ORDER FOR SUPPLIES OR SERVICES

IMPORTANT: Mark all packages and papers with contract and/or order numbers.

1. DATE OF ORDER	2. CONTRACT NO. *(If any)*		6. SHIP TO:
06/06/2017	CPSC-I-17-0015		**a. NAME OF CONSIGNEE**

3. ORDER NO.		4. REQUISITION/REFERENCE NO.	CONSUMER PRODUCT SAFETY COMMISSION
		REQ-4500-17-0002	

5. ISSUING OFFICE *(Address correspondence to)*
CONSUMER PRODUCT SAFETY COMMISSION
DIV OF PROCUREMENT SERVICES
4330 EAST WEST HWY
ROOM 523
BETHESDA MD 20814

b. STREET ADDRESS
DIRECTORATE FOR HEALTH SCIENCES
5 Research Place

c. CITY	d. STATE	e. ZIP CODE
Rockville	MD	20850

7. TO:

f SHIP VIA

a. NAME OF CONTRACTOR
CENTERS FOR DISEASE CONTROL AND PREVENTION

b. COMPANY NAME

8. TYPE OF ORDER

c. STREET ADDRESS
1600 CLIFTON RD NE

[X] **a. PURCHASE**

REFERENCE YOUR:

Please furnish the following on the terms and conditions specified on both sides of this order and on the attached sheet, if any, including delivery as indicated.

[ ] **b. DELIVERY**

Except for billing instructions on the reverse, this delivery order is subject to instructions contained on this side only of this form and is issued subject to the terms and conditions of the above-numbered contract.

d. CITY	e. STATE	f. ZIP CODE
ATLANTA	GA	30329-4018

9. ACCOUNTING AND APPROPRIATION DATA
See Schedule

10. REQUISITIONING OFFICE
CONSUMER PRODUCT SAFETY COMMISSION

11. BUSINESS CLASSIFICATION *(Check appropriate box(es))*

[ ] a. SMALL [ ] b. OTHER THAN SMALL [ ] c. DISADVANTAGED [ ] d. WOMEN-OWNED [ ] e. HUBZone

[ ] f. SERVICE-DISABLED VETERAN-OWNED [ ] g. WOMEN-OWNED SMALL BUSINESS (WOSB) ELIGIBLE UNDER THE WOSB PROGRAM [ ] h. EDWOSB

12. F.O.B. POINT
Destination

13. PLACE OF		14. GOVERNMENT B/L NO.	15. DELIVER TO F.O.B. POINT ON OR BEFORE *(Date)*	16. DISCOUNT TERMS
a. INSPECTION	**b. ACCEPTANCE**		12/31/2019	Net 30

17. SCHEDULE *(See reverse for Rejections)*

ITEM NO. (a)	SUPPLIES OR SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	DUNS Number: 927645465					
	COR: Kristina Hatlelid					
	Email: KHatlelid@cpsc.gov					
	Tel: 301-987-22258					
	This is an Inter-Agency Agreement between Continued ...					

18. SHIPPING POINT	19 GROSS SHIPPING WEIGHT	20 INVOICE NO.	17(h) TOTAL *(Cont. pages)* ◀

21. MAIL INVOICE TO:

SEE BILLING INSTRUCTIONS ON REVERSE

a. NAME	CPSC Accounts Payable Branch	$600,000.00
b. STREET ADDRESS (or P.O. Box)	AMZ 160 P.O. Box 25710	

17(i) GRAND TOTAL

c. CITY	d. STATE	e. ZIP CODE	$600,000.00 ◀
Oklahoma City	OK	73125	

22. UNITED STATES OF AMERICA BY *(Signature)*	23. NAME *(Typed)*
▶ *[signature]*	Cassandra C. Sterba TITLE: CONTRACTING/ORDERING OFFICER

AUTHORIZED FOR LOCAL REPRODUCTION
PREVIOUS EDITION NOT USABLE

OPTIONAL FORM 347 (Rev. 2/2012)
Prescribed by GSA/FAR 48 CFR 53 213(f)

IMPORTANT: Mark all packages and papers with contract and/or order numbers.

DATE OF ORDER	CONTRACT NO.		ORDER NO.
06/06/2017	CPSC-I-17-0015		

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	the Consumer Product Safety Commission and the Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.					
	The contractor shall conduct a case study of printer-emitted engineered nanoparticles (PEPs) for the performance period of June 26, 2017 through December 31, 2019 in accordance with the attached Inter-Agency Agreement. Accounting Info: 0100A17DSE-2017-2370400000-EXHR004000-255A0					
0001	This Interagency Agreement establishes the terms under which the National Institute for Occupational Safety and Health ("NIOSH"), the servicing agency, will conduct Phase 2 assessment to investigate environmental health and safety implications from engineered nanomaterials ("ENMs") released from nano-enabled products ("NEPs") during consumer use specific to - in vivo biokinetic, cardiovascular, other targeted organs, and genomic studies for the Consumer Product Safety Commission ("CPSC"), the requesting agency.	1	EA	600,000.00	600,000.00	
	The total amount of award: $600,000.00. The obligation for this award is shown in box 17(i).					
	TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H))	▷			$600,000.00	

AUTHORIZED FOR LOCAL REPODUCTION
PREVIOUS EDITION NOT USABLE

OPTIONAL FORM 348 (Rev 4/2006)
Prescribed by GSA FAR (48 CFR) 53 213(f)

UNITED STATES GOVERNMENT
INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
General Terms & Conditions (GT&C) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000	
	GT&C #	Order #	Amendment # / Mod #

DEPARTMENT AND/OR AGENCY		
1.	**Requesting Agency of Products / Services**	**Servicing Agency Providing Products / Services**
Name	U.S. Consumer Product Safety Commission	Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health
Address	4330 East West Highway Bethesda, MD 20814	1095 Willowdale Road, MS L-4020 Morgantown, WV 26505

2. Servicing Agency Tracking Number (Optional) : CDC IAA 17-NS17-07

3. Assisted Acquisition Agreement ☐ Yes ☑ No

4. GT&C Action (Check action being taken)

☑ **New**

☐ **Amendment** - Complete only the GT&C blocks being changed and explain the changes being made.

☐ **Cancellation** - Provide a brief explanation for the IAA cancellation and complete the effective End Date.

5. Agreement Period Start Date __08/26/2017__ End Date __12/31/2019__ of IAA or effective cancellation date
MM-DD-YYYY   MM-DD-YYYY

6. Recurring Agreement (Check One) A Recurring Agreement will continue, unless a notice to discontinue is received
☐ Yes   If Yes, this is an: Annual Renewal ☐
              Other Renewal ☐   State the other renewal period: _____
☑ No

7. Agreement Type (Check One)   ☑ Single Order IAA   ☐ Multiple Order IAA

8. Are Advance Payments Allowed for this IAA (Check One)   ☐ Yes   ☑ No

If Yes is checked, enter Requesting Agency's Statutory Authority Title and Citation

Note: Specific advance amounts will be captured on each related order.

PROGRAM CAN # 93908N1
CDCOH CAN   # 93908N2

UNITED STATES GOVERNMENT
INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
General Terms & Conditions (GT&C) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-1-17-0015	0000	
	GT&C #	Order #	Amendment # / Mod #

9. Estimated Agreement Amount (The Servicing Agency completes all information for the estimated agreement amount)

(Optional for assisted Acquisitions)

		Provide a general explanation of the Overhead Fees and Charges
Direct Cost	$559,440.00	CDC Overhead at 7.25%
Overhead Fees & Charges	$40,560.00	
Total Estimated Amount	$600,000.00	

10. STATUTORY AUTHORITY

a. Requesting Agency's Authority (Check One)

Franchise Fund	Revolving Fund	Working Capital Fund	Economy Act (31 U.S.C. 1535 / FAR 17.5)	Other Authority
☐	☐	☐	☐	☑

Fill in Statutory Authority Title and Citation for Franchise Fund, Revolving Fund, Working Capital Fund, or Other Authority

For CPSC: Section 27(6) of the Consumer Product Safety Act (15 U.S.S. 2076 (g)

b. Servicing Agency's Authority (Check One)

Franchise Fund	Revolving Fund	Working Capital Fund	Economy Act (31 U.S.C. 1535 / FAR 17.5)	Other Authority
☐	☐	☐	☐	☑

Fill in Statutory Authority Title and Citation for Franchise Fund, Revolving Fund, Working Capital Fund, or Other Authority

For CDC/NIOSH: Section 301(b)(1) of the Public Health Service Act of 1944 (42 U.S.C. 241(b)(1)), and 29 U.S.C.669

11. Requesting Agency's Scope (State and/or List Attachments that support Requesting Agency's Scope.)

Research to support project entitled "Phase 2: Case Study of printer-emitted engineered nanoparticles (PEPs) specific to in vivo biokinetic, cardiovascular, other targeted organs, and genomic studies."

12. Roles and Responsibilities for the Requesting Agency and the Servicing Agency (State and/or list attachments for the roles and responsibilities for the Requesting Agency and the Servicing Agency)

See attached Statement of Work.

UNITED STATES GOVERNMENT
INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
General Terms & Conditions (GT&C) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000	
	GT&C #	Order #	Amendment # / Mod #

13. Restrictions (Optional) (State and/or attach unique requirements and/or mission specific restrictions specific to this IAA).

14. Assisted Acquisition Small Business Credit Clause (The Servicing Agency will allocate the socio-economic credit to the Requesting Agency for any contract actions it has executed on behalf of the Requesting Agency).

15. Disputes: Disputes related to this IAA shall be resolved in accordance with instructions provided in the Treasury Financial Manual (TFM) Volume I, Part 2, Chapter 4700, Appendix 10; Intragovernmental Transaction (IGT) Guide.

16. Termination (Insert the number of days that this IAA may be terminated by written notice by either the Requesting or Servicing Agency.)

30 days
If this agreement is canceled, any implementing contract/order may also be canceled. If the IAA is terminated, the agencies shall agree to the terms of the termination, including costs attributable to each party and the disposition of awarded and pending actions.
If the Servicing Agency incurs costs due to the Requesting Agency's failure to give the requisite notice of its intent to terminate the IAA, the Requesting Agency shall pay any actual costs incurred by the Servicing Agency as a result of the delay in notification, provided such costs are directly attributable to the failure to give notice.

17. Assisted Acquisition Agreements - Requesting Agency's Organizations Authorized to Request Acquisition Assistance for this IAA (State or attach a list of Requesting Agency's organizations authorized to request acquisition assistance for this IAA.

N/A

18. Assisted Acquisition Agreements - Servicing Agency's Organizations Authorized to Request Acquisition Assistance for this IAA (State or attach a list of Servicing Agency's organizations authorized to request acquisition assistance for this IAA.)

N/A

19. Requesting Agency Clause(s) (Optional) (State and/or attach any additional Requesting Agency clauses.)

Clauses included on attached SOW.

20. Servicing Agency Clause(s) (Optional) (State and/or attach any additional Servicing Agency clauses.)

Equipment: CDC/NIOSH will retain title to any equipment procured in order to provide service.

UNITED STATES GOVERNMENT
INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
General Terms & Conditions (GT&C) Section

IAA Number	CPSC-I-17-0015	0000	
	GT&C #	Order #	Amendment # / Mod #

21. Additional Requesting Agency and/or Servicing Agency Attachments (Optional) (State and/or attach any additional Requesting Agency and/or Servicing Agency Attachments)

Confidentiality Requirements: To the extent permitted by law, all information reported to or otherwise obtained by CPSC or its agents under the Consumer Product Safety Act (CPSA) and provided to or share with NIOSH, which contains or relates to a trade secret or other matter referred to in Section 1905 of Title 18, United States Code, or subject to Section 552(b)(4) of the Title 5, United States Code, shall be held in confidence by NIOSH personnel.

There are additional terms, see SOW.

22. Annual Review of IAA

By signing this agreement, the parties agree to annually review the IAA if the agreement period exceeds one year. Appropriate changes will be made by amendment to the GT&C and/or modification to any affected Order(s).

AGENCY OFFICIAL

The Agency Official is the highest level accepting authority or official as designated by the Requesting Agency and Servicing Agency to sign this agreement. Each Agency Official must ensure that the general terms and conditions are properly defined, including the stated statutory authorities, and, that the scope of work can be fulfilled as per the agreement.

The Agreement Period Start Date (Block 5) must be the same as or later than the signature dates.

Actual work for this IAA may NOT begin until an Order has been signed by the appropriate individuals, as stated in the Instructions for Blocks 37 and 38.

23.	Requesting Agency	Servicing Agency
Name	Cassandra Sterba	Martha DiMuzio
Title	Contracting Officer	Associate Director for Fiscal Resources Mgmt
Telephone Number(s)	(301) 504-7837	(513) 533-6805
Fax Number		(513) 533-8371
Email Address	CSterba@cpsc.gov	MAD2@CDC.GOV
SIGNATURE	csterba@cpsc.gov	Martha A. Dimuzio -S
Approval Date		05/31/2017

UNITED STATES GOVERNMENT INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
Order Requirements and Funding Information (Order) Section

BUREAU OF THE Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000		Servicing Agency's Agreement Tracking Number (Optional)	17-NS17-07
	GT&C #	Order #	Amendment # / Mod #		

PRIMARY ORGANIZATION/OFFICE INFORMATION

24.	Requesting Agency	Servicing Agency
Primary Organization / Office Name	U.S. Consumer Product Safety Commission	Centers for Disease Control and Prevention/National Institute for ➕
Responsible Organization / Office Address	4330 East West Highway Bethesda, MD 20814	1095 Willowdale Road, MS L-4020 Morgantown, WV 26505

ORDER REQUIREMENTS INFORMATION

25. Order Action (Check One)

[✓] **New**

[ ] **Modification (Mod)** - List affected Order blocks being changed and explain the changes being made. For Example: for a performance period mod, state the new performance period for this Order in Block 27. **Fill out the Funding Modification Summary by Line (Block 26)** if the mod involves adding, deleting, or changing **Funding for an Order Line.**

[ ] **Cancellation** - Provide a brief explanation for Order cancellation and fill in the Performance Period End Date for the effective cancellation date.

26. Funding Modification Summary by Line	Line #	Line #	Line #	Total of All Other Lines (attach funding details)	Total
Original Line Funding					$0.00
Cumulative Funding Changes From Prior Mods [addition (+) or reduction (-)]					$0.00
Funding Change for This Mod					$0.00
TOTAL Modified Obligation	$0.00	$0.00	$0.00	$0.00	$0.00
Total Advance Amount (-)					$0.00
Net Modified Amount Due	$0.00	$0.00	$0.00	$0.00	$0.00

27. Performance Period	Start Date	06/26/2017	End Date	12/31/2019
For a performance period mod, insert the start and end dates that reflect the new performance period.		MM-DD-YYYY		MM-DD-YYYY

UNITED STATES GOVERNMENT INTERAGENCY
AGREEMENT (IAA)
Agreement Between Federal Agencies
Order Requirements and Funding Information (Order) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015		0000			Servicing Agency's Agreement	17-NS17-07
	GT&C #		Order #	Amendment # / Mod #		Tracking Number (Optional)	

28. Order Line / Funding Information

Line Number

		Requesting Agency Funding Information	Servicing Agency Funding Information

ALC	61-00-0001							75 090421							

Component	SP	ATA	AID	BPOA	EPOA	A	MAIN	SUB	SP	ATA	AID	BPOA	EPOA	A	MAIN	SUB
TAS (required by 10/1/2014)			61	2017	2017		0100				75	2017	2017		0943	000

and/or current TAS format	61-17-0100	75-17-0943
BETC	2370400000	COLL
Object Class Code (Optional)	255A0	2538
BPN	069287522 (DUNS)	9272645465 (DUNS)
BPN + 4 (Optional)	DISB	COLL
Additional Accounting Classification / Information (Optional)	0100A17DSE 2017 2370400000 EXHR004000 255A0	Fund Code: 09216020171RAD Adm Code:CCCC BA 5611 RF 1101 EIN 586051157

Requesting Agency Funding Expiration Date	Requesting Agency Funding Cancellation Date
09/30/2017	09/30/2022
MM-DD-YYYY	MM-DD-YYYY

Project Number & Title Phase 2: Case study of printer-emitted engineered nanoparticles (PEPs) specific to in vivo bi

Description of Products and/or Service, including the Bona Fide Need for this Order (State or attach a description of products/services, including the Bona Fide need for this Order.)

CPSC will provide $600,000 of FY 2017 funds to support collaborative research on a project entitled "Phase 2: Case study of printer-emitted engineered nanoparticles (PEPs) specific to in vivo biokinetic, cardiovascular, other targeted organs, and genomic studies," as described in the attached Statement of Work.

North American Industry Classification System (NCAIS) Number (Optional)

Breakdown of Reimbursable Line Costs		and/or	**Breakdown of Assisted Acquisition Line Cost:**	
Unit of Measure			Contract Cost	
Quantity	Unit Price	Total	Servicing Fees	
1	$559,440.00	$559,440.00	Total Obligated Cost	$0.00
Overhead Fees and Charges		$40,560.00	Advance for Line (-)	
Total Line Amount Obligated		$600,000.00	Net Total Cost	$0.00
Advance Line Amount (-)			Assisted Acquisition Servicing Fees Explanation	
Net Line Amount Due		$600,000.00	7.25% CDCOH RATE	

Type of Service Requirements

( ) Severable Service   (●) Non-Severable Service   ( ) Not Applicable

UNITED STATES GOVERNMENT INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
Order Requirements and Funding Information (Order) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000		Servicing Agency's Agreement	
	GT&C #	Order #	Amendment # / Mod #	Tracking Number (Optional)	17-NS17-07

29. Advance Information (Complete Block 29 if the Advance Payment for Products/Services was checked "Yes" on the GT&C)

Total Advance Amount for the Order _____ [All Order Line Advance amounts (Block 28) must sum to this total.]

Revenue Recognition Methodology (according to SFFAS 7)(Identify the Revenue Recognition Methodology that will be used to account for the Requesting Agency's expense and the Servicing Agency's revenue.)

- [ ] Straight-Line — Provide amount to be accrued _____ and Number of Months _____

- [ ] Accrual Per Work Completed — Identify the accounting post period:

  - [ ] Monthly per work completed & invoiced

  - [ ] Other — Explain other regular period (bimonthly, quarterly, etc.) for posting accruals and how the accrual amounts will be communicated if other than billed.

30. Total Net Order Amount: $600,000.00
[All Order Line Net Amounts Due for reimbursable agreements and Net Total Costs for Assisted Acquisition Agreements (Block 28) must sum to this total]

31. Attachments (State or list attachments)
- [ ] Key Project and/or acquisition milestones (Optional except for Assisted Acquisition Agreements)

- [x] Other Attachments (Optional)

  Statement of Work

BILLING AND PAYMENT INFORMATION

32. Payment Method (Check One) [Intra-governmental Payment and Collection (IPAC) is the Preferred Method.]

- [ ] Requesting Agency Initiated IPAC
- [x] Servicing Agency Initiated IPAC
- [ ] Credit Card
- [ ] Other — Explain other payment method and reasoning:

33. Billing Frequency (Check One)
[An Invoice must be submitted by the Servicing Agency and accepted by the Requesting Agency BEFORE funds are reimbursed (i.e., via IPAC transaction)]

Monthly invoicing via the IPAC system.

- [x] Monthly
- [ ] Quarterly
- [ ] Other Billing Frequency (include explanation): _____

34. Payment Terms (Check One)
- [ ] 7 Days [x] Other Payment Terms (include explanation): CDC will not IPAC customer nor will customer IPAC

within last 3 business days of the month.

UNITED STATES GOVERNMENT INTERAGENCY AGREEMENT (IAA)
Agreement Between Federal Agencies
Order Requirements and Funding Information (Order) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000		Servicing Agency's Agreement Tracking Number (Optional)	17-NS17-07
	GT&C #	Order #	Amendment # / Mod #		

35. Funding Clauses / Instructions (Optional) (State and/or list funding clauses/instructions)

36. Delivery / Shipping Information for Products (Optional)

Agency Name	U.S. Consumer Product Safety Commission
Point of Contact (POC) Name & Title	Treye Thomas
POC Email Address	tthomas@cpsc.gov
Delivery Address / Room Number	5 Research Place, Rockville, MD 20850
POC Telephone Number	(301) 987-2560
Special Shipping Information	

APPROVALS AND CONTACT INFORMATION

37. Program Officials
The Program Officials, as identified by the Requesting Agency and Servicing Agency, must ensure that the scope of work is properly defined and can be fulfilled for this Order. The Program Official may or may not be the Contracting Officer depending on each agency's IAA business process.

	Requesting Agency	Servicing Agency
Name	Joanna Matheson	Don Beezhold
Title	Contracting Officer Representative - N	Director, HELD
Telephone Number	(301) 987-2564	(304) 285-5963
Fax Number		(304) 285-6126
Email Address	JMatheson@cpsc.gov	ZEC1@CDC.GOV
SIGNATURE	jmatheson@cpsc.gov	
Date Signed		

38. Funding Officials — The Funds Approving Officials, as identified by the Requesting Agency and Servicing Agency, certify that the funds are accurately cited and can be properly accounted for per the purposes set forth in the Order. The Requesting Agency Funding Official signs to obligate funds. The Servicing Agency Funding Official signs to start the work, and to bill, collect, and properly account for funds from the Requesting Agency, in accordance with the agreement.

	Requesting Agency	Servicing Agency
Name	James Baker	Martha DiMuzio
Title	Director FMPS and Budget Officer	Assoc Dir for Fiscal Resources, NIOSH
Telephone Number	(301) 504-7575	(513) 533-6805
Fax Number		(513) 533-8371
Email Address	JBaker@cpsc.gov	MAD2@CDC.GOV
SIGNATURE	JAMES BAKER	Martha A. Dimuzio -S
Date Signed		

UNITED STATES GOVERNMENT INTERAGENCY AGREEMENT (IAA)

Agreement Between Federal Agencies
Order Requirements and Funding Information (Order) Section

BUREAU OF THE
Fiscal Service
LEAD. TRANSFORM. DELIVER.

IAA Number	CPSC-I-17-0015	0000		Servicing Agency's Agreement Tracking Number (Optional)	17-NS17-07
	GT&C #	Order #	Amendment # / Mod #		

CONTACT INFORMATION

39. FINANCE OFFICE Points of Contact (POCs)

	Requesting Agency (Payment Office)	Servicing Agency (Billing Office)
Name	Cindy Coszalter	Shelli Smalling, CDC/OCOO/OFR
Title	Agency Payment Officer, OFA, FAAA	Branch Chief, Debt Mgmt Branch
Office Address	P.O. Box 25710 Oklahoma City, OK 73125	KOGR Bldg COLUM Room 2085 MSs D48, Atlanta GA 30341
Telephone Number	(405) 954-8250	(678) 475-4595
Fax Number		
Email Address	cindy.coszalter@faa.gov	IBY0@CDC.GOV
Signature & Date (Optional)		

40. ADDITIONAL Points of Contact (POCs) (as determined by each Agency)
This may include CONTRACTING Office Points of Contact (POCs).

	Requesting Agency	Servicing Agency
Name	Kristina Hatlelid	Renee Dotson
Title	Contracting Officer Representative - Nano	Program Analyst, HELD
Office Address	5 Research Place Rockville, MD 20814	1095 Willowdale Road, MS 4020 Morgantown, WV 26505
Telephone Number	(301) 987-2258	(304) 285-6150
Fax Number		(304) 285-6126
Email Address	KHatlelid@cpsc.gov	CED9@CDC.GOV
Signature & Date (Optional)		*Renee Dotson* 5/24/17
Name	Cassandra Sterba	Sandra Stafford
Title	Contracting Officer	Budget Analyst, CDC/OCOO/OCFO
Office Address	4330 East West Highway Bethesda, MD 20814	1095 Willowdale Road, MS 4020 Morgantown WV 26505
Telephone Number	(301) 504-7837	(304) 285-6207
Fax Number		(304) 285-6126
Email Address	CSterba@cpsc.gov	SGT9@CDC.GOV
Signature & Date (Optional)	*Cassandra Sterba* 6-12-17	*Sandra Stafford* 5/24/17
Name		IAA UNIT
Title		ATTN: Kimberly Strickland
Office Address		KOGR Bldg COLUM Rm. 4023 Atlanta, GA 30341
Telephone Number		
Fax Number		
Email Address		IAAUNIT@CDC.GOV
Signature & Date (Optional)		Kimberly Strickland -S Digitally signed by Kimberly Strickland -S Date: 2017.06.05 15:50:06 -04'00'

Statement of Work
For
Interagency Agreement
CPSC# - CPSC-I-17-0015
CDC IAA # 17-NS17-07

Between

U.S. Consumer Product Safety Commission
And the
Centers for Disease Control and Prevention,
National Institute for Occupational Safety and Health

Title: Phase 2: Case study of printer-emitted engineered nanoparticles (PEPs) specific to- *in vivo* biokinetic, cardiovascular, other targeted organs, and genomic studies

I.   Background/Introduction

This Interagency Agreement establishes the terms under which the National Institute for Occupational Safety and Health ("NIOSH"), the servicing agency, will conduct Phase 2 assessment to investigate environmental health and safety implications from engineered nanomaterials ("ENMs") released from nano-enabled products ("NEPs") during consumer use specific to - *in vivo* biokinetic, cardiovascular, other targeted organs, and genomic studies for the Consumer Product Safety Commission ("CPSC"), the requesting agency.

NIOSH and the Harvard School of Public Health have been working together for the past four years to study the presence of ENMs in laser printer toner formulations as well as the release of nanoparticles during laser printer use. The purpose of these studies was to assess the safety of laser printer emissions and to generate data to determine a no observed adverse effect level (NOAEL). These Phase 1 studies consisted of short-term (8-day) in vivo exposures to assess mechanisms of inflammation and fibrosis to the lungs and alterations to peripheral vascular responses. In addition, in vitro screening assays were performed to assess whether laser printer generated nanoparticles directly or indirectly caused cellular injury. The proposed Phase 2 studies will use longer in vivo exposures (21-day) to assess the persistence of inhaled laser printer nanoparticles and to determine which organs these nanoparticles are transported to and the impact on those organs, including the assessment of systemic inflammation. The Phase 2 studies will also confirm in vivo, what was observed in the Phase 1 in vitro screening tests; to directly measure the impact on heart function as well as on neurogenic pathways. The results from the Phase 1 and Phase 2 studies together should provide a complete picture of potential consumer exposures and adverse health effects while laser printers are in use during short and longer-term exposures. The data from these studies is important to the agency mission since the comprehensive data from these "real-world" testing scenarios will provide CPSC staff with crucial information that can form the basis for a risk assessment to laser emitted particles.

II.   Project Summary and Statement of Work

In FY2016, the Phase 1 study consisted of the following research conducted by the servicing agency: particle generation, characterization and fractionation, in vitro dosimetric determination, in

1

vitro toxicity investigation in multiple cell lines, pilot study in in vivo evaluation of PEP-induced cardiovascular effects via inhalation. In the Phase 2 study, emphasis will be given on biokinetics of inhaled PEPs, other PEP-targeted organ toxicity, prolong PEP exposure-induced cardiopulmonary responses, and toxicological and disease prediction-based genomic studies.

The unique physicochemical properties of ENMs are being exploited for use in a growing variety of commercial NEPs, including electronics, cosmetics, and structural materials, as well as a wide variety of products for medical applications. Numerous *in vitro* and *in vivo* studies have investigated the possible adverse effects of inhalation exposure to pristine ENMs during synthesis and handling by workers and consumers. However, human exposure is not limited to pristine ENMs, but also includes a wide variety of particles released from NEPs across their life cycle, including consumer use and disposal. Therefore, these results cannot be correlated to exposures at consumer level since the test particles used are not representative of the "real world" exposure of consumers to PEPs. Indeed, the potential for exposure from such life cycle particulate matter ("LCPM") may exceed that of pristine engineered nanoparticles ("ENPs"). Moreover, the physico-chemical properties and toxicological profiles of LCPM may differ greatly from those of pristine ENPs. Despite the potential for LCPM exposure, most nanotoxicological studies have focused on pristine ENPs, and toxicological evaluation of LCPM has been limited. NIOSH and the EPA have therefore recommended life cycle analyses in their nanotechnology research programs. Most importantly, the servicing agency needs to develop a methodology that can link real world LCPM exposures to toxicology and cardiopulmonary risk. This study will specifically address this important research gap.

Numerous epidemiologic studies have shown strong associations between inhalation of ambient particulate matter ("PM") and cardiovascular mortality and morbidity, with mortality from cardiovascular causes exceeding that from respiratory causes. Animal studies also indicate that the cardiovascular system may be more sensitive to pulmonary exposure to PM than the lung. To date, however, most nanotoxicology studies have focused primarily on the pulmonary effects of ENP exposure. The Phase 2 study will address this research gap using a multi-tiered approach, including *in vitro*, *in vivo*, and genomic approaches, to evaluate pulmonary, cardiovascular, and other targeted organ effects of LCPM exposure. In vivo biokinetic studies will be performed to determine the biopersistence and the potential translocation of LCPM to other organs. Pathological analysis will be performed for these LCPM-targeted other organs. Gene signatures will be measured from cultured cells, lung tissue and blood to assess concordance in gene expression and identify mechanistic pathways. Moreover, these pathological assessments will be correlated with genomic-profiles of blood samples and will be compared with bioinformatics-based prediction of LCPM-induced toxicity and diseases in cardiopulmonary and other targeted organs.

This Phase 2 study will use state-of-the-art PEP exposure generation systems and in vivo bioassays combined with traditional pathological analysis and advanced computational genomic modeling to assess mechanisms of potential PEP-induced cardiopulmonary and other organ diseases and predict potential disease implications in humans. The integrated approach will identify mechanistic pathways predictive of LCPM-induced cardiopulmonary diseases in animal models, and by extrapolation, in humans. In addition, this Phase 2 study will identify biomarkers of early response and potential treatment targets. The recently developed particle generation and exposure methods will still be applied to create and characterize real-world PEP exposures for the assessment of cardiopulmonary effects. In vivo inhalation experiments will be performed to assess cardiopulmonary effects and biokinetics of inhaled PEPs and validate as well as extend the in vitro

findings of Phase 1. Particularly, the toxicological effects of PEPs will be measured under preexisting cardiovascular conditions, such as myocardial infarction (MI). Histopathological analysis will be performed for PEP-targeted other organs. Moreover, this Phase 2 study will identify PEP-induced gene signatures from the cultured cells (Phase 1), lung and blood samples from the in vivo exposure models and determine whether these gene signatures predictively match those for known pathways of cardiopulmonary and other organ diseases in humans. This multi-disciplinary screening approach, linking in vitro (Phase 1) and in vivo PEP exposures to in vitro and in vivo toxicological evaluation, as well as computational modeling results will provide a robust and useful framework for risk assessment of engineered nanomaterials released across the life cycle of NEPs. The development of genomic-based non-invasive diagnostic blood tests of cardiopulmonary and other targeted organ diseases can provide valuable predictive biomarkers for surveillance and early risk assessment of engineered nanomaterial exposures.

Researchers at Harvard Public Health's Center for Nanotechnology and Nanotoxicology developed an exposure generation system, Printer Exposure Generation System ("PEGS"), to monitor and assess nanoparticle emissions during printer use. NIOSH and Harvard have been working together for the last four years to study the presence of ENMs in toner formulations currently in use in laser printers and to assess safety implications during consumer use. Researchers at West Virginia University (WVU) have utilized computational toxicology approaches to identify signaling pathways related to multi-walled carbon nanotubes ("MWCNT")-induced lung fibrosis from gene expression profiles of MWCNT-treated mouse lungs. Currently, researchers at WVU are studying a genomic profiling pilot assay to explore PEP-induced genomic changes. The main objective of the research supported by this agreement is to study emitted ENMs-induced pulmonary, cardiovascular and other organ effects resulting from the use of laser printers.

III. Objective

This study will employ "real world" exposure systems to generate and characterize LCPM test particles. In addition, the study will develop and validate an in vivo and integrated multi-tiered toxicological screening approach suitable for evaluating a large number and variety of LCPM particles. Furthermore, the study will determine LCPM bioactivity and identify gene signatures predictive of early pulmonary and cardiovascular responses by combining in vitro, in vivo and computational genomic methods.

a. Hypothesis

Exposure to engineered nanomaterials (ENMs) released from nano-enabled products (NEPs) across their life cycle, namely PEPs, can induce adverse pulmonary and cardiovascular responses.

b. Experimental Design

In the Phase 2 study, emphasis will be given to cardiopulmonary responses, biokinetics of inhaled PEPs, other PEP-targeted organ toxicity, and toxicological and disease prediction-based genomic studies. A state-of-the-art PEP exposure generation system (Aim 1) will be applied and in vivo bioassays (Aim 2) combined with traditional pathological analysis and advanced computational genomic modeling to assess mechanisms of potential PEP-induced cardiopulmonary and other organ diseases and to predict potential disease implications in humans (Aim 3). The integrated approach will identify mechanistic pathways predictive of LCPM-induced cardiopulmonary
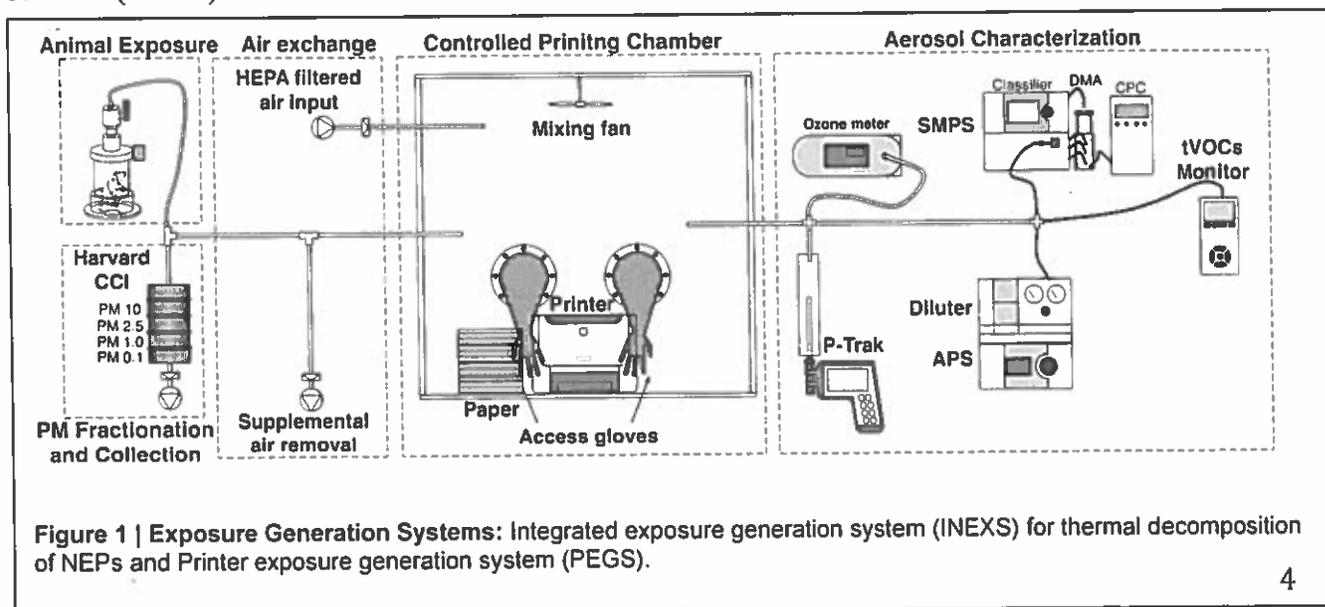
diseases in animal models, and by extrapolation, in humans. In addition, this study will identify biomarkers of early response and potential treatment targets. In Aim 1, the recently developed particle generation and exposure methods will be used to create and characterize real-world PEP exposures for the assessment of cardiopulmonary effects. In Aim 2, in vivo inhalation experiments will be performed to assess cardiopulmonary effects and biokinetics of inhaled PEPs and validate as well as extend the in vitro findings of Phase 1. In Aim 3, histopathological analysis will be conducted on other PEP-targeted organs (i.e., non-cardiopulmonary organs) and identify PEP-induced gene signatures from Phase 1 cultured cells, lung and blood samples from the Phase 2 in vivo models and determine whether these gene signatures predictively match those for known pathways of cardiopulmonary and other organ diseases in humans. It is anticipated that this multi-disciplinary screening approach, linking in vitro (Phase 1) and in vivo PEP exposures to in vitro and in vivo toxicological evaluation, as well as computational modeling results will provide a robust and useful framework for risk assessment of engineered nanomaterials released across the life cycle of NEPs. The development of genomic-based non-invasive diagnostic blood tests of cardiopulmonary and other targeted organ diseases will provide valuable predictive biomarkers for surveillance and early risk assessment of engineered nanomaterial exposures.

The Phase 2 study consists of two parts. The research components of Part A are: 1) generation and characterization of real-time particles released from nano-enabled printers during use for Part A studies; 2) in vivo biokinetic studies via intratracheal instillation; and 3) identification of PEP-induced gene signatures in lungs. The research components of Part B are: 1) generation and characterization of real-time particles released from nano-enabled printers during use for Part B studies; 2) determination of cardiovascular effects; 3) identification of other targeted organ toxicities; and 4) development of non-invasive blood-based genomic biomarkers for surveillance and risk assessment.

c. Methods

Aim 1: Generate and characterize real-time particles released from Nano-Enabled Printers during use (Parts A and B):
Real-time PEPs will be generated as outlined in Figure 1. Size fractionated samples will be collected for physicochemical and morphological characterization (size, shape and composition) using state-of-the-art analytical methods. The servicing agency will focus on the $PM_{0.1}$ size fraction of PEPs (Aim 2).

Figure 1 | Exposure Generation Systems: Integrated exposure generation system (INEXS) for thermal decomposition of NEPs and Printer exposure generation system (PEGS).

4

A suite of real-time instruments and time-integrated sampling methods will be used to measure size distribution and collect size fractionated airborne PM for post-sample physico-chemical, morphological, and toxicological characterization. Particle number concentration as a function of size will be measured in real-time using a Scanning Mobility Particle Sizer (SMPS Model 3091, TSI Inc.) and an Aerodynamic Particle Sizer (APS Model 3321, TSI Inc.). An electrostatic precipitator (ESP, Spokane Laboratories, NIOSH, WA) will be used to collect particles directly onto transmission electron microscopy (TEM) grids for electron microscopic analysis. The Harvard Cascade Impactor (CCI) will be used to size-fractionate and sample the PEPs. The CCI will operate with four stages corresponding to PM10, PM2.5, PM1.0 and PM0.1 (final filter) sizes. The major feature of this sampler is its ability to fractionate by size and collect large amounts of particles (mg quantities) onto inert polyurethane foam impaction substrates without the use of adhesives. The CCI PEP samples will be used for post-sampling gravimetric (mass concentration), chemical and morphological analysis. In addition to the thorough characterization, this study will monitor production of gaseous co-pollutants, such as volatile organic compounds (ToxiRae Plus photo ionization detector, RaeSystems, San Jose, CA). Other important environmental quality parameters (temperature, humidity, etc.) will also be monitored using the Q-Trak Model 8550 and 7565 (TSI, Inc. Shoreview, MN). Additional offline physico-chemical and morphological characterization of collected PEPs will also be performed. The latter characterization will entail the use of high-resolution instrumentation (e.g., ICP-MS, XPS, EELS, XRD, TEM-EDS, FTIR).

Aim 2: Perform biokinetic studies (Part A) and evaluate the cardiopulmonary activity of PEPs using rat exposure studies (Part B)

Clearance and Translocation - Biokinetic studies (Part A): The PEPs will be neutron-activated in order to measure their biopersistence and the potential of the particles or their constituents to translocate to other organs. A set of aliquots of the sampled PEPs will be neutron-activated at a Nuclear Reactor Laboratory. Samples will be irradiated with a thermal [slow] neutron flux of 5 x 10^{13} n/cm^{2}s for up to 120 hours. Long neutron activation of these particles will be necessary because the concentration of metals is relatively low. Depending on composition, the particles brought back from the Nuclear Reactor Laboratory will have the same physical and chemical properties, but will now contain radioactive gamma emitting metals such as Fe, Mn, Cr, Zn, and V. The specific activity will depend on the percent of that metal as well as the nuclear cross-section [the probability that an atom of that metal will capture a neutron] of that element. These radioisotopes have good gamma energies and reasonable half-lives. For example, neutron irradiation of Zn-containing PEPs will produce ^{65}Zn, which decays with a half-life of 244.26 days and emits gamma energies of 345, 770 and 1115 keV. The radioactive particles will be used in physiologically and geochemically based *in vitro* extractions and in pharmacokinetic studies in intact rats. The particles will be given by intratracheal instillation and the rats will be sacrificed at 2 hours, 1 day and 7 days post-exposure. The lungs and twenty other organs and tissues will be analyzed for gamma activity. These data will let us assess the solubility/retention of PEP particles in the lungs and the potential of metal constituents or intact particles to enter other organs of interest (e.g., brain, blood, liver, heart, and kidney). In the case that PEPs are difficult to neutron-activate due to the low per weight metal composition, we will explore identifying an organic chemical tracer molecule using NMR to assess bio-kinetics from inhaled PEPs.

Evaluate the cardiopulmonary activity of PEPs using rat exposure studies (Part B)
Sprague-Dawley rats will be exposed for 21 consecutive days (105 total hours) at 5 hours/day to both PEPs and gaseous co-pollutants released by the laser printer using the generation platform

described in Aim 1. Toxicological assessments will be performed on the following exposure days: 1, 5, 9, 13, 17 and 21 to evaluate the potential toxicological effects of the emissions from the tested laser printer. Particle concentration (mass/m^3), total particle (count/m^3), size distribution, and gases within the animal exposure chambers will be monitored in real-time throughout the study as described in Aim 1. Respiratory and cardiovascular function data will be collected continuously during the 21 days of exposure, and for 4-hour intervals for 5 consecutive days before and after the 21-day exposure. In more detail:

Electrocardiographic (ECG) data acquisition and analysis:
A subgroup of rats will undergo open heart surgery and have implanted a radio telemetry transmitter (DSI PhysioTel® Transmitter ETA-F20; Data Sciences International, Inc., Saint Paul, MN) for measurement of the ECG. Electrodes will be implanted subcutaneously in a Lead II configuration. At least 2 weeks post-implantation, left-ventricular myocardial infarction (MI) is induced by thermocoagulation. Real-time ECG waveforms will be continuously displayed and recorded using a PC-based system (Dataquest ART, Data Sciences International, Inc.). Offline, electrocardiogram (ECG) signals will be reviewed and analyzed using customized software scripts in Matlab (Mathworks, Inc., Natick, MA). Premature ventricular beats (PVBs) will be identified and annotated by an investigator blinded to the exposure status of each animal and the number of PVBs for each exposure hour recorded. A normal control exposed group of rats will be included in this study. If there are >50 PVBs in a given hour, the total number for that hour will be estimated based on review of three 1-min windows positioned at 5, 30, and 55-min into that hour.

To assess heart rate (HR) and heart rate variability (HRV), normal sinus beats will be automatically labeled and subsequently verified by an investigator. We will calculate heart rate as the reciprocal of the 3-min mean normal beat-to-beat interval, SDNN, a measure of total HRV, as the standard deviation of all normal beat-to-beat intervals within a 3-min interval, and RMSSD, a measure of HRV that reflects parasympathetic nervous system activity, as the root-mean-square of successive differences among all normal beat-to-beat intervals within a 3-min interval. HR and HRV will be assessed at 0, 60, 120, 180, and 240 min after the start of the exposure. If the ECG within 10 min of each time point could not be automatically labeled by the software or was otherwise of insufficient quality, no value will be reported for that time point for that rat.

ECG intervals will be estimated every 10 sec using Ponemah Physiology Platform version 4.8 (DSI Ponemah, Valley View, OH). Statistical analyses will be based on the mean PR interval and P wave duration for each rat for each hour.

Respiratory data collection: Briefly, rats will be exposed to PEPs or HEPA-filtered air (FA) in individual plethysmography chambers for 21 days. Continuous respiratory measures will be collected using an automated acquisition system (Buxco/Ponemah, DSI). Data will be reduced to 10 min. averages of the following parameters: frequency (f), tidal volume (TV), inspiratory time (Ti), expiratory time (Te), enhanced pause (Penh), accumulated volume (AV), minute volume (MV), peak inspiratory flow (PIF), peak expiratory flow (PEF), relaxation time (RT), end inspiratory pause (EIP), end expiratory pause (EEP), expiratory flow at 50 % (EF50), and pause (PAU). Other derived parameters will be calculated, including inspiratory duty cycle (IDC) and minute ventilation (Vi).

Lung injury and inflammation: BALF will be analyzed on day(s) 1, 5, 9, 13, 17 and 21 of exposure to assess cytotoxic lung injury (acellular LDH), neutrophil degranulation (myeloperoxidase, MPO) air-blood barrier damage (albumin and hemoglobin), and markers of inflammation and fibrogenesis (total and white blood cell differential counts, cytokine and chemokine concentrations). BALF collected on day(s) 1, 5, 9, 13, 17 and 21 of exposure will also be used to determine whether ROS production will be induced by the PEP exposure. The induction of oxidative stress will be measured by evaluating changes in the cellular content of glutathione (GSH), an important cellular antioxidant. In addition, at 24 hr after the final exposure, BALF will be collected and analyzed for neurotrophic factors, nerve growth factor (NGF) and Brain-derived neurotrophic factor (BDNF) using an ELISA assay. This will confirm the previous *in vitro* studies. At day(s) 1, 5, 9, 13, 17 and 21 of exposure, lung, heart and tracheobronchial lymph nodes tissue will be collected and examined histologically for inflammation and fibrosis; collagen deposition in lung tissue will be measured to further assess fibrogenesis. Chemiluminescence will be performed to assess ROS activity in both the heart and the lung. The rats will undergo the chemiluminescence procedure immediately after the end of the exposure on day(s) 1 and 5 to quantify the levels of *in vivo* chemiluminescence using a photon counter of the cardiac and pulmonary tissue for both PEPs and HEPA-filtered air (FA) exposed groups.

To monitor neurogenic pathways, 10 days prior to PEP exposure, a 5% solution of the fluorescent neural tracer Fast Blue will be instilled into the trachea. This intravital marker permeates the airway wall, is taken up by sensory nerve fibers in the airway epithelium and mucosa, and is transported to nerve cell bodies located in the nodose and jugular ganglia. One day post-exposure, nodose and jugular ganglia will be removed, dissociated using enzymatic digestion and enriched by removing non-neuronal components with centrifugation through 15% albumin. The enriched neuronal pellet will be resuspended in culture medium. Using fluorescence microscopy, fast blue labeled airway neurons will be collected using a cell picker. Non-airway neurons (unlabeled) will be collected as an internal control. The isolated airway neurons will be transferred to tubes and processed for quantitative RT-PCR measurement of trkA, trkB and TRRPV1 receptor and SP mRNA.

Collection of biological samples post-exposure to PEPs: In addition to BALF, blood, aorta, heart, liver, spleen, kidneys, lung lymph nodes and lung samples will be obtained. Also, serum samples will be taken at each time point to evaluate its potential to activate naïve endothelial cells in culture. Specifically, we will measure 1) relative mRNA changes for Vcam1, Icam1, Cxcl2, Ccl2 and IL6; 2) cell migration; and 3) proliferation, using methods previously described. Tissue and whole blood samples from each time point will also be used for gene induction analysis as described in Aim 3.

Evaluation of systemic inflammation: Heart, aorta, and liver tissue will be collected at Harvard, shipped to NIOSH, and evaluated for relative mRNA expression changes for mediators of inflammation (IL6, Cxcl2, Ccl2), acute phase response genes in the liver (Hp, Sap, Saa1), general stress response markers (Hif3a, Mt1, Mt2) and markers of endothelial cell activation (Sele, Vcam1). The panel of markers has been established from previous studies. From all exposures serum will be banked. If warranted and funding and resources are available, the ability of the serum from exposed rats to affect endothelial cell functionality (e.g., migration assays) will be evaluated.

<u>Genomic biomarker identification for risk assessment (Aim 3 see below):</u> The tissue as well as blood samples collected in the previous steps will be used for mRNA and miRNA profiling to identify genomic biomarkers. These biomarkers will be evaluated for potential utility in risk assessment of PEP-induced cardiopulmonary diseases. The following will be performed: 1) identify miRNA markers associated with cardiopulmonary disease in rat blood samples following exposures, and 2) examine whether the identified miRNA and mRNA markers are functionally related for the development of miRNA based non-invasive diagnostic blood tests of cardiopulmonary disease associated with exposure to particles. Further epigenetic changes in the specific genes measured may be done to understand gene expression and methylation level changes.

<u>Statistical analysis:</u> The servicing agency will employ semiparametric regression methods developed previously for continuous respiratory and cardiovascular data. Mean smooth trends will be derived for PEPs and FA-exposed animals, followed by testing using a 2-way ANOVA with Tukey post-hoc test for significant differences between individual groups, outcomes, or time points. Statistical power is estimated accounting for repeated measures using an effective sample size (ESS) $= n \times m/[1 + .5(m - 1)]$, where n is animals per group, and m is number of repeated measures, with a moderate longitudinal correlation of 0.5. Conservatively assuming an effect size of 0.3, with 4 animals per group and 2 measurements, we estimate $> 80\%$ statistical power for detecting significant differences between FA and PEP exposure groups.

Aim 3: Identify LCPM-induced gene signatures predictive of pulmonary and cardiovascular damage for risk analysis and early detection (Part A) and pathological analysis of other LCPM-targeted organs and predict potential disease implications in other organs (Part B):

<u>Part A</u>: 1) Identify and compare pathway-based gene signatures from *in vitro* and *in vivo* PEP-induced genome-wide mRNA expression profiles (Aims 2 and 3), and 2) evaluate the identified PEP-induced gene signatures as predictive indicators of pulmonary and cardiovascular damage for risk surveillance. To identify biomarkers for early detection of potential PEP-induced cardiopulmonary disease, we will: 1) identify miRNA markers associated with cardiopulmonary disease in rat blood samples following exposures (Aims 2 and 3) and examine whether identified miRNA and mRNA markers are functionally related for the development of miRNA based non-invasive diagnostic blood tests of cardiopulmonary disease associated with exposure to particles.

<u>Part B</u>: Heart tissue from rats exposed by whole-body inhalation to PEPs (and FA) will also be preserved for RT-PCR analysis to confirm the gene signatures and biomarkers previously identified from blood samples of rats intratracheally exposed to PEPs. Furthermore, this study will predict possible LCPM-induced disease implications in other tissue organs based on the analysis of genome-wide expression profiles (mRNA and miRNA) in blood samples from rats exposed to PEPs by whole-body inhalation. Bioinformatics analysis will be performed in the genome-wide profiles of the rat blood samples to identify significantly over-represented biological pathways, processes, molecular networks, diseases, and physiological functions. These results will be used to guide the design of future animal and genomic studies to confirm the predicted diseases and their pathogenesis. Tissues from other organs will be examined by certified pathologists to identify PEP-induced abnormalities. These pathological assessments will be correlated with genomic-profiles of

rat blood samples and will be compared with bioinformatic prediction of PEP-induced diseases and altered physiological functions.

Microarray expression profiling. Total RNA will be extracted from the cells (Aim 2) and the frozen rat lung tissue samples (Aim 3) in RNAlater using the RNeasy Fibrous Tissue Mini Kit (Qiagen, USA). Extracted RNA will be analyzed for mRNA profiling using Agilent Whole Genome Arrays (Agilent, Santa Clara, CA) with a universal reference design. Details of total RNA preparation, quality check, microarray hybridization, and image scanning will follow the standard protocols as described in our previous studies. Heart tissue from rats exposed by whole-body inhalation to PEPs (and FA) will also be preserved for RT-PCR analysis to confirm the gene signatures and biomarkers previously identified from blood samples of mice intratracheally exposed to PEPs.

Microarray data preprocessing and filtering. Missing data will be imputed using the K-means nearest neighbor algorithm with *Bioconductor*. For each dose and each time point, a set of differentially expressed genes will be identified by performing a two-class unpaired Significance Analysis of Microarrays (SAM) between the treated samples and zero dose samples from the corresponding time point. The list of significant probes will be subsequently filtered by restricting to those probes which are at least 1.5 fold up- or down-regulated. Additionally, a linear model will be fitted to the data, modeling the log expression of each gene in turn as a function of time, dose, and the interaction of time with dose. The t-statistic associated with the dose and interaction parameters will be moderated following the SAM algorithm and a threshold will be set to control for a false discovery rate of 1%, generating a list of genes whose expression values are significantly dependent on dose and a list of genes whose expression values are significantly dependent on dose in a time-dependent fashion. Significant time and dose interactions will be corroborated with 2-way ANOVA method.

Identification of PEP-induced gene signatures. From the pool of significant genes selected by pairwise-SAM analysis and linear modeling, the following methods will be used to identify Phase 1 *in vitro* particle-induced gene signatures: 1) Gene selection requires significant changes in at least two time points with more than a 1.5-fold change at all doses and significance in the linear model for the dose or the interaction of time and dose. This set of genes would be consistently differentially expressed following nanoparticle treatment; 2) Identify disease processes and biological functions associated with these significant genes using Ingenuity Pathway Analysis; 3) Identify significantly represented pathways relevant to PEP-induced cardiopulmonary disease. Similarly, *in vivo* PEP-induced gene signatures will be identified from genome-wide expression profiling of PEP particle-treated rats (Aim 2) and will be compared with co-cultured cells (Aim 2). Specifically, histopathological scores of *in vivo* lung damage in response to time and dose treatments will be used as phenotype patterns. Coefficients with these patterns will be generated for each gene using non-negative matrix factorization, Monte Carlo Markov Chain simulation, and simulated annealing. Significantly represented pathways relevant to pulmonary or cardiovascular patterns will be selected from comprehensive evaluation of pathways in the MSigDB database using Gene Set Enrichment Analysis. The most significant genes in each selected pathway will form a pathway-based gene signature. Concordant gene sets in the *in vivo* and *in vitro* experiments will be pinpointed. Pending the outcomes of the gene signature analyses, we will investigate whether inhibition of specific mediators (e.g., VEGF, MCP-1, ICAM-1, or TGF-β) by selective inhibitors or gene knock out/down will affect LCPM-induced gene expression profiles in the co-cultured cells, to determine functional involvements of the identified gene signatures. Our preliminary PEP co-culture studies suggest MCP-1 may play a role (Phase 1).

Evaluate the identified gene signatures in predicting human cardiopulmonary disease using patient data for risk assessment. The identified PEP-induced gene signatures will be evaluated in the prediction of risk for potential human cardiopulmonary diseases using published expression profiles in patient tissues. A nearest centroid classification method will be employed to predict these diseases and their progression, using animal-based or cell-based gene expression signatures, focusing on the concordant gene sets in the *in vitro* experiments (Aim 2) and the *in vivo* studies (Aim 3). Alternatively, support vector machine, decision trees, and Naïve Bayes could be used in the prediction. Overall accuracy, sensitivity, specificity, odds ratio, and hazard ratio will be used to evaluate the performance of the gene signatures in risk assessment. The identified cardiopulmonary disease-associated signature genes will be further validated with immunohistochemistry assays of PEP-treated animal tissues (Aim 2) and human patient tissues ($n = 50$) previously obtained from the NIH/NHLBI Biorepository.

Pathological analysis of other PEP-targeted organs. Once the *in vivo* biokinetic studies show the translocation of PEPs to other organs, pathological analysis will be performed for these other PEP-targeted organs by a certified pathologist to identify PEP-induced abnormalities.

miRNA extraction from rat blood samples. Approximately 700 µl of blood will be collected from each rat via cardiac puncture in Aim 3. Total RNA will be extracted using the Rat RiboPure-Blood RNA Isolation Kit (Ambion) according to the manufacturer's protocol.

miRNA expression profiling. Extracted total RNA will be analyzed for miRNA expression profiling by Ocean Ridge Biosciences (Palm Beach Gardens, FL) using custom microarrays incorporating all rat miRNAs present in the Sanger Institute mirBASE version 19.

miRNA data analysis and biomarker identification. Cardiopulmonary-associated miRNAs will be identified from miRNA expression profiles of particle-treated rat lung tissue and blood samples (Aim 3) using the same computational methods as described above. PEP-induced miRNA biomarkers in rat lung tissues and blood samples will be compared to select the miRNAs showing concordant expression patterns. The identified miRNA markers associated with PEP-induced cardiopulmonary damage will be compared with the miRNA expression profiles in human patient samples. The performance of the identified miRNAs in predicting cardiopulmonary disease risk in humans will be evaluated using the methods described above.

Integrated mRNA and miRNA analyses. From the identified miRNA and mRNA markers, significantly correlated miRNA-mRNA pairs will be selected for miRNA target prediction with TargetScan, PicTar, miRDB, and microRNA.org. In addition, TarBase will be used to retrieve experimentally validated miRNA targets. The pathway relevance and functional annotation of the identified miRNA markers will be evaluated with Ingenuity Pathway Analysis. Results from the integrated analysis of PEP-induced mRNA and miRNA expression changes from the rat model will be compared with those from human patients.

Comparative analysis of human and rodent genome. Rat and human genomes will be matched by gene name using the data mining tool Biomart based on Ensemble Genes. For ortholog genes defined as a "one-to-many" or "many-to-many" relationship between the rat and human genome, a randomly selected matched gene pair will be chosen to compute the genome-wide Pearson's coefficient. This process will be repeated 100 times, and the average correlation will be reported as

the final results. The whole genome-scale mRNA profile of each *in vitro* cell condition will be correlated with the global mRNA profile in each *in vivo* dose/time condition of the animal study, respectively. The correlation coefficients will be tested with statistical hypothesis testing and adjusted with multiple hypothesis testing, with a $P < 0.05$ defined as being statistically significant.

Predict possible disease implications in other PEP-targeted organs. The pathological assessments will be correlated with genomic-profiles of rat blood samples and will be compared with bioinformatics prediction of PEP-induced diseases and altered physiological functions. Moreover, pathway analysis will be used to evaluate significant diseases and physiological functions based on genome-wide expression profiles (mRNA and miRNA) of rat blood samples through the use of Ingenuity Pathway Analysis (IPA). The *p*-value determined by IPA is a measure of significance based upon the number of genes/molecules that map to a biological function, pathway, or network, which will be used to determine the statistical significance of disease relevance, physiological functions, biological pathways and processes. This analysis will predict possible PEP-induced disease implications in other tissue organs.

Statistical Assessment: For all biological outcomes, we will calculate descriptive statistics and histograms by exposure group, and construct bivariate scatter plots of response vs. exposure measurements. Multi-way ANOVA will be used to assess differences among exposure groups and interactions of exposure and potential effect modifiers, and linear regression will be used to assess dose-response relationships.

Power analysis for microarray experiments: To determine the number of microarrays needed for each experiment, we will follow the study of Dobbin and Simon. We will use a reference design with a two-color array system and no technical replicates. In each case, we will use a case-control design with equal numbers of arrays in each sample. We use equation 4.2 from Dobbin and Simon to calculate the number of arrays required. The analytical process described in SAM [84] also involves creating random permutations of the sample class labels. In order to generate at least 500 distinct permutations, we will require a minimum of 6 samples in each class, regardless of statistical power. For the cell line experiments, a total of 12 arrays (6 experimental + 6 controls) for each treatment condition will be required to achieve the minimal statistical power. For animal based experiments, 8 arrays per treatment condition will suffice for all but the smallest numbers of true differentially expressed genes on the array.

IV. Project Schedule

Part A
- Generate and characterize real-time particles released from nano-enabled printers during use for biokinetic and gene signature studies: months 1-6 upon execution of this agreement
- Biokinetic studies: months 6-18 upon execution of this agreement.
- Identify LCPM-induced gene signatures predictive of pulmonary and cardiovascular damage for risk analysis and early detection: months 1-24 upon execution of this agreement.

Part B
- Generate and characterize real-time particles released from nano-enabled printers during use to assess for cardiopulmonary and other organ toxicity: months 1-6 upon execution of this agreement

- Evaluate the cardiopulmonary activity of PEPs upon extended rat exposures: months 6-24 upon execution of this agreement
- Pathological analysis of other LCPM-targeted organs and predict potential disease implications in other organs with genomic approaches: months 6-24 upon execution of this agreement

V.   Reporting Requirements/Deliverables

NIOSH will provide preliminary test reports documenting the test protocols and resulting data at the completion of each task as documented in the Project Schedule outlined in paragraph IV, Project Schedule. Within 60 calendar days of completion of all the testing, NIOSH will issue a draft final report for CPSC staff review. Following CPSC staff review, NIOSH will have an additional 30 calendar days to deliver the final report summarizing the test data, including all photographs taken during the studies. NIOSH shall include in the final report any comments, edits or suggested changes requested by CPSC.

A. All recorded test data and findings	2 copies	December 31, 2019
B. Representative photographs	2 copies	December 31, 2019
C. Final report	2 copies	December 31, 2019

VI.   Resources

Under the Interagency Agreement, CPSC will provide funding of $600,000 of Fiscal Year 2017 funds to CDC/NIOSH. CDC/NIOSH shall submit monthly invoice via the IPAC system.

FY17: $600,000

Materials and Supplies	Cost (estimated budget)
Obj Code 25 - Services	
A) Contract (particle generation, characterization and fractionation, Biokinetic studies, *in vivo* evaluation of the bioactivity of PEPs via inhalation)	$270,000
B) Contract (identification of gene signature for PEP-induced cardiopulmonary damage and identification of PEP-induced other organ toxicity with genomic approaches)	$145,000
C) Contracts (mRNA and miRNA profiling)	$75,170.16
Obj Code 31 – Equipment	$49,500
Obj Code 26 - Supplies, Chemicals, Reagents, Assay Kits, labware	

Misc. Lab Supplies	$19,769.84
TOTAL Direct	**$ 559,440**
CDC Indirect Overhead charge (projected at 7.25%)	$40,560
TOTAL	$600,000

VII. Confidentiality Requirements and Data Sharing

a. To the extent permitted by law, all information reported to or otherwise obtained by CPSC or its agents under the Consumer Product Safety Act (CPSA) and provided to or shared with NIOSH, which contains or relates to a trade secret or other matter referred to in section 1905 of title 18, United States Code, or subject to section 552(b)(4) of title 5, United States Code, shall be held in confidence by NIOSH and any NIOSH contractor personnel.

b. To the extent permitted by law, including the Freedom of Information Act, NIOSH and any NIOSH contractor shall agree not to release the identity of any manufacturer or private labeler of any product being tested or reviewed in conjunction with this agreement. These provisions are consistent with and do not supersede, conflict with, or otherwise alter the employee obligations, rights, or liabilities created by existing statute or Executive Order relating to (1) classified information, (2) communications to Congress, (3) the reporting to an Inspector General of a violation of any law, rule, or regulation, or mismanagement, a gross waste of funds, an abuse of authority, or a substantial and specific danger to public health or safety, or (4) any other whistleblower protection. The definitions, requirements, obligations, rights, sanctions, and liabilities created by controlling Executive Orders and statutory provisions are incorporated into this agreement and are controlling.

c. All documents and other materials developed pursuant to this agreement shall have appropriate statements to indicate that the work was performed pursuant to the agreement by CPSC; that the documents and other materials produced are the views of the staff or members (present or past) of the servicing agency; and that although the documents and other materials may have been developed in conjunction with CPSC staff, the documents and other materials do not necessarily represent the views of the Consumer Product Safety Commission.

d. Any publications of or publicity pertaining to the work performed under this agreement shall include the following:

"This project was funded by CPSC. The content of this publication does not necessarily reflect the views of the Commission, nor does mention of trade names, commercial products, or organizations imply endorsement by the Commission."

e. NIOSH agrees that any report, manuscript or other document intended for publication or disclosure to the public and containing the results of work performed under this agreement

(the "Report") shall not identify a manufacturer, private labeler, or particular consumer product. To ensure that the Report does not identify a manufacturer, private labeler, or particular consumer product, NIOSH shall submit the Report to CPSC for review in accordance with section 6(b) of the Consumer Product Safety Act (15 U.S.C. § 2055(b), CPSC regulations at 16 C.F.R. part 1101, and CPSC Directives (Order No. 1450.2). In connection with this review, CPSC will redact from the Report the names of manufacturers and private labelers and the identities of consumer products. If the report contains information that reflects on the safety of a class of consumer products, CPSC will follow its procedures for review of that information.

    f. The servicing agency shall insure that the rights to all information, uses, processes, patents, and other developments resulting from this interagency agreement   will be made available to the public without charge on a nonexclusive basis.

VIII.   Scientific Integrity

CPSC has adopted the following principles on scientific integrity, which CPSC and NIOSH agree to adhere to while performing work pursuant to this agreement:

- Open communication among scientists and technical staff within and outside the Commission is encouraged.
- Professional growth and development of CPSC's scientific and technical staff are supported.
- The credibility of staff's scientific and technical work is encouraged, supported, and recognized.
- Accountability and transparency are expected and supported in communicating to the public the results of scientific and technical work.
- Scientific and technical staff are expected to adhere to a professional code of ethics.
- Protections exist and are expected to be followed to shield staff from undue influence or suppression.

IX.   Duration of Agreement and Amendments

This agreement will become effective on the last date of signature by the parties. The agreement will terminate 32 months after the last date of signature of the agreement by both parties, but may be amended at any time by mutual written consent of the parties.

X.   Disagreements

In the event that CPSC and NIOSH have a disagreement arising under this agreement, the parties shall cooperatively seek to resolve the disagreement by themselves. If the disagreement cannot be resolved between them, the parties agree to seek the assistance of a third party in resolving the disagreement.

XI.   NIOSH will provide personnel, laboratory support, and equipment to perform tasks described under this agreement.

XII. Contacts

    a. The contacts of each party to this agreement are:

        i. <u>CPSC COR</u>

            Kristina Hatelid
            U.S. Consumer Product Safety Commission
            Health Sciences Directorate
            5 Research Place
            Rockville, MD 20850
            301-987-2558
            KHatelid@cpsc.gov

   ii. <u>CPSC Project Officer</u>

            Joanna Matheson, Ph.D.
            U.S. Consumer Product Safety Commission
            Health Sciences Directorate
            5 Research Place
            Rockville, MD 20850
            301-987-2564
            jmatheson@cpsc.gov

        iii. <u>NIOSH Project Officer</u>

            Yong Qian, Ph.D.
            National Institute for Occupational Safety and Health
            1095 Willowdale Road, MS L-2015
            Morgantown, WV 26505
            304-285-6286
            YAQ2@cdc.gov

APPROVED AND ACCEPTED FOR THE NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH/CENTERS FOR DISEASE CONTROL AND PREVENTION:
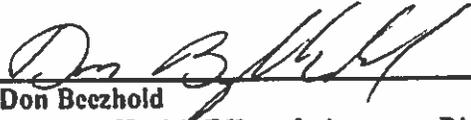
BY: _____   5/24/17
     Don Beezhold   Date
     Director, Health Effects Laboratory Division, NIOSH

APPROVED AND ACCEPTED FOR CONSUMER PRODUCT SAFETY COMMISSION

BY: csterba@cpsc.gov _____

     Cassandra Sterba
     CPSC Contracting Officer   **Date**