-charge-neutralization features to address these issues.

When developing procedures for cleaning up nanomaterial spills or contaminated surfaces, consideration should be given to the potential for exposure during cleanup. Inhalation exposure and dermal exposure will likely present the greatest risks. Consideration will therefore need to be given to appropriate levels of personal protective equipment. Inhalation exposure in particular will be influenced by the likelihood of material reaerosolization. In this context, it is likely that a hierarchy of potential exposures will exist, with dusts presenting a greater inhalation exposure potential than liquids, and liquids in turn presenting a greater potential risk than encapsulated or immobilized nanomaterials and structures.

As in the case of any material spill or cleaning of contaminated surfaces, the handling and disposal of the waste material should follow existing federal, state, or local regulations.

Table 8–2. Air-purifying particulate respirators

Respirator type	NIOSH assigned protection factor	Advantages	Disadvantages
Filtering facepiece (disposable)	10	– Lightweight – No maintenance or cleaning needed – No effect on mobility	– Provides no eye protection – Can add to heat burden – Inward leakage at gaps in face seal – Some do not have adjustable head straps – Difficult for a user to do a seal check – Level of protection varies greatly among models – Communication may be difficult

(continued)

Table 8–2 (Continued). Air-purifying particulate respirators

Respirator type	NIOSH assigned protection factor	Advantages	Disadvantages
Filtering facepiece (disposable) (continued)			– Fit testing required to select proper facepiece size – Some eyewear may interfere with the fit
Elastomeric half-facepiece	10	– Low maintenance – Reusable facepiece and replaceable filters and cartridges – No effect on mobility	– Provides no eye protection – Can add to heat burden – Inward leakage at gaps in face seal – Communication may be difficult – Fit testing required to select proper facepiece size – Some eyewear may interfere with the fit
Powered with loose-fitting facepiece	25	– Provides eye protection – Offers protection for people with beards, missing dentures or facial scars – Low breathing resistance – Flowing air creates cooling effect – Face seal leakage is generally outward – Fit testing is not required – Prescription glasses can be worn – Communication easier than with elastomeric half-facepiece or full-facepiece respirators – Reusable components and replaceable filters	– Added weight of battery and blower – Awkward for some tasks – Battery requires charging – Air flow must be tested with flow device before use

(continued)

Table 8–2 (Continued). Air-purifying particulate respirators

Respirator type	NIOSH assigned protection factor	Advantages	Disadvantages
Elastomeric full-facepiece with N-100, R-100, or P-100 filters	50	– Provides eye protection – Low maintenance – Reusable facepiece and replaceable filters and cartridges – No effect on mobility – More effective face seal than that of filtering facepiece or elastomeric half-facepiece respirators	– Can add to heat burden – Diminished field-of-vision compared to half-facepiece –Inward leakage at gaps in face seal –Fit testing required to select proper facepiece size –Facepiece lens can fog without nose cup or lens treatment –Spectacle kit needed for people who wear corrective glasses
Powered with tight-fitting half-facepiece or full-facepiece	50	–Provides eye protection with full-facepiece –Low breathing resistance –Face seal leakage is generally outward –Flowing air creates cooling effect –Reusable components and replaceable filters	–Added weight of battery and blower –Awkward for some tasks –No eye protection with half-facepiece –Fit testing required to select proper facepiece size –Battery requires charging –Communication may be difficult –Spectacle kit needed for people who wear corrective glasses with full face-piece respirators –Air flow must be tested with flow device before use

9 Occupational Health Surveillance

Occupational health surveillance is an essential component of an effective occupational safety and health program. The unique physical and chemical properties of nanomaterials, the increasing growth of nanotechnology in the workplace, and information suggesting that exposure to some engineered nanomaterials can cause adverse health effects in laboratory animals all support consideration of an occupational health surveillance program for workers potentially exposed to engineered nanomaterials [Schulte et al. 2008a]. Continued evaluation of toxicologic research and workers potentially exposed to engineered nanomaterials is needed to inform NIOSH and other groups regarding the appropriate components of occupational health surveillance for nanotechnology workers.

NIOSH has developed interim guidance relevant to medical screening (one component of an occupational health surveillance program) for nanotechnology workers (see NIOSH Current Intelligence Bulletin: *Interim Guidance on Medical Screening and Hazard Surveillance for Workers Potentially Exposed to Engineered Nanoparticles,* www.cdc.gov/niosh/review/public/115/). Medical screening is only part of a complete safety and health management program that follows the hierarchy of controls and involves various occupational health surveillance measures. Since specific medical screening of workers exposed to engineered nanoparticles has not been extensively discussed in the scientific literature, this document is intended to fill the knowledge gap on an interim basis.

Increasing evidence indicates that exposure to some engineered nanoparticles can cause adverse health effects in laboratory animals, but no studies of workers exposed to the few engineered nanoparticles tested in animals have been published. The current body of evidence about the possible health risks of occupational exposure to engineered nanoparticles is quite small. Insufficient scientific and medical evidence now exists to recommend the specific medical screening of workers potentially exposed to engineered nanoparticles. Nonetheless, the lack of evidence on which to recommend specific medical screening does not preclude its consideration by employers interested in taking precautions beyond standard industrial hygiene measures [Schulte et al. 2008b]. If medical screening recommendations exist for chemical or bulk materials of which nanoparticles are composed, they would apply to nanoparticles as well.

Ongoing research on the hazards of engineered nanoparticles is needed along with the continual reassessment of available data to determine whether specific medical screening is warranted for workers who are producing or using nanoparticles. In the meantime, the following recommendations are provided for the management of workplaces where employees may be exposed to engineered nanoparticles in the course of their work:

- Take prudent measure to control workers' exposures to nanoparticles.
- Conduct hazard surveillance as the basis for implementing controls.

- Continue use of established medical surveillance approaches.

NIOSH will continue to examine new research findings and update its recommendations about medical screening programs for workers exposed to nanoparticles. Additionally, NIOSH is seeking comments on the strengths and weaknesses of exposure registries for workers potentially exposed to engineered nanoparticles.

10 Research Needs

NIOSH has developed a strategic plan for research on several occupational safety and health aspects of nanotechnology. The plan is available at www.cdc.gov/niosh/topics/nanotech/strat_plan.html. NIOSH has focused its research efforts in the following 10 critical topic areas to guide in addressing knowledge gaps, developing strategies, and providing recommendations.

1. **Exposure Assessment**

   - Determine key factors that influence the production, dispersion, accumulation, and re-entry of nanomaterials into the workplace.
   - Determine how possible exposures to nanomaterials differ by work process.
   - Assess possible exposure when nanomaterials are inhaled or settle on the skin.

2. **Toxicity and Internal Dose**

   - Investigate and determine the physical and chemical properties (e.g., size, shape, solubility, surface area, oxidant generation potential, surface functionalization, surface charge, chemical composition) that influence the potential toxicity of nanomaterials.
   - Determine the deposition pattern of nanoparticles in the lung and their translocation to the interstitium and to extrapulmonary organs.
   - Evaluate short- and long-term effects of pulmonary exposure to nanomaterials in various organ systems and tissues (e.g., lungs, brain, cardiovascular).
   - Determine if intratracheal instillation or pharyngeal aspiration can mimic the biological response to inhalation exposure to nanomaterials.
   - Determine the dermal effects of topical exposure to nano-objects, whether these nano-objects can penetrate into the skin, and whether they can cause immune alterations.
   - Determine the genotoxic and carcinogenic potential of nano-objects.
   - Determine biological mechanisms for potential toxic effects.
   - Determine whether in vitro screening tests can be predictive on in vivo response.
   - Create and integrate models to help assess potential hazards.
   - Determine whether a measure other than mass is more appropriate for determining toxicity.

3. **Epidemiology and Surveillance**

   - Evaluate existing exposure and health data for workers employed in workplaces where nanomaterials are produced and used, with emphasis on improving our understanding of the value and

utility of establishing exposure registries for workers potentially exposed to engineered nanomaterials.

- Assess the feasibility of industry-wide exposure and epidemiological studies of workers exposed to engineered nanomaterials, with emphasis on workers potentially exposed to engineered carbonaceous nanomaterials.
- Integrate nanotechnology safety and health issues into existing hazard surveillance mechanisms and continue reassessing guidance related to occupational health surveillance for workers potentially exposed to engineered nanomaterials.
- Build on existing public health geographical information systems and infrastructure to enable effective and economic development of methods for sharing nanotechnology safety and health data.

4. Risk Assessment

- Determine how existing exposure-response data for fine and ultrafine particles (human or animal) may be used to identify the potential hazards and estimate the potential risks of occupational exposure to nanomaterials.
- Develop a framework for assessing the potential hazards and risks of occupational exposure to nanomaterials, using new toxicologic data on engineered nanomaterials and standard risk assessment models and methods.

5. Measurement Methods

- Evaluate methods used to measure the mass of respirable particles in the air and determine whether this measurement can be used to measure nanomaterials.
- Develop and field-test practical methods to accurately measure airborne nanomaterials in the workplace.
- Develop, test, and evaluate systems to compare and validate sampling.

6. Engineering Controls and Personal Protective Equipment

- Evaluate the effectiveness of engineering controls in reducing occupational exposures to nanoaerosols and developing new controls when needed.
- Evaluate the suitability of control-banding techniques when additional information is needed and evaluate the effectiveness of alternative materials.
- Evaluate and improve current personal protective equipment.
- Develop recommendations (e.g., use of respiratory protection) to prevent or limit occupational exposures to nanomaterials.

7. Fire and Explosion Safety

- Identify physical and chemical properties that contribute to dustiness, combustibility, flammability, and conductivity of nanomaterials.

- Recommend alternative work practices to eliminate or reduce work place exposures to nanomaterials.

8. Recommendations and Guidance

- Use the best available science to make interim recommendations for workplace safety and health practices during the production, use, and handling of nanomaterials.
- Evaluate and update mass-based occupational exposure limits for airborne particles to ensure good, continuing precautionary practices.

9. Communication and Information

- Establish partnerships to allow for identification and sharing of research needs, approaches, and results.
- Develop and disseminate training and education materials to workers, employers, and occupational safety and health professionals.

10. Applications

- Identify uses of nanotechnology for application in occupational safety and health.
- Evaluate and disseminate effective applications to workers, employers, and occupational safety and health professionals.

References

ACGIH [2001]. Industrial ventilation: a manual of recommended practice. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adams RJ, Bray D [1983]. Rapid transport of foreign particles microinjected into crab axons. Nature *303*:718–720.

Ambroise D, Wild P, Moulin J-J [2007]. Update of a meta-analysis on lung cancer and welding. Scand J Work Environ Health *32*(1):22–31.

Antonini JM [2003]. Health effects of welding. Crit Rev Toxicol *33*(1):61–103.

ASTM Subcommittee F23.40 [2003]. Standard test method for resistance of materials used in protective clothing to penetration by blood-borne pathogens using Phi–X174 bacteriophage penetration as a test system. West Conshohocken, PA: American Society for Testing and Materials, ASTM F1671–03.

ASTM Committee E2535–07 [2007]. Standard guide for handling unbound engineered nanoscale particles in occupational settings. West Conshohocken, PA: ASTM International.

Balazy A, Toivola M, Reponen T, Podgorski A, Zimmer A, Grinshpun SA [2006]. Manikin-based filtration performance evaluation of filtering-facepiece respirators challenged with nanoparticles. Ann Occup Hyg *50*(3):259–269.

Baltensperger U, Gaggeler HW, Jost DT [1988]. The epiphaniometer, a new device for continuous aerosol monitoring. J Aerosol Sci *19*(7):931–934.

Barlow PG, Clouter-Baker AC, Donaldson K, MacCallum J, Stone V [2005]. Carbon black nanoparticles induce type II epithelial cells to release chemotaxins for alveolar macrophages. Part Fiber Toxicol *2*(14).

Baron PA, Deye GJ, Chen B, Schwegler-Berry D, Shvedova AA, Castranova V [in press]. Aerosolization of single-walled carbon nanotubes for an inhalation study. Inhal Toxicol.

Bergman W, Garr J, Fearon D [1989]. Aerosol penetration measurements through protective clothing in small scale simulation tests. Lawrence Livermore National Laboratory. Presented at: 3rd International Symposium on Protection against Chemical Warfare Agents, UMEA, Sweden, June 11–16.

Borm PJA, Schins RPF, Albrecht C [2004]. Inhaled particles and lung cancer, Part B: Paradigms and risk assessment. Int J Cancer *110*:3–14.

Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, Koller W, Bowler RP, Mergler D, Bouchard M, Smith D, Gwiazda R, Doty RL [2007]. Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. Occup Environ Med *64*(3):167–177.

British Standards Institute [2008]. Nanotechnologies, Part 2: Guide to safe handling and disposal of manufactured nanomaterials. PD 6699–2:2007 [www.bsi-global.com/upload/Standards%20&%20Publications/Nanotechnologies/PD6699-2.pdf].

Brouwer DH, Gijsbers JHJ, Lurvink MWM [2004]. Personal exposure to ultrafine particles in the workplace: exploring sampling techniques and strategies. Ann Occup Hyg *48*(5):439–453.

Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K [2001]. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol App Pharmacol *175*(3):191–199.

Brown JS, Zeman KL, Bennett WD [2002]. Ultrafine particle deposition and clearance in the healthy and obstructed lung. Am J Respir Crit Care Med *166*:1240–1247.

Brunauer S, Emmett PH, Teller E [1938]. Adsorption of gases in multimolecular layers. J Am Chem Soc *60*:309.

Burton J [1997]. General methods for the control of airborne hazards. In: DiNardi SR, ed. The occupational environment—its evaluation and control. Fairfax, VA: American Industrial Hygiene Association.

Castranova V [2000]. From coal mine dust to quartz: mechanisms of pulmonary pathogenicity. Inhal Tox *12*(Suppl 3):7–14.

Daigle CC, Chalupa DC, Gibb FRMorrow PE, Oberdorster G, Utell MJ, Frampton MW [2003]. Ultrafine particle deposition in humans during rest and exercise. Inhal Toxicol *15*(6):539–552.

De Lorenzo AJD [1970]. The olfactory neuron and the blood-brain barrier. In: Wolstenholme GEW, Knight J, eds. Taste and smell in vertebrates. CIBA Foundation Symposium series. London: J&A Churchill, pp. 151–176.

Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG, Speizer BE [1993]. An association between air pollution and mortality in six U.S. cities. N Engl J Med *329*(24):1753–1759.

Donaldson K, Li XY, MacNee W [1998]. Ultrafine (nanometer) particle mediated lung injury. J Aerosol Sci *29*(5–6):553–560.

Donaldson K, Tran CL [2002]. Inflammation caused by particles and fibers. Inhal Toxicol *14*(1):5–27.

Donaldson K, Stone V [2003]. Current hypotheses on the mechanisms of toxicity of ultrafine particles. Ann 1st Super Sanita *39*(3):405–410.

Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, Stone V [2005]. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol. *2*(10):14.

Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A [2006]. Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol Sci *92*(1):5–22.

Driscoll KE [1996]. Role of inflammation in the development of rat lung tumors in response to chronic particle exposure. In: Mauderly JL, McCunney RJ, eds. Particle overload in the rat lung and lung cancer: implications for human risk assessment. Philadelphia, PA: Taylor & Francis, pp. 139–152.

Duffin R, Tran CL, Clouter A, Brown DM, MacNee W, Stone V, Donaldson K [2002]. The importance of surface area and specific reactivity in the acute pulmonary inflammatory response to particles. Ann Occup Hyg *46*:242–245.

Duffin R, Tran L, Brown D, Stone V, Donaldson K [2007]. Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity. Inhal Toxicol *19*(10):849–856.

Edlich RF, Suber F, Neal JG, Jackson EM, Williams FM [1999]. Integrity of powder-free gloves to bacteriophage penetration. J Biomed Mater Res B Appl Biomater *48*:755–758.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G [2006]. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect *114*:1172–1178.

Eninger RM, Honda T, Reponen T, McKay R, Grinshpun SA [2008a]. What does respirator certification tell us about filtration of ultrafine particles? J Occup Environ Hyg *5*:286–295.

Eninger RM, Honda T, Adhikari A, Heinonen-Tanski H, Reponen T, Grinshpun SA [2008b]. Filter performance of N99 and N95 facepiece respirators against viruses and ultrafine particles. Ann Occup Hyg *52*(5):385–396.

71 Fed. Reg. 50121–50192 [2006]. Occupational Safety and Health Administration: assigned protection factors, final rule [www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=FEDERAL_REGISTER&p_id=18846].

European Committee for Standardization [2002]. CEN 943–1:2002: Protective clothing against liquid and gaseous chemicals, aerosols and solid particles. Performance requirements for ventilated and non-ventilated "gas-tight" (Type 1) and "non-gas-tight" (Type 2) chemical protective suits. Brussels: European Committee for Standardization.

Evans DE, Heitbrink WA, Slavin TJ, Peters TM [2008]. Ultrafine and respirable particles in an automotive grey iron foundry. Ann Occup Hyg *52*(1):9–21.

Frampton MW, Stewart JC, Oberdorster G, Morrow PE, Chalupa D, Pietropaoli AP, Frasier LM, Speers DM, Cox C, Huang LS, Utell MJ [2006]. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. Environ Health Perspect *114*(1):51–58.

Fuchs NA [1964]. The mechanics of aerosols. Oxford, England: Pergamon Press.

Gao P, King WP, Shaffer R [2007]. Review of chamber design requirements for testing of personal protective clothing ensembles. J Occup & Environ Hyg *4*(8):562–571.

Gardiner K, van Tongeren M, Harrington M [2001]. Respiratory health effects from exposure to carbon black: results of the phase 2 and 3 cross sectional studies in the European carbon black manufacturing industry. Occup Environ Med *58*(8):496–503.

Gardner P, Hofacre K, Richardson A [2004]. Comparison of simulated respirator fit factors using aerosol and vapor challenges. J Occup and Environ Hyg *1*(1):29–38.

Garshick E, Laden F, Hart JE, Rosner B, Smith TJ, Dockery DW, Speizer FE [2004]. Lung cancer in railroad workers exposed to diesel exhaust. Environ Health Perspect *112*(15):1539–1543.

Garshick E, Laden F, Hart JE, Smith TJ, Rosner B [2006]. Smoking imputation and lung cancer in railroad workers exposed to diesel exhaust. Am J Ind Med *49*(9):709–718.

Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P [2005]. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect *113*(11):1555–1560.

Golanski L, Guiot A, Tardif F [2008]. Are conventional protective devices such as fibrous filter media, respirator cartridges, protective clothing and gloves also efficient for nanoaerosols? Brussels, Germany: European Community, Nanotech2 Project.

Goldstein M, Weiss H, Wade K, Penek J, Andrews L, Brandt-Rauf P [1987]. An outbreak of fume fever in an electronics instrument testing laboratory. J Occup Med *29*:746–749.

Granier JJ, Pantoya ML [2004]. Laser ignition of nanocomposite thermites. Combust Flame *138*:373–382.

Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, Lee S-B, Ji JH, Cho MH, Yu IJ [2008]. Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. Inhal Toxicol *20*(8):741–749.

Hart JE, Laden F, Schenker MB, Garshick E [2006]. Chronic obstructive pulmonary disease mortality in diesel-exposed railroad workers. Environ Health Perspect *114*(7):1013–1017.

HEI [2000]. Reanalysis of the Harvard Six Cities Study and the American Cancer Society Study of Particulate Air Pollution and Mortality. Cambridge, MA: Health Effects Institute.

Heim M, Mullins B, Wild M, Meyer J, Kasper, G [2005]. Filtration efficiency of aerosol particles below 20 nanometers. Aerosol Sci Tech *39*:782–789.

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levsen K [1995]. Chronic inhalation exposure of wistar rats and 2 different strains of mice to diesel-engine exhaust, carbon-black, and titanium-dioxide. Inhal Toxicol *7*(4):533–556.

Heitbrink WA, Evans DE, Peters TM, Slavin TJ [2007]. The characteristion and mapping of very fine particles in an engine machining and assembly facility. J Occup Environ Hyg *4*:341–351.

Heitbrink WA, Evans DE, Ku BK, Maynard AD, Slavin TJ, Peters TM [forthcoming]. Relationships between particle number, surface area, and respirable mass concentration in automotive engine manufacturing. J Occup Environ Hyg.

Hinds WC [1999]. Aerosol technology: properties, behavior, and measurement of airborne particles. 2nd ed. New York: Wiley-Interscience.

Hofacre KC [2006]. Aerosol penetration of fabric swatches. Unpublished paper presented at the Elevated Wind Aerosol Conference, Arlington, VA, September 2006.

Hoshino A, Fujioka K, Oku T, Suga M, Ssaki Y, Ohta T [2004]. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Lett *4*(11):2163–2169.

HSE [2004]. Horizon scannon information sheet on nanotechnology. Sudbury, Suffolk, United Kingdom: Health and Safety Executive [www.hse.gov/pubns/hsin1.pdf].

Huang SH, Chen CW, Chang CP, Lai CY, Chen CC [2007]. Penetration of 4.5 nm to 10 μm aerosol particles through fibrous filters. J Aero Sci *38*:719–727.

Hunter DD, Dey RD [1998]. Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium. Neuroscience *83*(2):591–599.

Ibald-Mulli A, Wichmann HE, Kreyling W, Peters A [2002]. Epidemiological evidence on health effects of ultrafine particles. J Aerosol Med Depos *15*(2):189–201.

International Council on Nanotechnology (ICON) [2006]. A survey of current practices in the nanotechnology workplace. [http://cohesion.rice.edu/CentersAndInst/ICON/emplibrary/ICONNanotechSurveyFullReduced.pdf].

ICRP [1994]. Human respiratory tract model for radiological protection. Oxford, England: Pergamon, Elsevier Science Ltd., International Commission on Radiological Protection, Publication No. 66.

ISO [2004a]. Protective clothing for use against solid particulates. Part 2: Test method of determination of inward leakage of aerosols of fine particles into suits. ISO Standard 13982–2. Geneva, Switzerland: International Organization for Standardization.

ISO [2004b]. ISO 16604:2004: Clothing for protection against contact with blood and body fluids: determination of resistance of protective clothing materials to penetration by blood-borne pathogens; test method using Phi-X 174 bacteriophage. Geneva, Switzerland: International Organization for Standardization.

ISO [1995]. Air quality; particle size fraction definitions for health-related sampling, ISO 7708. Geneva, Switzerland: International Organization for Standardization.

ISO [2006]. Workplace atmospheres; ultrafine, nanoparticle and nano-structured aerosols; exposure characterization and assessment. Geneva, Switzerland: International Standards Organization. Document no. ISO/TC 146/SC 2/WG1 N324, 32 pages.

Jaques PA, Kim CS [2000]. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. Inhal Toxicol *12*(8):715–731.

Jiang J, Oberdorster G, Elder A, Gelein R, Mercer P, Biswas P [2008]. Does nanoparticle activity depend upon size and crystal phase? Nanotox *2*(1):33–42.

Johnston CJ, Finkelstein JN, Mercer P, Corson N, Gelein R, Oberdorster G [2000]. Pulmonary effects induced by ultrafine PTFE particles. Toxicol Appl Pharmacol *168*:208–215.

Keskinen J, Pietarinen K, Lehtimaki M [1992]. Electrical low pressure impactor. J Aerosol Sci *23*:353–360.

Kim CS, Jaques PA [2004]. Analysis of total respiratory deposition of inhaled ultrafine particles in adult subjects at various breathing patterns. Aerosol Sci Technol *38*:525–540.

Kim CS, Bao L, Okuyama K, Shimada S, Niinuma H [2006]. Filtration efficiency of a fibrous filter for nanoparticles. J Nanopart Res *8*:215 –221.

Kim SE, Harrington MS, Pui DYH [2007]. Experimental study of nanoparticles penetration through commercial filter media. J Nanopart Res *9*:117–125.

Kreiss K, Mroz MM, Zhen B, Wiedemann H, Barna B [1997]. Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. Occup Environ Med *54*(8):605–612.

Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdorster G, Ziesenis A [2002]. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health *65*(20):1513–1530.

Kreyling WG, Semmler-Behnke M, Moller W [2006]. Ultrafine particle-lung interactions: does size matter? J Aerosol Med *19*(1):74–83.

Ku BK, Maynard AD [2005]. Comparing aerosol surface-area measurements of monodisperse ultrafine silver agglomerates by mobility analysis, transmission electron microscopy and diffusion charging. J Aerosol Sci *36*:1108–1124.

Ku BK, Maynard AD [2006]. Generation and investigation of airborne silver nanoparticles with specific size and morphology by homogeneous nucleation, coagulation and sintering. J Aerosol Sci *37*:452–470.

Kuhlbusch TAJ, Neumann S, Fissan H [2004]. Number size distribution, mass concentration, and particle composition of PM1, PM2.5, and PM10 in bag filling areas of carbon black production. J Occup Environ Hyg *1*(10):660–671.

Lam CW, James JT, McCluskey R, Hunter RL [2004]. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci *77*:126–134.

Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL [2006]. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit Rev Toxicol *36*:189–217.

Lee KP, Trochimowicz HJ, Reinhardt CF [1985]. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicol Appl Pharmacol *79*:179–192.

Lee CH, Guo YL, Tsai PJ, Chang HY, Chen CR, Chen CW, Hsiue TR [1997]. Fatal acute pulmonary oedema after inhalation of fumes from polytetrafluoroethylene (PTFE). Eur Res J *10*:1408–1411.

Lee SA, Grinshpun SA, Reponen T [2008]. Respiratory performance offered by N95 respirators and surgical masks: Human subject evaluation with NaCl aerosol representing bacterial and viral particle size range. Ann Occup Hyg *52*(3):177–85. Epub 2008 Mar 7.

Lippmann M [1977]. Regional deposition of particles in the human respiratory tract. In: Lee DHK, Murphy S, eds. Handbook of physiology; section IV, environmental physiology. Philadelphia, PA: Williams and Wilkins, pp. 213–232.

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang MY, Oberley T, Froines J, Nel A [2003]. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect *111*(4):455–460.

Li Z, Hulderman T, Salmen R, Chapman R, Leonard SS, Young S-H, Shvedova A, Luster MI, Simeonova PP [2007]. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ Health Perspect *115*:377–382.

Lison D, Lardot C, Huaux F, Zanetti G, Fubini B [1997]. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. Arch Toxicol *71*(12):725–729.

Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B [2006]. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci & Technol *40*(14):4346–4352. Epub 16 June 2006.

Lovric J, Bazzi HS, Cuie Y, fortin, GRA, Winnik FM, D Maysinger [2005]. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. J Mol Med *83*(5):377–385. Epub 2005 Feb 2.

Lux Research [2007]. The Nanotech Report, 5th Edition. New York: Lux Research.

Marple VA, Olson BA, Rubow KL [2001]. Inertial, gravitational, centrifugal, and thermal collection techniques. In: Baron PA, Willeke K, eds. Aerosol measurement: principles, techniques and applications. New York: John Wiley and Sons, pp. 229–260.

Martin S, Moyer E [2000]. Electrostatic respirator filter media: filter efficiency and most penetrating particle size effects. App Occup Environ Hyg *15*(8):609–617.

Maynard AD [2003]. Estimating aerosol surface area from number and mass concentration measurements. Ann Occup Hyg *47*(2):123–144.

Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V [2004]. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single walled carbon nanotube material. J Toxicol Environ Health *67*(1):87–107.

Maynard AM, Kuempel ED [2005]. Airborne nanostructured particles and occupational health. J Nanoparticle Research *7*(6):587–614.

Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, Shvedova AA, Castranova V [2008]. Alteration of depositon pattern and pulmonary response as a result of improved dispersion of aspirated single walled carbon nanotubes in a mouse model. Am J Physiol Lung Cell Mol Physiol *294*:L87–L97.

Methner MM, Birch ME, Evans DE, Ku BK, Crouch KG, Hoover MD [2007]. Mazzulxeli LF, ed: Case study: Identification and characterization of potential sources of worker exposure to carbon nanofibers during polymer composite laboratory operations. J Occup Environ Hyg *4*(12):D125–D130.

Methner MM [2008]. Engineering case reports: Old L, ed. Effectiveness of local exhaust ventilation (LEV) in controlling engineered nanomaterial emissions during reactor cleanout operations. J Occup Environ Hyg *5*(6):D63–D69.

Misra C, Singh M, Shen S, Sioutas C, Hall PM [2002]. Development and evaluation of a personal cascade impactor sampler (PCIS). J Aerosol Sci *33*(7):1027–1048.

Moller W, Hofer T, Ziesenis A, Karg E, Heyder J [2002]. Ultrafine particles cause cytoskeletal dysfunctions in macrophages. Toxicol Appl Pharmacol *182*(3):197–207.

Moller W, Brown DM, Kreyling WG, Stone V [2005]. Ultrafine particles cause cytoskeletal dysfunctions in macrophages: role of intracellular calcium. Part Fibre Toxicol *2*(7):12.

Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE [2005]. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. Toxicol Lett *155*(3):377–384.

Moshammer H, Neuberger M [2003]. The active surface of suspended particles as a predictor of lung function and pulmonary symptoms in Austrian school children. Atmos Environ *37*(13):1737–1744.

Mossman B, Churg A [1998]. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med *157*:1666–1680.

Muller J, Huaux F, Moreau N, Misson P, Heilier J-F, Delos M, Arras M, Fonseca A, Nagy JB, Lison D [2005]. Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol *207*:221–231.

Murray AR, Kisin E, Kommineni C, Kagan VE, Castranova V, Shvedova AA [2007]. Single-walled carbon nanotubes induce oxidative stress and inflammation in skin. Toxicologist *96*:A1406.

National Fire Protection Association (NFPA) [2007]. NFPA 1994 standard on protective ensembles for first responders to CBRN terrorism incidents.

Nel A, Xia T, Madlen L, Li W [2006]. Toxic potential of materials at the nanolevel. Science *311*:622–627.

Nemmar A, Hoet PHM, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B [2002]. Passage of inhaled particles into the blood circulation in humans. Circulation *105*:411–414.

NIOSH [1990]. NIOSH testimony on the Occupational Safety and Health Administration proposed rule on health standards: methods of compliance. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health.

NIOSH [1994]. NIOSH manual of analytical methods (NMAM®). 4th ed. By Schlecht PC, O'Connor PF, eds. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication 94–113 [www.cdc.gov/niosh/nmam/].

NIOSH [1996]. NIOSH guide to the selection and use of particulate respirators certified under 42 CFR 84. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 96–101.

NIOSH [2003]. Filtration and air-cleaning systems to protect building environments. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2003–136.

NIOSH [2004]. NIOSH respirator selection logic. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2005–100 [www.cdc.gov/niosh/docs/2005-100/].

NIOSH [2005a]. Procedure No. RCT–APR–STP–0057, 0058, 0059, Revision 1.1. Cincinnati, OH: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health [www.cdc.gov/niosh/npptl/stps/pdfs/RCT-APR-0057%2058%2059.pdf].

NIOSH [2005b]. Procedure No. RCT–APR–STP–0051, 0052, 0053, 0054, 0055, 0056, Revision 1.1. Cincinnati, OH: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health [www.cdc.gov/niosh/npptl/stps/pdfs/RCT-APR-0051%2052%2053%2054%2055%2056.pdf].

NIOSH [2009]. Current intelligence bulletin: interim guidance on medical screening of workers potentially exposed to engineered nanoparticles. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health [www.cdc.gov/niosh/docs/2009-116/].

NIOSH [2007]. Docket Number 036: Total Inward Leakage Test for Half-Mask Air-Purifying Particulate Respirators; Total Inward Leakage Test for Half-mask Air-purifying Particulate Respirators. Procedure No.

RCT–APR–STP–0068, January 31, 2007 [www.cdc.gov/niosh/docket/NIOSHdocket0036.html].

Oberdörster G, Yu [1990]. The carcinogenic potential of inhaled diesel exhaust: a particle effect? J Aerosol Sci *21*:S397–S401.

Oberdörster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J [1992]. Role of the alveolar macrophage in lung injury—studies with ultrafine particles. Environ Health Perspect *97*:193–199.

Oberdörster G, Ferin J, Lehnert BE [1994a]. Correlation between particle-size, in-vivo particle persistence, and lung injury. Environ Health Perspect *102*(S5):173–179.

Oberdörster G, Ferin J, Soderholm S, Gelein R, Cox C, Baggs R, Morrow PE [1994b]. Increased pulmonary toxicity of inhaled ultrafine particles: due to lung overload alone? Ann Occup Hyg *38*(Suppl 1):295–302.

Oberdörster G, Gelein RM, Ferin J, Weiss B [1995]. Association of particulate air pollution and acute mortality: involvement of ultrafine particles? Inhal Toxicol *7*(1):111–124.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C [2002]. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J Toxicol Environ Health *65* Part A(20):1531–1543.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C [2004]. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol *16*(6–7):437–445.

Oberdörster G, Oberdörster E, Oberdörster J [2005a]. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect *113*(7):823–839.

Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H [2005b]. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol *2*:8.

Park RM, Bowler RM, Eggerth DE, Diamond E, Spencer KJ, Smith D, Gwiazda R [2006]. Issues in neurological risk assessment for occupational exposures: the Bay Bridge welders. Neurotoxicology *27*(3):373–384.

Penttinen P, Timonen KL, Tiittanen P, Mirme A, Russkanen J, Pekkanen J [2001]. Ultrafine particles in urban air and respiratory health among adult asthmatics. Eur Respir J *17*(3):428–435.

Peters A, Dockery DW, Heinrich J, Wichmann HE [1997]. Short-term effects of particulate air pollution on respiratory morbidity in asthmatic children. Eur Respir J *10*(4):872–879.

Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, Lowel H [2004]. Exposure to traffic and the onset of myocardial infarction. N Engl J Med *351*(17):1721–1730.

Peters TM, Heitbrink WA, Evans DE, Slavin TJ, Maynard AD [2006]. The mapping of fine and ultrafine particle concentrations in an engine machining and assembly facility. Ann Occup Hyg *50*(3):249–257.

Plog BA, Niland J, Quinlan PJ, eds. [2002]. Fundamentals of industrial hygiene, 5th ed. Itasca, IL: National Safety Council.

Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K [2008]. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotech *3*(7):423–428. Epub 2008 May 20. [www.nature.com/naturenanotechnology].

Pope CA, Burnett RT, Thun MJ, Calle EE, Krewski E, Ito K, Thurston GD [2002]. Lung cancer, cardiopulmonary mortality and long term exposure to fine particulate air pollution. JAMA *287*(9):1132–1141.

Pope CA, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ [2004]. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation *109*(1):71–74.

Porter AE, Gass M, Muller K, Skepper JN, Midgley P, Welland M [2007a]. Direct imaging of single-walled carbon nanotubes in cells. Nat Nanotech *2*:713–717.

Porter AE, Gass M, Muller K, Skepper JN, Midgley P, Welland M [2007b]. Visualizing the uptake of C60 to the cytoplasm and nucleus of human monocyte-derived macrophage cells using energy-filtered

transmission electron microscopy and electron tomography. Environ Sci Technol *41*(8):3012–3017.

Pott F, Roller M [2005]. Carcinogenicity study with nineteen granular dusts in rats. Eur J Oncol *10*(4):249–281.

Pritchard DK [2004]. Literature review—explosion hazards associated with nanopowders. United Kingdom: Health and Safety Laboratory, HSL/2004/12.

Pui DYH, Kim SC [2006]. Penetration of nanoparticles through respirator filter media. Minneapolis, MN: University of Minnesota, Mechanical Engineering Department, Particle Technology Laboratory. NIOSH Contract No. 254–2005–M–11698 for National Personal Protective Technology Division.

Ratherman S [1996]. Methods of control. In: Plog B, ed. Fundamentals of industrial hygiene. Itasca, IL: National Safety Council.

Rengasamy S, Verbofsky R, King WP, Shaffer RE [2007]. Nanoparticle penetration through NIOSH-approved N95 filtering-facepiece respirators. J Int Soc Res Prot *24*:49–59.

Rengasamy S, King WP, Eimer B, Shaffer RE [2008a]. Filtration performance of NIOSH-approved N95 and P100 filtering-facepiece respirators against 4–30 nanometer size nanoparticles. J Occup Environ Hyg *5*(9):556–564.

Rengasamy S, Eimer BC, Shaffer RE [2008b]. Nanoparticle filtration performance of commercially available dust masks. J Int Soc Res Prot *25*:27.

Renwick LC, Brown D, Clouter A, Donaldson K [2004]. Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particles. Occup Environ Med *61*:442–447.

Richardson, AW, Eshbaugh, JP, Hofacre, KC, Gardner PD [2005]. Respirator filter efficiency testing against particulate and biological aerosols under moderate to high flow rates. Columbus, OH: Battelle Memorial Institue. Contract Report No. SP0700–00–D–3180.

Ruckerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann HE, Peters A [2006]. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med *173*(4):432–441.

Ryman-Rasmussen JP, JE Riviere JE, NA Monteiro-Riviere [2006]. Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicol Sci *91*(1):159–165.

Sager T, Porter D, Castranova V [2008]. Pulmonary response to intratracheal instillation of fine or ultrafine carbon black or titanium dioxide: Role of surface area. Toxicologist *102*:A1491.

Sayes CM, Fortner JD, Guo W, Lyon D, Boyd AM, Ausman KD, Tao YJ, Sitharaman B, Wilson LJ, Hughes JB, West JL, Colvin VL [2004]. The differential cytotoxicity of water-soluble fullerenes. Nano Letters *4*(10):1881–1887.

Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, Beach JM, Moore VC, Doyle CD, West JL, Billups WE, Ausman KD, Colvin VL [2005]. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicol Lett *161*(2):135–142.

Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL [2006]. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci *92*(1):174–185.

Schneider T, Cherrie JW, Vermeulen R, Kromhout H [1999]. Conceptual model for assessment of dermal exposure. J Occup Environ Med *56*:765–773.

Schneider T, Cherrie JW, Vermeulen R, Kromhout H [2000]. Dermal exposure assessment. Ann Occup Hyg *44*(7):493–499.

Schulte P, Geraci C, Zumwalde R, Hoover M, Kuempel E [2008a]. Occupational risk management of engineered nanoparticles. J Occup Environ Hyg 5: 239–249.

Schulte PA, Trout D, Zumwalde R, Kuempel E, Geraci C, Castranova V, Mundt DJ, Mundt KA, Halperin WE [2008b]. Options for occupational health surveillance of workers potentially exposed to engineered nanoparticles: state of the science. J Occup Environ Med *50*:517–526.

Seinfeld JA, Pandis SN [1998]. Atmospheric chemistry and physics. New York: John Wiley and Sons.

Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdorster G, Kreyling WG [2004]. Long-term

clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. Inhal Toxicol *16*(6–7):453–459.

Shalev I, Barker RL, McCord MG, Tucker PA, Lisk BR [2000]. Protective textile particulate penetration screening. Performance of protective clothing: 7th Symposium, ASTM STP 1386, West Conshohocken, PA: American Society for Testing and Materials, ASTM pp. 155–161.

Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K [2004]. On the cytotoxicity of quantum dots. Microbiol Immunol *48*(9):669–675.

Shvedova AA, Kisin ER, AR Murray, Gandelsman VZ, Maynard AD, Baron PA, Castranova V [2003]. Exposure to carbon nanotube material: assessment of the biological effects of nanotube materials using human keratinocyte cells. J Toxicol Environ Health *66*(20):1909–1926.

Shvedova AA, Kisin ER, Murray AR, Schwegler-Berry D, Gandelsman VZ, Baron P, Maynard A, Gunther MR, Castranova V [2004]. Exposure of human bronchial epithelial cells to carbon nanotubes cause oxidative stress and cytotoxicity. In: Proceedings of the Society for Free Radical Research Meeting, Paris, France: Society for Free Radical Research International, European Section, June 26–29, 2003.

Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D [2005]. Unusual inflammatory and fibrogenic pulmonary responses to single walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol *289*(5):L698–708. Epub 2005 Jun 10.

Shvedova AA, Sager T, Murray A, Kisin E, Porter DW, Leonard SS, Schwegler-Berry D, Robinson VA, Castranova V [2007]. Critical issues in the evaluation of possible effects resulting from airborne nanoparticles. In: Monteiro-Riviere N and Tran L (eds). Nanotechnology: characterization, dosing and health effects. Philadelphia, PA: Informa Healthcare, pp. 221–232.

Shvedova AA, Kisin E, Murray AR, Johnson V, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Stone S, Frazer D, Chen T, Deye G, Maybnard A, Baron P, Mason R, Kadiiska M, Stadler K, Mouithys-Mickalad A, Castranova V, Kaagan VE [2008]. Inhalation of carbon nanotubes induces oxidative stress and cytokine response causing respiratory impairment and pulmonary fibrosis in mice. Toxicologist *102*:A1497.

Sriram K, Porter DW, Tsuruoka S, Endo M, Jefferson AM, Wolfarth MG, Rogers GM, Castranova V, Luster MI [2007]. Neuroinflammatory response following exposure to engineered nanomaterials. Toxicologist *96*:A1390.

Steenland K, Deddens J, Stayner L [1998]. Diesel exhaust and lung cancer in the trucking industry: exposure-response analyses and risk assessment. Am J Ind Med *34*(3):220–228.

Stefaniak AB, Hoover MD, Dickerson RM, Peterson EJ, Day GA, Breysse PN, Kent MS, Scripsick RC [2003]. Surface area of respirable beryllium metal, oxide, and copper alloy aerosols and implications for assessment of exposure risk of chronic beryllium disease. Am Ind Hyg Assoc J *64*:297–305.

Stevens G, Moyer E [1989]. Worst case aerosol testing parameters: I. sodium chloride and dioctyl phthalate aerosol filter efficiency as a function of particle size and flow rate. Am Ind Hyg Assoc J *50*(5):257–264.

Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J [2008]. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. J Toxicol Sci *33*(1):105–16.

Takenaka S, D Karg, C Roth, H Schulz, A Ziesenis, U Heinzmann, P Chramel, Heyder J [2001]. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Persp *109*(suppl. 4):547–551.

The Royal Society, The Royal Academy of Engineering [2004]. Nanoscience and nanotechnologies. London, UK: The Royal Society and The Royal Academy of Engineering [www.nanotec.org.uk/finalReport.htm].

Thomas K, Aguar P, Kawasaki H, Morris J, Nakanish J, Savage N [2006]. Research strategies for safety evaluation of nanomaterials, part VIII: international efforts to develop risk-based safety evaluations for nanomaterials. Toxic Sci *92*(1):23–32.

Timonen KL, G Hoek, J Heinrich, A Bernard, B Brunekreef, J de Hartog, K Hameri, A Ibald-Mulli, A Mirme, A Peters, P Tiittanen, WB Kreyling, J Pekkanen [2004]. Daily variation in fine and ultrafine particulate air pollution and urinary concentrations

of lung Clara cell protein CC16. Occup Environ Med *61*(11):908–914.

Tinkle SS, Antonini JM, Rich BA, Robert JR, Salmen R, DePree K, Adkins EJ [2003]. Skin as a route of exposure and sensitization in chronic beryllium disease. Environ Health Perspect *111*(9):1202–1208.

Törnqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, Macnee W, Donaldson K, Söderberg S, Newby DE, Sandström T, Blomberg A [2007]. Persistent endothelial dysfunction in humans after diesel exhaust inhalation. Am J Respir Crit Care Med *176*(4):395–400.

Tran CL, Cullen RT, Buchanan D, Jones AD, Miller BG, Searl A, Davis JMG, Donaldson K [1999]. Investigation and prediction of pulmonary responses to dust, part II. In: Investigations into the pulmonary effects of low toxicity dusts. Contract Research Report 216/1999. Suffolk, UK: Health and Safety Executive.

Tran C, Buchanan LD, Cullen RT, Searl A, Jones AD, Donaldson K [2000]. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. Inhal Toxicol *12*(12):1113–1126.

TSI [2005]. Mechanisms of filtration for high efficiency fibrous filters. Application Note ITI–041, TSI Incorporated [www.tsi.com/AppNotes/appnotes.aspx?Cid=24&Cid2=195&Pid=33&lid=439&file=iti_041#mech].

US DOE [2007]. Approach to Nanomaterial ES&H, U.S. Department of Energy's Nanoscale Science Research Centers. Washington, DC: U.S. Department of Energy.

VanOsdell DW, Liu BYH, Rubow KL, Pui DYH [1990]. Experimental-study of submicrometer and ultrafine particle penetration and pressure-drop for high-efficiency filters. Aerosol Sci Technol *12*(4):911–925.

Vaughan NP, Milligan BD, Ogden TL [1989]. Filter weighing reproducibility and the gravimetric detection limit. Ann Occup Hyg *33*(3):331–337.

Wang ZM, Gao P [2007]. A study on magnetic passive aerosol sampler for measuring aerosol particle penetration through protective ensembles. Unpublished paper presented at the 26th Annual Conference of the American Association for Aerosol Research. Reno, NV, September 24–28.

Wang L, Castranova V, Rojanasakul Y, Lu Y, Scabilloni J, Mercer RR [2008]. Direct fibrogenic effects of dispersed single walled carbon nanotubes on human lung fibroblasts. Toxicologist *102*:A1499.

Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR [2004]. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicol Sci *77*:117–125.

Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL [2006]. Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci *91*(1):227–236.

Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM [2007]. Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: differential responses related to surface properties. Toxicology *230*:90–104.

Wiebel A, Bouchet R, Boulch F, Knauth P [2005]. The big problem of particle size: a comparison of methods for determination of particle size in nanocrystalline anatase powers. Chem Mater *17*:2378–2385.

Woo KS, Chen DR, Pui DYH, Wilson WE [2001]. Use of continuous measurements of integral aerosol parameters to estimate particle surface area. Aerosol Sci Technol *34*:57–65.

Wood JP [2001]. Containment in the pharmaceutical industry. New York: Marcel Dekker, Inc.

Sources of Additional Information

Aitken RJ, Creely KS, Tran CL [2004]. Nanoparticles: an occupational hygiene review. HSE Research Report 274. UK Health and Safety Executive [www.hse.gov.uk/research/rrhtm/rr274.htm].

Balazy A, Podgórski A, Grado L [2004]. Filtration of nanosized aerosol particles in fibrous filters: I. Experimental results. Warsaw Poland: Warsaw University of Technology, Department of Chemical and Process Engineering.

Baron PA, Willeke K [2001]. Aerosol measurement. Principles, techniques and applications. New York: Wiley-Interscience.

Blake T, Castranova V, Schwegler-Berry D, Baron P, Deye GJ, Li CH, Jones W [1998]. Effect of fiber length on glass microfiber cytotoxicity. J Toxicol Environ Health *54*(Part A)(4):243–259.

Borm PJA [2002]. Particle toxicology: from coal mining to nanotechnology. Inhal Toxicology *14*:311–324.

Byrd DM, Cothern CR [2000]. Introduction to risk analysis. Rockville, MD: Government Institutes.

Castranova V [1998]. Particles and airways: basic biological mechanisms of pulmonary pathogenicity. Appl Occup Environ Hyg *13*(8):613–616.

Conhaim RL, Eaton A, Staub NC, Heath TD [1988]. Equivalent pore estimate for the alveolar-airway barrier in isolated dog lung. J Appl Physiol *64*(3):1134–1142.

Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdörster G, Salem H, Schlesinger RB [2000]. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. Toxicol Sci *55*(1):24–35.

EC [2004]. Nanotechnologies: a preliminary risk analysis on the basis of a workshop organized in Brussels, March 1–2, 2004, by the Health and Consumer Protection Directorate General of the European Commission. Brussels, Germany: European Commission [http://www.certh.gr/dat/7CF3F1C6/file.pdf?633556363252496250].

EPA [1992]. Guidelines for exposure assessment. Washington, DC: U.S. Environmental Protection Agency.

Faux SP, Tran CL, Miller BG, Jones AD, Monteiller C, Donaldson K [2003]. In vitro determinants of particulate toxicity: the dose-metric for poorly soluble dusts. Research report 154. Suffolk, UK: Health and Safety Executive.

Ferin J, Oberdörster G [1992]. Translocation of particles from pulmonary alveoli into the interstitium. J Aerosol Med *5*(3):179–187.

Gibbs JP, Crump KS, Houck DP, Warren PA, Mosley WS [1999]. Focused medical surveillance: a search for subclinical movement disorders in a cohort of U.S. workers exposed to low levels of manganese dust. Neurotoxicol *20*(2–3):299–313.

Hardman R [2006]. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect *114*(2):165–172.

Hayes AW, ed. [2001]. Principles and methods of toxicology. 4th ed. Philadelphia, PA: Taylor and Francis.

Hoet PHM, Bruske-Hohlfeld I, Salata OV [2004]. Nanoparticles-known and unknown health risks. J Nanobiotech *2*(12):1–15.

Hofmann W, Sturm R, Winkler-Heil R, Pawlak E [2003]. Stochastic model of ultrafine particle deposition and clearance in the human respiratory tract. Radiat Prot Dosim *105*(1–4):77–80.

Hood E [2004]. Nanotechnology: looking as we leap. Environ Health Perspect *112*(13):A741–A749.

Huckzko A, Lange H [2001]. Carbon nanotubes: experimental evidence for a null risk of skin irritation and allergy. Fullerene Sci Tech *9*(2):247–250.

Huckzko A, Lange H, Calko E, Grubeck-Jaworska H, Droszez P [2001]. Physiological testing of carbon nanotubes: are they asbestos-like? Fullerene Sci Tech *9*(2):251–254.

Keller A, Fierz M, Siegmann K, Siegmann HC, Filippov A [2001]. Surface science with nanosized particles in a carrier gas. J Vac Sci Technol A *19*(1):1–8.

Klaassen CD, Amdur MO, Doull J [1986]. Casarett and Doull's toxicology: the basic science of poisons. New York: Macmillan Publishing Company.

Last JM [1983]. A dictionary of epidemiology. New York: Oxford University Press.

Lee KW, Liu BYH [1981]. Experimental study of aerosol filtration by fibrous filters. Aerosol Sci Technol *1*(1):35–46.

Lee KW, Liu BYH [1982]. Theoretical study of aerosol filtration by fibrous filters. Aerosol Sci Technol *1*(2):147–162.

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang MY, Oberley T, Froines J, Nel A [2003]. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect *111*(4):455–460.

Maynard AD [2001]. Experimental determination of ultrafine TiO_2 de-agglomeration in surrogate pulmonary surfactant—preliminary results. Inhaled Particles IX, Cambridge, UK: The British Occupational Hygiene Society.

Maynard AD [2002]. Experimental determination of ultrafine TiO_2 de-agglomeration in surrogate pulmonary surfactant—preliminary results. Ann Occup Hyg *46*(Suppl 1):197–202.

NCI [1979]. Bioassay of titanium dioxide for possible carcinogenicity. Technical Report Series No. 97. Bethesda, MD: National Cancer Institute.

Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PHM, Verbruggen A, Nemery BS [2001]. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med *164*(9):1665–1668.

NFPA [1994]. Standard on protective ensembles for first responders to CBRN terrorism incidents. Quincy, MA: National Fire Protection Association.

Nichols G, Byard S, Bloxham MJ, Botterill J, Dawson NJ, Dennis A, Diart V, North NC, Sherwood JD [2002]. A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization. J Pharm Sci *91*(10):2103–2109.

Nel A, Xia T, Madler L, Li N [2006]. Toxic potential of materials at the nanolevel. Science *311*:622–627.

NIOSH [1987]. NIOSH guide to industrial respiratory protection. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 87–116, Appendix B.

NRC [1994]. Science and judgement in risk assessment. Washington D.C.: National Academy Press.

Oberdörster E [2004]. Manufactured nanomaterials (Fullerenes, C60) induce oxidative stress in brain of juvenile largemouth bass. Environ Health Perspect *112*(10):1058–1062.

Oberdörster G, Ferin J, Finkelstein J, Soderholm S [1992]. Thermal-degradation events as health-hazards—Particle Vs gas-phase effects, mechanistic studies with particles. Acta Astronaut *27*:251–256.

OSHA [1998]. Occupational Safety and Health Administration, OSHA 3143 (revised): industrial hygiene. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration.

Patty [2000]. Patty's industrial hygiene and toxicology. New York: John Wiley and Sons.

Pflucker F, Wendel V, Hohenberg H, Gärtner E, Will T, Pfeiffer S, Wept F, Gers-Barlag H [2001]. The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. Skin Pharmacol Appl Skin Physiol *14*(Suppl 1):92–97.

Pui DYH, Chen D [2002]. Experimental and modeling studies of nanometer aerosol filtration. Sponsored by the Department of Energy. Minneapolis, MN: University of Minnesota, Particle Technology Laboratory. Grant No. DOEIDE–FG02–98ER1490.

Rao GVS, Tinkle S, Weissman DN, Antonini JM, Kashon ML, Salmen R, Battelli LA, Willard PA, Hoover MD, Hubbs AF [2003]. Efficacy of a technique for exposing the mouse lung to particles aspirated from the pharynx. J Toxicol Environ Health *66*(15):1441–1445.

Renwick LC, Donaldson K, Clouter A [2001]. Impairment of alveolar macrophage phagocytosis by ultrafine particles. Toxicol Appl Pharmacol *172*(2):119–127.

Roels H, Lauwerys R, Buchet JP, Genet P, Sarhan MJ, Hanotiau I, Defays M, Bernard A, Stanescu D [1987]. Epidemiologic survey among workers exposed to manganese—effects on lung, central-nervous-system, and some biological indexes. Am J Ind Med *11*(3):307–327.

Schultz J, Hohenberg H, Plücker F, Gärtner E, Will T, Pfeiffer S, Wepf R, Wendel V, Gers-Barlag H, Wittern KP [2002]. Distribution of sunscreens on skin. Adv Drug Deliv Rev *54*(Suppl 1):S157–S163.

Scott R [1997]. Basic concepts of industrial hygiene. Boca Raton, LA: Lewis Publishers.

Stefaniak AB, Hoover MD, Dickerson RM, Peterson EJ, Day GA, Breysse PN, Kent MS, Scripsick RC [2003]. Surface area of respirable beryllium metal, oxide, and copper alloy aerosols and implications for assessment of exposure risk of chronic beryllium disease. Am Ind Hyg Assoc J *64*(3):297–305.

Thomas K, Sayre P [2005]. Research strategies for safety evaluation of nanomaterials, Part 1: evaluation the human health implications of exposure to nanoscale materials. Toxicol Sci *87*(2):316–321.

Wang HC [1996]. Comparison of thermal rebound theory with penetration measurements of nanometer particles through wire screens. Aerosol Sci Technol *24*(3):129–134.

Wang HC, Kasper G [1991]. Filtration efficiency of nanometer-size aerosol-particles. J Aerosol Sci *22*(1):31–41.

Wennberg A, Iregren A, Struwe G, Cizinsky G, Hagman M, Johansson L [1991]. Manganese exposure in steel smelters a health-hazard to the nervous-system. Scand J Work Environ Health 17(4):255–262.

Appendix

Nanoparticle Emission Assessment Technique for Identification of Sources and Releases of Engineered Nanomaterials

1.0 Introduction

This appendix describes a technique that can be used by industrial hygienists for conducting initial workplace assessments for possible nanoparticle emissions. It allows a semi-quantitative evaluation of processes and tasks in the workplace where releases of engineered nanoparticles may occur. NIOSH uses several sampling approaches simultaneously with the goal of obtaining key physicochemical particle metrics: number concentration, qualitative size, shape, degree of agglomeration, and mass concentration of elemental constituents of interest.

2.0 Scope

Employers, workers, and researchers engaged in the production and use of engineered nanomaterials have expressed an interest in determining whether these nanomaterials are hazardous and if the potential for worker exposure exists. NIOSH has an active toxicology program to assess the potential hazards of engineered nanoparticles. Unfortunately these studies require long time periods and fall behind the pace of production and use of these nanomaterials. To assist in answering the latter of these questions, NIOSH established a nanotechnology field research team tasked with visiting facilities and collecting information about the potential for release of nanomaterials and worker exposure at those facilities. The initial challenges that the field research team encountered were: 1) determining which exposure metric (e.g., mass, particle number concentration, particle surface area) for engineered nanoparticles would provide a consistent body of knowledge to align with the toxicological results observed in experimental animal studies; and 2) selecting a sampling method based on metrics that were practical and would provide reproducible results. Engineered nanomaterials can be measured in the workplace using a variety of instrumentation including: condensation particle counter (CPC); optical particle counter (OPC); scanning mobility particle sizer (SMPS); electric low pressure impactor (ELPI); aerosol diffusion charger; and tapered element oscillating microbalance (TOEM), which vary in complexity and field portability. Unfortunately, relatively few of the above instruments are readily applicable to routine exposure monitoring due to non-specificity, lack of portability, difficulty of use, and high cost. NIOSH researchers have developed and used a field assessment strategy for determining exposures to engineered nanoparticles that could be adopted by other health and safety professionals in the evaluation of occupational exposures [Methner, et. al. 2007; Methner, 2008].

Since there are currently no exposure limits specific to engineered nanomaterials, this technique is used to determine whether

airborne releases of engineered nanomaterials occur. This assessment, which compares particle number concentrations and relative particle size at the potential emission source to background particle number concentrations and particle size, provides a semi-quantitative means for determining the effectiveness of existing control measures in reducing engineered nanoparticle exposures. This procedure utilizes portable direct-reading instrumentation supplemented by filter-based air samples (source-specific and personal breathing zone [PBZ]). The use of filter samples is crucial for particle identification because direct-reading instruments used for determining particle number concentrations are incapable of identifying the composition of the particles.

3.0 Summary of the On-Site Initial Assessment

The initial assessment uses a combination of direct-reading, handheld instruments (CPC and OPC) and filter-based sampling (e.g. 37-mm diameter filter cassettes) for subsequent chemical and microscopic analyses (Figure 1). This semi-quantitative approach was first described by Maynard et al. [2004] and NIOSH has adopted a similar approach. The technique includes determining particle number concentration using direct-reading, handheld particle counters at potential emission sources and comparing those data to background particle number concentrations. If elevated concentrations of suspected nanoparticles are detected at potential emission sources, relative to the background particle number concentrations, then a pair of filter-based, source-specific air samples are collected with one sample analyzed by transmission electron microscopy (TEM) or scanning electron microscopy (SEM) for particle identification and characterization, and the other used for determining the elemental mass concentration (Figure 2). A second pair of filter-based air samples may also be collected in the personal breathing zone of workers. Breathing zone samples are analyzed in the same manner as the area air samples (i.e., by TEM and elemental mass).

4.0 Air Sampling Instrumentation and Filter Media Used in the Initial Assessment

The following instrumentation is used by NIOSH; however, use does not constitute endorsement.

4.1 TSI model 3007 (or model 8525) (TSI Inc, Shoreview, MN), handheld condensation particle counter (CPC), which uses isopropanol to condense on particles so they can be counted

The TSI units provide a non-specific measure of the total number of particles independent of chemical identity per cubic centimeter of air (P/cm^3). The measureable range is between 10–1,000 nm for model 3007, or between 20–1,000 nm for model 8525. The range of detection for these instruments is reported by the manufacturer to be 0–100,000 P/cm^3.

4.2 ART Instruments Hand Held Particle Counter (HHPC-6, ART Instruments, Grants Pass, Oregon), which operates on optical counting principles using laser light scattering.

The HHPC-6 optical particle counter (OPC) can measure the total number of particles per liter (P/L)

of air independent of chemical identity within six specific size ranges. The OPC used by the NIOSH field research team provides particle counts in the following size cut-points: 300 nm; 500 nm; 1,000 nm; 3,000 nm; 5,000 nm; and 10,000 nm. The range of detection for this instrument is reported by the manufacturer to be 0–70,000 P/L. Different manufacturers' OPCs may have slightly different particle size ranges and could be substituted.

4.3 Appropriate air sampling filter media (e.g. mixed cellulose ester, quartz fiber filter) are selected depending on nanoparticle type and desired analytical information (e.g., determination of particle morphology using TEM or SEM, elemental analysis for metals, elemental analysis for carbon)

4.4 Air sampling pumps capable of sampling at high flow rates (e.g., 7 liters per minute or other flow rate depending upon the duration of the task and the appropriate NIOSH method, if a method is available)

4.5 Sampling pump flow calibrator

4.6 If desired, personal cascade impactor or respirable cyclone (see 5.3.3)

4.7 If desired, cassette conductive cowl (see 5.3.3)

4.8 Optional research-grade particle analyzers for expanded surveys (see 5.6.1)

4.9 Optional surface sampling supplies such as substrate (e.g., Ghost Wipes™), disposable 10 cm × 10 cm templates, sterile containers, and nitrile gloves for handling media (see 5.6.2)

5.0 Evaluation of Potential Releases of Engineered Nanomaterials

5.1 Identify Potential Sources of Emissions

The overall purpose of this step is to develop a list of target areas and tasks that will be evaluated with the particle analyzers.

The initial assessment involves identifying the potential sources of engineered nanomaterial emissions by reviewing the type of process, process flow, material inputs and discharges, tasks, and work practices. When available, literature (e.g., MSDS, records of feedstock materials) is reviewed to gain an understanding of the engineered nanomaterials being produced or used, including their physicochemical properties such as size, shape, solubility, and reactivity. Once the potential sources of emissions have been identified from the process review, the industrial hygienist (or other qualified person):

- Conducts an observational walk-through survey of the production area and processes to locate potential sources of emissions.
- Determines the frequency and duration of each operation and the type of equipment used for handling and containment of the material.

- Determines the presence/absence of general and local exhaust ventilation and other engineering controls. (This initial assessment includes identifying points of potential system failure that could result in emission from the containment/control system [e.g., hole in duct, deteriorated sealing gasket]).
- Determines the process points where containment is deliberately breached (e.g., opening system for product retrieval or for cleaning).

5.2 Conduct Particle Concentration Sampling

5.2.1 Background measurements

Determining the contribution of background particle concentrations on measurements made for the particles of interest (e.g., engineered nanoparticles) is an important evaluation of assessing the possible airborne release of engineered nanoparticles.

Ideally, during the initial assessment, the industrial hygienist (or other qualified person), will determine the average airborne particle concentration at various processes and adjacent work areas with the CPC and OPC *before* the processing or handling of nanomaterials begins. If the background particle concentrations are high (values are relative and will vary with processes and facilities), an assessment is made as to whether there may be a source of incidental nanoparticles in the area. Incidental nanoparticles may be generated from a variety of sources, including vacuum pumps, natural gas heating units, gasoline/propane/diesel powered fork lift trucks, or other combustion activities such as welding, soldering, or heat-sealing. The CPC and OPC can be used to check these sources for incidental nanoparticle releases. Outdoor or re-circulated air supply from the building ventilation system should also be considered as a possible source of nanoparticles [Peters et al. 2006].

Measurements of background particle concentrations are repeated after the active processing, manufacturing, or handling of the nanomaterial has ended. An average background concentration is then computed and subtracted from the measurements made during processing, manufacturing, or the handling of engineered nanomaterials. This approach is acceptable only if background particle counts remain relatively stable throughout the measurement period and particle emissions from the process under investigation are sufficiently elevated above background. For other situations, correcting for particle background concentrations becomes more complex requiring additional sampling over an extended time period to determine the source(s) and magnitude of background particle concentrations. This type of evaluation is generally outside the scope of the initial assessment described here.

5.2.2 Area sampling

Once initial background particle concentrations have been determined, measurements of airborne particle concentrations and size ranges are

made with the CPC and OPC simultaneously at locations near the suspected or likely emission source (e.g., opening a reactor, handling product, potential leak points in the ventilation system). Airborne particle concentrations are determined before, during, and after each task or operation to identify those factors (e.g., controls, worker interaction, work practices) that may affect airborne particle concentrations. This information is used to identify processes, locations, and personnel for filter-based air sampling (5.3).

5.3 Conduct Filter-based Area and Personal Air Sampling

5.3.1 *Area air sampling*

A pair of filter-based, air samples are collected at process/task locations and/or workers engaged in process operations where suspected engineered nanomaterial emissions may occur, based on air sampling results using the CPC and OPC.

Filter-based area air samples provide more specific information on the engineered nanomaterial of interest (e.g., size, shape, mass). The pair of air samples includes one sample analyzed for elemental mass and one sample analyzed by electron microscopy. For example, one sample might be collected for metals determination (e.g., NIOSH Method 7300, 7303) or elemental carbon (e.g., NIOSH Method 5040) depending on the composition of the engineered nanomaterial. The other sample would be collected for particle characterization (e.g., size, shape, dimension, degree of agglomeration) by TEM or SEM using the measurement techniques specified in NIOSH Methods 7402, 7404, or other equivalent methods [NIOSH 1994].

The source-specific air samples are collected as close as possible to the suspected emission source but outside of any existing containment, to increase the probability of detecting any possible release of engineered nanomaterials. Sampling duration generally matches the length of time in which the potential exposure to the engineered nanomaterial exists at the task or specific process. In cases where the duration of the tasks associated with the potential airborne release of nanomaterials is short (e.g., minutes), a relatively high air sampling flow rate may be required (approximately 7 liters per minute) to ensure adequate particle loading on the filter media. If specific information is desired on the worker's potential exposure to the engineered nanomaterial then PBZ samples should be collected using the two- sample filter-based sampling strategy described above.

If the particle number concentrations (using CPC or OPC) are substantially high, then shorter sampling times for the TEM or SEM sample may be necessary to avoid overloading the filter and interfering with particle characterization. The specific sampling time should be based on direct-reading instrument results and professional judgment of the industrial hygienist. In general, filter samples are collected for the duration of a given task, normally 15–30 minutes. If the

direct-reading instruments indicate a high particle number concentration the sampling time can be shortened to 5–10 minutes, or both a short- and long-duration sample may be collected to ensure an adequate sample for electron microscopy analysis. See Table 1 for additional sampling time guidance. However, the sampling times in Table 1 were based on collection of asbestos fibers by NIOSH Method 7402 and may not be applicable for much smaller engineered nanoparticles. See Figures 3–5 for example TEM micrographs.

A minimum of 2 background filter samples are collected distant from the potential sources of engineered nanoparticle exposure to serve as an indicator of ambient particle identification and concentration.

5.3.2 *Personal air samples*

When possible, personal breathing zone (PBZ) air samples are collected on workers likely to be exposed to engineered nanomaterials (e.g., engaged in active handling of nanomaterials or operating equipment previously identified as emitting nanoparticles). If measurements obtained with the CPC and OPC indicate that nanoparticles are being emitted at a specific process where a worker is located, then the collection of PBZ samples may be warranted.

PBZ samples are analyzed in the same manner as the area air samples (i.e., by TEM and elemental mass). It may be necessary to collect samples at a relatively high flow rate (e.g., 7 liters per minute) if the duration of the task and the resulting potential exposure is short.

5.3.3 *Optional sample collection*

In the event that measurements made by the OPC indicate a large fraction (over 50%) of particles exceeding 1,000 nm in size, the use of a personal cascade impactor or respirable cyclone sampler in tandem with a filter-based air sampling cassette may be required for both the mass and TEM/SEM analyses to eliminate large particles that may interfere with analysis and be of limited interest. The use of an impactor or cyclone will require using a flow rate appropriate for the particle cut size and is usually in the range of 1.7–2.5 liters per minute. Open-face, and impactor or cyclone samples may be collected side by side to allow a more thorough interpretation of analytical results. Additionally, if it is anticipated that the nanoparticles of interest will have a tendency to be electrostatically attracted to the sides of the plastic air sampling cassette, a conductive cowl may be necessary to eliminate particle loss and subsequent underestimation of the airborne nanoparticle concentration. The use of a personal cascade impactor, respirable cyclone, or conductive cowl is made at the discretion of the industrial hygienist (or other qualified person).

If the facility is manufacturing or using TiO_2, then the sampling should include the sampling recommendations found in the NIOSH *Draft Document: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide* (www.cdc.gov/

niosh/review/public/TiO2/default.html),which recommends collecting a mass-based airborne measurement using NIOSH Method 0600.

5.4 Quality Assurance and Quality Control

To ensure valid emission measurements, the following quality assurance and control steps should be taken:

- Use factory calibrated direct-reading particle analyzers
- Perform daily zero-checks on all particle counters before each use
- Calibrate pumps before and after each sampling day
- Submit for analysis any process, background, and bulk material samples along with field and media blanks to a laboratory accredited by the American Industrial Hygiene Association (AIHA)

5.5 Data Interpretation

Since the size of airborne engineered nanoparticles and the degree of agglomeration may be unknown at the time of sample collection, the use of direct-reading, particle sizing/counting instruments may provide a semi-quantitative indication of the magnitude of potential emissions, provided background particle number subtraction can be successfully accomplished. The particle number concentration measurements taken with the CPC and OPC will provide a measurement of particles larger than the ASTM definition of nanoparticles (1–100 nm) [ASTM 2006]. However, the two particle counters can be used simultaneously to obtain a semi-quantitative size differential evaluation of the aerosol being sampled. The CPC provides a measure of total particles per cm^3 in the size range of 10–1,000 nm (or 20–1,000 nm). The OPC provides the total number of particles per liter of air within six specific size ranges: 300 nm; 500 nm; 1,000 nm, 3,000 nm, 5,000 nm and > 10,000 nm. If necessary, the data from the CPC and OPC can be used together to determine the number concentration of nanoscale particles. For example, a high particle number concentration on the CPC, in combination with a high particle number concentration in the small size ranges (300–500 nm) on the OPC, may indicate the possible presence of nanoscale particles. Conversely, a low CPC particle number concentration, in combination with a high OPC particle number concentration in the larger size ranges (> 1,000 nm) may indicate the presence of larger particles and/or engineered nanoparticle agglomerates. These assumptions of nanoparticles versus larger particles and/or nanoparticle agglomerates may be verified by TEM or SEM analysis.

5.5.1 *Selectivity*

Selectivity is a critical issue when characterizing exposure using airborne particle number concentration. Airborne nanoparticles are present in many workplaces and often originate from multiple sources such as combustion, vehicle emissions, and

infiltration of outside air. Particle counters are generally not selective to particle source or composition, making it difficult to differentiate between incidental and process-related nanoparticles using number concentration alone. The CPC and OPC are used to identify sources of nanoparticles and the filter-based samples are used to verify the size, shape, and chemical composition of the nanoparticles with the goal of differentiating between incidental and engineered nanoparticles.

5.5.2 *Limitations*

The exposure assessment technique does have some limitations including:

- Although this issue is not unique to particle number concentration measurements, orders of magnitude difference can exist in aerosol number concentrations, depending on the number and types of sources of particle emissions. Monitoring over several days and during different seasons can provide a better understanding of the variability that might exist in airborne particle number concentrations found in background measurements and in measurements made at sources where engineered nanomaterials are handled.
- The upper dynamic range of the CPC is 100,000 P/cm^3. A dilutor, consisting of a modified HEPA filter cartridge placed upstream of the inlet, can extend the range of the CPC when particle number concentrations are greater than 100,000 P/cm^3 [Peters et al. 2006; Heitbrink et al. 2007; Evans et al. 2008].
- The analysis of air samples by TEM or SEM with energy dispersive X-ray spectrometry can provide information on the elemental composition of the nanomaterials. However, TEM and SEM analysis can be compromised if there is particle overload on the filter. Alternatively, if the loading is too sparse, an accurate assessment of particle characteristics may not be possible (see 5.3.1).
- Note that area samples are collected as closely as possible to the source of emission to allow for more accurate determination of a nanoparticle release and to identify locations most likely to result in worker exposure. **Therefore, results from this type of sampling should not be interpreted as representative of worker exposure.** However, samples collected in such a fashion should serve as an indicator of material release and the possible need for controls.

5.6 Expanded Research (In Depth Assessments)

5.6.1 *Research instrumentation*

A major obstacle in conducting more specific measurement of engineered nanomaterials in the workplace is a lack of field-portable instruments that can be easily maneuvered within

a facility or easily worn by a worker to provide an indication of PBZ exposure. Additionally, there is no single instrument capable of measuring the numerous potential exposure metrics associated with engineered nanomaterials (e.g., number concentration, surface area, size, shape, mass concentration) [Maynard and Aitken 2007]. Although the following instruments lack field portability and ease of use, they can measure many of the desirable exposure metrics and provide information about the particle size distribution. These research-grade particle analyzers are not usually part of the initial assessment but are used when additional knowledge about the nanoscale particle temporal or spatial exposure variation or size distribution is desired.

5.6.1.1 *Particle Surface-Area Analyzers*

Toxicology studies have indicated that surface area of nanoparticles may be an important exposure dose metric. Portable aerosol diffusion chargers may be used to provide estimates of external aerosol surface area when airborne particles are smaller than 100 nm in diameter, but these may tend to overestimate external surface area when particles are larger than 100 nm in diameter. These instruments are based on diffusion charging followed by detection of the charged aerosol using an electrometer.

The TSI Aerotrak™ 9000 Nanoparticle Aerosol Monitor does not measure total active surface area but indicates the surface area of particles which may be deposited in the lung in units of square micrometers per cubic centimeter, corresponding to either the tracheobronchial or alveolar regions of the lung. The Ecochem DC 2000-CE measures the total particle surface area. These devices are currently being evaluated as part of the process used by NIOSH to conduct initial assessments. These particle surface analyzers are used as area samplers.

5.6.1.2. *Scanning Mobility Particle Sizer*

More specific depictions of particles by size (diameter) and number can greatly improve the ability to evaluate possible releases of engineered nanoparticles. One particular instrument, the Scanning Mobility Particle Sizer (SMPS) measures particle diameters from 2.5–1,000 nm and can display data as a size and number distribution using up to 167 size channels. The SMPS is widely used as a research tool for characterizing nanoscale aerosols. The SMPS employs a continuous, fast-scanning technique to provide high-resolution measurements. However, the SMPS may take 2–3 minutes to scan which may not be useful for the process screening in workplaces with highly variable aerosol size distributions. Its applicability for use in the workplace may be limited because of its size, cost, and use of an internal radioactive source.

The Fast Mobility Particle Sizer (FMPS) is similar to the SMPS but has a much faster response time (approximately 1 second). However, because it has fewer particle size channels, it does not include the same level of detail on particle size distributions that can be determined with the SMPS.

The FMPS and SMPS are used as area samplers.

5.6.1.3 *Low Pressure Impactors*

The Electrical Low Pressure Impactor (ELPI) combines diffusion charging and a cascade impactor to provide aerosol size distributions by aerodynamic diameter as determined real time by mass and number collected on a series of plates.

Low pressure cascade impactors offer the ability to size particles and then conduct secondary analyses (e.g., metals analysis). However, these instruments are sensitive to harsh field conditions and are not considered portable. The ELPI is used as an area sampler.

5.6.1.4 *Tapered Element Oscillating Microbalance*

The tapered element oscillating microbalance (TEOM) is commonly used for sampling aerosols less than 1 μm in diameter, however, the sampling inlet can be set to select different size fractions. The TEOM determines mass by detecting a change in vibration frequency across a particle-collecting substrate. The TEOM can be configured to provide size-differentiated mass measurements and is used as an area sampler.

5.6.2 *Surface sampling*

Surface sampling to detect the presence of engineered nanomaterials is not routinely part of the initial assessment but may be conducted to determine if surface contamination exists. Surface sampling does not provide size-specific information but may be useful for determining whether engineered nanomaterials have migrated away from active production or handling areas and have contaminated nonproduction work areas. The decision to collect surface samples is made in the field at the discretion of the industrial hygienist (or other qualified person), and is dependent on direct observation and the nanomaterial of interest. For example, surface sampling was completed at a quantum dot facility after observing dusty surfaces in areas adjacent to the production area. In order to determine if the dust was contaminated with quantum dots, surface samples were collected and analyzed for the chemical components of the quantum dots produced by that facility.

Surface wipe samples are collected using a pre-moistened substrate such as Ghost Wipe™ towelettes in accordance with NIOSH Method 9102 for elements or the NIOSH method for specific elements (e.g., NIOSH Method 9100 for lead). When collecting wipe samples, the following steps should be followed:

- Don a pair of nitrile disposable gloves
- Wipe the surface within a disposable 10 cc × 10 cc template using four horizontal s-shaped strokes
- Fold the exposed side of the wipe in and wiping the same area with four vertical s-shaped strokes
- Fold the wipe, exposed side in, and placing it into a sterile container

Gloves and template are discarded after each sample collection to eliminate the possibility of cross-contaminating successive samples. Wipe samples may be collected from undisturbed horizontal surfaces throughout the facility at locations suspected to be contaminated and in areas expected to be free of engineered nanomaterials. Wipe samples are analyzed following the appropriate NIOSH method for the chemical substance of interest.

6.0 Conclusions

The NIOSH initial assessment technique uses complimentary approaches to semi-quantitatively evaluate the potential releases of engineered nanoparticles. Two different particle counters are used in a parallel and differential manner to evaluate the total particle number relative to background and the relative size distribution of the particles. If this initial evaluation indicates an elevated number of small particles, which could potentially be the engineered nanoparticle of interest, then the particle counters are used to detect the source of the emissions. If nanoparticles are found and determined to be emitted from a specific process (versus background incidental nanoscale particles), then additional samples are collected for qualitative measurement of particle size and shape, (by TEM or SEM analysis) and for determination of elemental mass concentration (by chemical analysis).

The initial assessment technique is useful for determining whether airborne releases of engineered nanomaterials are occurring at potential emission sources. This assessment provides a semi-quantitative means for determining whether existing measures are adequate for controlling nanomaterial emissions or if additional controls may be required.

The NIOSH emission assessment technique may be useful to health and safety professionals who are interested in determining whether release of nanomaterials occurs in the workplace. Where possible, use of the technique should be repeated in workplaces of interest to gain a better understanding of the daily fluctuations in airborne exposures at processes and tasks in which engineered nanomaterials occur and for determining potential sources of background particle number concentrations. A more systematic and routine assessment of the workplace can provide more definitive information on the performance of control measures and if additional actions are needed to reduce worker exposure.

The initial assessment technique can be expanded or modified to determine additional metrics (Figure 6). Research initiatives addressing more comprehensive process monitoring, particle metrics, personal exposure monitoring, and method/approach development and validation are currently underway within NIOSH. As this information becomes available, revisions to the Approaches to Safe Nanotechnology document will be made.

Information about contacting the nanotechnology field research team is available at: [www.cdc.gov/niosh/

docs/2008-121], see the Fact Sheet: NIOSH Nanotechnology Field Research Effort [NIOSH 2008].

7.0 References

ASTM International [2006] ASTM-E2456–06 Standard terminology relating to nanotechnology

Evans DE, Heitbrink WA, Slavin TJ, Peters TM [2008]. Ultrafine and respirable particles in an automotive grey iron foundry. Ann Occup Hyg *52*(1):9–21.

Heitbrink WA, Evans DE, Peters TM, Slavin TJ [2007]. The characterization and mapping of very fine particles in an engine machining and assembly facility. J Occup Environ Hyg *4*:341–351.

NIOSH [2008]. NIOSH Fact Sheet: NIOSH Nanotechnology Field Research Effort, Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 2008–121 [http://www.cdc.gov/niosh/docs/2008-121/].

NIOSH [1994]. NIOSH manual of analytical methods (NMAM®), 4th ed. By Schlecht PC, O'Conner PF, eds. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 94–113 [www.cdc.gov/niosh/nmam/].

Maynard A, Aitken R [2007]. Assessing exposure to airborne nanomaterials: current abilities and future requirements. Nanotoxicology *1*(1):26–41.

Maynard A, Baron P, Foley M, Shvedova A, Kisin E, Castranova V [2004] Exposure to carbon nanotube material: Aerosol release during the handling of unrefined single walled carbon nanotubes material. J Toxicology. Environmental Health Part A, *67*: 1; 87–107.

Methner MM, Birch ME, Evans DE, Ku BK, Crouch KG, Hoover MD [2007]. Mazzukelli LF, ed: Case study: Identification and characterization of potential sources of worker exposure to carbon nanofibers during polymer composite laboratory operations. J Occup Environ Hyg *4*(12): D125–D130.

Methner M [2008]. Engineering case reports. Old L, ed. Effectiveness of local exhaust ventilation in controlling engineered nanomaterial emissions during reactor cleanout operations. J Occup Environ Hyg *5*(6): D63–D69.

Peters T, Heitbrink W, Evans D, Slavin T, Maynard A [2006]. The mapping of fine and ultrafine particle concentrations in an engine machining and assembly facility. Ann Occup Hyg *50*(3):1–9.

Table 1. Approximate sampling times for TEM grid based on particle number concentrations*.

		Open-faced cassettes		
	TEM grid	**25-mm**	**37-mm**	**47-mm**
Diameter (mm)	3.0	25.0	37.0	47.0
Effective diameter (mm)	3.0	22.2	34.2	44.2
Effective collection area (mm^2)	7	385	916	1531
Flow (L/min)	0.1	7	7	7
Desired Loading ($\#/mm^2$)	1.E+06	1.E+06	1.E+06	1.E+06
Air concentration ($\#/cm^3$)	**Time (min)**			
250	282.7	220.2	523.4	874.8
500	141.4	110.1	261.7	437.4
1,000	70.7	55.0	130.8	218.7
2,000	35.3	27.5	65.4	109.4
4,000	17.7	13.8	32.7	54.7
8,000	8.8	6.9	16.4	27.3
16,000	4.4	3.4	8.2	13.7
32,000	2.2	1.7	4.1	6.8
64,000	1.1	0.9	2.0	3.4
128,000	0.6	0.4	1.0	1.7

*NIOSH NMAM Method 7402 Asbestos by TEM and personal communication with Dr. Aleksandr Stefaniak (NIOSH)

Figure 1. A demonstration of the initial assessment technique with side-by-side sampling using (from left to right) the OPC, co-located open-face filter cassettes, and the CPC: examples of PBZ and source-specific filter-based sampling setup.

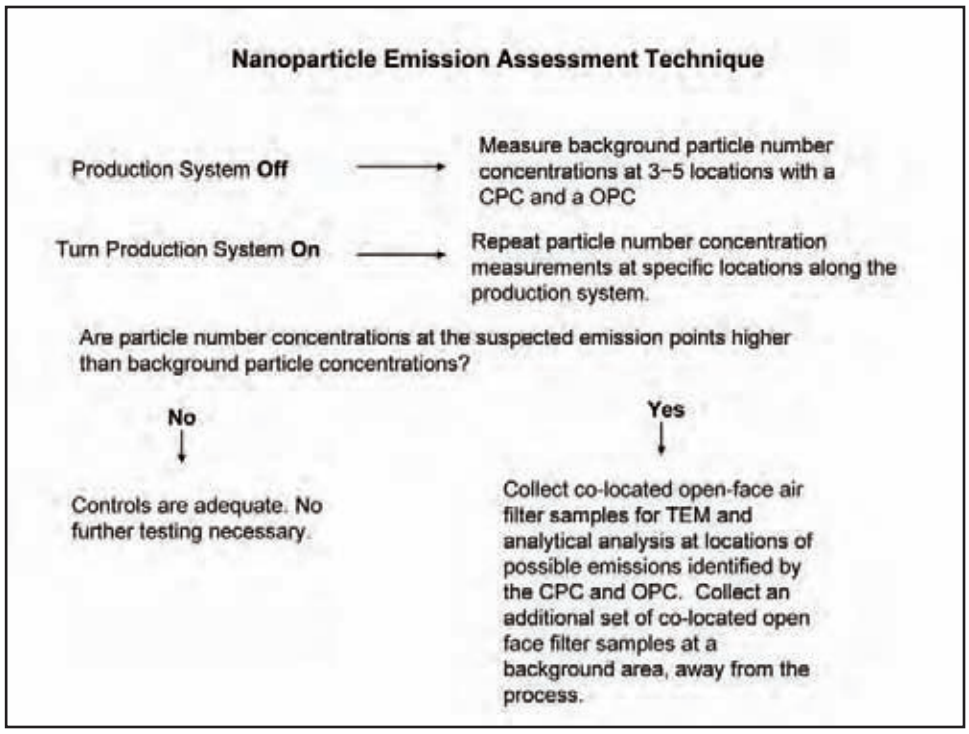

Figure 2. Summary of the initial assessment technique

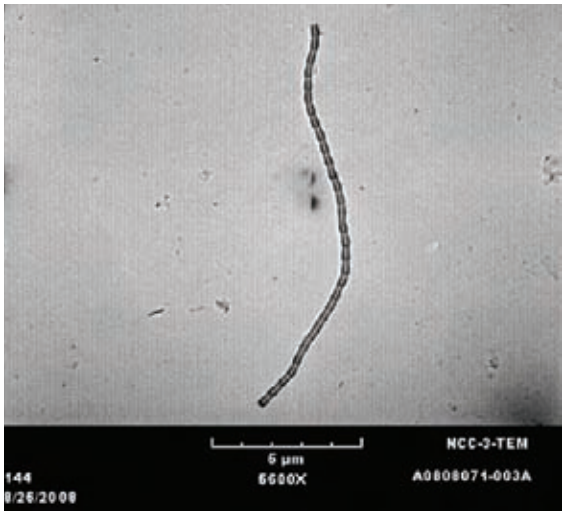

Figure 3. Electron microscopy micrograph of a carbon nanofiber

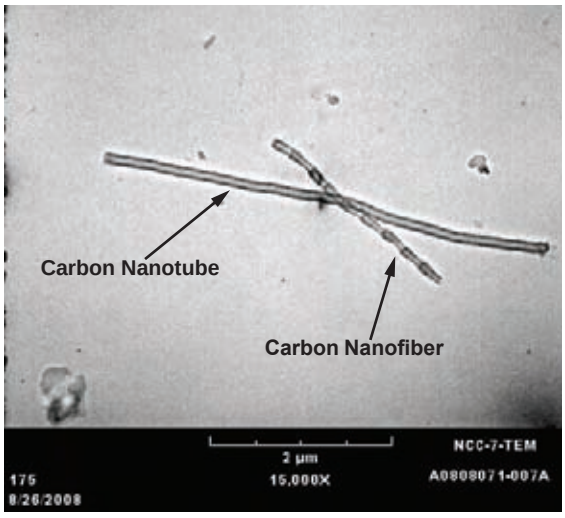

Figure 4. Electron microscopy micrograph of a carbon nanofiber and carbon nanotube

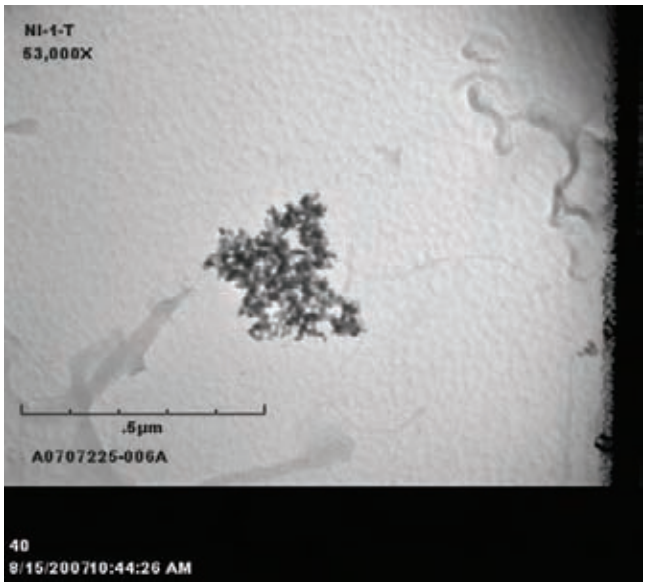

Figure 5. Electron microscopy micrograph of an agglomerated nanoparticle of nickel oxide

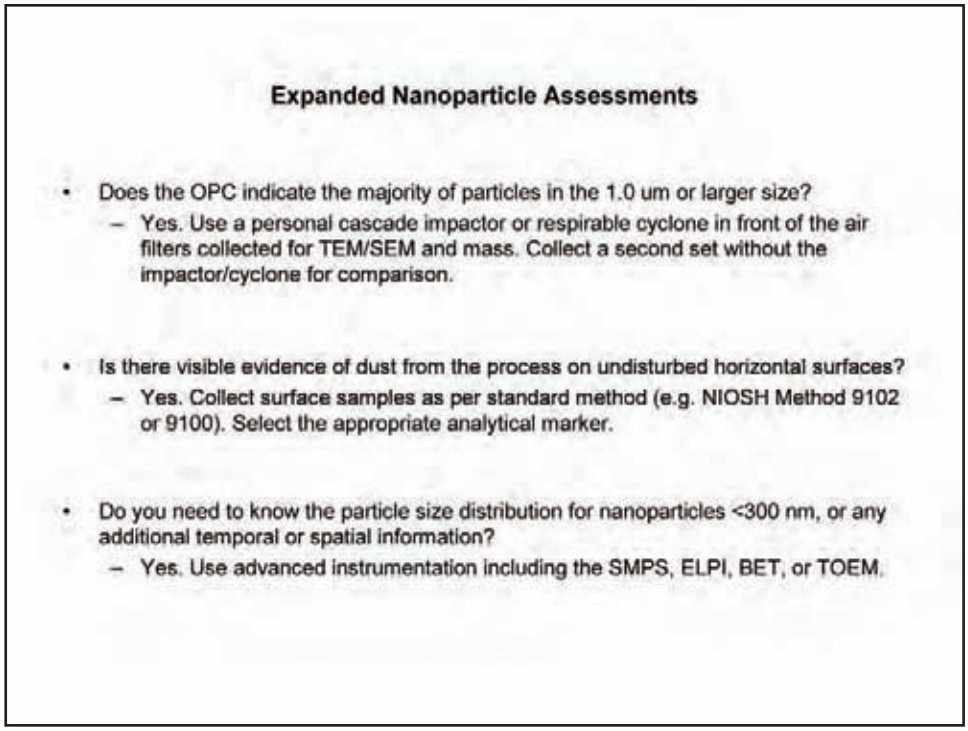

Figure 6. Considerations for expanded nanomaterial assessments

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DHHS (NIOSH) Publication No. 2009–125

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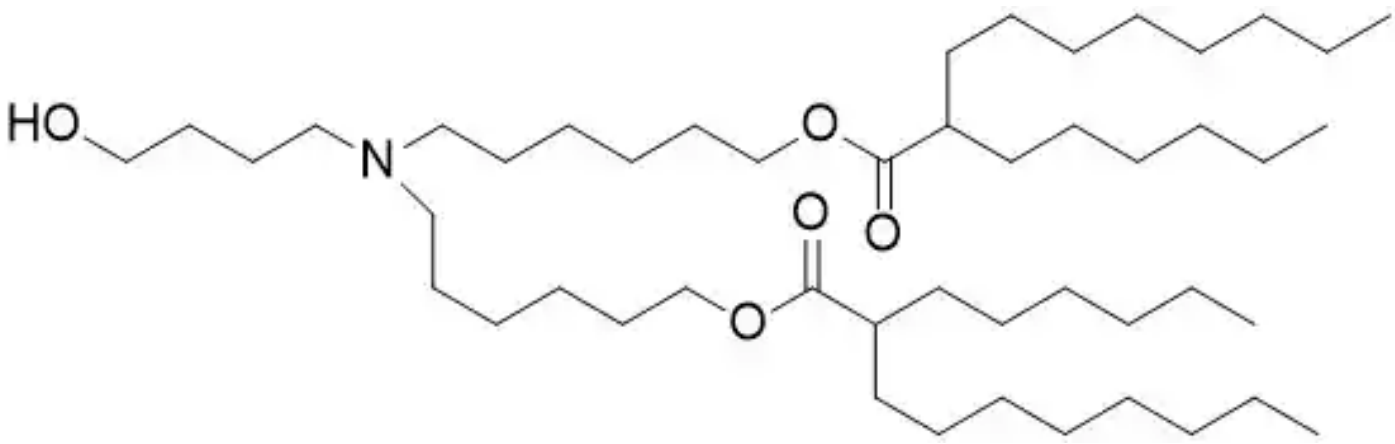

ALC-0315

Product Number: N-1020

$75.00 – $390.00

-	0	+	5mg (N-1020)	$75.00
-	0	+	10mg (N-1020)	$125.00
-	0	+	50mg (N-1020)	$390.00

Add to cart

SKU: N-1020

Category: Lipids

Tag: nanoparticles

Description Additional Information Documentation

ALC-0315 is an ionizable lipid which has been used to form lipid nanoparticles for delivery of RNA. ALC-0315 is one of the components in the BNT162b2 vaccine against SARS-CoV-2 in addition to ALC-0159, DSPC, and cholesterol. This product is for research use only and not for human use.

References

1) R. Tenchov, R. Bird, A. E. Curtze, Q. Zhou (2021) "Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement" ACS Nano, DOI: 10.1021/acsnano.1c04996.
2) K.H. Moss, P. Popova, et al. (2019) "Lipid Nanoparticles for Delivery of Therapeutic RNA Oligonucleotides" Mol. Pharmaceutics 16, 2265–2277, DOI: 10.1021/acs.molpharmaceut.8b01290.
3) Y. Duan, A. Dhar, et al. (2020) "A brief review on solid lipid nanoparticles: part and parcel of contemporary dru delivery systems" RSC Adv.,10, 26777-26791.

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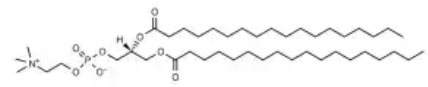

Lipids

DSPC (18:0/18:0 PC)

Product Number: L-1118

94 / 100
Bioz Stars

$30.00 – $240.00

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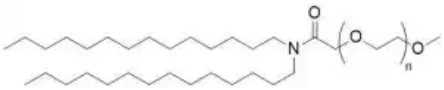

Lipids

ALC-0159

Product Number: N-2010

$125.00 – $495.00

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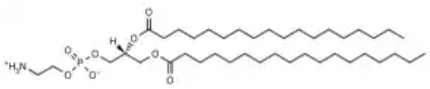

Lipids

DSPE (18:0/18:0 PE)

Product Number: L-2118

91 / 100
Bioz Stars

$94.00 – $340.00

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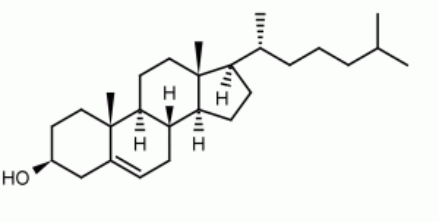

Lipids

Cholesterol

Product Number: L-6012

93 / 100

Bioz Stars
$30.00 – $150.00

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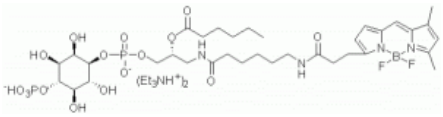
Related products

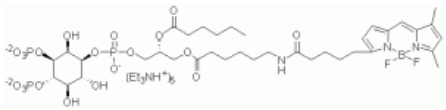


Lipids

GloPIPs BODIPY FL-PI(4)P

Product Number: C-04F6A

View products

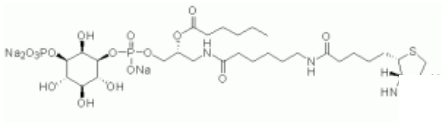


Lipids

BODIPY FL PI(3,4)P2

Product Number: C-34F6
$448.00 – $770.00

View products



Lipids

GloPIPs Biotin-PI(3)P

Product Number: C-03B6A

85 / 100
Bioz Stars

$277.00 – $846.00

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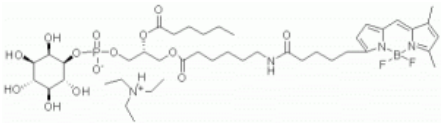

Lipids

BODIPY FL Phosphatidylinositol

Product Number: C-00F6

91 / 100
Bioz Stars

$280.00 – $446.00

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EXHIBIT 5
G Chem 0315

Safety Data Sheet

Revision Date: Mar.-23-2021
Print Date: Jun.-28-2021

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifier

Product name : ALC-0315

Catalog No. : CS-0145622

CAS No. : 2036272-55-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, manufacture of substances.

1.3 Details of the supplier of the safety data sheet

Company: ChemScene LLC

Tel: 732-484-9848

Fax: 888-484-5008

E-mail: sales@chemscene.com

1.4 Emergency telephone number

Emergency Phone #: 732-484-9848

· 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Skin corrosion/irritation (Category 2),H315

Serious eye damage/eye irritation (Category 2A),H319

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Warning

Hazard statement(s)

H315 Causes skin irritation

H319 Causes serious eye irritation

Precautionary statement(s)

P264 Wash hands thoroughly after handling

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 IF ON SKIN: Wash with plenty of soap and water.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P313 Get medical advice/attention.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

P362 Take off contaminated clothing and wash before reuse.

2.3 Other hazards

None.

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula:	$C_{48}H_{95}NO_5$
Molecular Weight:	766.27
CAS No. :	2036272-55-4

4. FIRST AID MEASURES

4.1 Description of first aid measures

Eye contact

Remove any contact lenses, locate eye-wash station, and flush eyes immediately with large amounts of water. Separate eyelids with fingers to ensure adequate flushing. Promptly call a physician.

Skin contact

Rinse skin thoroughly with large amounts of water. Remove contaminated clothing and shoes and call a physician.

Inhalation

Immediately relocate self or casualty to fresh air. If breathing is difficult, give cardiopulmonary resuscitation (CPR). Avoid mouth-to-mouth resuscitation.

Ingestion

Wash out mouth with water; Do NOT induce vomiting; call a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2).

4.3 Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

5. FIRE FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, dry chemical, foam, and carbon dioxide fire extinguisher.

5.2 Special hazards arising from the substance or mixture

During combustion, may emit irritant fumes.

5.3 Advice for firefighters

Wear self-contained breathing apparatus and protective clothing.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use full personal protective equipment. Avoid breathing vapors, mist, dust or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.

Refer to protective measures listed in sections 8.

6.2 Environmental precautions

Try to prevent further leakage or spillage. Keep the product away from drains or water courses.

6.3 Methods and materials for containment and cleaning up

Absorb solutions with finely-powdered liquid-binding material (diatomite, universal binders); Decontaminate surfaces and equipment by scrubbing with alcohol; Dispose of contaminated material according to Section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Avoid inhalation, contact with eyes and skin. Avoid dust and aerosol formation. Use only in areas with appropriate exhaust ventilation.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly sealed in cool, well-ventilated area. Keep away from direct sunlight and sources of ignition.

Recommended storage temperature: 2-8°C, protect from light

Shipping at room temperature if less than 2 weeks.

7.3 Specific end use(s)

No data available.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Engineering controls

Ensure adequate ventilation. Provide accessible safety shower and eye wash station.

Personal protective equipment

Eye protection	Safety goggles with side-shields.
Hand protection	Protective gloves.
Skin and body protection	Impervious clothing.
Respiratory protection	Suitable respirator.
Environmental exposure controls	Keep the product away from drains, water courses or the soil. Clean spillages in a safe way as soon as possible.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Viscous liquid
Odor	No data available
Odor threshold	No data available
pH	No data available
Melting/freezing point	No data available
Boiling point/range	No data available
Flash point	No data available
Evaporation rate	No data available
Flammability (solid, gas)	No data available
Upper/lower flammability or explosive limits	No data available
Vapor pressure	No data available
Vapor density	No data available
Relative density	No data available
Water Solubility	No data available

Partition coefficient	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidizing properties	No data available

9.2 Other safety information

No data available.

10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available.

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to avoid

No data available.

10.5 Incompatible materials

Strong acids/alkalis, strong oxidising/reducing agents.

10.6 Hazardous decomposition products

Under fire conditions, may decompose and emit toxic fumes.

Other decomposition products - no data available.

11.TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

Classified based on available data. For more details, see section 2

Skin corrosion/irritation

Classified based on available data. For more details, see section 2

Serious eye damage/irritation

Classified based on available data. For more details, see section 2

Respiratory or skin sensitization

Classified based on available data. For more details, see section 2

Germ cell mutagenicity

Classified based on available data. For more details, see section 2

Carcinogenicity

IARC: No component of this product present at a level equal to or greater than 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by ACGIH.

NTP: No component of this product present at a level equal to or greater than 0.1% is identified as a anticipated or confirmed carcinogen by NTP.

OSHA: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed

carcinogen by OSHA.

Reproductive toxicity

Classified based on available data. For more details, see section 2

Specific target organ toxicity - single exposure

Classified based on available data. For more details, see section 2

Specific target organ toxicity - repeated exposure

Classified based on available data. For more details, see section 2

Aspiration hazard

Classified based on available data. For more details, see section 2

Additional information

This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity

No data available.

12.2 Persistence and degradability

No data available.

12.3 Bioaccumlative potential

No data available.

12.4 Mobility in soil

No data available.

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment unavailable as chemical safety assessment not required or not conducted.

12.6 Other adverse effects

No data available.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Dispose substance in accordance with prevailing country, federal, state and local regulations.

Contaminated packaging

Conduct recycling or disposal in accordance with prevailing country, federal, state and local regulations.

14. TRANSPORT INFORMATION

DOT (US)

This substance is considered to be non-hazardous for transport.

IMDG

This substance is considered to be non-hazardous for transport.

IATA

This substance is considered to be non-hazardous for transport.

15. REGULATORY INFORMATION

SARA 302 Components:

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components:

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards:

No SARA Hazards.

Massachusetts Right To Know Components:

No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components:

No components are subject to the Pennsylvania Right to Know Act.

New Jersey Right To Know Components:

No components are subject to the New Jersey Right to Know Act.

California Prop. 65 Components:

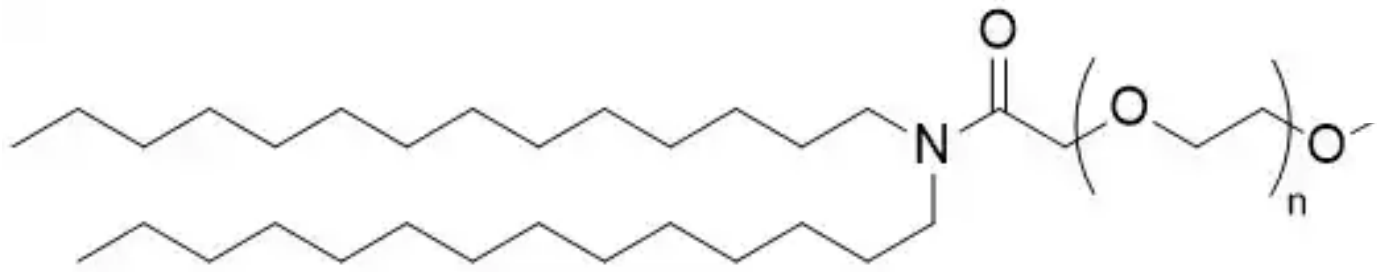
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or anyother reproductive harm.

16. OTHER INFORMATION

 The above information is correct to the best of our present knowledge but does not purport to be all inclusive and should be used only as a guide. The product is for research use only and for experienced personnel. It must only be handled by suitably qualified experienced scientists in appropriately equipped and authorized facilities. The burden of safe use of this material rests entirely with the user. ChemScene disclaims all liability for any damage resulting from handling or from contact with this product.

EXHIBIT 5
H Echelon 0159

Search ...



ALC-0159

Product Number: N-2010

$125.00 – $495.00

-	0	+	5mg (N-2010)	$125.00
-	0	+	10mg (N-2010)	$225.00
-	0	+	25mg (N-2010)	$495.00

Add to cart

SKU: N-2010

Category: Lipids

Tag: nanoparticles

Description Additional Information Documentation

ALC-0159 is a PEGylated lipid which has been used to form lipid nanoparticles for delivery of RNA. ALC-0159 is one of the components in the BNT162b2 vaccine against SARS-CoV-2 in addition to ALC-0315, DSPC, and cholesterol.

This product is for research use only and not for human use.

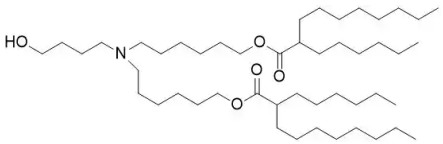
References

1) R. Tenchov, R. Bird, A. E. Curtze, Q. Zhou (2021) "Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement" ACS Nano, DOI: 10.1021/acsnano.1c04996.

2) K.H. Moss, P. Popova, et al. (2019) "Lipid Nanoparticles for Delivery of Therapeutic RNA Oligonucleotides" Mol. Pharmaceutics 16, 2265–2277, DOI: 10.1021/acs.molpharmaceut.8b01290.

3) Y. Duan, A. Dhar, et al. (2020) "A brief review on solid lipid nanoparticles: part and parcel of contemporary drug delivery systems" RSC Adv.,10, 26777-26791.

You may also like…

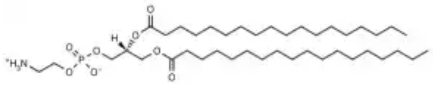


Lipids

ALC-0315

Product Number: N-1020
$75.00 – $390.00

View products



Lipids

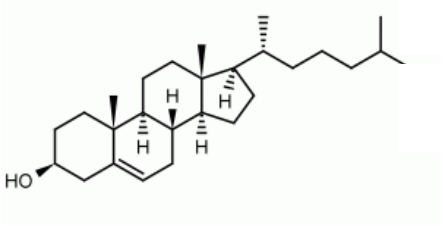
DSPE (18:0/18:0 PE)

Product Number: L-2118

91 / 100
Bioz Stars

$94.00 – $340.00

View products



Lipids

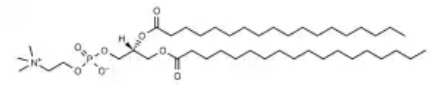
Cholesterol

Product Number: L-6012

93 / 100
Bioz Stars

$30.00 – $150.00

View products



Lipids

DSPC (18:0/18:0 PC)

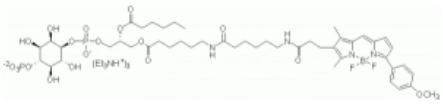
Product Number: L-1118

94 / 100
Bioz Stars

$30.00 – $240.00

View products

Related products



Lipids

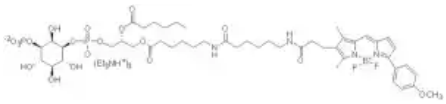
BODIPY TMR PI(4)P

Product Number: C-04M6

85 / 100
Bioz Stars

$430.00 – $739.00

View products

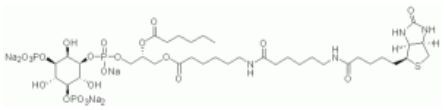


Lipids

BODIPY TMR PI(3)P

Product Number: C-03M6
$448.00 – $770.00

View products



Lipids

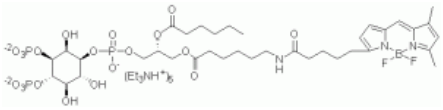
Biotin Phosphatidylinositol 3,5-bisphosphate

Product Number: C-35B6

93 / 100
Bioz Stars

$255.00 – $783.00

View products



Lipids

BODIPY FL PI(3,4)P2

Product Number: C-34F6
$448.00 – $770.00

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675 Arapeen Dr. Ste 302 Salt Lake City, UT 84108 - 801-588-0455

EXHIBIT 5
I MEC 0159

MCE® MedChemExpress

Safety Data Sheet

Revision Date: May.-18-2021
Print Date: Jul.-31-2021

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifier

Product name : ALC-0159
Catalog No. : HY-138300
CAS No. : 1849616-42-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, manufacture of substances.

1.3 Details of the supplier of the safety data sheet

Company: MedChemExpress USA
Tel: 609-228-6898
Fax: 609-228-5909
E-mail: sales@medchemexpress.com

1.4 Emergency telephone number

Emergency Phone #: 609-228-6898

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

2.3 Other hazards

None.

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula: $(C_2H_4O)nC_{31}H_{63}NO_2$
Molecular Weight: N/A
CAS No. : 1849616-42-7

4. FIRST AID MEASURES

4.1 Description of first aid measures

Eye contact

Remove any contact lenses, locate eye-wash station, and flush eyes immediately with large amounts of water. Separate eyelids with fingers to ensure adequate flushing. Promptly call a physician.

Skin contact

Rinse skin thoroughly with large amounts of water. Remove contaminated clothing and shoes and call a physician.

Inhalation

Immediately relocate self or casualty to fresh air. If breathing is difficult, give cardiopulmonary resuscitation (CPR). Avoid mouth-to-mouth resuscitation.

Ingestion

Wash out mouth with water; Do NOT induce vomiting; call a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2).

4.3 Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

5. FIRE FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, dry chemical, foam, and carbon dioxide fire extinguisher.

5.2 Special hazards arising from the substance or mixture

During combustion, may emit irritant fumes.

5.3 Advice for firefighters

Wear self-contained breathing apparatus and protective clothing.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use full personal protective equipment. Avoid breathing vapors, mist, dust or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.

Refer to protective measures listed in sections 8.

6.2 Environmental precautions

Try to prevent further leakage or spillage. Keep the product away from drains or water courses.

6.3 Methods and materials for containment and cleaning up

Absorb solutions with finely-powdered liquid-binding material (diatomite, universal binders); Decontaminate surfaces and equipment by scrubbing with alcohol; Dispose of contaminated material according to Section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Avoid inhalation, contact with eyes and skin. Avoid dust and aerosol formation. Use only in areas with appropriate exhaust ventilation.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly sealed in cool, well-ventilated area. Keep away from direct sunlight and sources of ignition.

Recommended storage temperature:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month

Shipping at room temperature if less than 2 weeks.

7.3 Specific end use(s)

No data available.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Engineering controls

Ensure adequate ventilation. Provide accessible safety shower and eye wash station.

Personal protective equipment

Eye protection	Safety goggles with side-shields.
Hand protection	Protective gloves.
Skin and body protection	Impervious clothing.
Respiratory protection	Suitable respirator.
Environmental exposure controls	Keep the product away from drains, water courses or the soil. Clean spillages in a safe way as soon as possible.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Solid
Odor	No data available
Odor threshold	No data available
pH	No data available
Melting/freezing point	No data available
Boiling point/range	No data available
Flash point	No data available
Evaporation rate	No data available
Flammability (solid, gas)	No data available
Upper/lower flammability or explosive limits	No data available
Vapor pressure	No data available
Vapor density	No data available
Relative density	No data available
Water Solubility	No data available
Partition coefficient	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidizing properties	No data available

9.2 Other safety information

No data available.

10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available.

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to avoid

No data available.

10.5 Incompatible materials

Strong acids/alkalis, strong oxidising/reducing agents.

10.6 Hazardous decomposition products

Under fire conditions, may decompose and emit toxic fumes.

Other decomposition products - no data available.

11.TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

Classified based on available data. For more details, see section 2

Skin corrosion/irritation

Classified based on available data. For more details, see section 2

Serious eye damage/irritation

Classified based on available data. For more details, see section 2

Respiratory or skin sensitization

Classified based on available data. For more details, see section 2

Germ cell mutagenicity

Classified based on available data. For more details, see section 2

Carcinogenicity

IARC: No component of this product present at a level equal to or greater than 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by ACGIH.

NTP: No component of this product present at a level equal to or greater than 0.1% is identified as a anticipated or confirmed carcinogen by NTP.

OSHA: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by OSHA.

Reproductive toxicity

Classified based on available data. For more details, see section 2

Specific target organ toxicity - single exposure

Classified based on available data. For more details, see section 2

Specific target organ toxicity - repeated exposure

Classified based on available data. For more details, see section 2

Aspiration hazard

Classified based on available data. For more details, see section 2

Additional information

This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity

No data available.

12.2 Persistence and degradability

No data available.

12.3 Bioaccumlative potential

No data available.

12.4 Mobility in soil

No data available.

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment unavailable as chemical safety assessment not required or not conducted.

12.6 Other adverse effects

No data available.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Dispose substance in accordance with prevailing country, federal, state and local regulations.

Contaminated packaging

Conduct recycling or disposal in accordance with prevailing country, federal, state and local regulations.

14. TRANSPORT INFORMATION

DOT (US)

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IMDG

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IATA

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

15. REGULATORY INFORMATION

SARA 302 Components:

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components:

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards:

No SARA Hazards.

Massachusetts Right To Know Components:

No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components:

No components are subject to the Pennsylvania Right to Know Act.

New Jersey Right To Know Components:

No components are subject to the New Jersey Right to Know Act.

California Prop. 65 Components:

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or anyother reproductive harm.

16. OTHER INFORMATION

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA

EXHIBIT 5
J DSPC

SAFETY DATA SHEET
1,2-Distearoyl-sn-glycero-3-PC

Revision: 09/02/2018
Supersedes Revision: 05/28/2014

according to Regulation (EC) No. 1907/2006 as amended by (EC) No. 2015/830 and US OSHA HCS 2015

Section 1. Identification of the Substance/Mixture and of the Company/Undertaking

1.1 Product Code: 15100

Product Name: 1,2-Distearoyl-sn-glycero-3-PC

Synonyms: 1,2-distearoyl-sn-glycero-3-phosphatidylcholine; Coatsome MC 8080; 1,2-Distearoyl-sn-glycero-3-Phosphocholine; 1,2-DSPC;

1.2 Relevant identified uses of the substance or mixture and uses advised against:

Relevant identified uses: For research use only, not for human or veterinary use.

1.3 Details of the Supplier of the Safety Data Sheet:

Company Name: Cayman Chemical Company
1180 E. Ellsworth Rd.
Ann Arbor, MI 48108

Web site address: www.caymanchem.com

Information: Cayman Chemical Company +1 (734)971-3335

1.4 Emergency telephone number:

Emergency Contact: CHEMTREC Within USA and Canada: +1 (800)424-9300
CHEMTREC Outside USA and Canada: +1 (703)527-3887

Section 2. Hazards Identification

2.1 Classification of the Substance or Mixture:

2.2 Label Elements:

GHS Signal Word: **None**

GHS Hazard Phrases:

Based on evaluation of currently available data this substance or mixture is not classifiable according to GHS.

GHS Precaution Phrases:

No phrases apply.

GHS Response Phrases:

No phrases apply.

GHS Storage and Disposal Phrases:

Please refer to Section 7 for Storage and Section 13 for Disposal information.

2.3 Adverse Human Health Effects and Symptoms: Material may be irritating to the mucous membranes and upper respiratory tract. May be harmful by inhalation, ingestion, or skin absorption. May cause eye, skin, or respiratory system irritation. To the best of our knowledge, the toxicological properties have not been thoroughly investigated.

Section 3. Composition/Information on Ingredients

CAS # / RTECS #	Hazardous Components (Chemical Name)/ REACH Registration No.	Concentration	EC No./ EC Index No.	GHS Classification
816-94-4 NA	1,2-Distearoyl-sn-glycero-3-PC	100.0 %	212-440-2 NA	No data available.

Cayman CHEMICAL

SAFETY DATA SHEET
1,2-Distearoyl-sn-glycero-3-PC

Revision: 09/02/2018
Supersedes Revision: 05/28/2014

Section 4. First Aid Measures

4.1 Description of First Aid Measures:

In Case of Inhalation: Remove to fresh air. If not breathing, give artificial respiration or give oxygen by trained personnel. Get immediate medical attention.

In Case of Skin Contact: Immediately wash skin with soap and plenty of water for at least 15 minutes. Remove contaminated clothing. Get medical attention if symptoms occur. Wash clothing before reuse.

In Case of Eye Contact: Hold eyelids apart and flush eyes with plenty of water for at least 15 minutes. Have eyes examined and tested by medical personnel.

In Case of Ingestion: Wash out mouth with water provided person is conscious. Never give anything by mouth to an unconscious person. Get medical attention. Do NOT induce vomiting unless directed to do so by medical personnel.

Section 5. Fire Fighting Measures

5.1 Suitable Extinguishing Media: Use alcohol-resistant foam, carbon dioxide, water, or dry chemical spray.
Use water spray to cool fire-exposed containers.

Unsuitable Extinguishing Media: A solid water stream may be inefficient.

5.2 Flammable Properties and Hazards: No data available.

No data available.

Flash Pt: No data.

Explosive Limits: LEL: No data. UEL: No data.

Autoignition Pt: No data.

5.3 Fire Fighting Instructions: As in any fire, wear self-contained breathing apparatus pressure-demand (NIOSH approved or equivalent), and full protective gear to prevent contact with skin and eyes.

Section 6. Accidental Release Measures

6.1 Protective Precautions, Protective Equipment and Emergency Procedures: Avoid raising and breathing dust, and provide adequate ventilation.
As conditions warrant, wear a NIOSH approved self-contained breathing apparatus, or respirator, and appropriate personal protection (rubber boots, safety goggles, and heavy rubber gloves).

6.2 Environmental Precautions: Take steps to avoid release into the environment, if safe to do so.

6.3 Methods and Material For Containment and Cleaning Up: Contain spill and collect, as appropriate.
Transfer to a chemical waste container for disposal in accordance with local regulations.

Section 7. Handling and Storage

7.1 Precautions To Be Taken in Handling: Avoid breathing dust/fume/gas/mist/vapours/spray.
Avoid prolonged or repeated exposure.

7.2 Precautions To Be Taken in Storing: Keep container tightly closed.
Store in accordance with information listed on the product insert.

Section 8. Exposure Controls/Personal Protection

8.1 Exposure Parameters:

8.2 Exposure Controls:

8.2.1 Engineering Controls (Ventilation etc.): Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below recommended exposure limits.

8.2.2 Personal protection equipment:

Eye Protection: Safety glasses

Protective Gloves: Compatible chemical-resistant gloves

Other Protective Clothing: Lab coat

Respiratory Equipment (Specify Type): NIOSH approved respirator, as conditions warrant.

Work/Hygienic/Maintenance Practices: Do not take internally.
Facilities storing or utilizing this material should be equipped with an eyewash and a safety shower.
Wash thoroughly after handling.

No data available.

Section 9. Physical and Chemical Properties

9.1 Information on Basic Physical and Chemical Properties

Physical States:	[ ] Gas [ ] Liquid [X] Solid
Appearance and Odor:	A crystalline solid
pH:	No data.
Melting Point:	No data.
Boiling Point:	No data.
Flash Pt:	No data.
Evaporation Rate:	No data.
Flammability (solid, gas):	No data available.
Explosive Limits:	LEL: No data. UEL: No data.
Vapor Pressure (vs. Air or mm Hg):	No data.
Vapor Density (vs. Air = 1):	No data.
Specific Gravity (Water = 1):	No data.
Solubility in Water:	No data.
Solubility Notes:	~25 mg/ml in EtOH;
Octanol/Water Partition Coefficient:	No data.
Autoignition Pt:	No data.
Decomposition Temperature:	No data.
Viscosity:	No data.

9.2 Other Information

Percent Volatile:	No data.
Molecular Formula & Weight:	C44H88NO8P 790.2

SAFETY DATA SHEET
1,2-Distearoyl-sn-glycero-3-PC

Revision: 09/02/2018
Supersedes Revision: 05/28/2014

Section 10. Stability and Reactivity

10.1 Reactivity: No data available.

10.2 Stability: Unstable [ ] Stable [X]

10.3 Stability Note(s): Stable if stored in accordance with information listed on the product insert.

Polymerization: Will occur [ ] Will not occur [X]

10.4 Conditions To Avoid: No data available.

10.5 Incompatibility - Materials To Avoid: strong oxidizing agents

10.6 Hazardous Decomposition or Byproducts: carbon dioxide
carbon monoxide
nitrogen oxides
phosphorous oxides

Section 11. Toxicological Information

11.1 Information on Toxicological Effects: The toxicological effects of this product have not been thoroughly studied.

CAS #	Hazardous Components (Chemical Name)	NTP	IARC	ACGIH	OSHA
816-94-4	1,2-Distearoyl-sn-glycero-3-PC	n.a.	n.a.	n.a.	n.a.

Section 12. Ecological Information

12.1 Toxicity: Avoid release into the environment.
Runoff from fire control or dilution water may cause pollution.

12.2 Persistence and Degradability: No data available.

12.3 Bioaccumulative Potential: No data available.

12.4 Mobility in Soil: No data available.

12.5 Results of PBT and vPvB assessment: No data available.

12.6 Other adverse effects: No data available.

Section 13. Disposal Considerations

13.1 Waste Disposal Method: Dispose in accordance with local, state, and federal regulations.

Section 14. Transport Information

14.1 LAND TRANSPORT (US DOT):

DOT Proper Shipping Name: Not dangerous goods.
DOT Hazard Class:
UN/NA Number:

14.1 LAND TRANSPORT (European ADR/RID):

ADR/RID Shipping Name: Not dangerous goods.
UN Number:
Hazard Class:

14.3 AIR TRANSPORT (ICAO/IATA):

ICAO/IATA Shipping Name: Not dangerous goods.

Additional Transport Information: Transport in accordance with local, state, and federal regulations.

Section 15. Regulatory Information

EPA SARA (Superfund Amendments and Reauthorization Act of 1986) Lists

CAS #	Hazardous Components (Chemical Name)	S. 302 (EHS)	S. 304 RQ	S. 313 (TRI)
816-94-4	1,2-Distearoyl-sn-glycero-3-PC	No	No	No

CAS #	Hazardous Components (Chemical Name)	Other US EPA or State Lists
816-94-4	1,2-Distearoyl-sn-glycero-3-PC	CAA HAP,ODC: No; CWA NPDES: No; TSCA: No; CA PROP.65: No

Regulatory Information Statement: This SDS was prepared in accordance with 29 CFR 1910.1200 and Regulation (EC) No.1272/2008.

Section 16. Other Information

Revision Date: 09/02/2018

Additional Information About This Product: No data available.

Company Policy or Disclaimer: DISCLAIMER: This information is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes.

EXHIBIT 6
Grams Affidavit

UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF COLORADO

DANIEL ROBERT	*	
SSGT, U.S. ARMY	*	
	*	
HOLLI MULVIHILL	*	
SSGT, USMC	*	
	*	
Plaintiffs,	*	
	*	
v.	*	
	*	Civil Action No. 21-02228
LLOYD AUSTIN Secretary of Defense,	*	*
U.S. DEPARTMENT OF DEFENSE	*	
Washington, D.C. 20301	*	
	*	
and	*	
	*	
XAVIER BECERRA	*	
Secretary of the U.S. Department of		*
Health and Human Services	*	
U.S. DEPARTMENT OF HEALTH	*	
AND HUMAN SERVICES	*	
	*	
and	*	
	*	

JANET WOODCOCK, Acting *
Commissioner of the Food & Drug *
Administration *
U.S. FOOD AND *
DRUG ADMINISTRATION *
*
UNITED STATES OF AMERICA *
*
Defendants. *

* * * * * * * * * * * *

Exhibit 2 of Motion for Temporary Restraining Order and Amended Complaint

AFFIDAVIT OF DR. RALPH GRAMS IN SUPPORT OF TEMPORARY RESTRAINING ORDER MOTION

I, Doctor **Ralph Grams**, MD, FCAP, FACMI being duly sworn, depose and state as follows:

1. I make this affidavit in support of the above referenced MOTION as expert testimony in support thereof.

2. The expert opinions expressed here are my own and arrived at from my persons, professional and educational experiences taken in context, where appropriate, by scientific data, publications, treatises, opinions, documents, reports and other information relevant to the subject matter.

Experience & Credentials

3. I am competent to testify to the facts and matters set forth herein. I have provided written testimony previously to this Court, wherein I provided my credentials and bona fides to render this and other opinions.

4. May it please the Court, I will provide said CV, evidence of my expertise and bona fides as requested or directed.

5. Said experience and expertise in pathology and work in the biological and chemical weapons field is the basis upon which I am rendering this opinion

6. Since the last sworn affidavit that I provided in this case, I was asked to conduct further analysis of the same samples, using the same equipment in the same laboratory and per the same protocols and procedures. In fact, the mass spectrometry that was relied upon in my last statement is effectively the same for purposes of this sworn statement.

7. In particular, I was asked to look at the publicly available documents attached hereto as they relate to a key ingredient in both the Pfizer and Moderna Covid 19 vaccines Appendices A & B respectively, attached and annexed hereto. I did not test the Johnson & Johnson samples, so there is no further discussion of that EAU Covid 19 Vaccine and I express no opinion about it. Of importance to note is that the Pfizer sample is that of BioNTech and not the FDA approved Comirnaty, because Pfizer has not yet started production of Comirnaty for sale or distribution into the United States. Accordingly the BioNTech remains for Emergency Use only and to my knowledge is the only Covid 19 vaccine being provided members of the Armed Services per Secretary Austin's orders for mandatory inoculations for all Services dated August 19, 2021.

8. The key ingredient in each of the samples is a compound used by the different manufacturers to achieve the same result, which is delivery of RNA fragments to a broad distribution of cells in the user's genome using lipid nanoparticles. The main difference between the two different sets of lipid nano particles are the composition of some ingredients.

Pfizer's BioNTech

9. Pfizer uses Acuitas Therapeutics Inc. "Acuitas LNP Technology" under Intellectual Property licensing agreements[1], also commonly referred to simply as "hydrogel,' which has a chemical composition of:

[1] See: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836001/ & https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836001/

a. 4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), 2 [(polyethylene glycol)-2000]- N,N-ditetradecylacetamide, 1,2-distearoyl-sn-glycero-3- phosphocholine, and cholesterol; also known as
b. ALC 1035, ALC-0159 and DSCP[2]

10. The UK government in its Health Safety Executive office states the following about the ALC 3015 ingredient, as echoed by the NARH states the following:

a. The ALC-0315 is a hexane containing compound and these are known to be potentially neurotoxic. ALC-0159 contains polyethylene glycol (PEG) that is associated with hypersensitivity and allergenic reactions. The toxicological profile of the mRNA delivery system cannot be determined because neither have the concentrations been declared, nor has the nanoparticle delivery system, surface charges and other physicochemical characteristics been declared. These may dramatically increase the toxicological profile. [3]
b. Regarding it's other toxicity, the Safety Data Page reflects the terms "unknown" or "Classified" thereby making a complete assessment of its toxicity impossible to know absent significant scientific study, which has not been completed as of this date.

11. Furthermore the Safety Data Sheet states in the very heading, "**Danger**" and it additionally cautions "Evidence for human carcinogenicity Current classification: **Group 1 a** "

12. In studying the contents of Pfizer's key Lipid Nanoparticle ingredient by utilizing a MALDI TOF MS (Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer) laboratory instrument, I was able to observe the spectrographic data provided by this instrument with the use of standards and controls; which reveal that this ingredient does appear in the 767.33 range as demonstrated in the spectrometry results attached hereto as Appendix C (pages 5 & 7)

Moderna Vaccine

[2] See: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1016212/Temporary_Authorisation_Patient_Information_BNT162__-_09-09-2021.pdf

[3] See: https://www.hsl.gov.uk/media/480242/material%20safety%20data%20sheet%20quartz%20final%20v2%202019.pdf *https://www.anhinternational.org/news/have-you-decided-what-youll-do-or-say-if-offered-a-covid-vaccine/*

13. Moderna, on the other hand, delivers its RNA fragments through a slightly different Lipid Nanoparticle called "SM-102" and it's scientific composition is: polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG], cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC]

14. According to its (SM-102) patent, WO2020/160397, the compound designed and described, in pertinent part:

> The present disclosure provides novel methods of producing nucleic acid lipid Nanoparticle (LNP) formulations ,the produced formulations thereof, and the related therapeutic and/or diagnostic uses, such as methods involving the nucleic acid lipid nanoparticles to deliver one or more therapeutics and/or prophylactics, such as a nucleic acid, to and/or produce polypeptides in mammalian cells or organs.

The Patent is attached and annexed as a part hereto as Exhibit C.

15. The safety Data Sheet for SM-102 describes it as:

a. "not for human or veterinary use"
b. "GHS06 Skull and crossbones"
c. "H310 Fatal in contact with skin "
d. "GHS08 Health hazard "
e. "H351 Suspected of causing cancer"
f. "H361 Suspected of damaging fertility or the unborn child"
g. "H372 Causes damage to the central nervous system, the kidneys, the liver and the
respiratory system through prolonged or repeated exposure"

16. In studying the contents of Moderna's key Lipid Nanoparticle ingredient I used the same spectrographic instruments to provide the data with the use of standards and controls; which reveal that this Lipid Nanoparticle ingredient (SM-102) does appear in the 711.08 range also demonstrated in Appendix C (page 11). In each such case, the spectrometry demonstrates significant prevalence as a key ingredient.

17. Given that these Covid 19 Vaccines were both Investigational New Drugs and Emergency Use Authorization vaccines, manufacturers are allowed to substitute ingredients during the testing process because the IND's are experimental and therefore not necessarily the final product that will be approved. On this note, the FDA's prospective approval of Comirnaty may or may not be accurate and will not be dispositive until such time as the Comirnaty product has been manufactured and all ingredients disclosed in accordance with FDA labeling regulations.

18. For this reason, it is impossible to characterize BioNTech as being interchangeable with the Comirnaty approved drug until such time as the phase III clinical studies being conducted at this moment under the current IND/EUA regulations are completed. These tests are not scheduled for completion until 2025, at which time we will then be able to re-test the contents of the drug to verify if the ingredients are the same, substantially the same or different. As such, at no time should the DOD or any other agency presume that BioNTech is an approved drug; it is not and this is why it continues to carry the characterization of an Investigational New Drug for Emergency Use only.

Opinion

19. I have reviewed the second Motion for Temporary Restraining Order and Amended Complaint, which delineates the subject matter relating to studies I performed and conclude as follows:

a) The key Lipid Nanoparticle RNA delivery system ingredients of Moderna's vaccine are pathological toxins and dangerous or deadly to humans and should therefore be considered allergens to all humans;
b) The Key Lipid Nanoparticle RNA delivery system in and Pfizer's BioNTech vaccine are also pathological toxins and dangerous or deadly to humans
c) The amount of each such ingredient is not divulged at this time and by virtue of being in the Investigational state of the IND process, may change between lots and batches, so it is impossible to know how much of these toxins are being delivered to the users without mass spectrometry analysis for each such batch and lot.
d) The only difference between the two Moderna and Pfizer Covid 19 Vaccines is the slight difference in composition of the Lipid Nanoparticles and amount of each other nearly identical ingredient together with the actual composition and sequencing of the RNA

fragments being delivered to cause cell mutation and production of abnormal cells ("Spike Proteins.'

e) Each such Covid 19 Vaccine is potentially dangerous or deadly to the users.

f) Each such Covid 19 Vaccine contains known allergens whereby effectively all humans are allergic to some of the key ingredients.

g) Each such Covid 19 Vaccine is demonstrably dangerous or deadly as demonstrated by the notoriously high fatalities and Serious Adverse Events published by the VAERS system.

h) Each such Covid 19 Vaccine should be immediately recalled and all authorization for use should immediately be terminated or cancelled.

i) All unused supplies of the said Covid 19 Vaccines should be treated as hazardous materials, accounted for and disposed of in accordance with the terms of the OSHA or other responsible body's disposal guidelines.

20. I am competent to opine on the medical aspects of these allegations based upon my above-referenced education and professional medical experience and the basis of my opinions are formed as a result of my education and experience.

21. As a Medical Doctor and scientist in the biological health and treatment of human beings, I confirm and attest to the accuracy and truthfulness of my foregoing statements, analysis and attachments hereto:

_____/s/_____
Ralph Grams, MD

State of Florida §
§
County of Flagler §

The undersigned, being duly sworn, deposes and says:

I, Ralph Grams, MD, declare under the penalty of perjury of the laws of the United States of America, and state upon personal knowledge that:

I am an adult of sound mind, ___ years old, and declare that the information herein is true, correct and complete and that I have voluntarily affirmed this affidavit based upon my own personal knowledge, education, and experience, and under the penalty of perjury of the laws of the United States of America.

SUBSCRIBED AND SWORN TO BEFORE ME on the 23 day of September 2021, to certify which witness my hand and official seal.

/S/

Kay Kanter

Notary Public for the State of Colorado

My Commission Expires: _____

APPENDIX A

List of Pfizer BioNTech ALC 0315 Safety Data Sheet

MCE® MedChemExpress

Safety Data Sheet

Revision Date: Mar.-23-2021
Print Date: Sep.-9-2021

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifier

Product name : ALC-0315

Catalog No. : HY-138170

CAS No. : 2036272-55-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, manufacture of substances.

1.3 Details of the supplier of the safety data sheet

Company: MedChemExpress USA

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: sales@medchemexpress.com

1.4 Emergency telephone number

Emergency Phone #: 609-228-6898

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Skin corrosion/irritation (Category 2),H315

Serious eye damage/eye irritation (Category 2A),H319

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Warning

Hazard statement(s)

H315 Causes skin irritation

H319 Causes serious eye irritation

Precautionary statement(s)

P264 Wash hands thoroughly after handling

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352 IF ON SKIN: Wash with plenty of soap and water.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P313 Get medical advice/attention.

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

P362 Take off contaminated clothing and wash before reuse.

2.3 Other hazards

None.

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula:	$C_{48}H_{95}NO_5$
Molecular Weight:	766.27
CAS No. :	2036272-55-4

4. FIRST AID MEASURES

4.1 Description of first aid measures

Eye contact

Remove any contact lenses, locate eye-wash station, and flush eyes immediately with large amounts of water. Separate eyelids with fingers to ensure adequate flushing. Promptly call a physician.

Skin contact

Rinse skin thoroughly with large amounts of water. Remove contaminated clothing and shoes and call a physician.

Inhalation

Immediately relocate self or casualty to fresh air. If breathing is difficult, give cardiopulmonary resuscitation (CPR). Avoid mouth-to-mouth resuscitation.

Ingestion

Wash out mouth with water; Do NOT induce vomiting; call a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2).

4.3 Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

5. FIRE FIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media

Use water spray, dry chemical, foam, and carbon dioxide fire extinguisher.

5.2 Special hazards arising from the substance or mixture

During combustion, may emit irritant fumes.

5.3 Advice for firefighters

Wear self-contained breathing apparatus and protective clothing.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Use full personal protective equipment. Avoid breathing vapors, mist, dust or gas. Ensure adequate ventilation. Evacuate

personnel to safe areas.

Refer to protective measures listed in sections 8.

6.2 Environmental precautions

Try to prevent further leakage or spillage. Keep the product away from drains or water courses.

6.3 Methods and materials for containment and cleaning up

Absorb solutions with finely-powdered liquid-binding material (diatomite, universal binders); Decontaminate surfaces and equipment by scrubbing with alcohol; Dispose of contaminated material according to Section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Avoid inhalation, contact with eyes and skin. Avoid dust and aerosol formation. Use only in areas with appropriate exhaust ventilation.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly sealed in cool, well-ventilated area. Keep away from direct sunlight and sources of ignition.

Recommended storage temperature: 4°C, protect from light

* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)

Shipping at room temperature if less than 2 weeks.

7.3 Specific end use(s)

No data available.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Engineering controls

Ensure adequate ventilation. Provide accessible safety shower and eye wash station.

Personal protective equipment

Eye protection	Safety goggles with side-shields.
Hand protection	Protective gloves.
Skin and body protection	Impervious clothing.
Respiratory protection	Suitable respirator.
Environmental exposure controls	Keep the product away from drains, water courses or the soil. Clean spillages in a safe way as soon as possible.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Viscous liquid
Odor	No data available
Odor threshold	No data available

pH	No data available
Melting/freezing point	No data available
Boiling point/range	No data available
Flash point	No data available
Evaporation rate	No data available
Flammability (solid, gas)	No data available
Upper/lower flammability or explosive limits	No data available
Vapor pressure	No data available
Vapor density	No data available
Relative density	No data available
Water Solubility	No data available
Partition coefficient	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidizing properties	No data available

9.2 Other safety information

No data available.

10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available.

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to avoid

No data available.

10.5 Incompatible materials

Strong acids/alkalis, strong oxidising/reducing agents.

10.6 Hazardous decomposition products

Under fire conditions, may decompose and emit toxic fumes.

Other decomposition products - no data available.

11.TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

Classified based on available data. For more details, see section 2

Skin corrosion/irritation

Classified based on available data. For more details, see section 2

Serious eye damage/irritation

Classified based on available data. For more details, see section 2

Respiratory or skin sensitization

Classified based on available data. For more details, see section 2

Germ cell mutagenicity

Classified based on available data. For more details, see section 2

Carcinogenicity

IARC: No component of this product present at a level equal to or greater than 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by ACGIH.

NTP: No component of this product present at a level equal to or greater than 0.1% is identified as a anticipated or confirmed carcinogen by NTP.

OSHA: No component of this product present at a level equal to or greater than 0.1% is identified as a potential or confirmed carcinogen by OSHA.

Reproductive toxicity

Classified based on available data. For more details, see section 2

Specific target organ toxicity - single exposure

Classified based on available data. For more details, see section 2

Specific target organ toxicity - repeated exposure

Classified based on available data. For more details, see section 2

Aspiration hazard

Classified based on available data. For more details, see section 2

Additional information

This information is based on our current knowledge. However the chemical, physical, and toxicological properties have not been completely investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity

No data available.

12.2 Persistence and degradability

No data available.

12.3 Bioaccumlative potential

No data available.

12.4 Mobility in soil

No data available.

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment unavailable as chemical safety assessment not required or not conducted.

12.6 Other adverse effects

No data available.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product

Dispose substance in accordance with prevailing country, federal, state and local regulations.

Contaminated packaging

Conduct recycling or disposal in accordance with prevailing country, federal, state and local regulations.

14. TRANSPORT INFORMATION

DOT (US)

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IMDG

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

IATA

Proper shipping name: Not dangerous goods

UN number: -

Class: -

Packing group: -

15. REGULATORY INFORMATION

SARA 302 Components:

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components:

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards:

No SARA Hazards.

Massachusetts Right To Know Components:

No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components:

No components are subject to the Pennsylvania Right to Know Act.

New Jersey Right To Know Components:

No components are subject to the New Jersey Right to Know Act.

California Prop. 65 Components:

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or anyother reproductive harm.

16. OTHER INFORMATION

APPENDIX B

List of Moderna Covid 19 Vaccine SM-102 Safety Data Sheet

Safety Data Sheet
acc. to OSHA HCS

Printing date 04/11/2021 Revision date 04/11/2021

1 Identification

· **Product identifier**

· **Trade name: SM-102**
· **Synonym** 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester

· **Article number:** 33474
· **Application of the substance / the mixture** For research use only, not for human or veterinary use.

· **Details of the supplier of the safety data sheet**
· **Manufacturer/Supplier:**
Cayman Chemical Co.
1180 E. Ellsworth Rd.
Ann Arbor, MI 48108
USA

· **Information department:** Product safety department
· **Emergency telephone number:**
During normal opening times: +1 (734) 971-3335
US/CANADA: 800-424-9300
Outside US/CANADA: 703-741-5970

2 Hazard(s) identification

· **Classification of the substance or mixture**


GHS02 Flame

Flam. Liq. 2 H225 Highly flammable liquid and vapor.


GHS06 Skull and crossbones

Acute Tox. 2 H310 Fatal in contact with skin.


GHS08 Health hazard

Carc. 2 H351 Suspected of causing cancer.
Repr. 2 H361 Suspected of damaging fertility or the unborn child.
STOT RE 1 H372 Causes damage to the central nervous system, the kidneys, the liver and the respiratory system through prolonged or repeated exposure.


GHS09 Environment

Aquatic Chronic 1 H410 Very toxic to aquatic life with long lasting effects.


GHS07

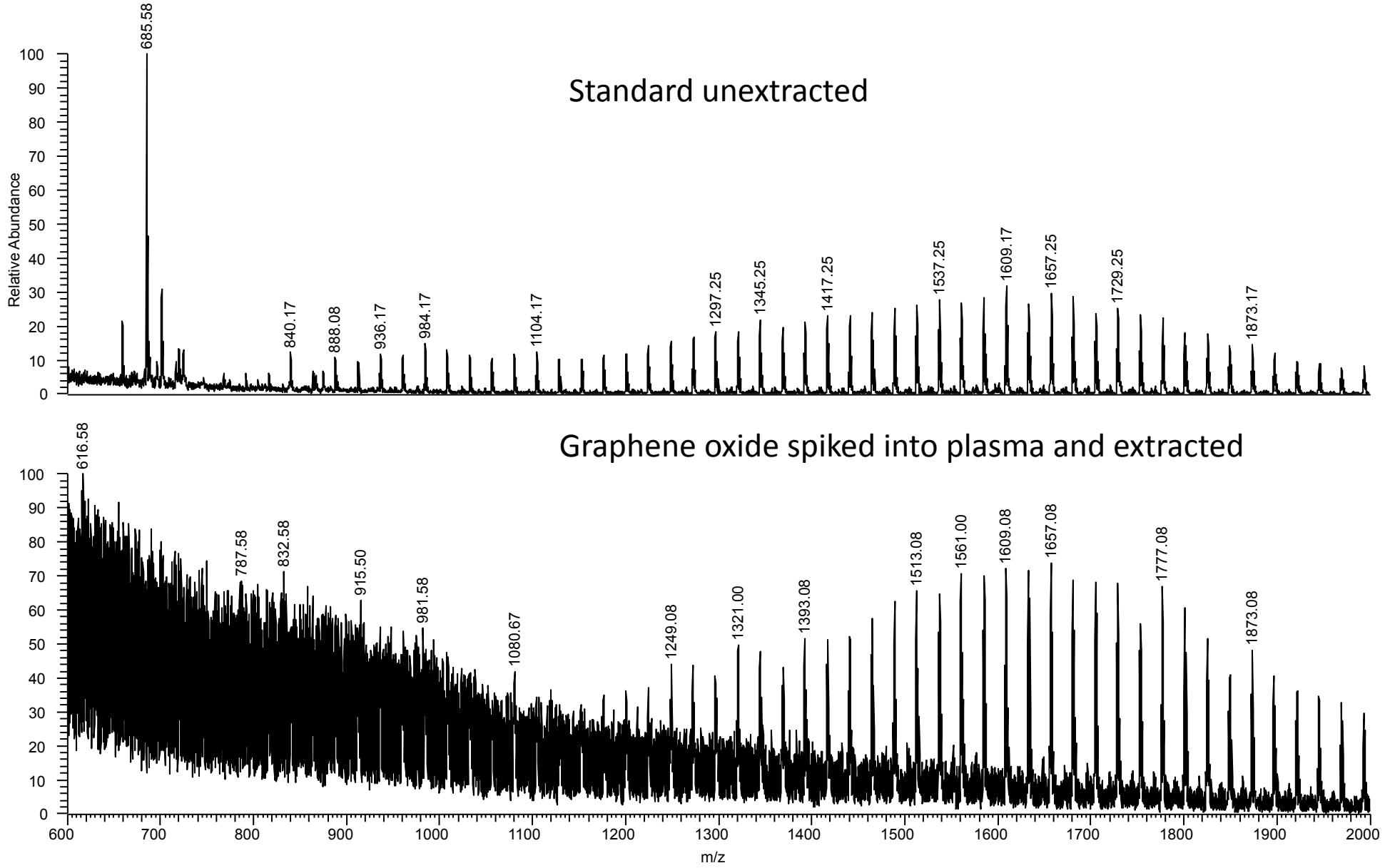
(Contd. on page 2)

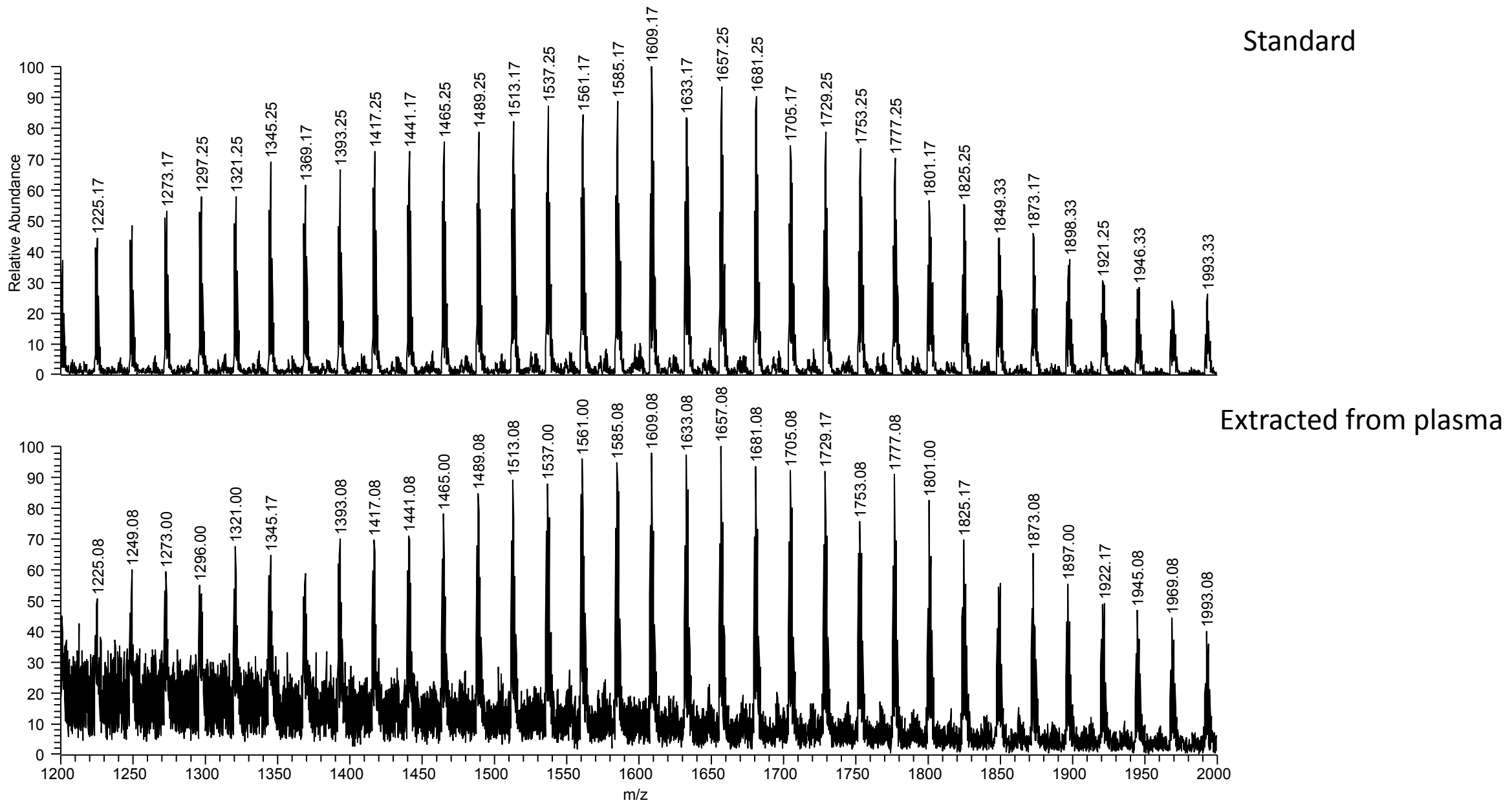
APPENDIX C

Mass Spectrometry Results

Graphene oxide analysis

- A method was developed to analyze Graphene oxide using MALDI ion trap mass spectrometry
- Graphene oxide readily absorbs the laser energy and so analysis can be conducted without the addition of a traditional MALDI matrix
  - This enables reduction of ionization from other molecules that do not absorb the laser energy
- We tested Graphene oxide as a standard
- We tested Graphene oxide spiked into plasma and whole blood
  - We successfully extracted and analyzed Graphene oxide from both matrices
- We tested for the presence of Graphene Oxide in the vaccines provided
  - We observed a large polymeric background in both unextracted and extracted samples
  - We did not observe Graphene oxide in the tested samples


Standard unextracted
Relative Abundance
685.58
840.17
888.08
936.17
984.17
1104.17
1297.25
1345.25
1417.25
1537.25
1609.17
1657.25
1729.25
1873.17
Graphene oxide spiked into plasma and extracted
616.58
787.58
832.58
915.50
981.58
1080.67
1249.08
1321.00
1393.08
1513.08
1561.00
1609.08
1657.08
1777.08
1873.08
m/z


Standard
Extracted from plasma
Relative Abundance
m/z
1225.17
1273.17
1297.25
1321.25
1345.25
1369.17
1393.25
1417.25
1441.17
1465.25
1489.25
1513.17
1537.25
1561.17
1585.17
1609.17
1633.17
1657.25
1681.25
1705.17
1729.25
1753.25
1777.25
1801.17
1825.25
1849.33
1873.17
1898.33
1921.25
1946.33
1993.33
1225.08
1249.08
1273.00
1296.00
1321.00
1345.17
1393.08
1417.08
1441.08
1465.00
1489.08
1513.08
1537.00
1561.00
1585.08
1609.08
1633.08
1657.08
1681.08
1705.08
1729.17
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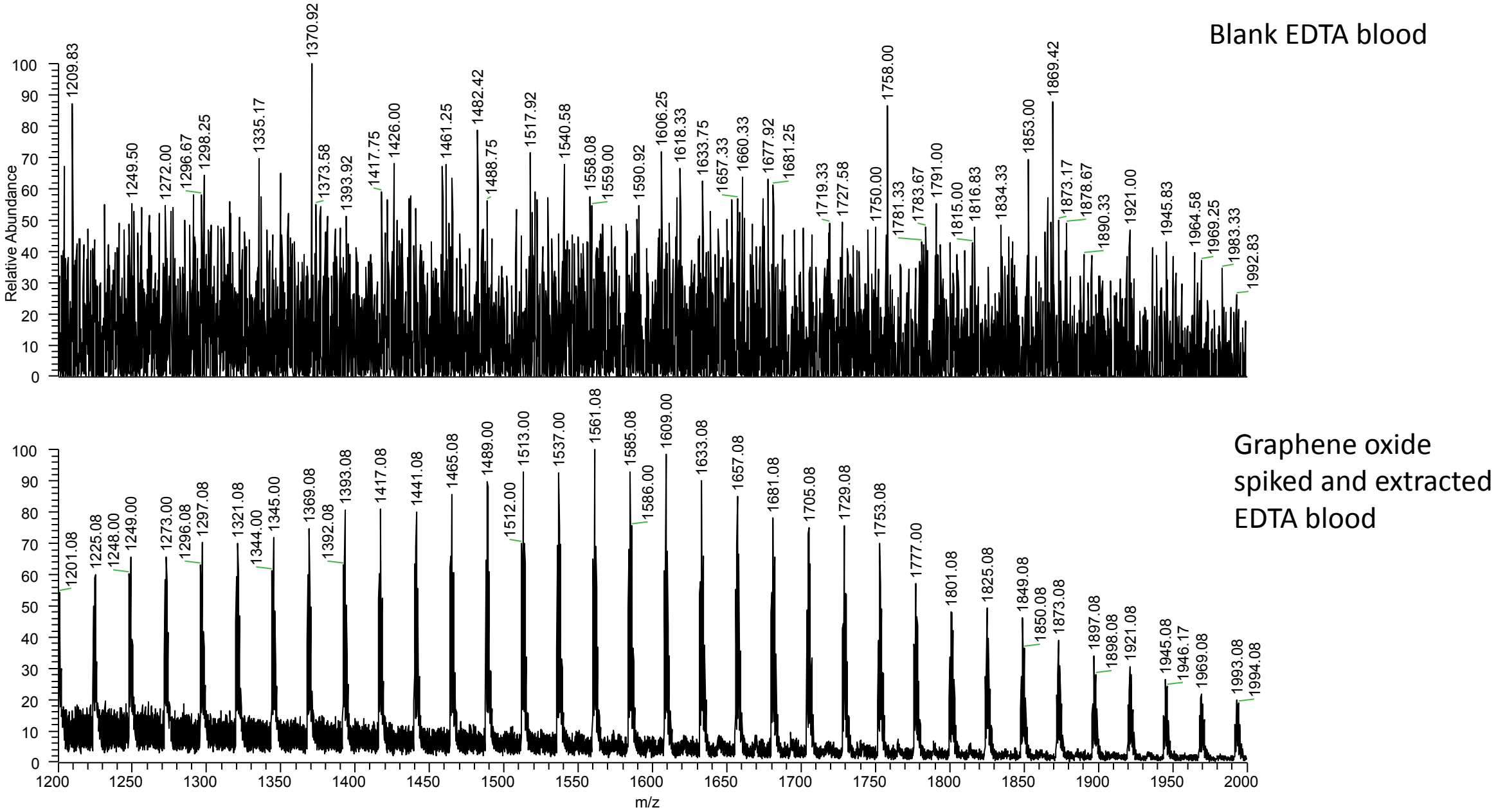
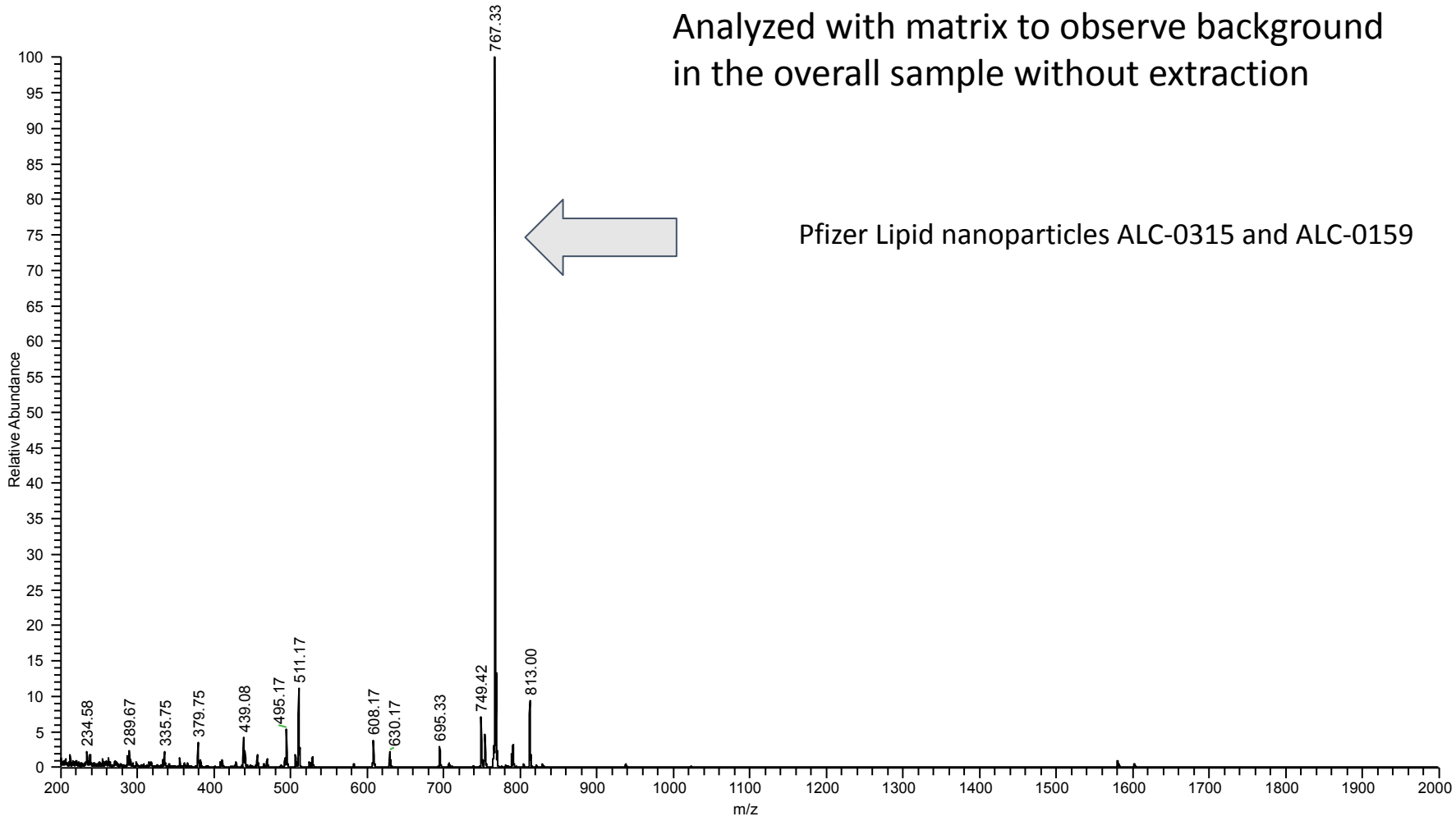
1801.00
1825.17
1873.08
1897.00
1922.17
1945.08
1969.08
1993.08


Blank EDTA blood
Graphene oxide spiked and extracted EDTA blood
Relative Abundance
m/z
1209.83
1249.50
1272.00
1296.67
1298.25
1335.17
1370.92
1373.58
1393.92
1417.75
1426.00
1461.25
1482.42
1488.75
1517.92
1540.58
1558.08
1559.00
1590.92
1606.25
1618.33
1633.75
1657.33
1660.33
1677.92
1681.25
1719.33
1727.58
1750.00
1758.00
1781.33
1783.67
1791.00
1815.00
1816.83
1834.33
1853.00
1869.42
1873.17
1878.67
1890.33
1921.00
1945.83
1964.58
1969.25
1983.33
1992.83
1201.08
1225.08
1248.00
1249.00
1273.00
1296.08
1297.08
1321.08
1344.00
1345.00
1369.08
1392.08
1393.08
1417.08
1441.08
1465.08
1489.00
1512.00
1513.00
1537.00
1561.08
1585.08
1586.00
1609.00
1633.08
1657.08
1681.08
1705.08
1729.08
1753.08
1777.00
1801.08
1825.08
1849.08
1850.08
1873.08
1897.08
1898.08
1921.08
1945.08
1946.17
1969.08
1993.08
1994.08

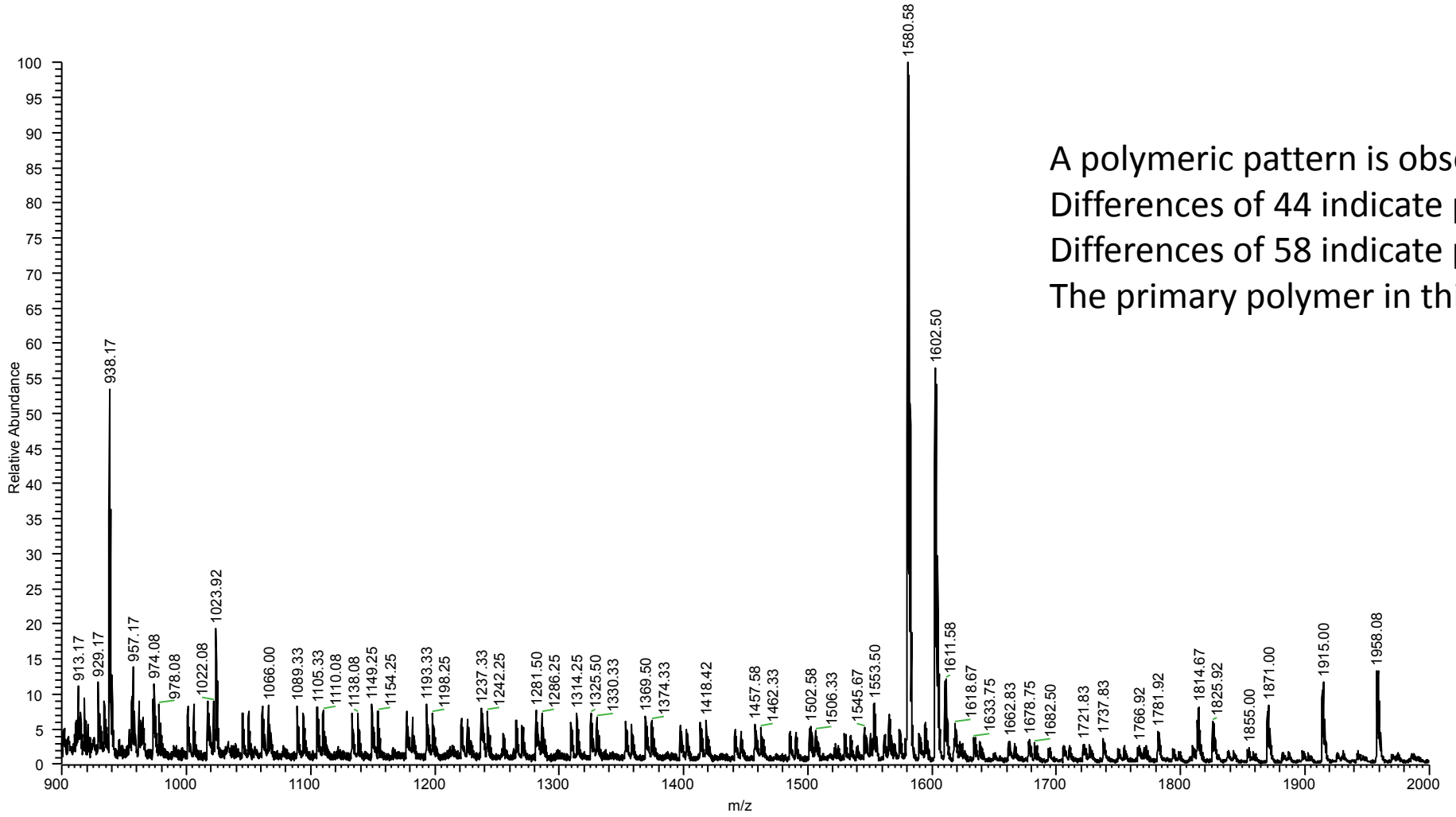
EF123 analyzed with MALDI matrix, unextracted



Analyzed with matrix to observe background in the overall sample without extraction

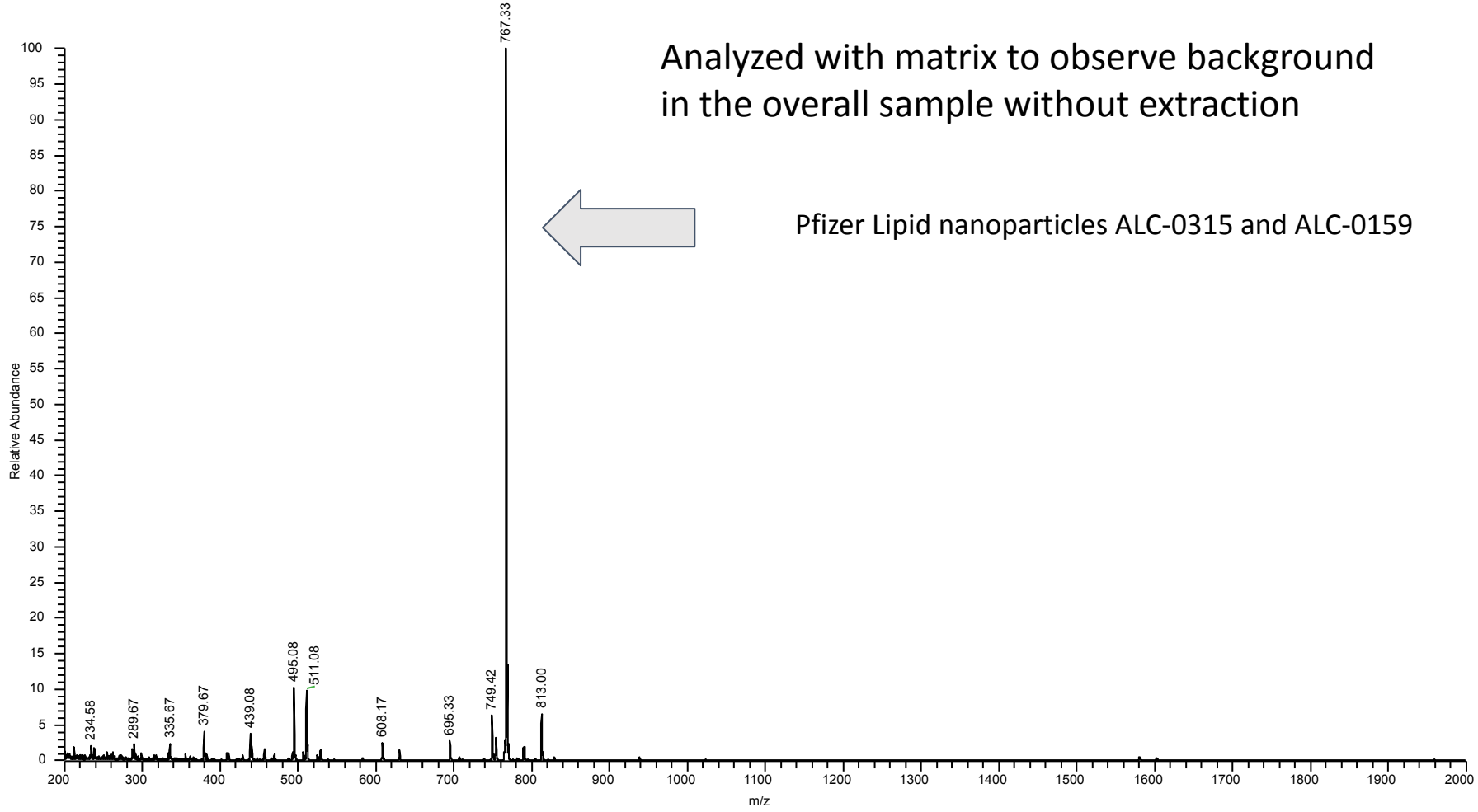
Pfizer Lipid nanoparticles ALC-0315 and ALC-0159

EF123, HCCA matrix, unextracted, 900-2000 mass range



A polymeric pattern is observed
Differences of 44 indicate polyethylene glycol
Differences of 58 indicate polypropylene glycol
The primary polymer in this vaccine is PEG

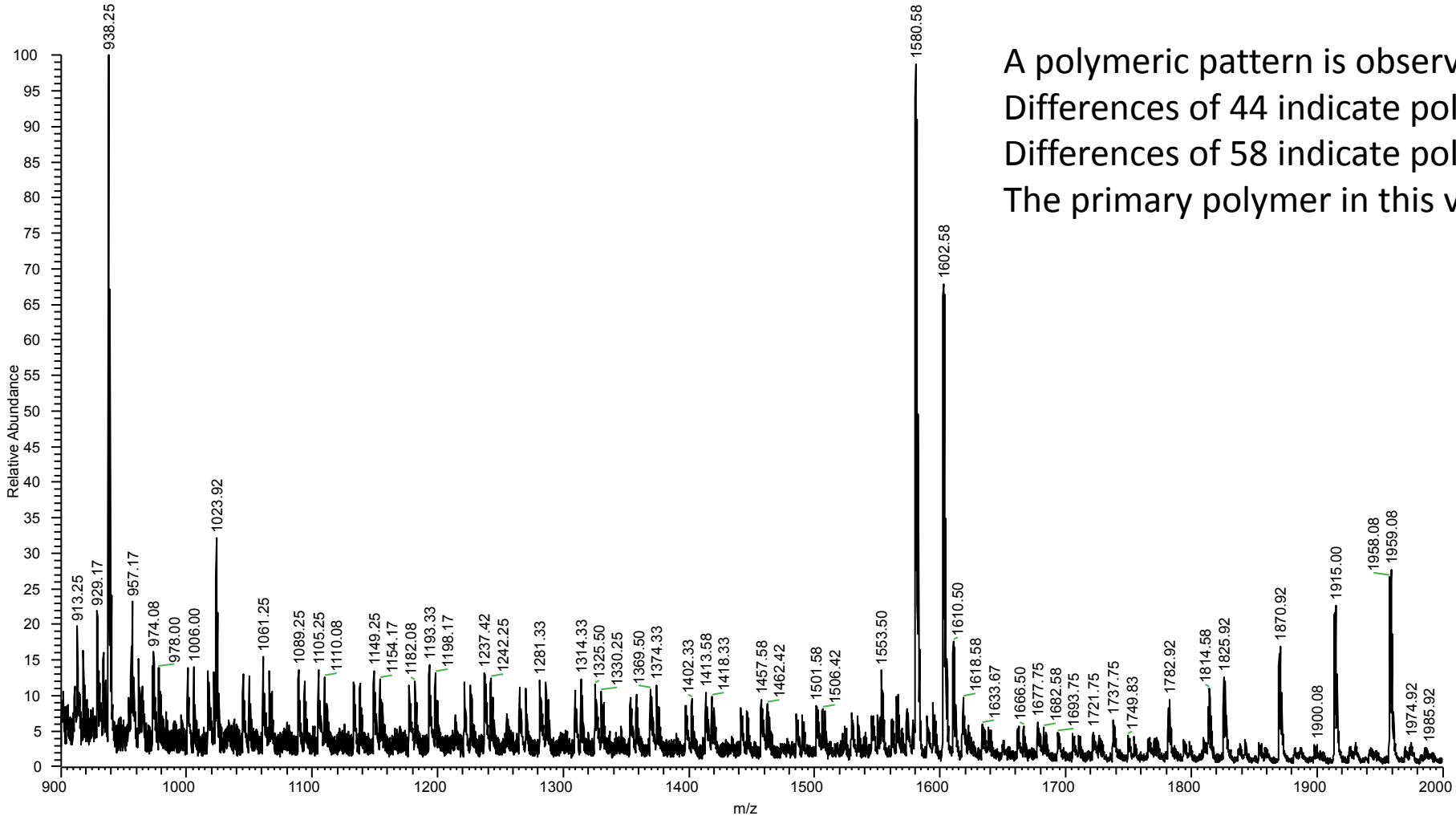
EF456, unextracted, HCCA matrix



Analyzed with matrix to observe background in the overall sample without extraction

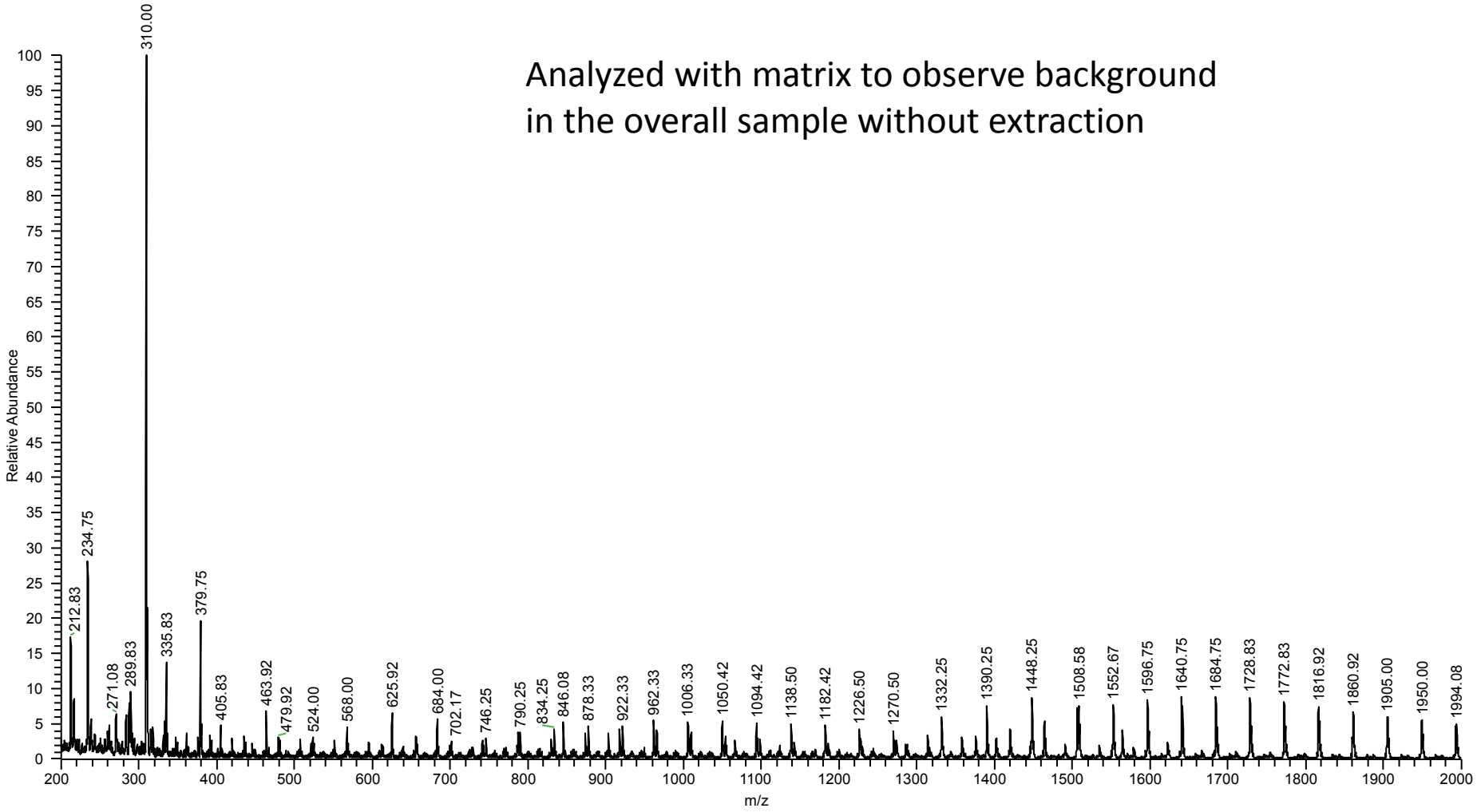
Pfizer Lipid nanoparticles ALC-0315 and ALC-0159

EF456, unextracted, mass 900-2000



A polymeric pattern is observed as with EF123
Differences of 44 indicate polyethylene glycol
Differences of 58 indicate polypropylene glycol
The primary polymer in this vaccine is PEG

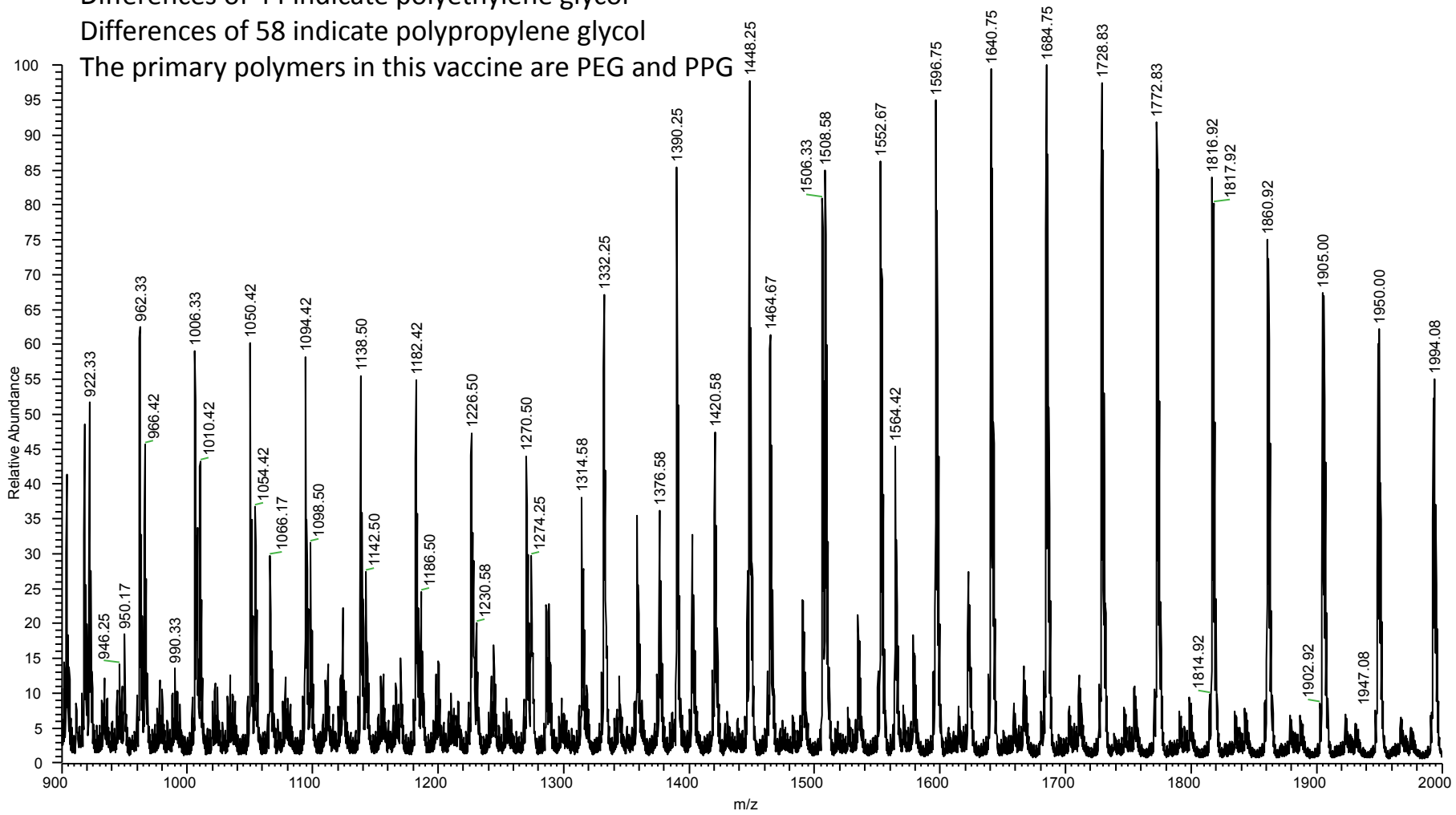
J2, unextracted, HCCA matrix



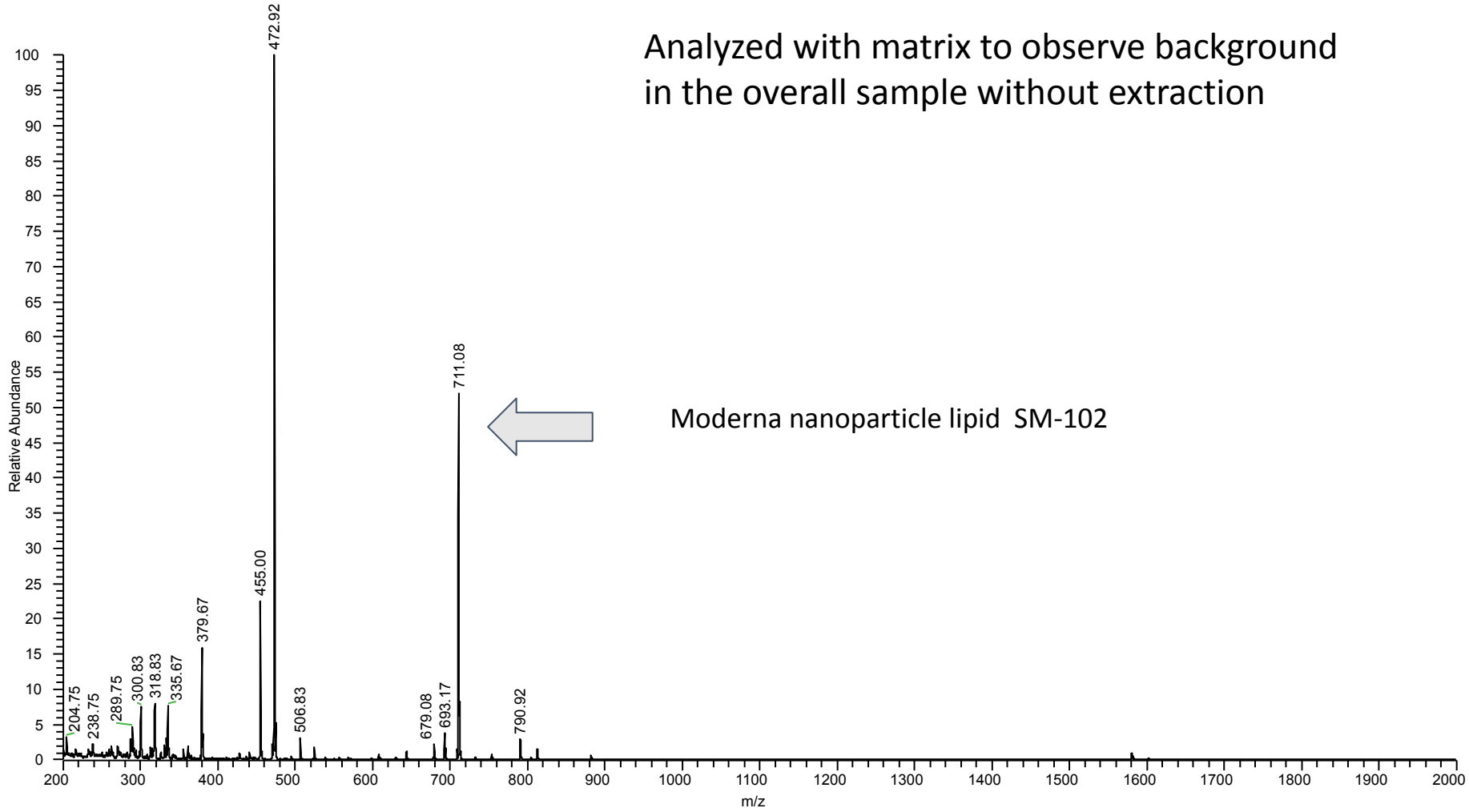
Analyzed with matrix to observe background in the overall sample without extraction

J2, unextracted, mass 900-2000

A polymeric pattern is observed as with EF123
Differences of 44 indicate polyethylene glycol
Differences of 58 indicate polypropylene glycol
The primary polymers in this vaccine are PEG and PPG



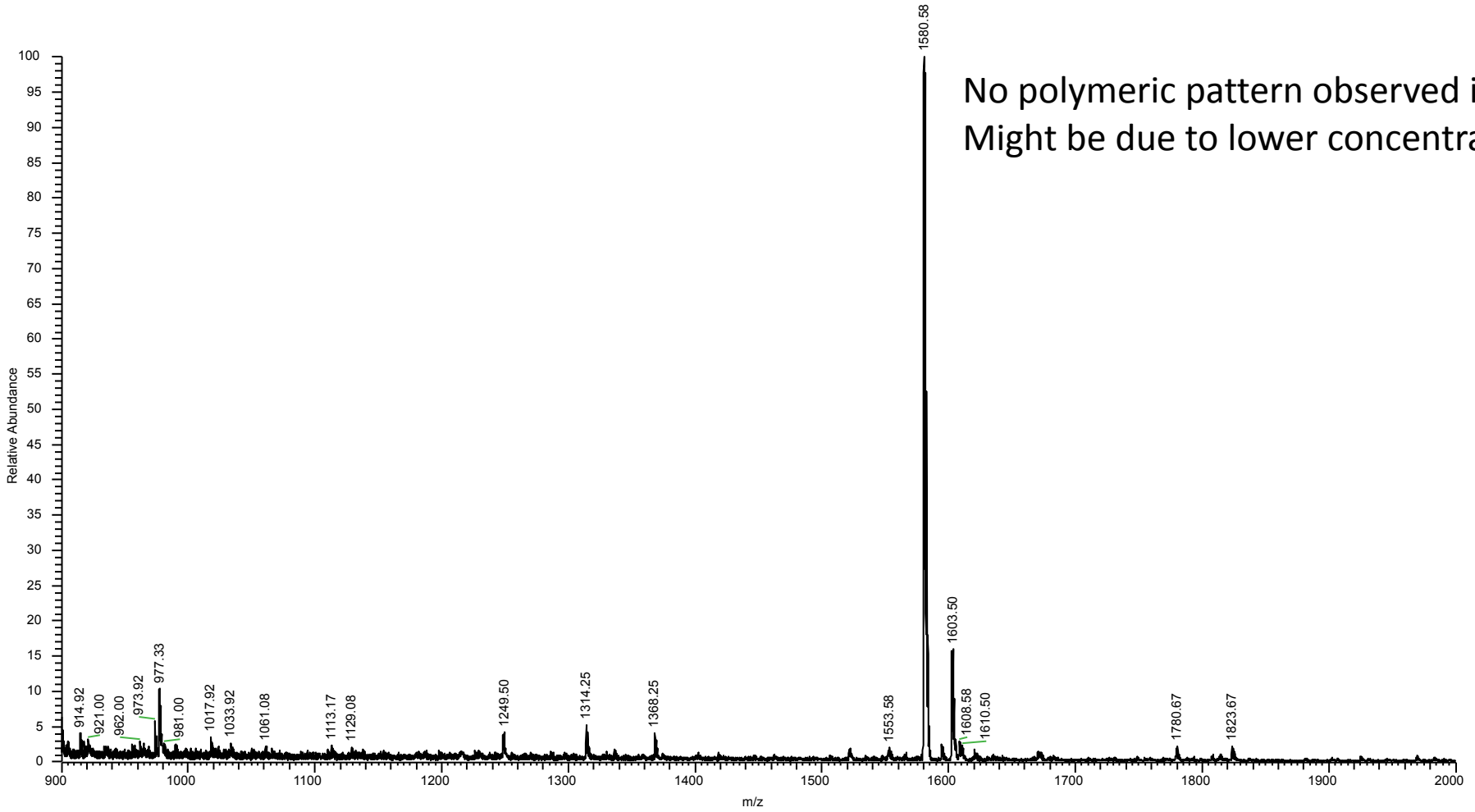
M2, unextracted, HCCA matrix



Analyzed with matrix to observe background in the overall sample without extraction

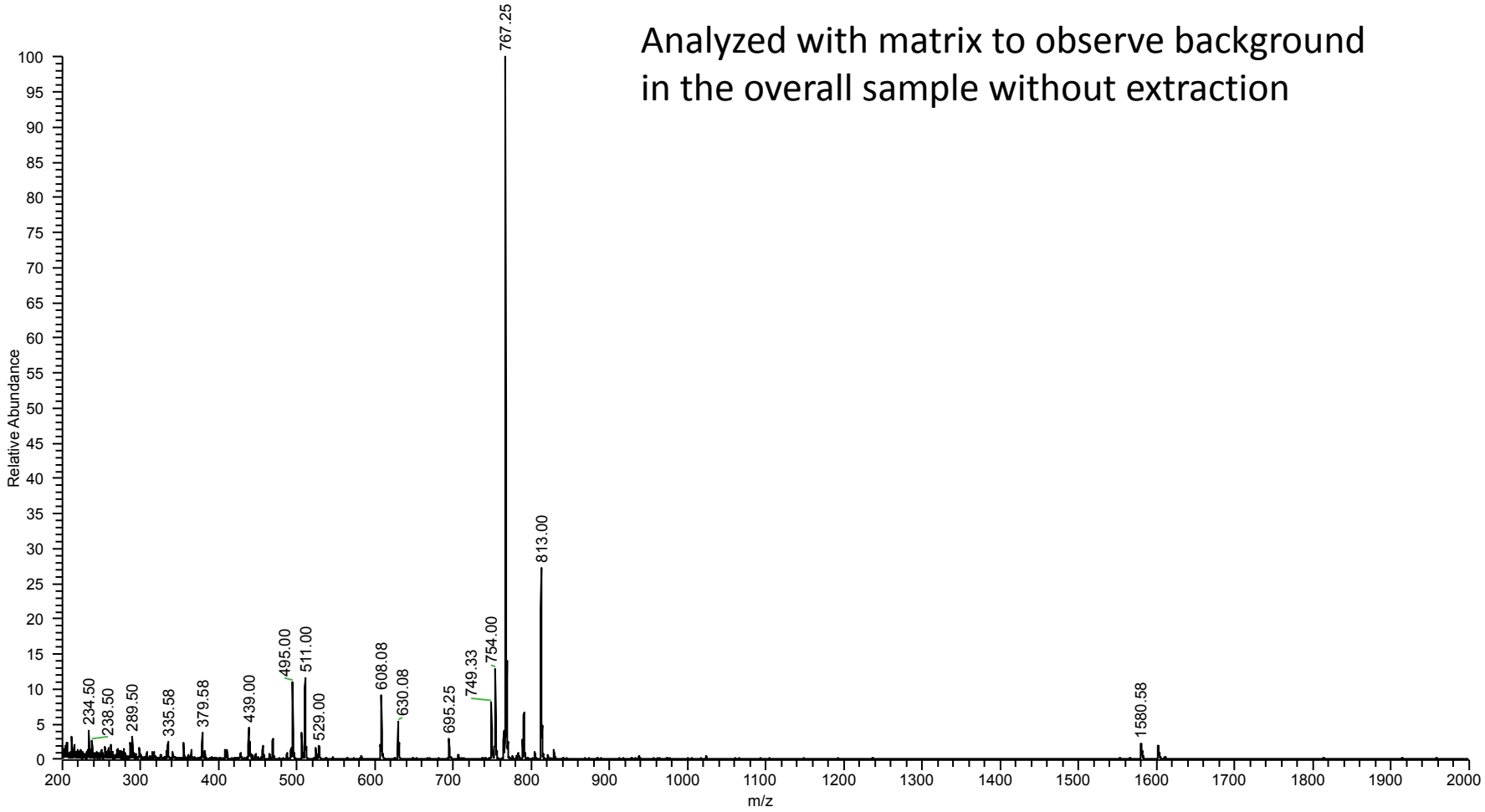
Moderna nanoparticle lipid SM-102

M2, unextracted, mass range 900-2000



No polymeric pattern observed in this sample
Might be due to lower concentration of polymer

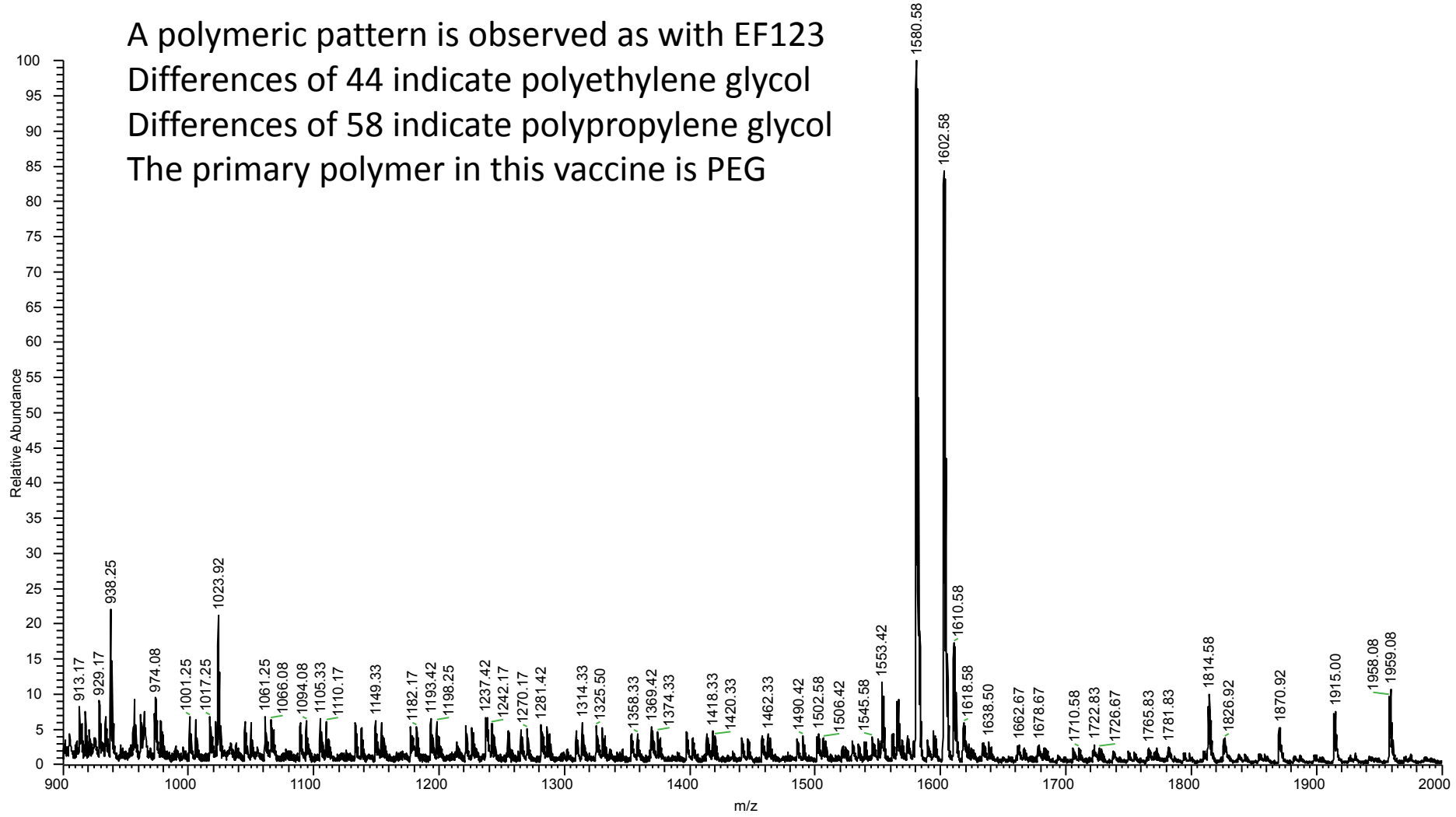
PKI, unextracted, HCCA matrix



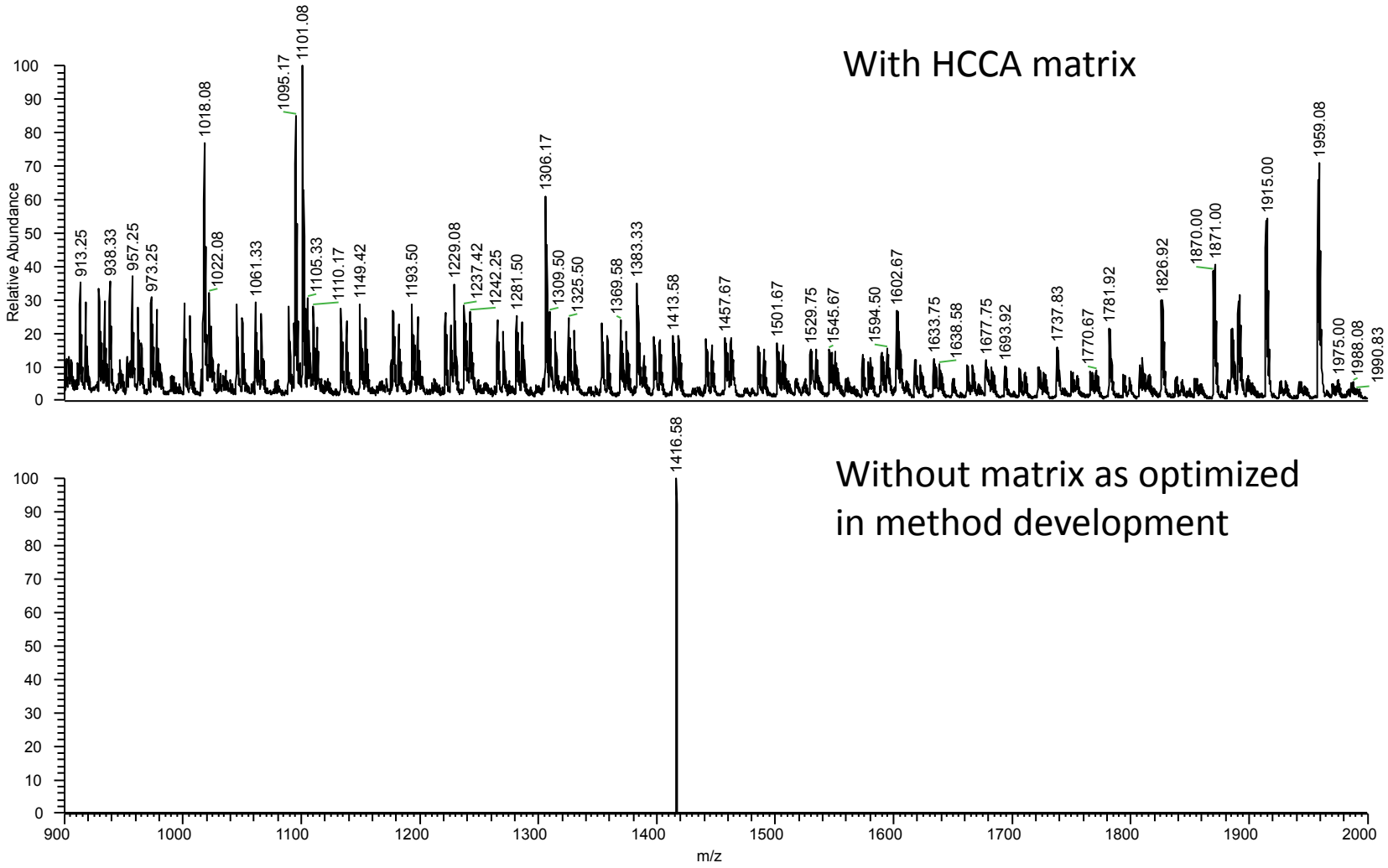
Analyzed with matrix to observe background in the overall sample without extraction

PKI, unextracted, mass range 900-2000

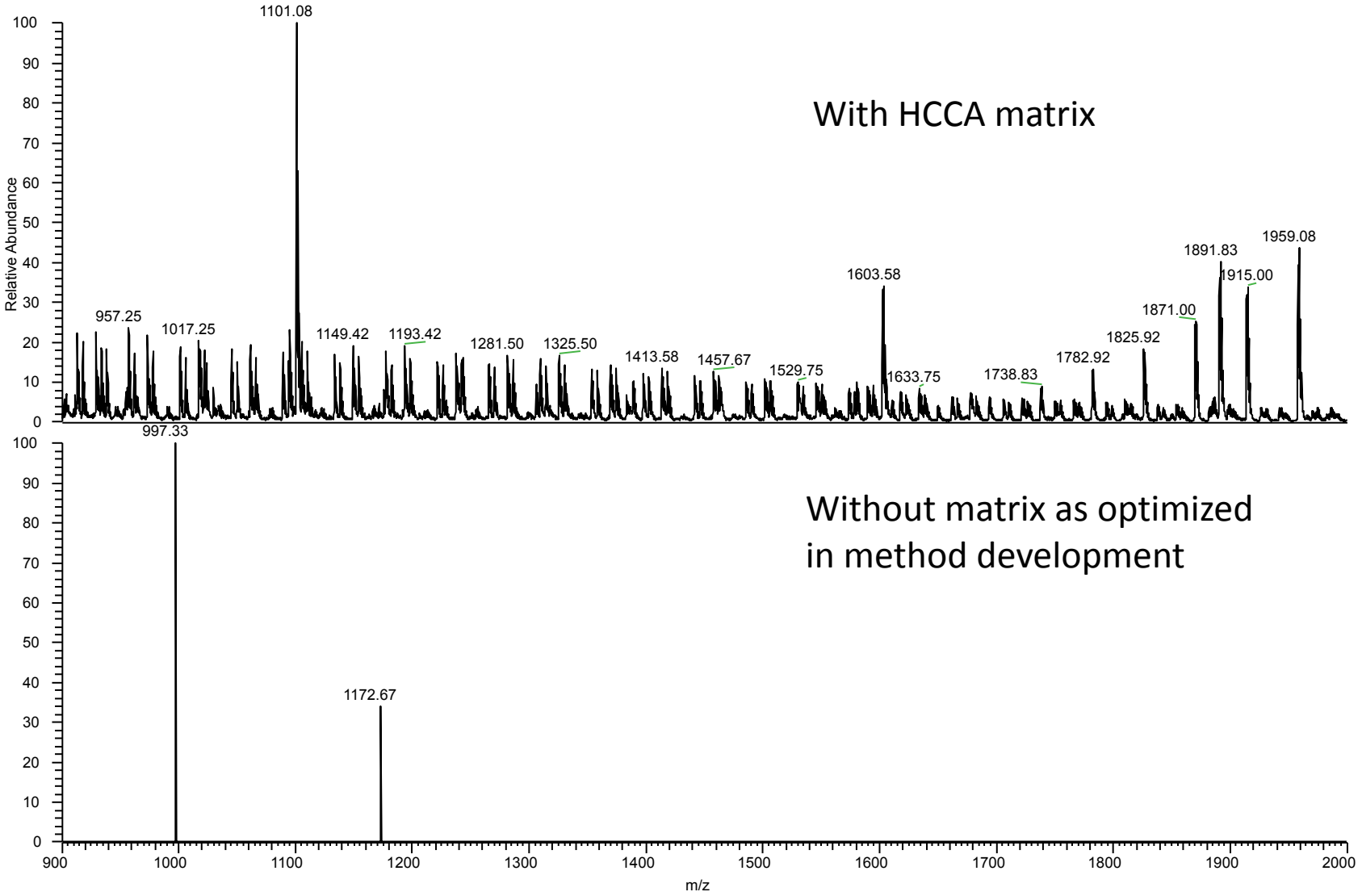
A polymeric pattern is observed as with EF123
Differences of 44 indicate polyethylene glycol
Differences of 58 indicate polypropylene glycol
The primary polymer in this vaccine is PEG



EF123, extracted



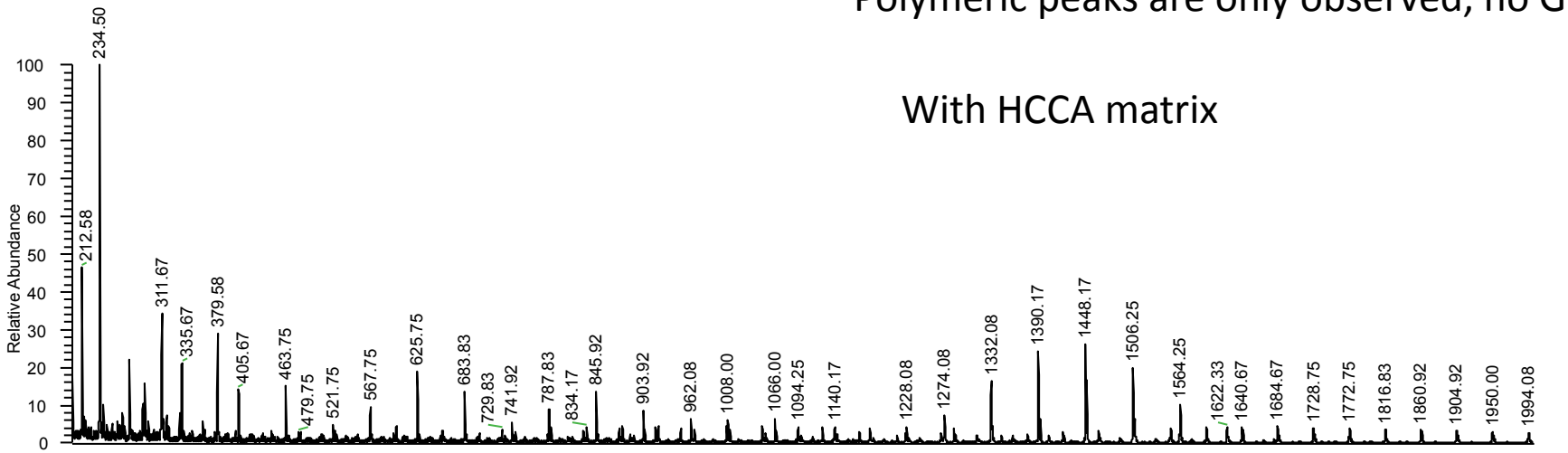
EF456, extracted



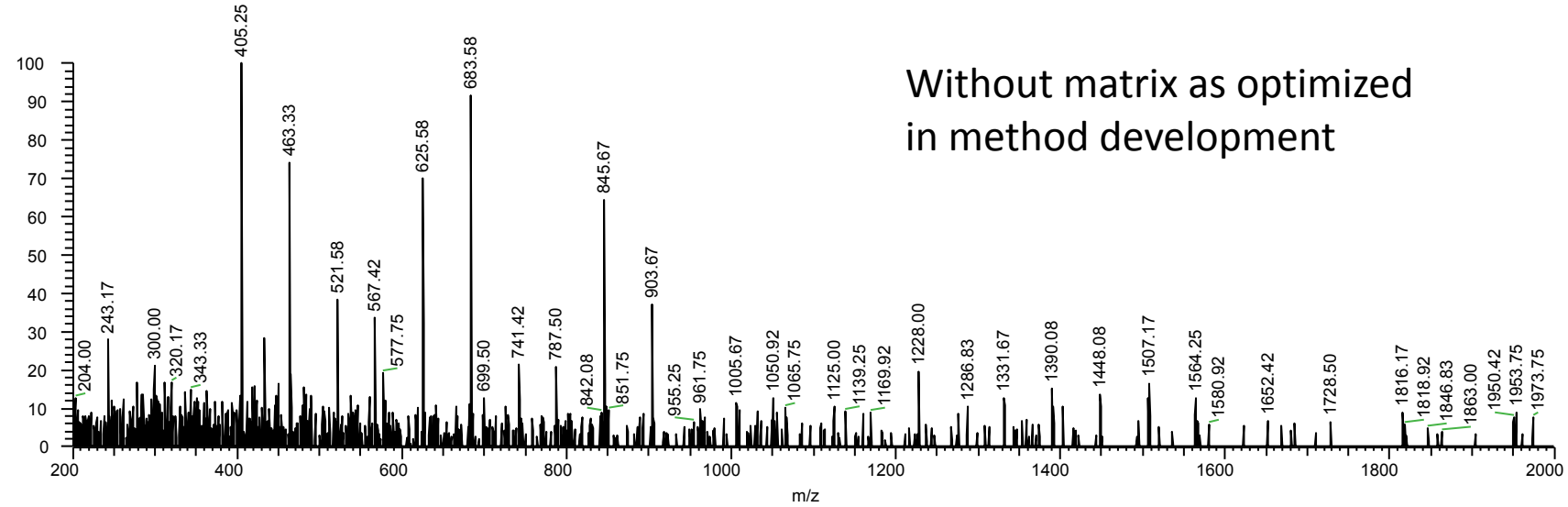
J2, extracted

Polymeric peaks are only observed, no Graphene oxide

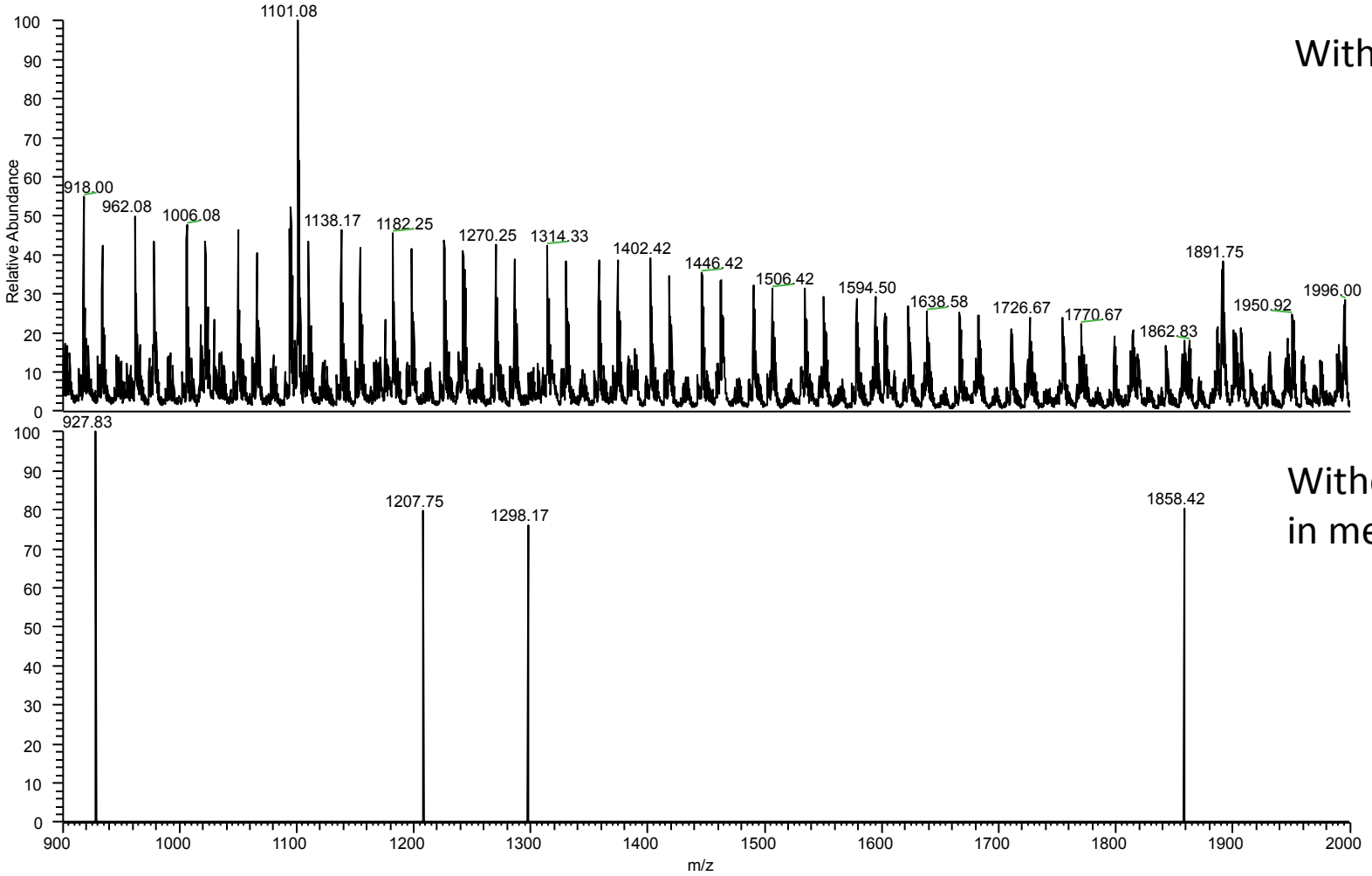
With HCCA matrix



Without matrix as optimized in method development



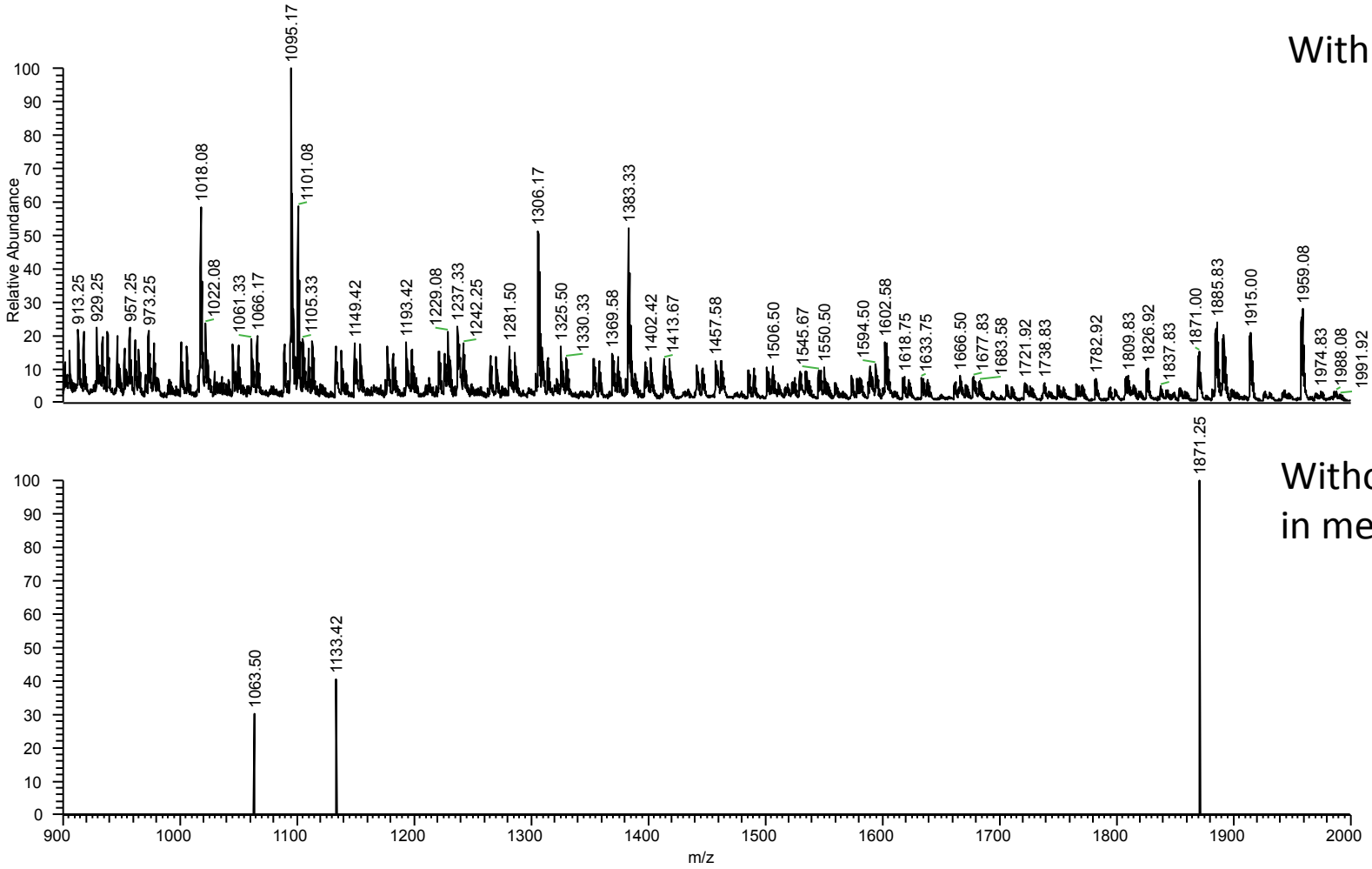
M2, extracted



With HCCA matrix

Without matrix as optimized in method development

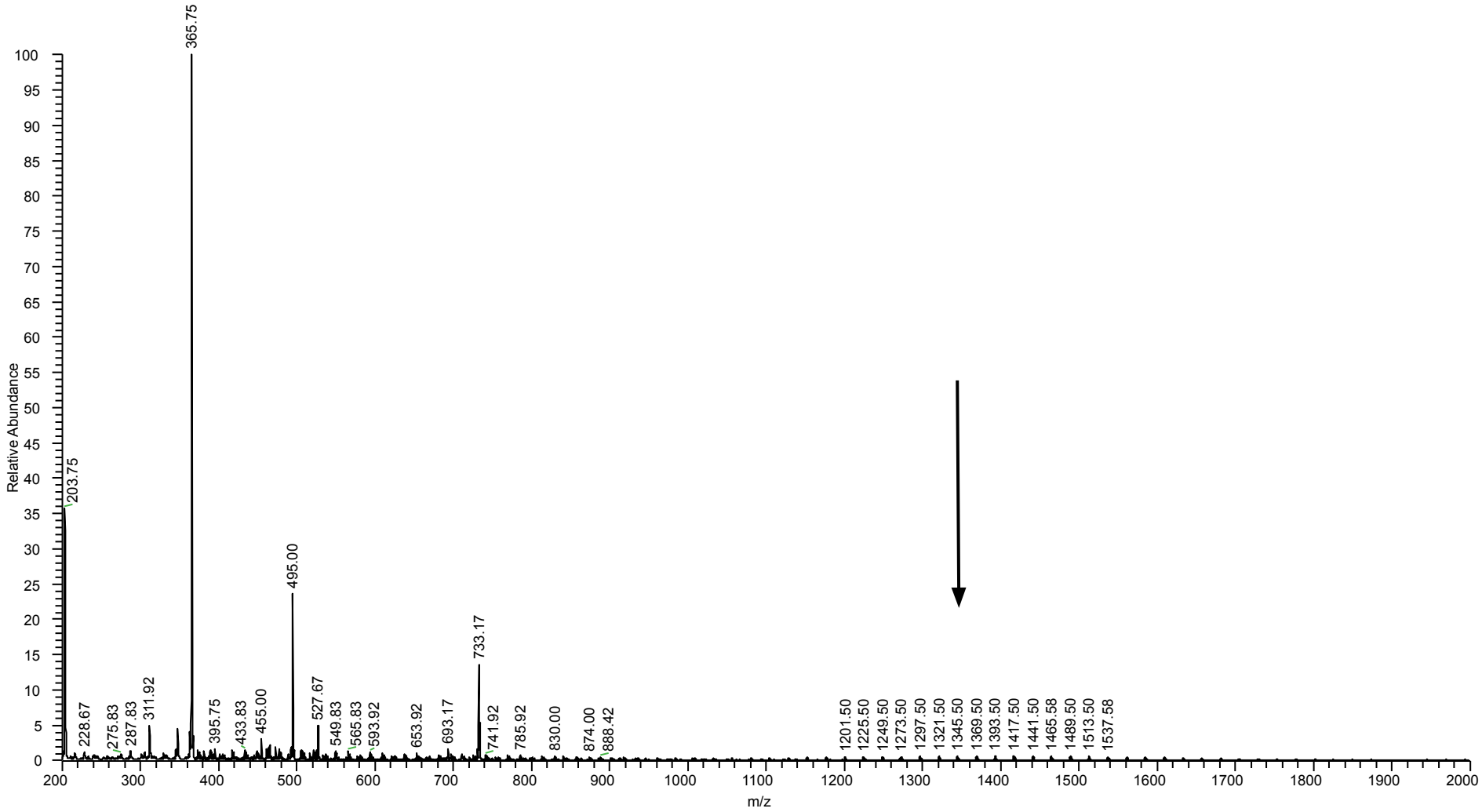
PKI, extracted



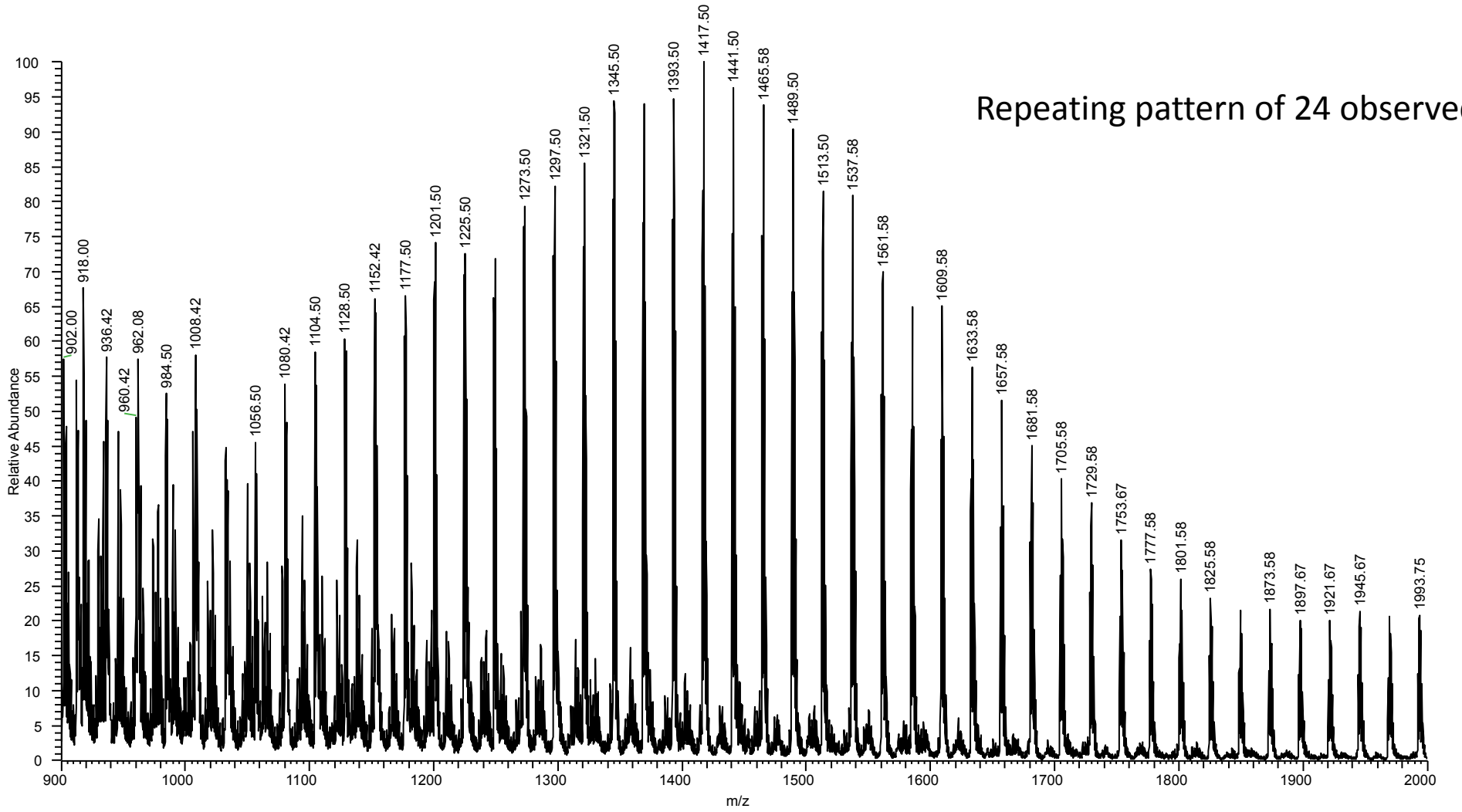
With HCCA matrix

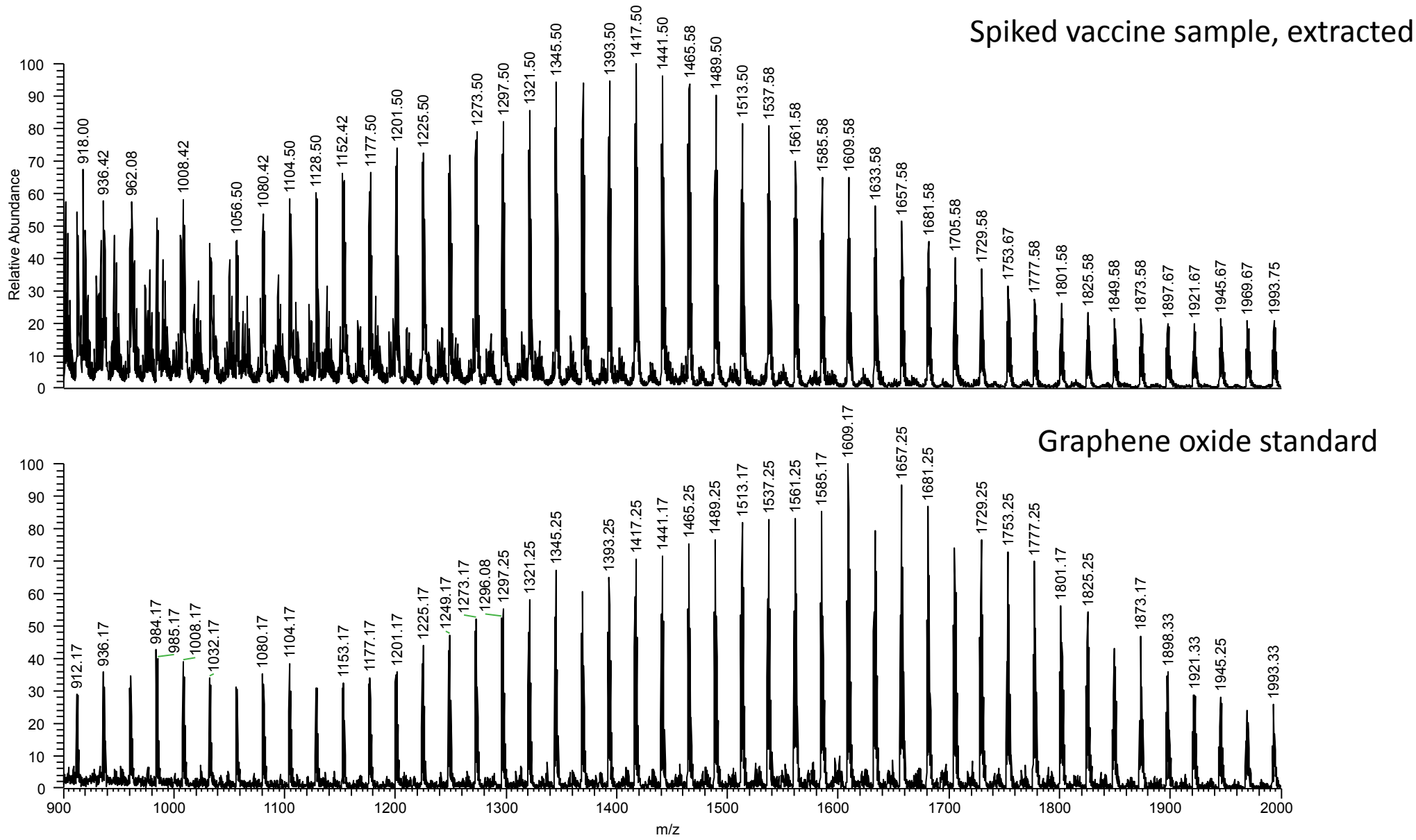
Without matrix as optimized in method development

M2 Vaccine spiked with GO



M2 vaccine spiked with GO, mass range 900-2000




Spiked vaccine sample, extracted
Relative Abundance
100
90
80
70
60
50
40
30
20
10
0
918.00
936.42
962.08
1008.42
1056.50
1080.42
1104.50
1128.50
1152.42
1177.50
1201.50
1225.50
1273.50
1297.50
1321.50
1345.50
1393.50
1417.50
1441.50
1465.58
1489.50
1513.50
1537.58
1561.58
1585.58
1609.58
1633.58
1657.58
1681.58
1705.58
1729.58
1753.67
1777.58
1801.58
1825.58
1849.58
1873.58
1897.67
1921.67
1945.67
1969.67
1993.75
Graphene oxide standard
912.17
936.17
984.17
985.17
1008.17
1032.17
1080.17
1104.17
1153.17
1177.17
1201.17
1225.17
1249.17
1273.17
1296.08
1297.25
1321.25
1345.25
1393.25
1417.25
1441.17
1465.25
1489.25
1513.17
1537.25
1561.25
1585.17
1609.17
1657.25
1681.25
1729.25
1753.25
1777.25
1801.17
1825.25
1873.17
1898.33
1921.33
1945.25
1993.33
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
m/z