Forensic Chemistry

Net Weights: Visualizing and Quantifying their Contribution to Drug Background Levels in Forensic Laboratories

--Manuscript Draft--

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Abstract:	While the drug background in forensic laboratories has been quantified, the processes that contribute to the background have not been extensively researched. This work presents both qualitative visualization and quantitative analysis of the spread of simulant drug particulate during the process of taking net weights. The process was modeled using three masses of powder (0.2 g, 2 g, and 100 g). The net weight process, in which the mixture was poured onto weighing paper, was mimicked and the resulting aerosolized particulate was allowed to settle. Wetted cotton swabs were then used to sample 6.45 cm 2 (1 in 2) squares extending up to 61 cm (24 in) away from the weigh paper. The swabs were then extracted and quantified using LC-MS/MS and two-dimensional color plots were created to visualize the magnitude of particulate spread. Qualitative flow visualization of the process, accomplished using laser light sheet videography, was also completed to support the quantitative extraction experiments and provide a visual representation of the mechanism of particulate spread. Surface concentrations were found to be highest in the area immediately surrounding the weigh paper, though transport as far as 61 cm (24 in) was observed with all mass loadings. The amount of the material aerosolized and transported on the bench surrounding the weigh paper was dependent upon the mass of material being poured. These results highlight that weighing activities encountered in forensic labs may be a primary contributor to drug background and may be a potential source of inhalation exposure for chemists.
Suggested Reviewers:	Daniel Katz, PhD Laboratory Director, Maryland State Police Forensic Science Division daniel.katz@maryland.gov Laboratory director with knowledge of drug chemistry.
	Rebecca Mead, MS Supervisory Chemist, Vermont Forensic Laboratory rebecca.mead@vermont.gov Supervisory drug chemist with extensive experience.
	Robert Kirkby, CIH Industrial Hygienist, Michigan State Police Forensic Science Division kirkbyr@michigan.gov Forensic industrial hygienist with an extensive background in chemist safety,
Opposed Reviewers:	
Response to Reviewers:	

March 24, 2020

Dr. Jose Almirall & Dr. Glen Jackson
Elsevier*, Forensic Chemistry*

Dear Dr. Almiral, Dr. Jackson, Associate Editor(s) and Referees:

We are pleased to submit our manuscript entitled “Net Weights: Visualizing and Quantifying their Contribution to Drug Background Levels in Forensic Laboratories” by Edward Sisco, Matthew Staymates, and Laura Watt for consideration by *Forensic Chemistry*. In this article, we build upon previous work, including “An Easy to Implement Approach for Laboratories to Visualize Particle Spread During the Handling and Analysis of Drug Evidence”, to provide both qualitative and quantitative data to demonstrate particle spread during the net weight process. A combination of laser light sheet imaging and quantitative analysis of a simulated drug mixture by LC-MS/MS highlights the spread of trace particulate more than two feet from the point where the net weight is taken. This work builds upon previous publications that established drug background levels in forensic laboratories by providing forensic chemists insight into the processes that cause particle spread and potentially contribute to background.

This work aims to provide forensic chemists with tools to better understand processes that contribute to elevated drug background levels through a combination of qualitative and quantitative data. Because of the direct implementation to the forensic chemist, we feel this work warrants consideration for publication in *Forensic Chemistry*. The manuscript has been neither published nor submitted elsewhere for publication.

Thank you for your efforts on behalf of the review process. We look forward to your recommendation regarding our manuscript.

Sincerely,

Edward Sisco, Ph.D.

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June 2nd, 2020

Dr. Glen Jackson, Editor-in-Chief

Elsevier*, Forensic Chemistry*

Re: Manuscript ID. FORC-D-20-00037, "Net Weights: Visualizing and Quantifying their Contribution to Drug Background Levels in Forensic Laboratories"

Dear Editor and Referees,

Thank you very much for your letter concerning the above referenced manuscript and the enclosed referees' comments. We find the referees' comments very constructive, and we appreciate the effort they put into thoughtfully reviewing our paper. These comments allowed us to improve the quality of the material presentation and to expand on several specific points that were identified in the review. The specific actions we have taken to address the referees' questions and concerns, keyed to their specific comments, are described in the attached summary of revisions.

We have uploaded the revised manuscript and this revisions letter to the *Forensic Chemistry* website. Please convey our thanks to the reviewers for their helpful comments and efforts on behalf of the review process. Thank you again for your review and consideration of our manuscript for publication.

Sincerely,

Edward Sisco

Manuscript ID. FORC-D-20-00037

SPECIFIC RESPONSES TO THE REVIEWERS

Responses to the reviewer can be found in red text

We would like to thank both the referees for their suggestions and comments. We feel that their comments and questions have made this work substantially better. Responses to the reviewer's specific bullets are presented below in red text.

Reviewer #1:

General Comments:

Well written and well organized paper. Valuable, original research with practical applications.

Specific Comments:

Page 2, Line 41: "Combing" is this supposed to be "Combining"?

Yes, this has been updated in the text.

Page 3, Line 20: Why 5 minutes of settling time prior to sampling? Is this for convenience? Is it driven by knowledge of settling rate of the various particle sizes? Would sampling after a longer or shorter settling time have an impact on the amount of recovered substances? What real world applications does this have for the forensic science laboratory?

The five-minute settling time prior to sampling was chosen mostly for convenience. From the laser light sheet imaging most of the settling occurs within the first minute after the pour is complete. Sampling any time thereafter that would produce similar results regardless of whether it was five minutes, ten minutes, etc. In a practicing forensic laboratory, the five-minute wait period is likely not applicable since the chemist will then move the weigh paper containing the powder onto and off of the balance which will again release particulate. The dumping of the material back into the bag or container would represent another process where particulate release will occur.

Page 3: I recommend including a statement regarding the fact that room air flow was not controlled in your materials and methods section. This was noted in the conclusion, but it should be noted earlier as this was one of my main questions during my initial manuscript review.

The statement "Air flow around the sample collection area was not controlled during the experiments, however standard air conditioning and ventilation within the larger laboratory space was operating at normal conditions. This laboratory is considerably large, with a footprint of roughly 93 m^3 (1000 ft^3) and a ceiling height of almost 6 m (20 ft). The experimental setup for these measurements was located on table that was not located directly under a supply vent." was added to the materials and method section within the paragraph explaining the pouring of the samples.

Page 4, Line 6: "Only minor components of the mixture were quantified as many seized drug samples are minor components in the presence of a cutting agent." I recommend expanding on this concept as it may not be familiar to all readers.

This concept has been further clarified with the following text:

"Only the minor components of the mixture (acetaminophen and benzocaine) were quantified. This was completed because many seized drug samples, especially those containing synthetic opioids, consist of one or more cutting agents in a high weight percentage relative to the actual drug which is present in a low (often single digit) weight percentage."

Page 4, Line 10: Why piracetam?

The analytical method that was used in this work was previously developed for another application that required the use of non-deuterated compounds as internal standards. Piracetam is also a readily available, inexpensive chemical that could be employed by laboratories if they wanted to complete such a study.

Page 11 (Conclusion): General comment - The study assumes air flow is directed away from the individual conducting the pouring (although this was not controlled in the study design). From figures 2, 4, and 6 it can be inferred that there may be substantial particulate settling on the leading edge of the sampling surface. This would result in significant settling on the lap of a chemist seated in front of the weigh station. From the employee's health perspective this would likely be of more consequence than settling on the bench top.
The reviewer is absolutely correct that the possibility exists for the particle to settle into the lap of the examiner. While we failed to call that out specifically, we have considered that, and it is one of the things we are working to measure in the next set of experiments as it could be more consequential to chemist's health and safety. This has been expanded upon in the conclusion.

Reviewer #2:
The authors present a well written, original, interesting and relevant study providing insight in the risk of drug residue spreading in forensic laboratories.
The work is suitable for publication in Forensic Chemistry.
My advice to the Editor : accept with minor revisions.
Regarding these minor revisions please find my comments and suggestions below.

Comments and Suggestions :

1) Materials and Methods, page 2 : It is understandable that the authors do not work with real illicit drug samples but how are they sure that this model system is representative for illicit drug powders (cocaine HCl, amphetamine, etc). Are the particle characteristics similar enough (particle size distribution, electrostatic properties)? In my opinion the authors could and should reflect on this in more detail. For instance a forensic drug expert told me that in his experience amphetamine sulphate will hardly spread whereas heroin base will spread out very easily and that this is density related.
   A significant effort was made to try to ensure that the materials chosen were representative of "real-world" samples, however, there is minimal literature that discusses these crucial characteristics for seized drug samples. Because of the lack of available information, the materials were chosen as they were safe to handle in bulk quantities and would be reasonable to be encountered in a forensic setting. To help address the lack of available information on real-world samples we are currently developing a project to measure and understand some of these fundamental properties (particle size, electrostatic characteristics, particle shape, etc.) with some of our collaborating forensic laboratories.

2) Figure 2, page 6 : I would highly recommend to provide a color indication to differentiate areas which were not analyzed from areas were the amount was below the detection limit?
   We have attempted to display the results in this manner in the past but have received feedback that inclusion of an additional color to differentiate the cells that were not sampled made it even more confusing. Because of this we have removed that information from the figures but provided it in the Supplemental Information. Reference to the respective Supplemental Figure has been added to each figure caption.

3) Table 2, page 7 : What is meant with 'total mass'? Is this the total mass of the squares sampled? If so this is not representative for the overall amount because not all squares

are sampled. Or is this the total amount of an overall sampling of the entire area? If so this sampling process needs to be specified.
The value represented the total mass of material that was recovered. This has been more clearly stated in the caption: “Maximum recovered surface concentration from a single grid square and total mass of material that was recovered from all sample grid squares that were sampled for all replicates and experiments in the study.”

4) Page 7, 2gr experiments : There seems to be a contradiction in the follwing two sentences: "The high level of benzocaine in the single sample was likely the result of several large particles falling off of the weigh paper and onto the grid square closest to the weigh paper since concentrations of hundreds of micrograms per square centimeter were more commonly observed" "Benzocaine, however, was observed at higher concentrations further away from the weigh paper due to its smaller particle size” Or does benzocaine consists of small particles in combination with bigger aggregates? In that case one could doubt whether this is a good reference material.
Our apologies for the confusion between these sentences. What we intended to say was that for one replicate a small cluster of powder escaped the weigh paper and ended up on the benchtop next to the weigh paper. This occurred as part of the pouring process and not as the result of air transport. To clarify, the sentence has been updated to read “The high level of benzocaine in the single sample (Replicate 2, Figure 4E and Supplement Figure 5) was likely the result of several large particles falling off of the weigh paper, during the pouring of the powder, and winding up on the grid square closest to the weigh paper – explaining why concentrations in the range of hundreds of micrograms per square centimeter were observed.”

5) Figure 4, page 8 : Why is the benzocaine vs acetaminophen comparison in terms of level and spread reversed vs the 0.2 gr experiment?
We are unclear what the reviewer is referring to in this point. The axes are consistent for all three plots.

6) Page 9, 100gr experiments : What is the relative effect? If one would take the total mass sampled what fraction would that represent of the total mass weighed and what trend does that represent? I think such a comparison of relative amounts spread makes for a valuable comparison. How would handling 100 gr compare to handling 100 times 1 gr?
The reviewer makes a good point in that the discussion of the relative amounts is beneficial. The relative effects have been added to Table 2 as percentages of material recovered and a short discussion of the stark difference between the 100 g and the 0.2 g / 2g experiments has been added to the 100 g results discussion.

7) Conclusions, page 11 : What do the authors recommend for minimizing sample spread when weighing case work samples in the lab? Could the authors deliberate a bit more what their findings mean for a high volume forensic illicit drug analysis laboratory? In future work the authors could test the efficacy of a number of measures to minimize contamination and spread of illicit drug powder. Another idea for future studies is to sample by taping/stubbing a sample grid and studying the particles on the tape with a technique like SEM. This could give insight in the effect of particle size and type on the spreading behavior.
We feel that without the appropriate additional studies it would be too early to provide recommendations on how to limit particle spread. Another set of experiments is planned

for when the laboratory reopens to look at different mitigation strategies (*i.e.*, working in a hood, working behind a shield, use of larger weight paper, use of a weigh boat, etc.) and different sampling strategies (*i.e.*, taking net weights by pouring into a secondary bag or container instead of directly onto weigh paper) which would allow us to provide recommendations backed by data. This work was presented to provide a framework for how those future studies would take place.

The use of an SEM stub / tape to aid in studying particle size is a potentially unique solution that we will be certain to investigate, and if successful, incorporate into our next set of experiments.

Minor points :

1) Materials and Methods, page 4 : Is there no regular LC column in the system? This must mean that there is no separation of the compounds and that the authors directly analyze the compounds in the MS. Is this correct?
   This is correct, samples were analyzed with only a guard column. This has been explicitly stated in that paragraph by incorporating the following edit to the sentence regarding runtime: "**Since there was no LC column, and therefore no separation, a** run time of 2.5 min was used with a blank run completed between each sample to ensure there was no carryover."

2) Materials and Methods, page 4 : Did the authors check whether the IS had affinity for the cotton swab? Ideally the IS response is not affected by the presence of the swab. Also for the other compounds ideally all material on the swab is fully transferred to the extraction solvent. Is this the case?
   We did not explicit try to separate the differences in collection efficiency (how well the cotton swab collected material) and extraction efficiency (how well the cotton swab released the material). Instead, it was incorporated into a single measurement of process efficiency. Given that there was an almost 90 % efficiency, it is likely that the extraction efficiency is near 100 %. This would be in line with other experiments we have completed with similar analyte.

3) Materials and Methods. page 4 : Was a solution deposited on the surface to study the process efficiency? If so how sure are the authors that this sufficiently mimics the sampling process of powder residue?
   While there is a possibility that solution deposition does not completely mimic powder deposition, it is the only way we had available to deposit a known mass of material onto the surface.

4) Materials and Methods, page 4 : The authors report a sample efficiency of roughly 85%, what is causing the 15% loss? Did the authors do a 2nd extraction of the sampled surface to check whether active ingredient still remains on the surface after sampling? Why is the variation so substantial?
   We did not complete multiple extractions of the cotton swab or multiple samplings of the phenolic resin to attempt to identify the cause of the 15 % loss. Unfortunately, given lab closures this is not something we can accomplish at this time.

5) Materials and Methods, page 4 : Are there there any cooling vans inside the equipment? If the laser is only 1 meter above the table this might affect the air flow? In general there

is hardly information on the air ventilation system, closed doors, the size of the room etc. Is the set-up representative for a standard lab situation?

There is a small cooling fan in the laser head, and the exhaust flow was directed up away from the sampling table. The aerodynamic reach of inlet flow into the fan is negligible at 1 meter, especially considering that the buoyant flow generated by the two experimentalists is over 20 liters/second each (https://doi.org/10.1115/1.2353274). The two researchers performing these visualization experiments were as still as possible before and immediately after the bag pours to try and minimize unnecessary air movement. We have added text in the manuscript that explains the size of the laboratory and comments on the HVAC system.

"Air flow around the sample collection area was not controlled during the experiments, however standard air conditioning and ventilation within the larger laboratory space was operating at normal conditions. This laboratory is considerably large, with a footprint of roughly 93 m^3 (1000 ft^3) and a ceiling height of almost 6 m (20 ft). The experimental setup for these measurements was located on table that was not located directly under a supply vent."

6) Reference 12 : Reference needs to be adjusted, inconsistent use of capitals.

Reference 12 has been updated.

Net Weights: Visualizing and Quantifying their Contribution to Drug Background Levels in Forensic Laboratories

Edward Sisco[a], Matthew E. Staymates[a], Laura M. Watt[a]

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Abstract

While the drug background in forensic laboratories has been quantified, the processes that most contribute to the background have not been extensively researched. This work presents both qualitative visualization and quantitative analysis of the spread of simulant drug particulate during the process of taking net weights. The process was modeled using three masses of powder (0.2 g, 2 g, and 100 g). The net weight process, in which the mixture was poured onto weighing paper, was mimicked and the resulting aerosolized particulate was allowed to settle. Wetted cotton swabs were then used to sample 6.45 cm^2 (1 in^2) squares extending up to 61 cm (24 in) away from the weigh paper. The swabs were then extracted and quantified using LC-MS/MS and two-dimensional color plots were created to visualize the magnitude of particulate spread. Qualitative flow visualization of the process, accomplished using laser light sheet videography, was also completed to support the quantitative extraction experiments and provide a visual representation of the mechanism of particulate spread. Surface concentrations were found to be highest in the area immediately surrounding the weigh paper, though transport as far as 61 cm (24 in) was observed with all mass loadings. The amount of the material aerosolized and transported on the bench surrounding the weigh paper was dependent upon the mass of material being poured. These results highlight that weighing activities encountered in forensic labs may be a primary contributor to drug background and may be a potential source of inhalation exposure for chemists.

Keywords

Drug Analysis; Net Weights; Visualization; Exposure

Introduction

As novel psychoactive substances (NPSs) and synthetic opioids continue to be seen in casework, the push for forensic chemists to perform a safer analysis persists. In order to establish safer protocols, laboratories must first identify practices that lead to increased exposure risks to forensic chemists or technicians and then develop policies and/or procedures to reduce/mitigate these risks. Identification of these practices may be complicated because the contribution of a specific process can be difficult to measure or visualize. Additionally, once practices that pose potential safety risks are identified, relaying the risk to non-scientists, such as attorneys, lawmakers, or management, can be problematic as they are often unfamiliar with the work practices. Because of this, it is critical to develop tools that allow for the creation of easily accessible and digestible forms of such information.

One area where this approach has already occurred is in the measurement and understanding of the trace drug background in drug chemistry units and other areas within forensic laboratories. This work, which involved wipe sampling of surfaces and subsequent quantitation of a panel of drugs, has shown that trace drug residue is present on the large majority of surfaces in forensic laboratories [1–3]. Areas where elevated surface concentrations were observed were typically within the drug chemistry unit and consisted of surfaces such as balances, benches, heat sealers, and microscopes [2,4]. While it is unreasonable to expect laboratories to eliminate surface background due to the nature of their work, measuring and monitoring drug levels can allow laboratory staff to identify where to focus cleaning efforts, develop protocols that could lower background levels, and ensure data quality. Similar efforts have also taken place in police stations [5,6].

Forensic DNA analysis is another area where there has been substantial work completed in understanding factors or processes that contribute to, in this case, DNA background. Work by Finnebraaten *et al* found that individuals speaking in a DNA unit without a mask could transmit DNA up to 120 cm away from where they were standing [7]. Other works identified surfaces where DNA accumulates [8], eliminate air as a potential source of background [9], and measure how well robotic workstations reduce contamination [10]. The factors affecting fiber recovery and contamination in trace evidence examinations have also been investigated [11].

While most of these studies have used real materials to either measure or evaluate these processes, this approach can be problematic in drug analysis due to the high toxicity of materials. The use of simulant materials, however, allows for minimization of potential exposure hazards. This approach was used in recent work to visualize contamination of work surfaces, utensils, and Personal Protective Equipment (PPE) in the analysis of drug evidence [4]. Using fluorescent material and UV-light visualization, the spread of trace drug simulant was observed throughout a drug chemist's workstation because of PPE-contamination from opening and handling the evidence. This type of approach, though purely qualitative, allows for a complimentary understanding of background levels when coupled with the prior quantitative drug background work.

The use of fluorescent material coupled with UV-light visualization may be useful to other forensic disciplines and has been employed in a number of other sectors including the medical [12–14] and food handling [15] fields. A similar particle visualization technique that can be leveraged is laser light sheet imaging [16], where a visible laser sheet is used to visualize the transport of microparticles in the air. The benefit of laser light sheet imaging is that real-time video of particle movement can be captured as opposed to fluorescent material, where imaging of where particulates settle is commonly completed.

In both the visualization study and the trace background study the process of weighing drug evidence was hypothesized as a potential major contributor to drug background levels and has been highlighted as a potential concerning practice by the National Institute of Occupational Safety and Health (NIOSH)[17]. Taking a net weight, where the evidence, presumably a powder, is poured out of its original container onto weigh paper to obtain a weight of just the powder, is one of the most common ways drug evidence is weighed. However, the pouring of the material out of its original container leads to aerosolization of the drug particulate. This work aims to provide a holistic (quantitative and qualitative) understanding of the spread of aerosolized drug particulate during the net weight process. Combining laser light sheet flow visualization with quantitative measurements by liquid chromatography mass spectrometry (LC-MS/MS), the particle spread of a simulant drug mixture was measured up to 61 cm (24 in) from where the material was poured. The results of this work highlight the potential risks of pouring drug evidence in an un-controlled manner while also providing a new methodology to study other work processes common in a forensics laboratory.

Materials and Methods

Chemicals and Materials

The simulant drug mixture that was used for this study consisted of 70 % w/w lactose (Honeywell, Charlotte, NC), 25 % w/w acetaminophen (Sigma, St. Louis, MO), and 5 % w/w benzocaine (Sigma). Particle size distributions for the compounds were determined using sieving (Supplemental Information, Figure S1) and were found to be centered between 150 µm and 300 µm for lactose, >850 µm for acetaminophen, and 300 µm and 600 µm for benzocaine. Three different masses of the mixtures were studied (approximately 0.2 g, 2 g, and 100 g) to identify the effects of total mass on particle spread and to represent different types of evidence that drug chemists may have to handle. Appropriate masses of each compound were weighed directly into 90 mm by 62 mm plastic bags for 0.2 g and 2.0 g experiments or 250 mm by 150 mm plastic bags for 100 g experiments. Prior to pouring, the mixture was agitated in the bag for five minutes. This process was completed in a separate room to ensure it did not contribute to contamination of the test surface.

A phenolic resin benchtop (76 cm by 152 cm) (Global Industrial, Port Washington, NY) was used as the test surface. The surface was scored with a razor blade to create a grid of 24 by 28 squares, with each square measuring 2.54 cm by 2.54 cm (1 in by 1 in) to create a guide for sample collection. The weigh paper was placed at one end of the grid in order to maximize the possible distance from the pouring of the powder. A picture of this setup is shown in Figure 1.

Prior to an experiment being completed, the lab bench was thoroughly cleaned with 200 proof ethanol (Decon Laboratories, King of Prussia, PA) and allowed to dry. Three to five randomly chosen squares were then sampled and extracted to ensure that the bench was clean prior to the experiment. The weigh paper was taped, using double-sided tape, to the phenolic lab bench and the respective amount of the simulant mixture was poured out of the plastic bag and onto the weigh paper from a distance of approximately 7.6 cm (3 in). Gentle tapping of the plastic bag was used to completely empty its contents (as shown in the Supplemental Videos). After pouring, the aerosolized particles were allowed to settle for 5 min prior to sample collection. ~~Air flow within the room was not controlled during the experiments~~ Air flow around the sample collection area was not controlled during the experiments, however standard air conditioning and ventilation within the larger laboratory space was operating at normal conditions. This laboratory is considerably large, with a footprint of roughly 93 m^3 (1000 ft^3) and a ceiling height of almost 6 m (20 ft). The experimental setup for these measurements was located on table that was not located directly under a supply vent.~~.~~ The weigh paper containing the simulant powder was not moved prior to sample collection to minimize the risk of accidental release of more aerosolized particles or spilling of the bulk powder.

Cotton swabs (VWR, Radnor, PA) wetted with 200 proof ethanol were used for sample collection. Collection was completed by wetting the cotton swabs with ethanol, removing excess ethanol with an absorbent towel, and swabbing a single grid square with firm pressure in a unidirectional motion. One swab was used per square. Due to the large number (656) of grid squares, only select squares (Figure S2 and Figure S3) were sampled. In the immediate area around the weigh paper all squares were sampled with striated sampling being used further away. For 0.2 g and 2 g experiments 190 of the 656 grid squares were sampled while for the 100 g experiments 222 of the 656 grid squares were sampled. The sampling pattern was modified for the 100 g experiments because of the need to accommodate larger weigh paper.

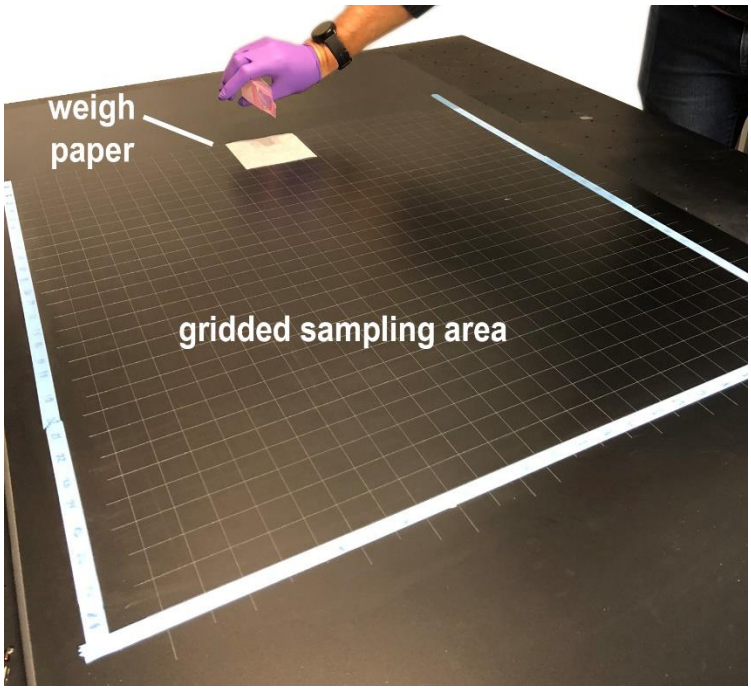

Figure 1. Photograph on the configuration for the net weight experiments.

Mass Spectral Analysis

Quantitation of the distribution of the simulant drug mixture was completed via extraction of the cotton swabs and analysis by LC-MS/MS. Only the minor components of the mixture (acetaminophen and benzocaine) were quantified. This was completed because many seized drug samples, especially those containing synthetic opioids, consist of one or more cutting agents in a high weight percentage relative to the actual drug which is present in a low (typically single digit) weight percentage. ~~as many seized drug samples are minor components in the presence of a cutting agent.~~ Extraction was completed by cutting off the head of the cotton swab and placing it in a 2 mL amber glass vial (Restek, Bellefonte, PA). A 0.5 mL aliquot of methanol (Sigma, Chromasolv-grade) containing piracetam (Sigma) (approximate concentration of 5 µg mL^{-1}) was added, the vial was capped and then vortexed for 10 s. Analysis was completed using a Thermo UltiMate 3000 (Waltham, MA, USA) liquid chromatography (LC) system coupled to a Sciex 4000 QTrap (Framingham, MA, USA) mass spectrometer (MS/MS). LC parameters included a 15 µL injection volume, an isocratic mobile phase consisting of 95 % methanol and 5 % water (both containing 0.1 % v/v formic acid) at a flow rate of 0.7 mL min^{-1}, and the use of a Restek Raptor Biphenyl guard column (2.7 µm x 5 mm x 3 mm). MS parameters included a curtain gas of 10 a.u., an ionspray voltage of +5500 V, a source temperature of 550 °C, an ion source gas 1 of 50 a.u., and an ion source gas 2 of 50 a.u. All analyses were completed in multiple reaction monitoring (MRM) mode, the details of which are shown in Table 1. For each compound (piracetam, acetaminophen, and benzocaine), two MRM transitions were monitored – one for quantitative analysis and a second for confirmation. Since there was no LC column, and therefore no separation, a~~A~~ run time of 2.5 min was used with a blank run completed between each sample to ensure there was no carryover. The peak areas for acetaminophen and benzocaine were ratioed to that of piracetam and compared to an 11-point calibration curve to determine the amount of material recovered and, therefore, the surface concentration of a particular grid square.

Table 1. MRM transition parameters for the quantitative experiments. For all transitions an entrance potential and a cell exit potential of 10 V was used. The top, bolded, transition for each compound was the one used for quantitative analyses.

Compound	Q1 (*m/z*)	Q3 (*m/z*)	Declustering Potential (V)	Collision Energy (V)
Piracetam	**143**	**98**	**40**	**20**
	143	69	40	40
Acetaminophen	**152**	**65**	**50**	**43**
	152	93	50	30
Benzocaine	**166**	**65**	**25**	**55**
	166	77	25	41

Process Efficiency and Background

An additional study was completed to establish the efficiency (both collection and extraction) of the sample collection and extraction process. This was completed by solution depositing 10 µg of acetaminophen and benzocaine in 2.54 cm by 2.54 cm (1 in by 1 in) squares on a separate piece of phenolic resin laboratory bench. These squares were then sampled and extracted using the above protocols. Ten replicates were completed and process efficiencies of 87.5 % (±22.0 %) and 86.7 % (±25.9 %) were found for acetaminophen and benzocaine, respectively. Extractions of the benchtop surface without any material present were also completed to identify if there was any background signal from the collection and extraction process. A slight signal was observed in the benzocaine channel, and therefore a low mass cutoff of 0.10 µg mL^{-1} was employed for benzocaine. This cutoff value was above the limit of quantitation for the method, which was 0.01 µg mL^{-1} for both compounds. No background signal was observed at the acetaminophen or piracetam channels.

Visualization of the Process

A 5 Watt, 532 nm continuous laser (civillaser.com) was used for particle illumination during the pouring experiments. A custom laser sheet generator was built for this laser that consisted of a 5 mm cylindrical glass rod and a 3D printed mounting bracket that registered the glass rod along the laser axis. The laser was positioned via tripod about 1 m above the pour area and at 45° from horizontal to minimize shadowing.

A high-definition video camera (Sony Handycam, Sony, Toyko, Japan) was used to record the pouring events.

A custom LabVIEW code was developed to aid in post-processing of the quantitative data. We chose a color mapping approach to illustrate the magnitude of contamination spread for each bag pour. The LabVIEW code inputed the extraction data as a series of X and Y positions, and then converted the extraction values into a color value based on a predetermined color scale. A bilinear interpolation algorithm was used to smooth the data and help with interpretation of the results. The final export from the LabVIEW code was a 2-dimensional color plot and scale bar. Adobe Photoshop was then used to map this color plot onto an aerial photograph of the actual baggie pour location, resulting in a birds-eye-view of the bag pour and subsequent spread of aerosolized drug particulate during the net weight process.

Results and Discussion

0.2 g Experiments

Three different mass loadings (0.2 g, 2 g, and 100 g) were chosen for these experiments to represent different amounts of powder that may be received as evidence in a forensic laboratory. For each mass loading three replicate experiments were completed. The quantitative data from the grid samples containing acetaminophen and benzocaine were then taken to create two-dimensional color plots that allowed for visualization of particle spread over the benchtop. The resulting color plots of the 0.2 g replicates are shown in Figure 2 and Figure S4. Still images taken during the laser light sheet visualization of one of these replicates are shown in Figure 3 and the corresponding video of the bag pour can be found in Supplemental Information (Supplemental Video 1).

For the 0.2 g experiments particulate spread was detected up to 61 cm (24 in away) from where the material was poured (Figure 2). For all three replicates, the immediate area (approximately 10 cm, or 4 in) surrounding the weigh paper had the highest surface concentrations for both acetaminophen and benzocaine. Concentrations greater than 0.78 µg cm^{-2} (5 µg in^{-2}) were exclusive to this area as evidenced by the black color in the 2-D colorplot (Figure 2). Maximum surface concentrations from a single grid square were 3.6 µg cm^{-2} (23 µg in^{-2}) and 9.6 µg cm^{-2} (62 µg in^{-2}) for acetaminophen and benzocaine, respectively. The maximum recovered surface concentrations and total mass extracted for all replicates can be found in Table 2. In the immediate area around the weigh paper recovery of acetaminophen and benzocaine was well co-located though divergence of the two at farther distances was observed and may be a function of differences in particle size. Outside of the 10 cm (4 in) area around the weigh paper surface concentrations dropped significantly and individual samples were, largely, at or below 0.25 µg cm^{-2} (1.5 µg in^{-2}). Several individual grid squares did have higher concentrations, presumably caused by the settling of larger particles. The spread of benzocaine in all replicates was further than that of acetaminophen, even though it was the minor component, which is likely the result of the small average particle size for benzocaine, as compared to acetaminophen.

Interestingly, there was a large amount of variation in where the particulate settled between replicates. In Replicate 1 (Figure 2 A. and D.) much of the particulate settled directly in front of the weigh paper, whereas Replicate 2 and Replicate 3 trended towards the right and left sides of the weigh paper. This is likely a combination of effects from both the exact location on the weigh paper where the bulk powder was poured and the positioning of the hand during the pouring process. Differences may also be attributed to the force with which the plastic bag had to be agitated to completely remove the powder. Additionally, no special efforts were made to control or mitigate airflow within the laboratory during these experiments, so it is possible that the ventilation system created air perturbances that contributed to differences between replicates.

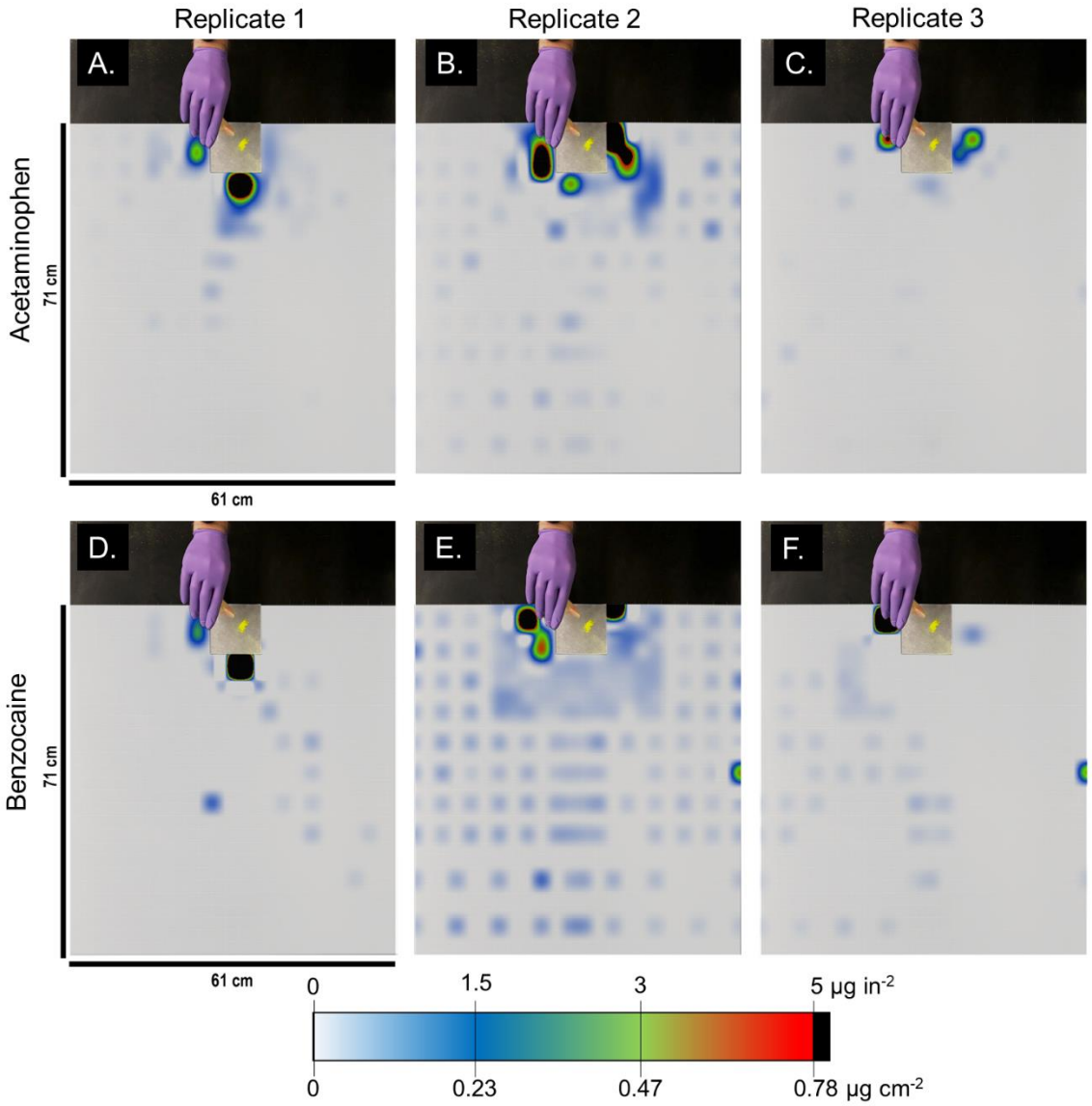

Figure 2. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (D., E., and F.) from the three replicates of the 0.2 g experiment. Areas of white indicate either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 2. Areas of black indicate grid squares where the surface concentration was in excess of 0.78 µg cm^{-2} (5 µg in^{-2}). This cutoff was used to allow for better visualization of the areas with lower surface concentration. Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S4).

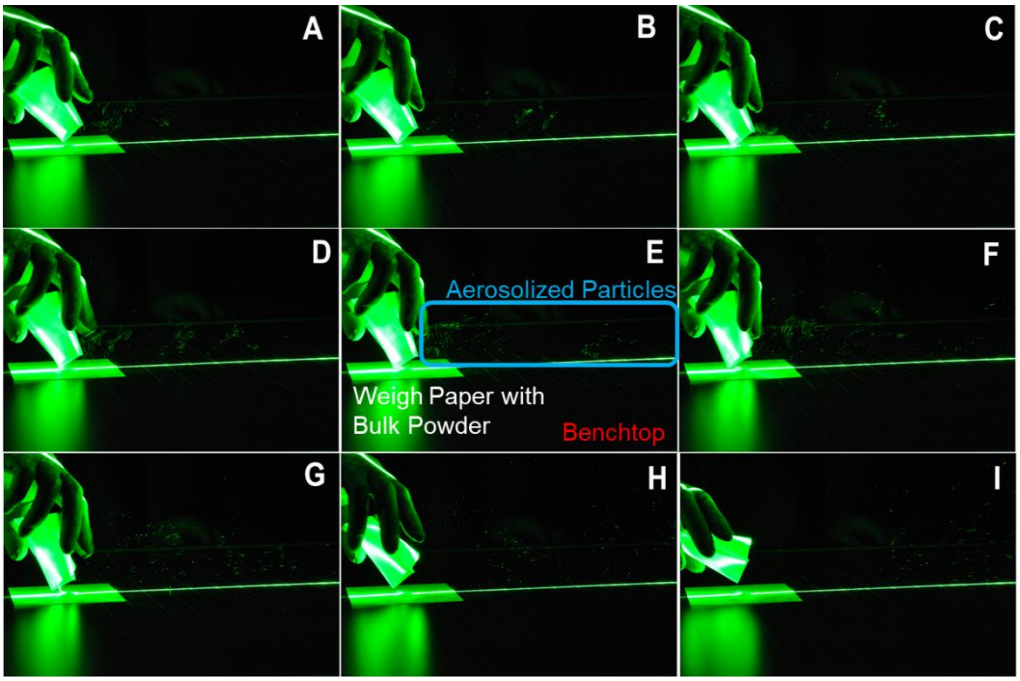

Figure 3. Still images from the laser light sheet visualization of a 0.2 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 1).

Table 2. Maximum recovered surface concentration from a single grid square and total mass of material that was recovered from all ~~sample~~ grid squares that were sampled for all replicates and experiments in the study. Also provided is the percentage of compound recovered from all grid squares that were sampled, relative to the initial mass of each compound for each experiment.

		Max Surface Concentration (µg cm^{-2})			Total Amount Recovered (µg)			Percentage of Compound Recovered (%)		
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0.2 g	Acetaminophen	2.9	3.5	0.8	47.0	104.9	15.3	0.094	0.210	0.031
	Benzocaine	9.7	4.8	7.1	74.5	121.7	58.4	0.745	1.217	0.584
2 g	Acetaminophen	8.4	30.6	24.2	232.1	522.4	403.5	0.046	0.104	0.081
	Benzocaine	40.5	216.4	21.3	925.8	2338.1	536.7	0.925	2.338	0.537
100 g	Acetaminophen	38.8	37.5	43.0	1014.7	1012.7	594.0	0.004	0.004	0.002
	Benzocaine	35.2	33.1	19.9	1731.3	1612.3	445.0	0.034	0.032	0.009

2.0 g Experiments

Figures 4 and 5 show the results of using approximately 2 g of the mixture. Like the 0.2 g experiments, the bulk of the aerosolized particulate fell within the immediate area surrounding the weigh paper though detectable amounts were found up to 61 cm (24 in) away. The increase in total mass poured translated to an increase in average surface concentration, as expected. Maximum recovered samples for single samples of acetaminophen and benzocaine were 30.6 µg cm^{-2} (197.2 µg in^{-2}) and 216 µg cm^{-2} (1396.5 µg in^{-2}), respectively. The high level of benzocaine in the single sample (Replicate 2, Figure 4E and Supplement Figure 5) was likely the result of several large particles falling off of the weigh paper, during the pouring of the powder, and winding up on~~to~~ the grid square closest to the weigh paper – ~~since~~explaining why concentrations in the range of hundreds of micrograms per square centimeter were ~~more commonly~~ observed.

For the 2 g experiments the majority of the aerosolized particles fell directly in front of the weigh paper. As with the 0.2 g experiments, high levels of acetaminophen were co-located with high levels of benzocaine.

Benzocaine, however, was observed at higher concentrations further away from the weigh paper due to its smaller particle size. The overall lower surface concentrations of acetaminophen, compared to benzocaine, highlight the importance of particle size on aerosol release. There was five times the amount of acetaminophen, compared to benzocaine, in the starting powder, yet for all 0.2 g and 2 g replicates a lower overall mass of acetaminophen was recovered from the surface.

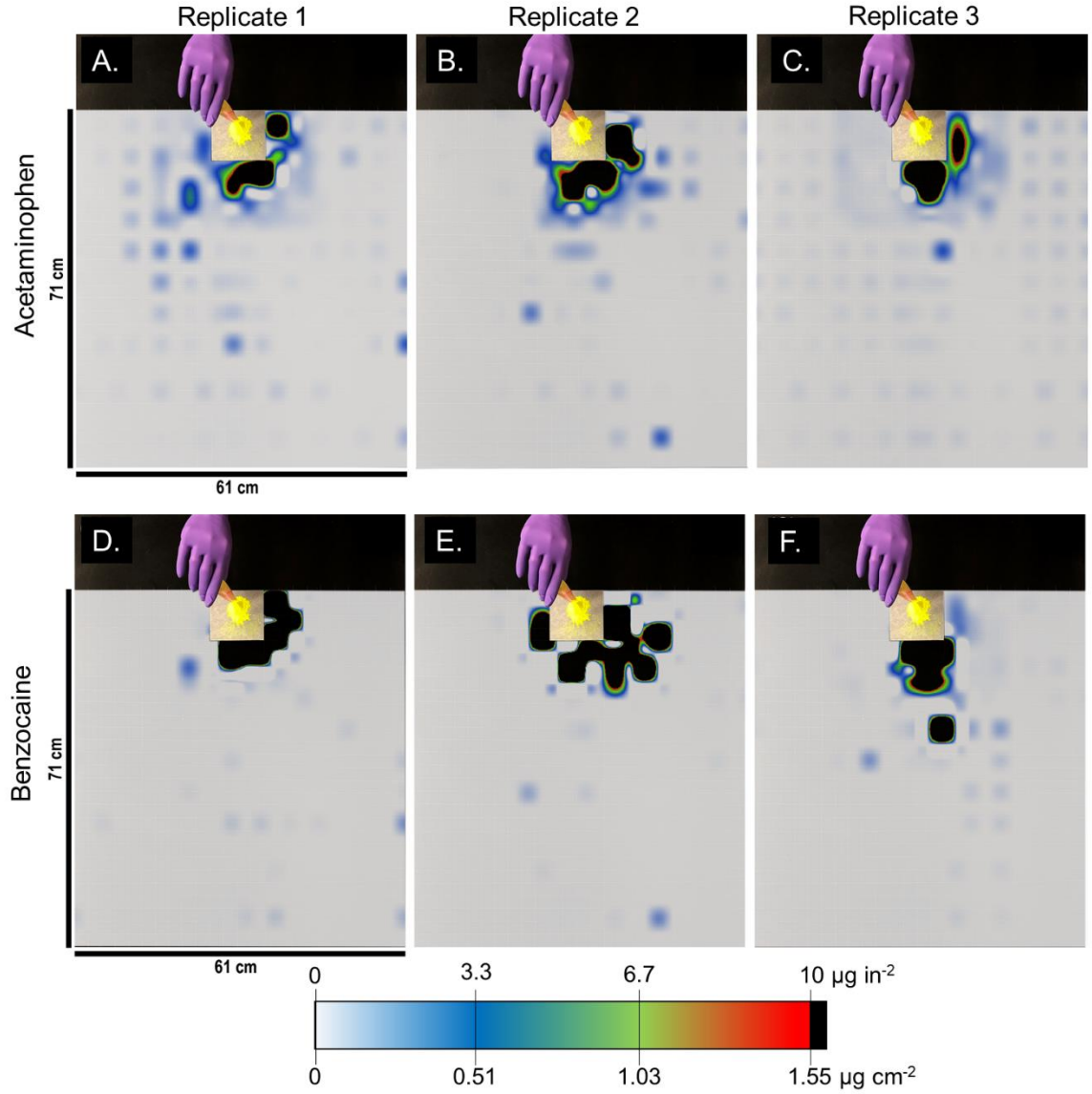

Figure 4. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (D., E., and F.) from the three replicates of the 2 g experiment. Areas of white indicate either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 2. Areas of black indicate grid squares where the surface concentration was in excess of 1.55 µg cm^{-2} (10 µg in^{-2}). The cutoff was used to allow for better visualization of the areas with lower surface concentration. Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S5).

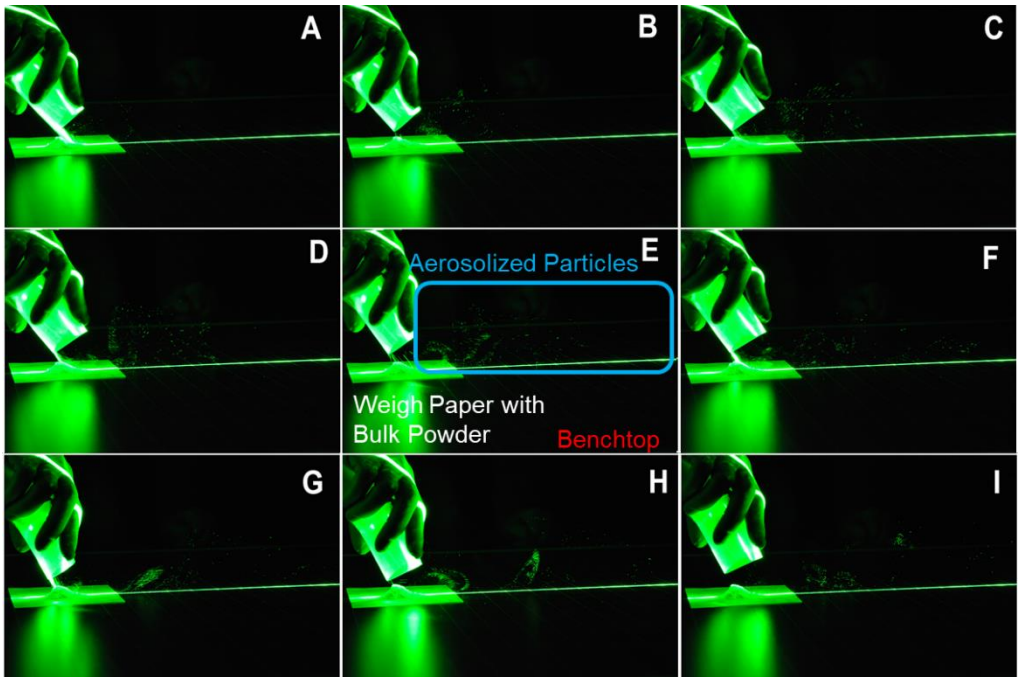

Figure 5. Still images from laser light sheet visualization of a 2 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 2).

100 g Mixture Simulant

Figures 6 and 7 show the quantitative and qualitative results from the 100 g net weight experiments. Because of the higher mass of material being poured, a larger sheet of weigh paper (14 cm by 20 cm, 5.5 in by 8 in) was used to contain the material which required a modified sampling scheme (Figure S2). Interestingly, the results from the 100 g experiments show substantially different trends compared to the 0.2 g and 2 g experiments. Lower acetaminophen and benzocaine surface concentration in the area immediately surrounding the weigh paper were measured compared to high concentration pockets of the two compounds throughout the entire sampling surface. One of the replicates (Replicate 1) did have a high concentration sample near the weigh paper, but the large area of high surface concentration was not observed. While the color plots in Figure 6 (A., B., C., E., F., and G.) appear to show minimal levels of benzocaine or acetaminophen in the area around the weigh paper, this is largely an artifact of the scaling. Figure 6 (D. and H.) shows the Replicate 2 data scaled to the same values as the 2 g experiments (Figure 4). Under this scaling it is clear that the area immediately around the weigh paper has substantial levels of both compounds as would be expected from the 0.2 g and 2 g experiments.

Overall, surface concentrations were significantly higher for the 100 g experiments, compared to the 0.2 g, but were not substantially higher than the 2 g experiments. Table 2 also presents a similar trend when looking at percentage of material recovered. For the 0.2 g and 2 g experiments, similar percentages of acetaminophen (approximately 0.1 %) and benzocaine (approximately 1 %) were recovered. However, the percentage recovered was substantial lower for the 100 g experiments (approximately 0.003 % and 0.025 % for acetaminophen and benzocaine, respectively). This difference may be due to the larger piece of weigh paper that was used but may also be a factor of greater spread further from the weigh paper for the 100 g experiments, as observed with high concentration samples at distances greater than 45 cm (18 in) away. As with the previous experiments, benzocaine was found to travel further than acetaminophen. However, for Replicate 3 of the 100 g experiments, a higher recovered mass of acetaminophen was observed, when compared to benzocaine. Given the two-dimensional color plot of this replicate, it appears as though that was driven by a single grid square with an abnormally high concentration.

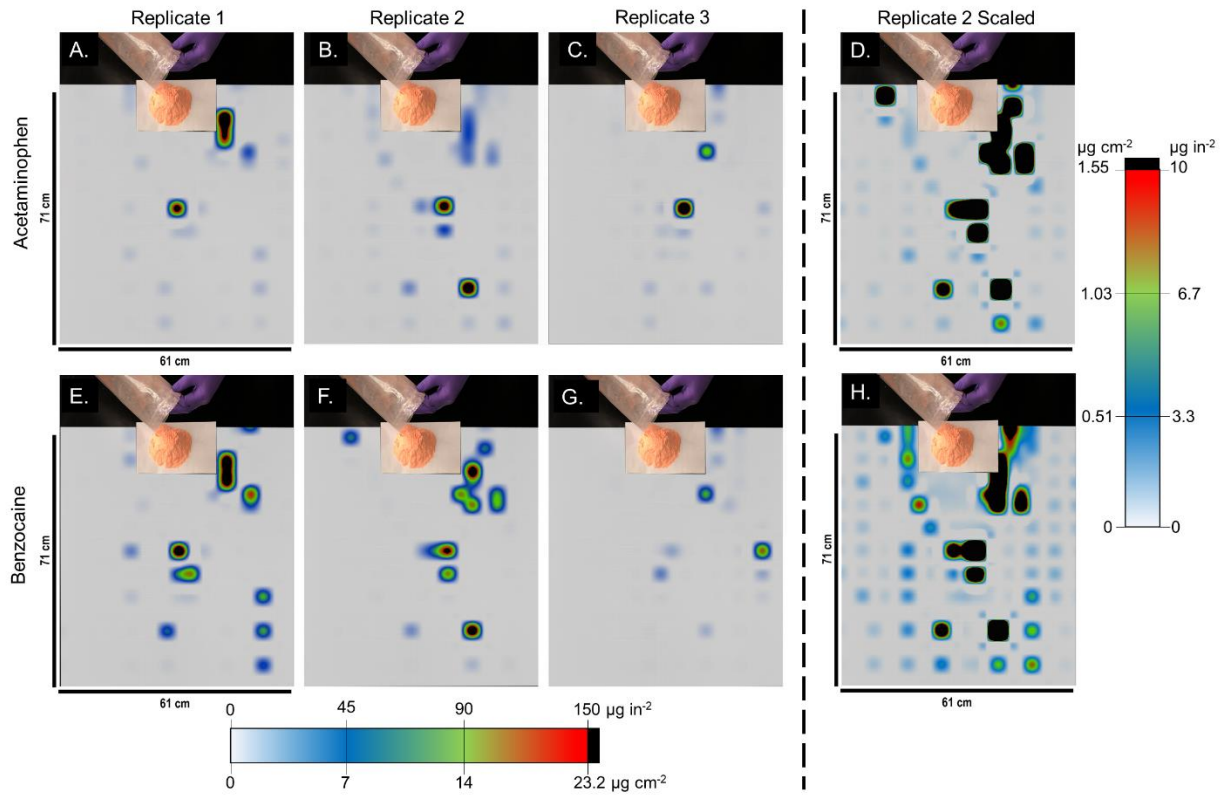

Figure 6. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (E., F., and G.) from the three replicates of the 100 g experiment. Areas of white indicate that either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 3. Areas of black indicate grid squares where the surface concentration was in excess of 23.2 μg cm^{-2} (150 μg in^{-2}). The cutoff was used to allow for better visualization of the areas with lower surface concentration. A second color plot of Replicate 2, scaled to a cutoff concentration of 1.55 μg cm^{-2} (10 μg in^{-2}), is shown for acetaminophen (D.) and benzocaine (H.). Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S6).

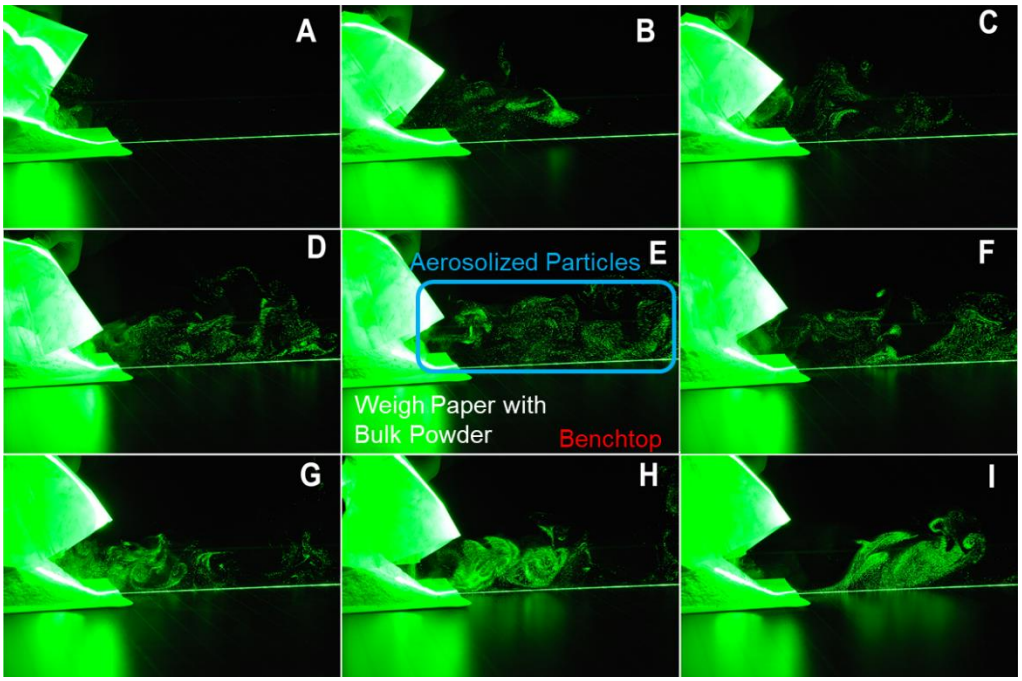

Figure 7. Still images from laser light sheet visualization of a 100 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 3).

Conclusion

This study presents a quantitative approach for measuring drug background levels on planar surfaces after a common net weight exercise. A combination of wet swab sampling and LC-MS/MS provided quantitative data for two-dimensional particle spread from the net weight activity. Three mass loadings were evaluated (0.2g, 2g, and 100g), and each loading level demonstrated significant particle transport from the weigh paper to the surrounding surfaces. Generally, background levels increased with increasing mass of material. Background levels as high as 23 µg cm^{-2} (150 µg in^{-2}) were measured at distances 61 cm (24 in) from the weigh paper.

This study also presents a qualitative flow visualization approach for understanding the mechanisms driving background ~~contamination~~ on surfaces. This visualization methodology complements the quantitative extraction process by uncovering how microparticles are transported in the air during net weight activities. During a common dumping activity, bulk powder is emptied from original container (usually a baggie) onto weigh paper. This serves to aerosolize some fraction of the particulate sample, and natural airflows in the room then transport this material away from the source where it eventually settles onto surrounding surfaces. This flow visualization tool may be useful for chemists and other personnel that handle drug samples that want to fully understand the impact of handling bulk powders in laboratory settings. Given the long distances at which particles were found to spread, it is conceivable that particle will settle on other surfaces besides the laboratory bench (*i.e.*, the chemist's lap, the floor, surrounding work areas). Current work is focused on understanding the possibility and probability of these types of spread, measuring the effectiveness of strategies to mitigate particulate spread, ~~-~~as well as quantitating the net weight~~is~~ process in the third dimension to provide a better understanding of the direct inhalation risk to a chemist.

Disclaimer

Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

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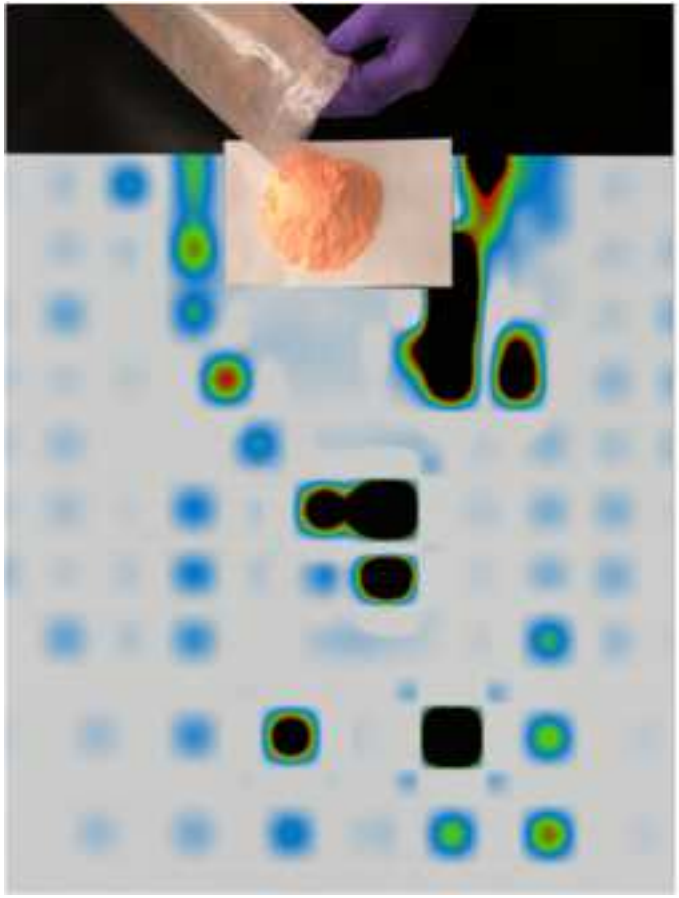
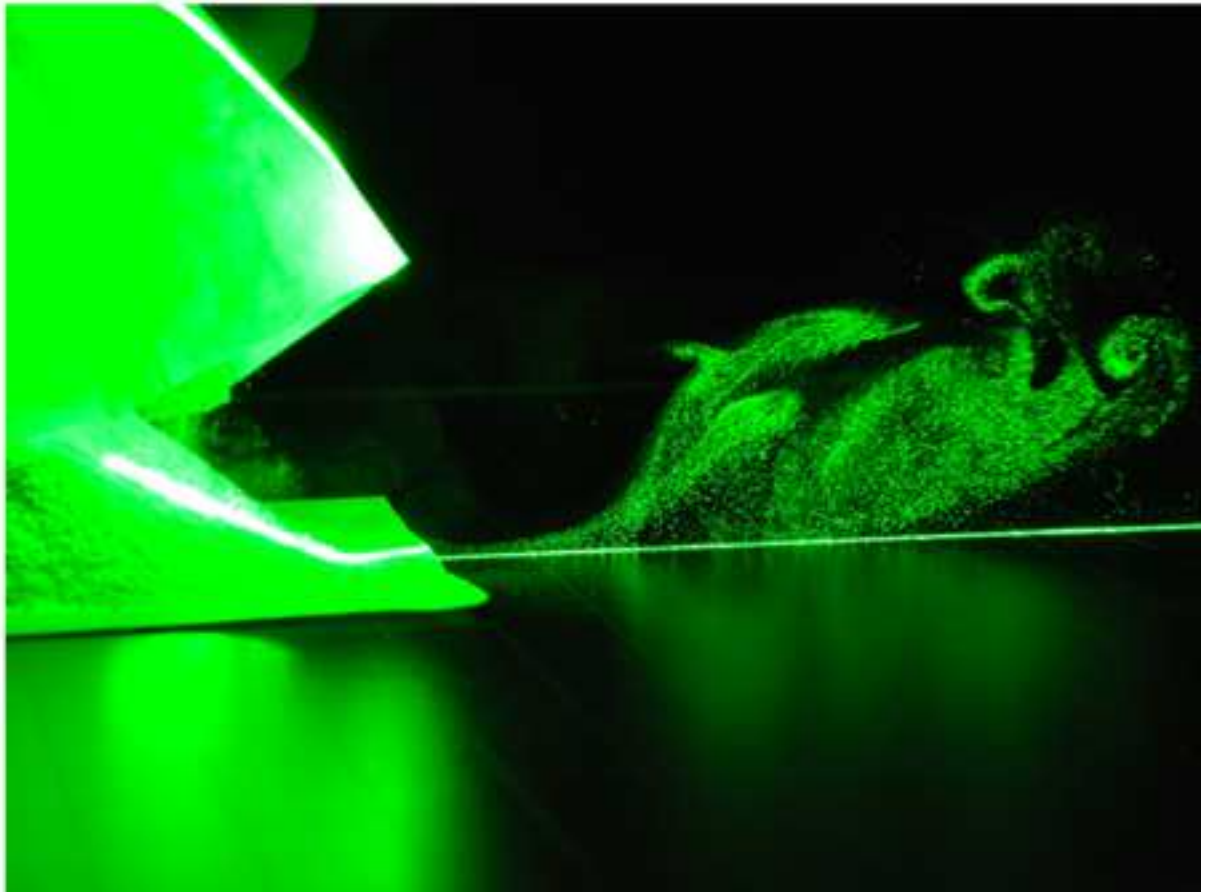
Highlights

- Qualitative and quantitative analysis of net weight particulate spread completed.
- Three masses (0.2 g, 2 g, and 100 g) investigated.
- Particle spread found up to 61 cm away.
- Background levels up to 216 µg cm^{-2} observed.

Graphical Abstract





Net Weights: Visualizing and Quantifying their Contribution to Drug Background Levels in Forensic Laboratories

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Abstract

While the drug background in forensic laboratories has been quantified, the processes that most contribute to the background have not been extensively researched. This work presents both qualitative visualization and quantitative analysis of the spread of simulant drug particulate during the process of taking net weights. The process was modeled using three masses of powder (0.2 g, 2 g, and 100 g). The net weight process, in which the mixture was poured onto weighing paper, was mimicked and the resulting aerosolized particulate was allowed to settle. Wetted cotton swabs were then used to sample 6.45 cm^2 (1 in^2) squares extending up to 61 cm (24 in) away from the weigh paper. The swabs were then extracted and quantified using LC-MS/MS and two-dimensional color plots were created to visualize the magnitude of particulate spread. Qualitative flow visualization of the process, accomplished using laser light sheet videography, was also completed to support the quantitative extraction experiments and provide a visual representation of the mechanism of particulate spread. Surface concentrations were found to be highest in the area immediately surrounding the weigh paper, though transport as far as 61 cm (24 in) was observed with all mass loadings. The amount of the material aerosolized and transported on the bench surrounding the weigh paper was dependent upon the mass of material being poured. These results highlight that weighing activities encountered in forensic labs may be a primary contributor to drug background and may be a potential source of inhalation exposure for chemists.

Keywords

Drug Analysis; Net Weights; Visualization; Exposure

Introduction

As novel psychoactive substances (NPSs) and synthetic opioids continue to be seen in casework, the push for forensic chemists to perform a safer analysis persists. In order to establish safer protocols, laboratories must first identify practices that lead to increased exposure risks to forensic chemists or technicians and then develop policies and/or procedures to reduce/mitigate these risks. Identification of these practices may be complicated because the contribution of a specific process can be difficult to measure or visualize. Additionally, once practices that pose potential safety risks are identified, relaying the risk to non-scientists, such as attorneys, lawmakers, or management, can be problematic as they are often unfamiliar with the work practices. Because of this, it is critical to develop tools that allow for the creation of easily accessible and digestible forms of such information.

One area where this approach has already occurred is in the measurement and understanding of the trace drug background in drug chemistry units and other areas within forensic laboratories. This work, which involved wipe sampling of surfaces and subsequent quantitation of a panel of drugs, has shown that trace drug residue is present on the large majority of surfaces in forensic laboratories [1–3]. Areas where elevated surface concentrations were observed were typically within the drug chemistry unit and consisted of surfaces such as balances, benches, heat sealers, and microscopes [2,4]. While it is unreasonable to expect laboratories to eliminate surface background due to the nature of their work, measuring and monitoring drug levels can allow laboratory staff to identify where to focus cleaning efforts, develop protocols that could lower background levels, and ensure data quality. Similar efforts have also taken place in police stations [5,6].

Forensic DNA analysis is another area where there has been substantial work completed in understanding factors or processes that contribute to, in this case, DNA background. Work by Finnebraaten *et al* found that individuals speaking in a DNA unit without a mask could transmit DNA up to 120 cm away from where they were standing [7]. Other works identified surfaces where DNA accumulates [8], eliminate air as a potential source of background [9], and measure how well robotic workstations reduce contamination [10]. The factors affecting fiber recovery and contamination in trace evidence examinations have also been investigated [11].

While most of these studies have used real materials to either measure or evaluate these processes, this approach can be problematic in drug analysis due to the high toxicity of materials. The use of simulant materials, however, allows for minimization of potential exposure hazards. This approach was used in recent work to visualize contamination of work surfaces, utensils, and Personal Protective Equipment (PPE) in the analysis of drug evidence [4]. Using fluorescent material and UV-light visualization, the spread of trace drug simulant was observed throughout a drug chemist's workstation because of PPE-contamination from opening and handling the evidence. This type of approach, though purely qualitative, allows for a complimentary understanding of background levels when coupled with the prior quantitative drug background work.

The use of fluorescent material coupled with UV-light visualization may be useful to other forensic disciplines and has been employed in a number of other sectors including the medical [12–14] and food handling [15] fields. A similar particle visualization technique that can be leveraged is laser light sheet imaging [16], where a visible laser sheet is used to visualize the transport of microparticles in the air. The benefit of laser light sheet imaging is that real-time video of particle movement can be captured as opposed to fluorescent material, where imaging of where particulates settle is commonly completed.

In both the visualization study and the trace background study the process of weighing drug evidence was hypothesized as a potential major contributor to drug background levels and has been highlighted as a potential concerning practice by the National Institute of Occupational Safety and Health (NIOSH)[17]. Taking a net weight, where the evidence, presumably a powder, is poured out of its original container onto weigh paper to obtain a weight of just the powder, is one of the most common ways drug evidence is weighed. However, the pouring of the material out of its original container leads to aerosolization of the drug particulate. This work aims to provide a holistic (quantitative and qualitative) understanding of the spread of aerosolized drug particulate during the net weight process. Combining laser light sheet flow visualization with quantitative measurements by liquid chromatography mass spectrometry (LC-MS/MS), the particle spread of a simulant drug mixture was measured up to 61 cm (24 in) from where the material was poured. The results of this work highlight the potential risks of pouring drug evidence in an un-controlled manner while also providing a new methodology to study other work processes common in a forensics laboratory.

Materials and Methods

Chemicals and Materials

The simulant drug mixture that was used for this study consisted of 70 % w/w lactose (Honeywell, Charlotte, NC), 25 % w/w acetaminophen (Sigma, St. Louis, MO), and 5 % w/w benzocaine (Sigma). Particle size distributions for the compounds were determined using sieving (Supplemental Information, Figure S1) and were found to be centered between 150 µm and 300 µm for lactose, >850 µm for acetaminophen, and 300 µm and 600 µm for benzocaine. Three different masses of the mixtures were studied (approximately 0.2 g, 2 g, and 100 g) to identify the effects of total mass on particle spread and to represent different types of evidence that drug chemists may have to handle. Appropriate masses of each compound were weighed directly into 90 mm by 62 mm plastic bags for 0.2 g and 2.0 g experiments or 250 mm by 150 mm plastic bags for 100 g experiments. Prior to pouring, the mixture was agitated in the bag for five minutes. This process was completed in a separate room to ensure it did not contribute to contamination of the test surface.

A phenolic resin benchtop (76 cm by 152 cm) (Global Industrial, Port Washington, NY) was used as the test surface. The surface was scored with a razor blade to create a grid of 24 by 28 squares, with each square measuring 2.54 cm by 2.54 cm (1 in by 1 in) to create a guide for sample collection. The weigh paper was placed at one end of the grid in order to maximize the possible distance from the pouring of the powder. A picture of this setup is shown in Figure 1.

Prior to an experiment being completed, the lab bench was thoroughly cleaned with 200 proof ethanol (Decon Laboratories, King of Prussia, PA) and allowed to dry. Three to five randomly chosen squares were then sampled and extracted to ensure that the bench was clean prior to the experiment. The weigh paper was taped, using double-sided tape, to the phenolic lab bench and the respective amount of the simulant mixture was poured out of the plastic bag and onto the weigh paper from a distance of approximately 7.6 cm (3 in). Gentle tapping of the plastic bag was used to completely empty its contents (as shown in the Supplemental Videos). After pouring, the aerosolized particles were allowed to settle for 5 min prior to sample collection. Air flow around the sample collection area was not controlled during the experiments, however standard air conditioning and ventilation within the larger laboratory space was operating at normal conditions. This laboratory is considerably large, with a footprint of roughly 93 m^3 (1000 ft^3) and a ceiling height of almost 6 m (20 ft). The experimental setup for these measurements was located on table that was not located directly under a supply vent. The weigh paper containing the simulant powder was not moved prior to sample collection to minimize the risk of accidental release of more aerosolized particles or spilling of the bulk powder.

Cotton swabs (VWR, Radnor, PA) wetted with 200 proof ethanol were used for sample collection. Collection was completed by wetting the cotton swabs with ethanol, removing excess ethanol with an absorbent towel, and swabbing a single grid square with firm pressure in a unidirectional motion. One swab was used per square. Due to the large number (656) of grid squares, only select squares (Figure S2 and Figure S3) were sampled. In the immediate area around the weigh paper all squares were sampled with striated sampling being used further away. For 0.2 g and 2 g experiments 190 of the 656 grid squares were sampled while for the 100 g experiments 222 of the 656 grid squares were sampled. The sampling pattern was modified for the 100 g experiments because of the need to accommodate larger weigh paper.

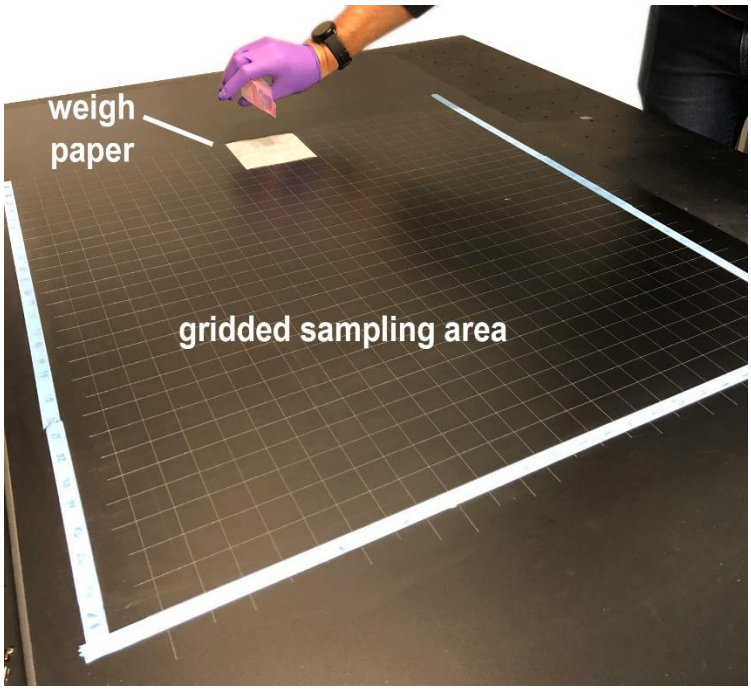

Figure 1. Photograph on the configuration for the net weight experiments.

Mass Spectral Analysis

Quantitation of the distribution of the simulant drug mixture was completed via extraction of the cotton swabs and analysis by LC-MS/MS. Only the minor components of the mixture (acetaminophen and benzocaine) were quantified. This was completed because many seized drug samples, especially those containing synthetic opioids, consist of one or more cutting agents in a high weight percentage relative to the actual drug which is present in a low (typically single digit) weight percentage. Extraction was completed by cutting off the head of the cotton swab and placing it in a 2 mL amber glass vial (Restek, Bellefonte, PA). A 0.5 mL aliquot of methanol (Sigma, Chromasolv-grade) containing piracetam (Sigma) (approximate concentration of 5 µg mL^{-1}) was added, the vial was capped and then vortexed for 10 s. Analysis was completed using a Thermo UltiMate 3000 (Waltham, MA, USA) liquid chromatography (LC) system coupled to a Sciex 4000 QTrap (Framingham, MA, USA) mass spectrometer (MS/MS). LC parameters included a 15 µL injection volume, an isocratic mobile phase consisting of 95 % methanol and 5 % water (both containing 0.1 % v/v formic acid) at a flow rate of 0.7 mL min^{-1}, and the use of a Restek Raptor Biphenyl guard column (2.7 µm x 5 mm x 3 mm). MS parameters included a curtain gas of 10 a.u., an ionspray voltage of +5500 V, a source temperature of 550 °C, an ion source gas 1 of 50 a.u., and an ion source gas 2 of 50 a.u. All analyses were completed in multiple reaction monitoring (MRM) mode, the details of which are shown in Table 1. For each compound (piracetam, acetaminophen, and benzocaine), two MRM transitions were monitored – one for quantitative analysis and a second for confirmation. Since there was no LC column, and therefore no separation, a run time of 2.5 min was used with a blank run completed between each sample to ensure there was no carryover. The peak areas for acetaminophen and benzocaine were ratioed to that of piracetam and compared to an 11-point calibration curve to determine the amount of material recovered and, therefore, the surface concentration of a particular grid square.

Table 1. MRM transition parameters for the quantitative experiments. For all transitions an entrance potential and a cell exit potential of 10 V was used. The top, bolded, transition for each compound was the one used for quantitative analyses.

Compound	Q1 (*m/z*)	Q3 (*m/z*)	Declustering Potential (V)	Collision Energy (V)
Piracetam	**143**	**98**	**40**	**20**
	143	69	40	40
Acetaminophen	**152**	**65**	**50**	**43**
	152	93	50	30
Benzocaine	**166**	**65**	**25**	**55**
	166	77	25	41

Process Efficiency and Background

An additional study was completed to establish the efficiency (both collection and extraction) of the sample collection and extraction process. This was completed by solution depositing 10 µg of acetaminophen and benzocaine in 2.54 cm by 2.54 cm (1 in by 1 in) squares on a separate piece of phenolic resin laboratory bench. These squares were then sampled and extracted using the above protocols. Ten replicates were completed and process efficiencies of 87.5 % (±22.0 %) and 86.7 % (±25.9 %) were found for acetaminophen and benzocaine, respectively. Extractions of the benchtop surface without any material present were also completed to identify if there was any background signal from the collection and extraction process. A slight signal was observed in the benzocaine channel, and therefore a low mass cutoff of 0.10 µg mL^{-1} was employed for benzocaine. This cutoff value was above the limit of quantitation for the method, which was 0.01 µg mL^{-1} for both compounds. No background signal was observed at the acetaminophen or piracetam channels.

Visualization of the Process

A 5 Watt, 532 nm continuous laser (civillaser.com) was used for particle illumination during the pouring experiments. A custom laser sheet generator was built for this laser that consisted of a 5 mm cylindrical glass rod and a 3D printed mounting bracket that registered the glass rod along the laser axis. The laser was positioned via tripod about 1 m above the pour area and at 45° from horizontal to minimize shadowing. A high-definition video camera (Sony Handycam, Sony, Toyko, Japan) was used to record the pouring events.

A custom LabVIEW code was developed to aid in post-processing of the quantitative data. We chose a color mapping approach to illustrate the magnitude of contamination spread for each bag pour. The LabVIEW code inputed the extraction data as a series of X and Y positions, and then converted the extraction values into a color value based on a predetermined color scale. A bilinear interpolation algorithm was used to smooth the data and help with interpretation of the results. The final export from the LabVIEW code was a 2-dimensional color plot and scale bar. Adobe Photoshop was then used to map this color plot onto an aerial photograph of the actual baggie pour location, resulting in a birds-eye-view of the bag pour and subsequent spread of aerosolized drug particulate during the net weight process.

Results and Discussion

0.2 g Experiments

Three different mass loadings (0.2 g, 2 g, and 100 g) were chosen for these experiments to represent different amounts of powder that may be received as evidence in a forensic laboratory. For each mass loading three replicate experiments were completed. The quantitative data from the grid samples containing acetaminophen and benzocaine were then taken to create two-dimensional color plots that allowed for visualization of particle spread over the benchtop. The resulting color plots of the 0.2 g replicates are shown in Figure 2 and Figure S4. Still images taken during the laser light sheet visualization of one of these replicates are shown in Figure 3 and the corresponding video of the bag pour can be found in Supplemental Information (Supplemental Video 1).

For the 0.2 g experiments particulate spread was detected up to 61 cm (24 in away) from where the material was poured (Figure 2). For all three replicates, the immediate area (approximately 10 cm, or 4 in) surrounding the weigh paper had the highest surface concentrations for both acetaminophen and benzocaine. Concentrations greater than 0.78 µg cm^{-2} (5 µg in^{-2}) were exclusive to this area as evidenced by the black color in the 2-D colorplot (Figure 2). Maximum surface concentrations from a single grid square were 3.6 µg cm^{-2} (23 µg in^{-2}) and 9.6 µg cm^{-2} (62 µg in^{-2}) for acetaminophen and benzocaine, respectively. The maximum recovered surface concentrations and total mass extracted for all replicates can be found in Table 2. In the immediate area around the weigh paper recovery of acetaminophen and benzocaine was well co-located though divergence of the two at farther distances was observed and may be a function of differences in particle size. Outside of the 10 cm (4 in) area around the weigh paper surface concentrations dropped significantly and individual samples were, largely, at or below 0.25 µg cm^{-2} (1.5 µg in^{-2}). Several individual grid squares did have higher concentrations, presumably caused by the settling of larger particles. The spread of benzocaine in all replicates was further than that of acetaminophen, even though it was the minor component, which is likely the result of the small average particle size for benzocaine, as compared to acetaminophen.

Interestingly, there was a large amount of variation in where the particulate settled between replicates. In Replicate 1 (Figure 2 A. and D.) much of the particulate settled directly in front of the weigh paper, whereas Replicate 2 and Replicate 3 trended towards the right and left sides of the weigh paper. This is likely a combination of effects from both the exact location on the weigh paper where the bulk powder was poured and the positioning of the hand during the pouring process. Differences may also be attributed to the force with which the plastic bag had to be agitated to completely remove the powder. Additionally, no special efforts were made to control or mitigate airflow within the laboratory during these experiments, so it is possible that the ventilation system created air perturbances that contributed to differences between replicates.

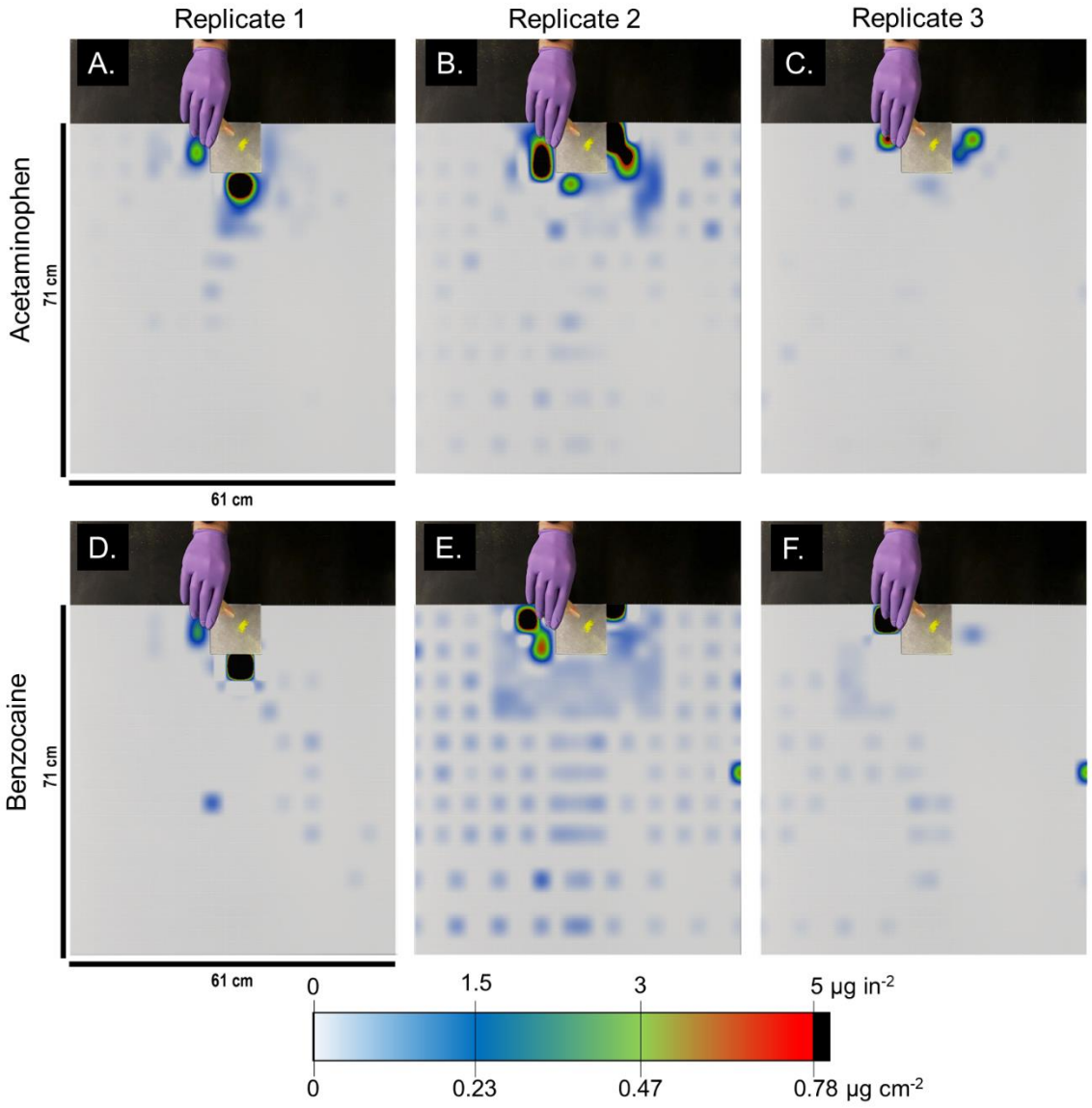

Figure 2. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (D., E., and F.) from the three replicates of the 0.2 g experiment. Areas of white indicate either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 2. Areas of black indicate grid squares where the surface concentration was in excess of 0.78 µg cm^{-2} (5 µg in^{-2}). This cutoff was used to allow for better visualization of the areas with lower surface concentration. Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S4).

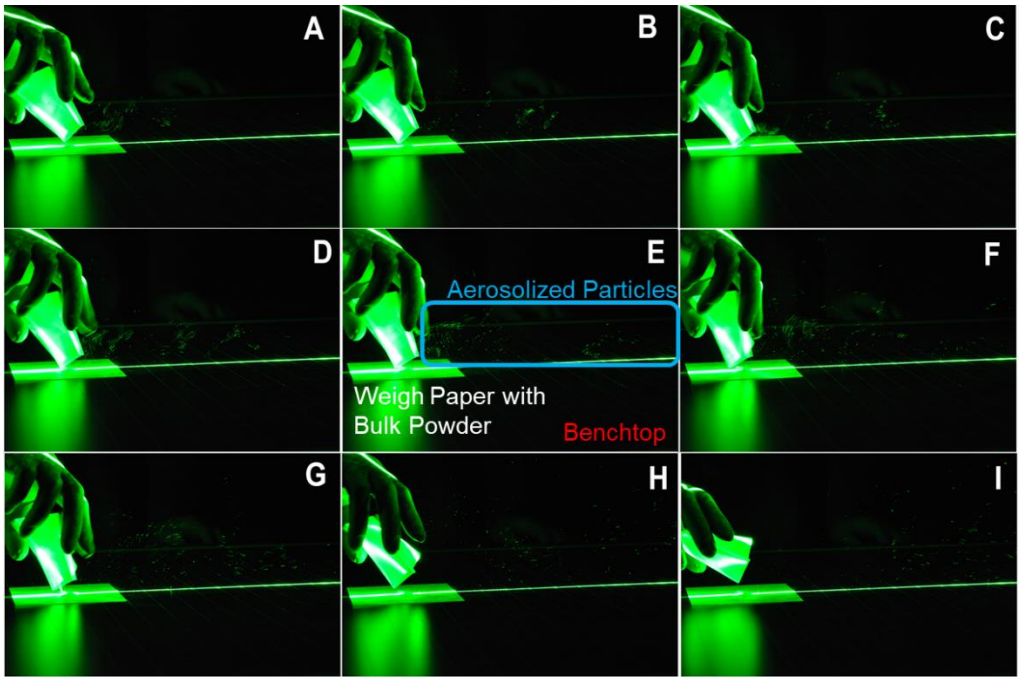

Figure 3. Still images from the laser light sheet visualization of a 0.2 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 1).

Table 2. Maximum recovered surface concentration from a single grid square and total mass of material that was recovered from all grid squares that were sampled for all replicates and experiments in the study. Also provided is the percentage of compound recovered from all grid squares that were sampled, relative to the initial mass of each compound for each experiment.

		Max Surface Concentration (µg cm^{-2})			Total Amount Recovered (µg)			Percentage of Compound Recovered (%)		
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0.2 g	Acetaminophen	2.9	3.5	0.8	47.0	104.9	15.3	0.094	0.210	0.031
	Benzocaine	9.7	4.8	7.1	74.5	121.7	58.4	0.745	1.217	0.584
2 g	Acetaminophen	8.4	30.6	24.2	232.1	522.4	403.5	0.046	0.104	0.081
	Benzocaine	40.5	216.4	21.3	925.8	2338.1	536.7	0.925	2.338	0.537
100 g	Acetaminophen	38.8	37.5	43.0	1014.7	1012.7	594.0	0.004	0.004	0.002
	Benzocaine	35.2	33.1	19.9	1731.3	1612.3	445.0	0.034	0.032	0.009

2.0 g Experiments

Figures 4 and 5 show the results of using approximately 2 g of the mixture. Like the 0.2 g experiments, the bulk of the aerosolized particulate fell within the immediate area surrounding the weigh paper though detectable amounts were found up to 61 cm (24 in) away. The increase in total mass poured translated to an increase in average surface concentration, as expected. Maximum recovered samples for single samples of acetaminophen and benzocaine were 30.6 µg cm^{-2} (197.2 µg in^{-2}) and 216 µg cm^{-2} (1396.5 µg in^{-2}), respectively. The high level of benzocaine in the single sample (Replicate 2, Figure 4E and Supplement Figure 5) was likely the result of several large particles falling off of the weigh paper, during the pouring of the powder, and winding up on the grid square closest to the weigh paper – explaining why concentrations in the range of hundreds of micrograms per square centimeter were observed.

For the 2 g experiments the majority of the aerosolized particles fell directly in front of the weigh paper. As with the 0.2 g experiments, high levels of acetaminophen were co-located with high levels of benzocaine. Benzocaine, however, was observed at higher concentrations further away from the weigh paper due to its

smaller particle size. The overall lower surface concentrations of acetaminophen, compared to benzocaine, highlight the importance of particle size on aerosol release. There was five times the amount of acetaminophen, compared to benzocaine, in the starting powder, yet for all 0.2 g and 2 g replicates a lower overall mass of acetaminophen was recovered from the surface.

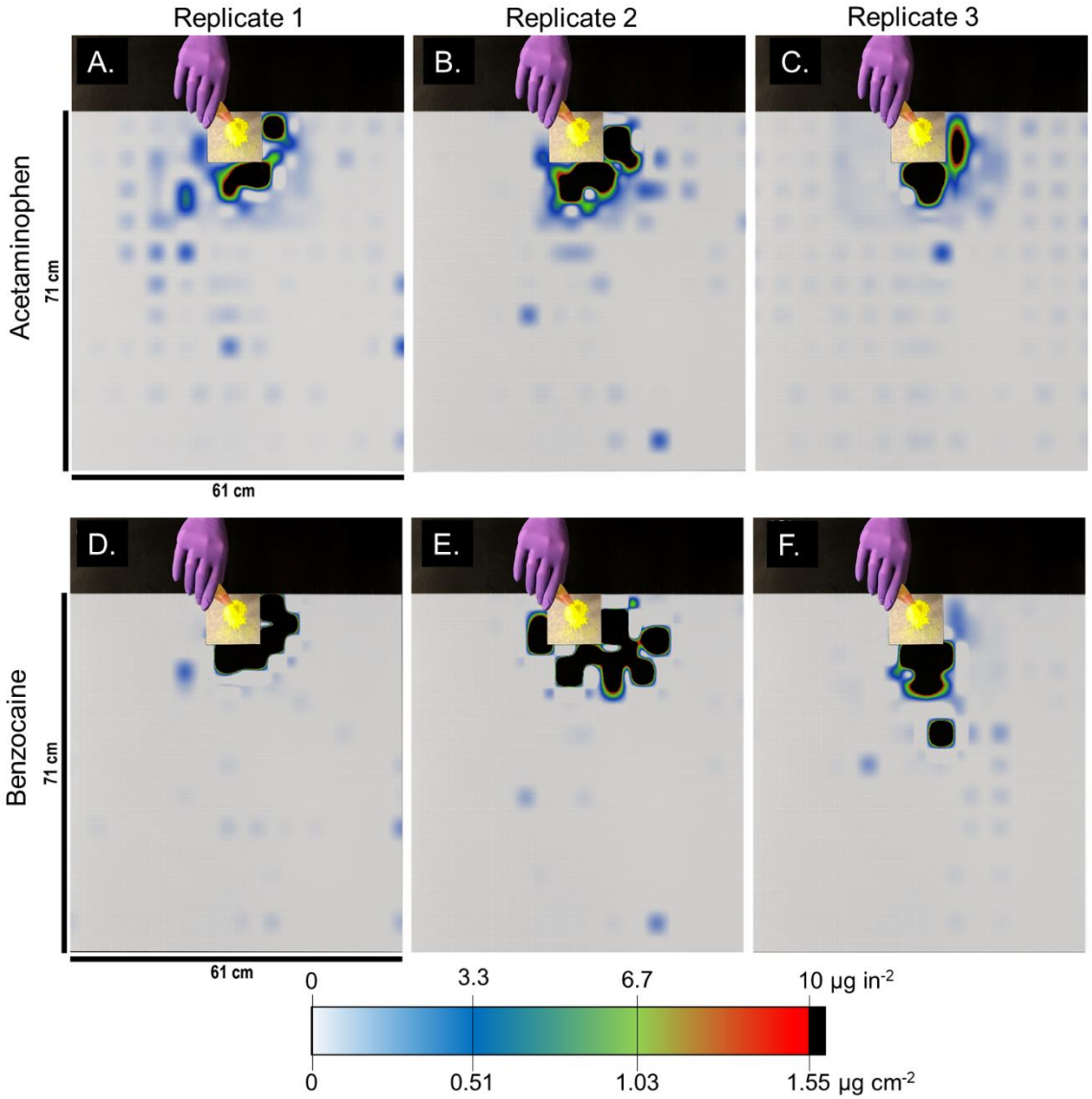

Figure 4. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (D., E., and F.) from the three replicates of the 2 g experiment. Areas of white indicate either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 2. Areas of black indicate grid squares where the surface concentration was in excess of 1.55 µg cm^{-2} (10 µg in^{-2}). The cutoff was used to allow for better visualization of the areas with lower surface concentration. Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S5).

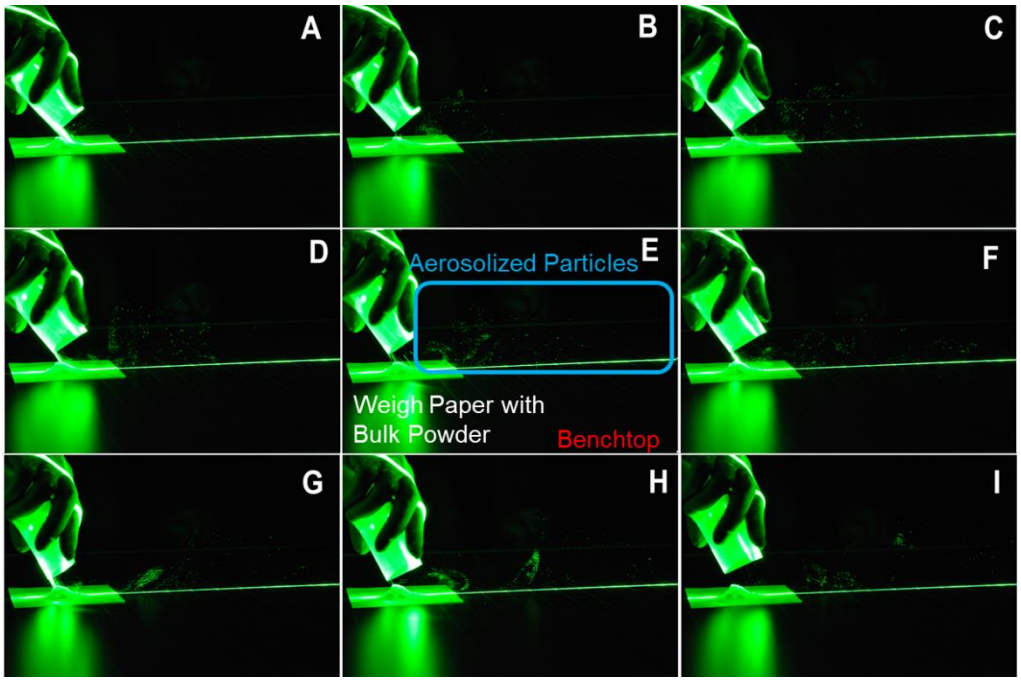

Figure 5. Still images from laser light sheet visualization of a 2 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 2).

100 g Mixture Simulant

Figures 6 and 7 show the quantitative and qualitative results from the 100 g net weight experiments. Because of the higher mass of material being poured, a larger sheet of weigh paper (14 cm by 20 cm, 5.5 in by 8 in) was used to contain the material which required a modified sampling scheme (Figure S2). Interestingly, the results from the 100 g experiments show substantially different trends compared to the 0.2 g and 2 g experiments. Lower acetaminophen and benzocaine surface concentration in the area immediately surrounding the weigh paper were measured compared to high concentration pockets of the two compounds throughout the entire sampling surface. One of the replicates (Replicate 1) did have a high concentration sample near the weigh paper, but the large area of high surface concentration was not observed. While the color plots in Figure 6 (A., B., C., E., F., and G.) appear to show minimal levels of benzocaine or acetaminophen in the area around the weigh paper, this is largely an artifact of the scaling. Figure 6 (D. and H.) shows the Replicate 2 data scaled to the same values as the 2 g experiments (Figure 4). Under this scaling it is clear that the area immediately around the weigh paper has substantial levels of both compounds as would be expected from the 0.2 g and 2 g experiments.

Overall, surface concentrations were significantly higher for the 100 g experiments, compared to the 0.2 g, but were not substantially higher than the 2 g experiments. Table 2 also presents a similar trend when looking at percentage of material recovered. For the 0.2 g and 2 g experiments, similar percentages of acetaminophen (approximately 0.1 %) and benzocaine (approximately 1 %) were recovered. However, the percentage recovered was substantial lower for the 100 g experiments (approximately 0.003 % and 0.025 % for acetaminophen and benzocaine, respectively). This difference may be due to the larger piece of weigh paper that was used but may also be a factor of greater spread further from the weigh paper for the 100 g experiments, as observed with high concentration samples at distances greater than 45 cm (18 in) away. As with the previous experiments, benzocaine was found to travel further than acetaminophen. However, for Replicate 3 of the 100 g experiments, a higher recovered mass of acetaminophen was observed, when compared to benzocaine. Given the two-dimensional color plot of this replicate, it appears as though that was driven by a single grid square with an abnormally high concentration.

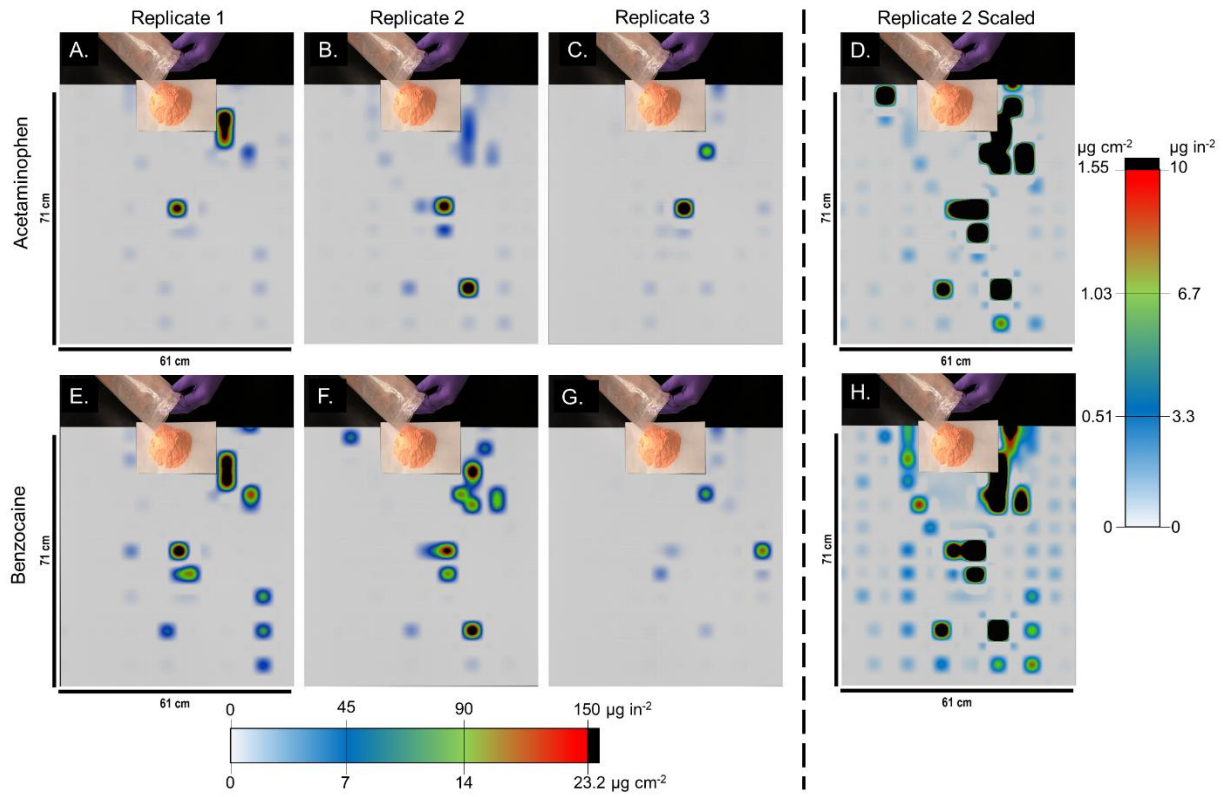

Figure 6. Color plots showing the surface concentration measurements for acetaminophen (A., B., and C.) and benzocaine (E., F., and G.) from the three replicates of the 100 g experiment. Areas of white indicate that either no quantifiable amount was recovered, or the area was not sampled. A picture of the sampled versus unsampled squares can be found in Supplemental Figure 3. Areas of black indicate grid squares where the surface concentration was in excess of 23.2 μg cm^{-2} (150 μg in^{-2}). The cutoff was used to allow for better visualization of the areas with lower surface concentration. A second color plot of Replicate 2, scaled to a cutoff concentration of 1.55 μg cm^{-2} (10 μg in^{-2}), is shown for acetaminophen (D.) and benzocaine (H.). Maximum surface concentrations for these experiments can be found in Table 2 and Supplemental Information (Figure S6).

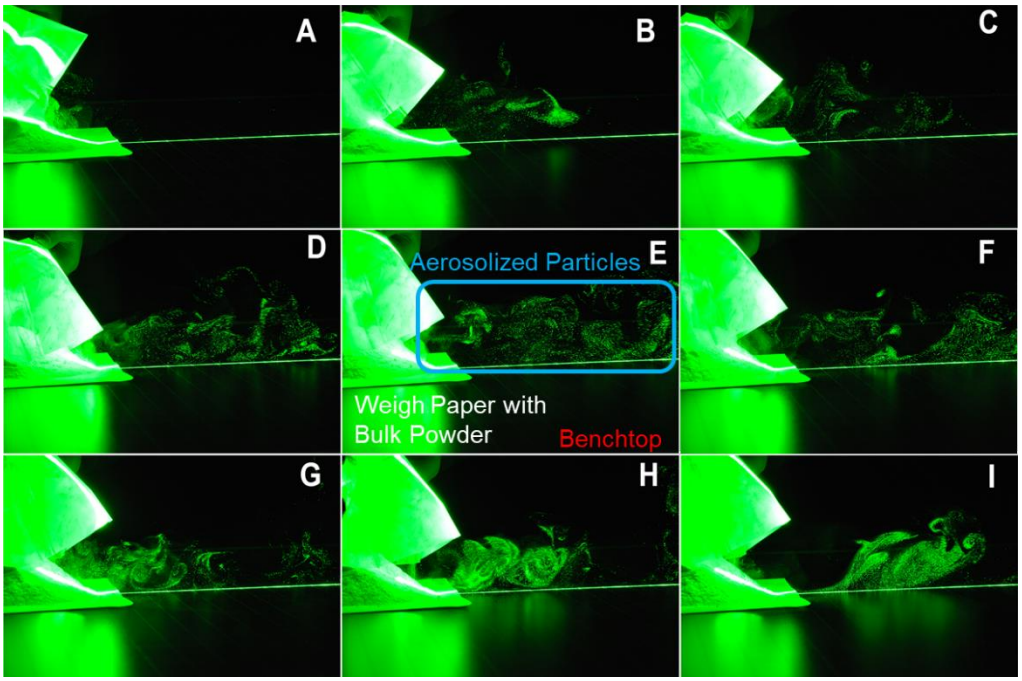

Figure 7. Still images from laser light sheet visualization of a 100 g experiment. Aerosolized particles from the simulant drug mixture can be observed in green in the still images and are highlighted in still image E. The corresponding video to these still images can be found in the Supplemental Information (Supplemental Video 3).

Conclusion

This study presents a quantitative approach for measuring drug background levels on planar surfaces after a common net weight exercise. A combination of wet swab sampling and LC-MS/MS provided quantitative data for two-dimensional particle spread from the net weight activity. Three mass loadings were evaluated (0.2g, 2g, and 100g), and each loading level demonstrated significant particle transport from the weigh paper to the surrounding surfaces. Generally, background levels increased with increasing mass of material. Background levels as high as 23 µg cm^{-2} (150 µg in^{-2}) were measured at distances 61 cm (24 in) from the weigh paper.

This study also presents a qualitative flow visualization approach for understanding the mechanisms driving background on surfaces. This visualization methodology complements the quantitative extraction process by uncovering how microparticles are transported in the air during net weight activities. During a common dumping activity, bulk powder is emptied from original container (usually a baggie) onto weigh paper. This serves to aerosolize some fraction of the particulate sample, and natural airflows in the room then transport this material away from the source where it eventually settles onto surrounding surfaces. This flow visualization tool may be useful for chemists and other personnel that handle drug samples that want to fully understand the impact of handling bulk powders in laboratory settings. Given the long distances at which particles were found to spread, it is conceivable that particle will settle on other surfaces besides the laboratory bench (*i.e.*, the chemist's lap, the floor, surrounding work areas). Current work is focused on understanding the possibility and probability of these types of spread, measuring the effectiveness of strategies to mitigate particulate spread, as well as quantitating the net weight process in the third dimension to provide a better understanding of the direct inhalation risk to a chemist.

Disclaimer

Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

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Figure 1

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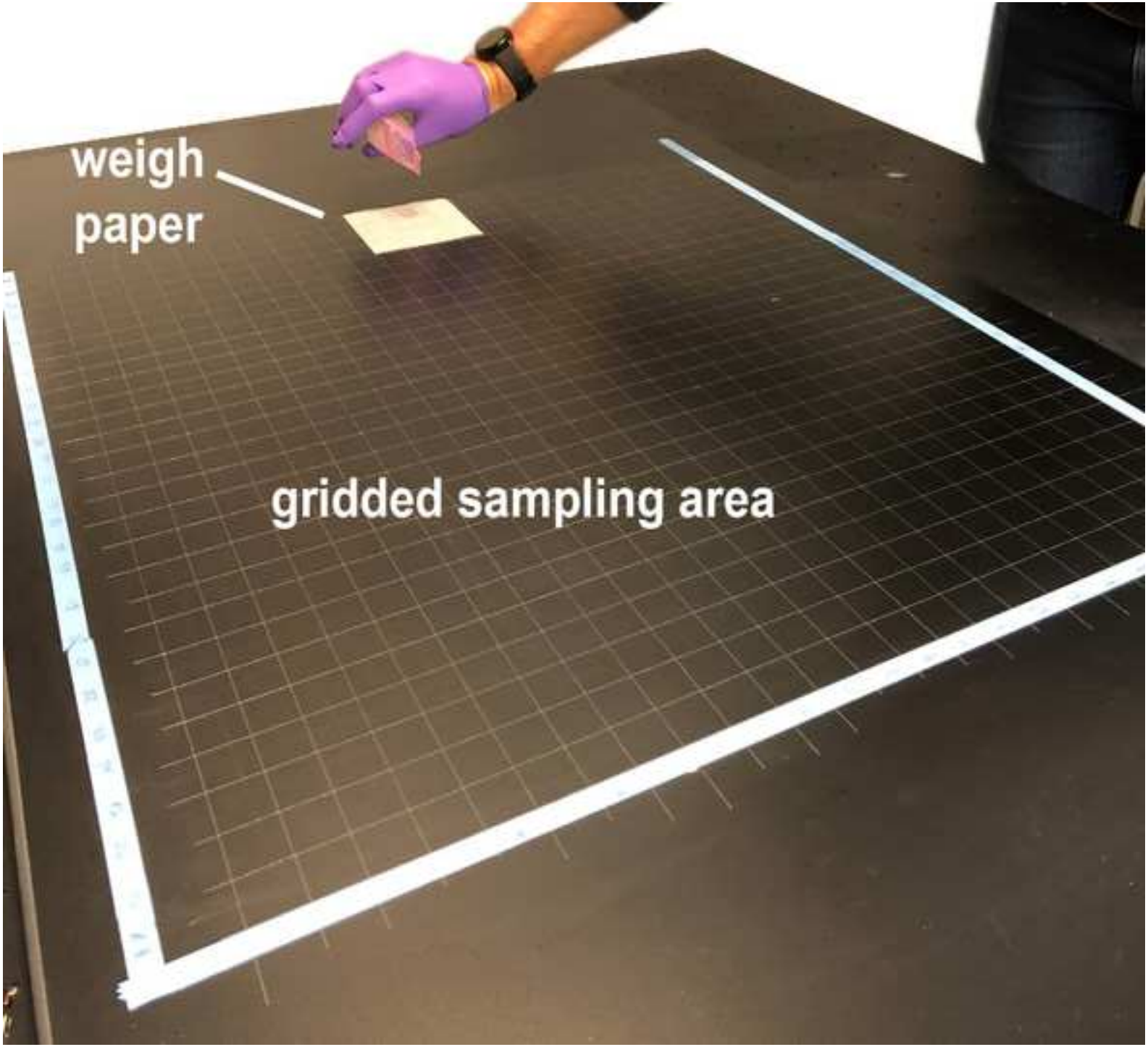
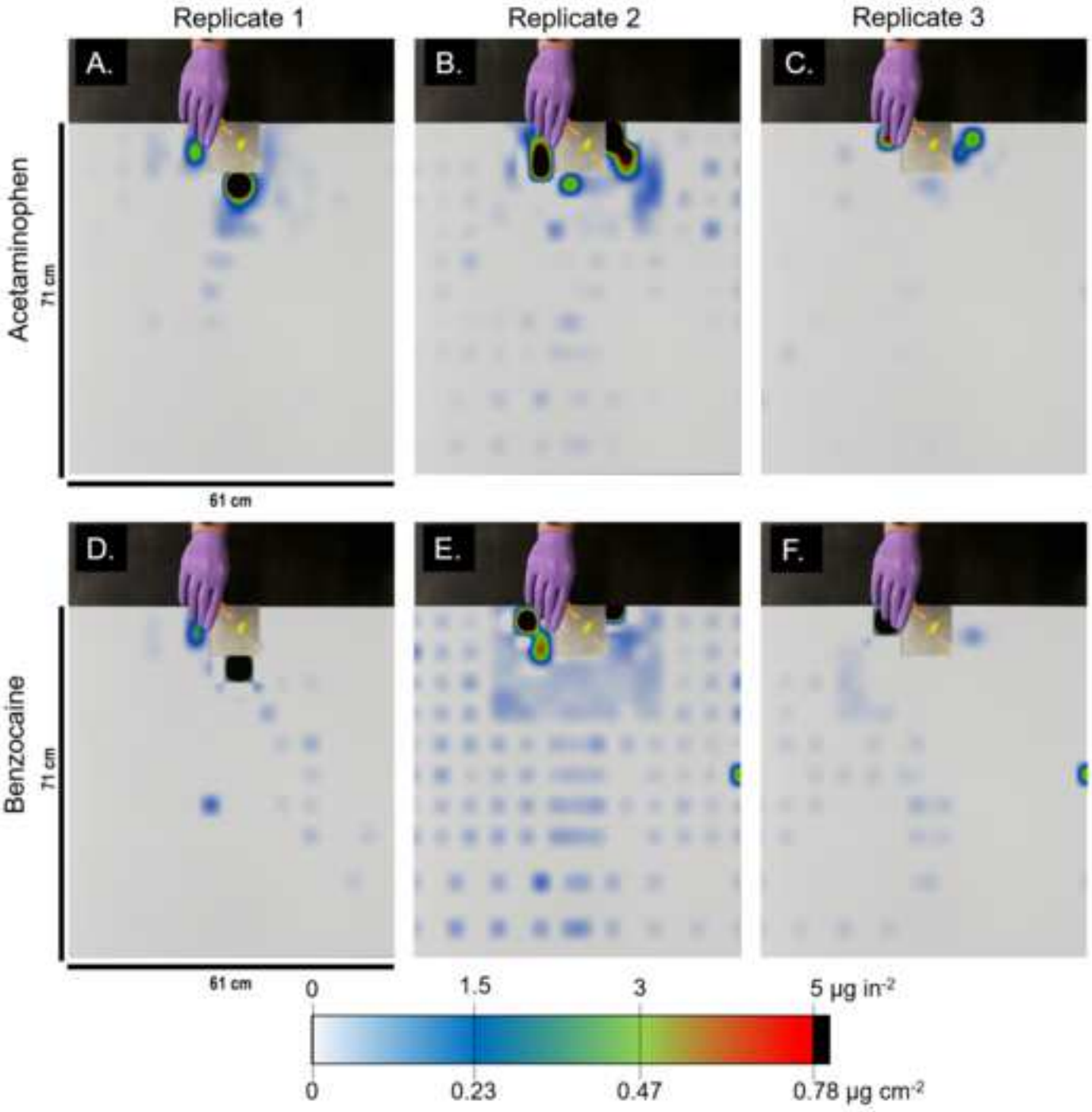


Figure 2



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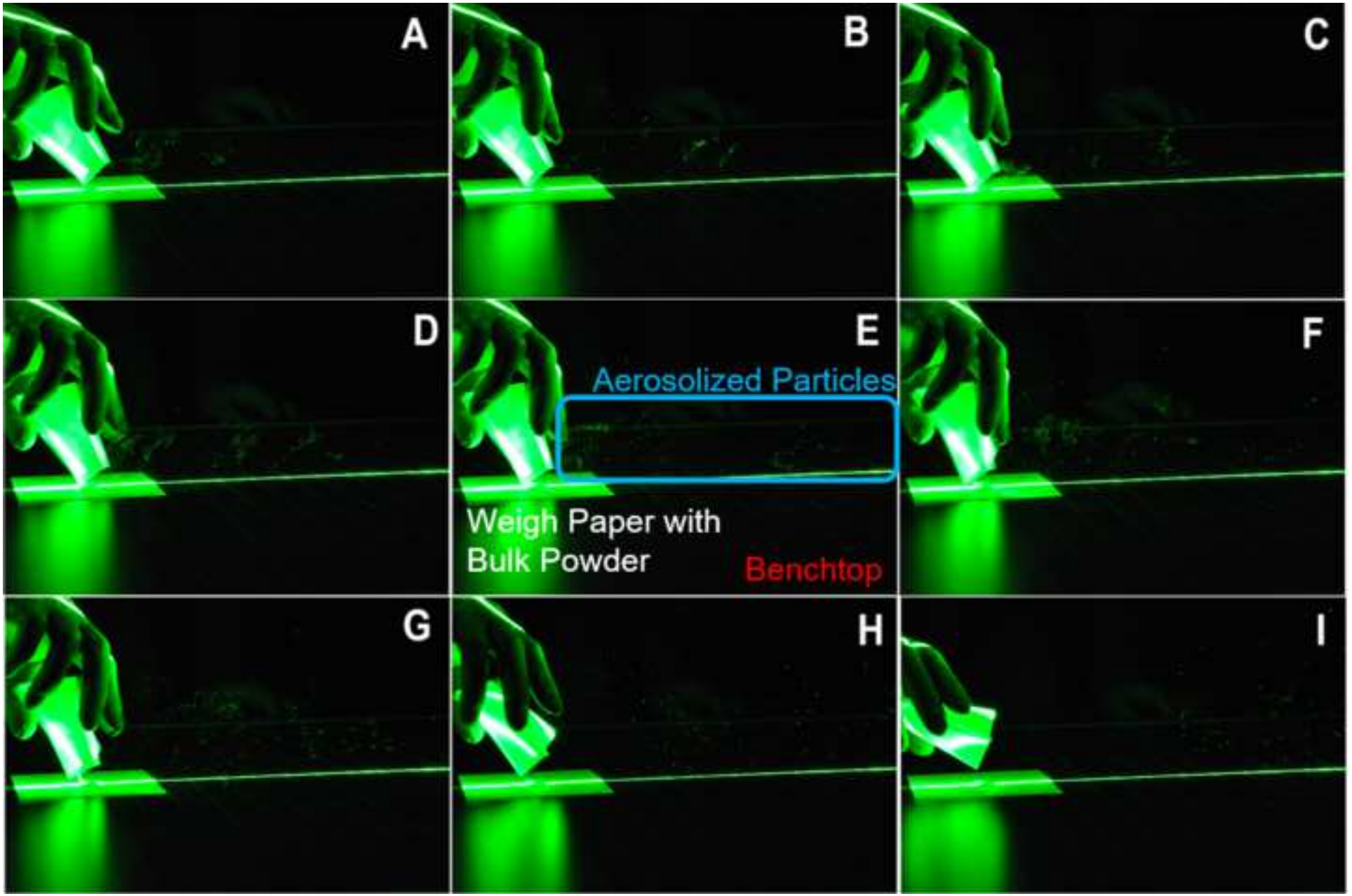

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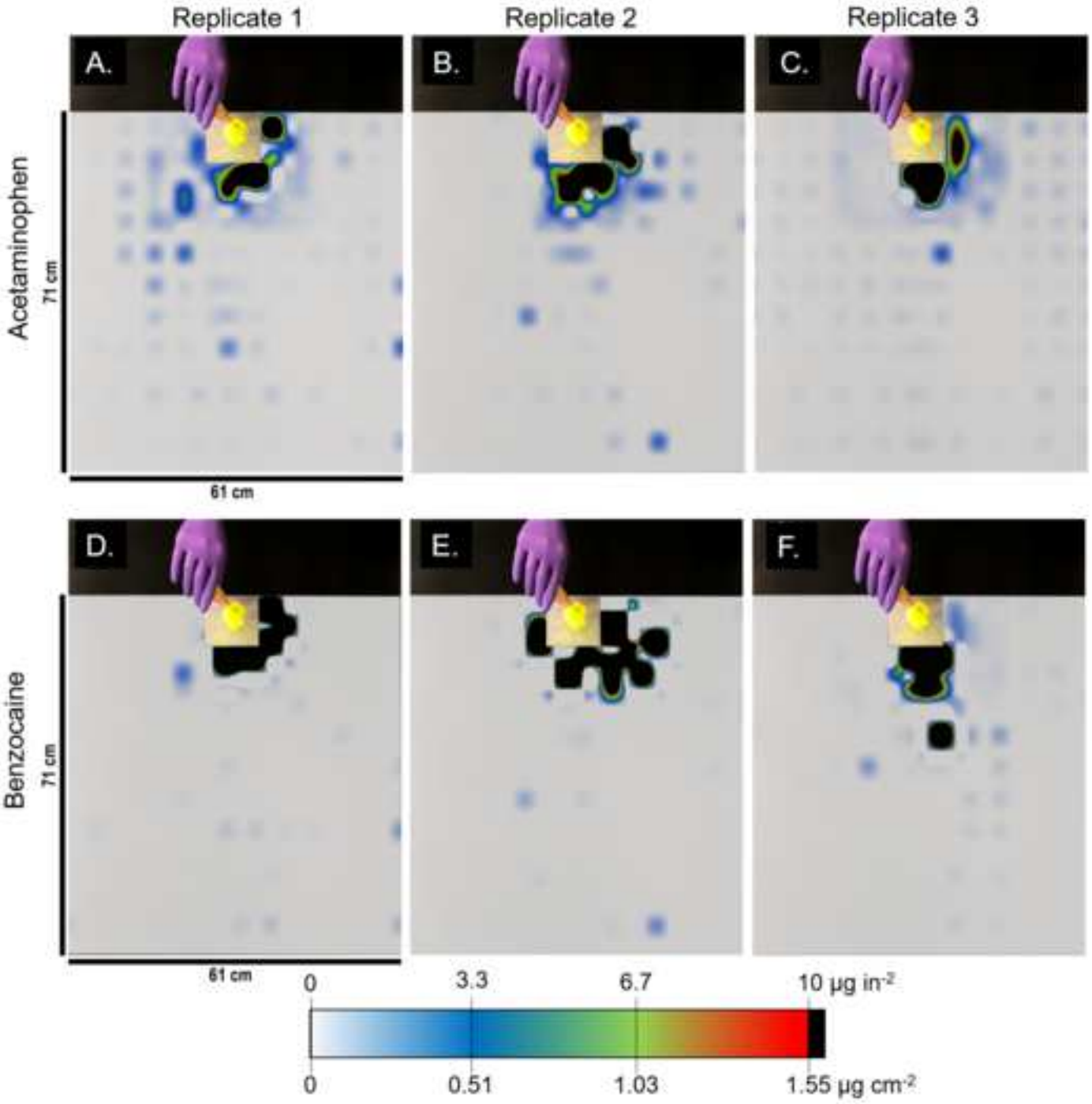

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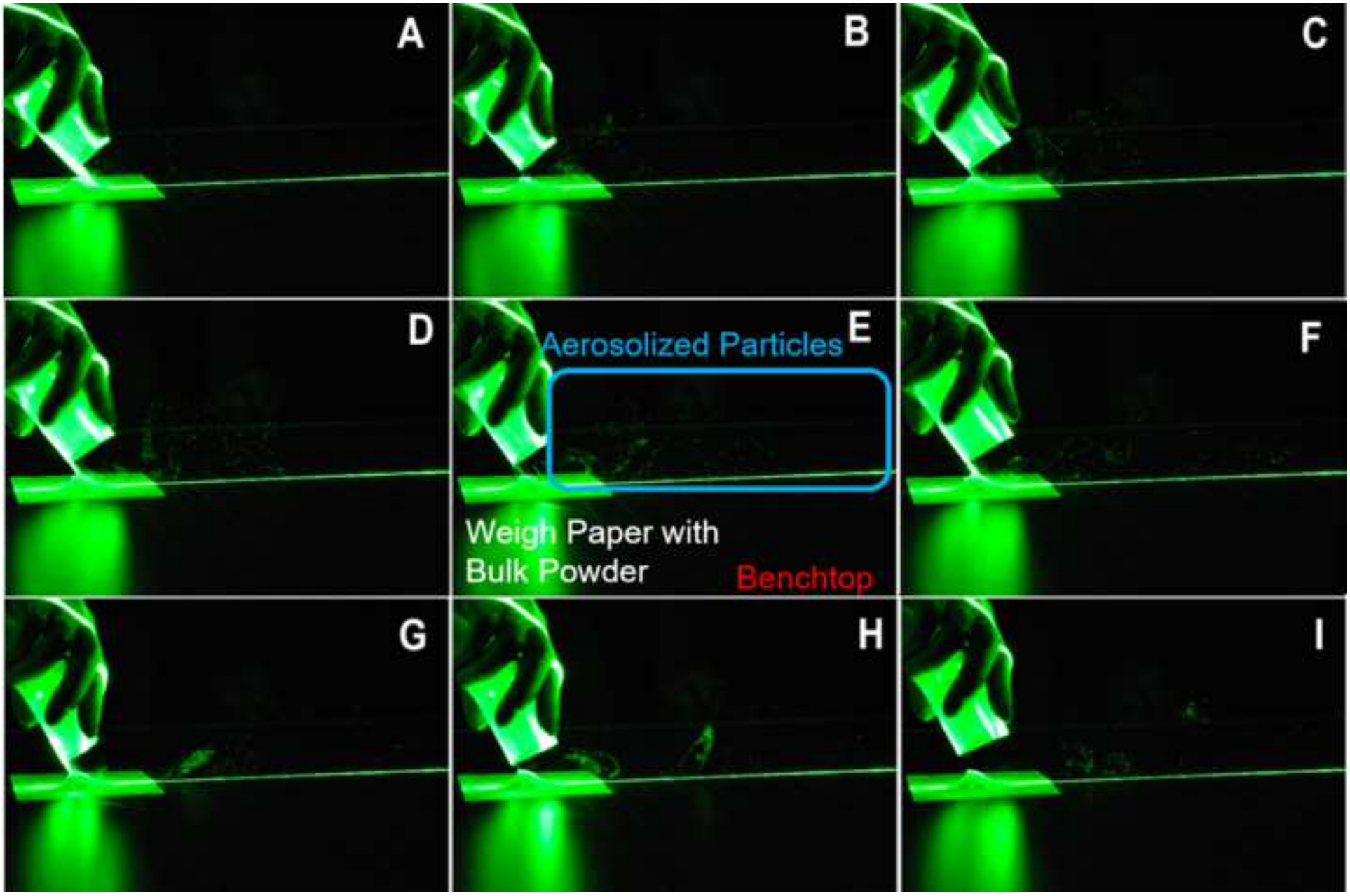

Figure 6

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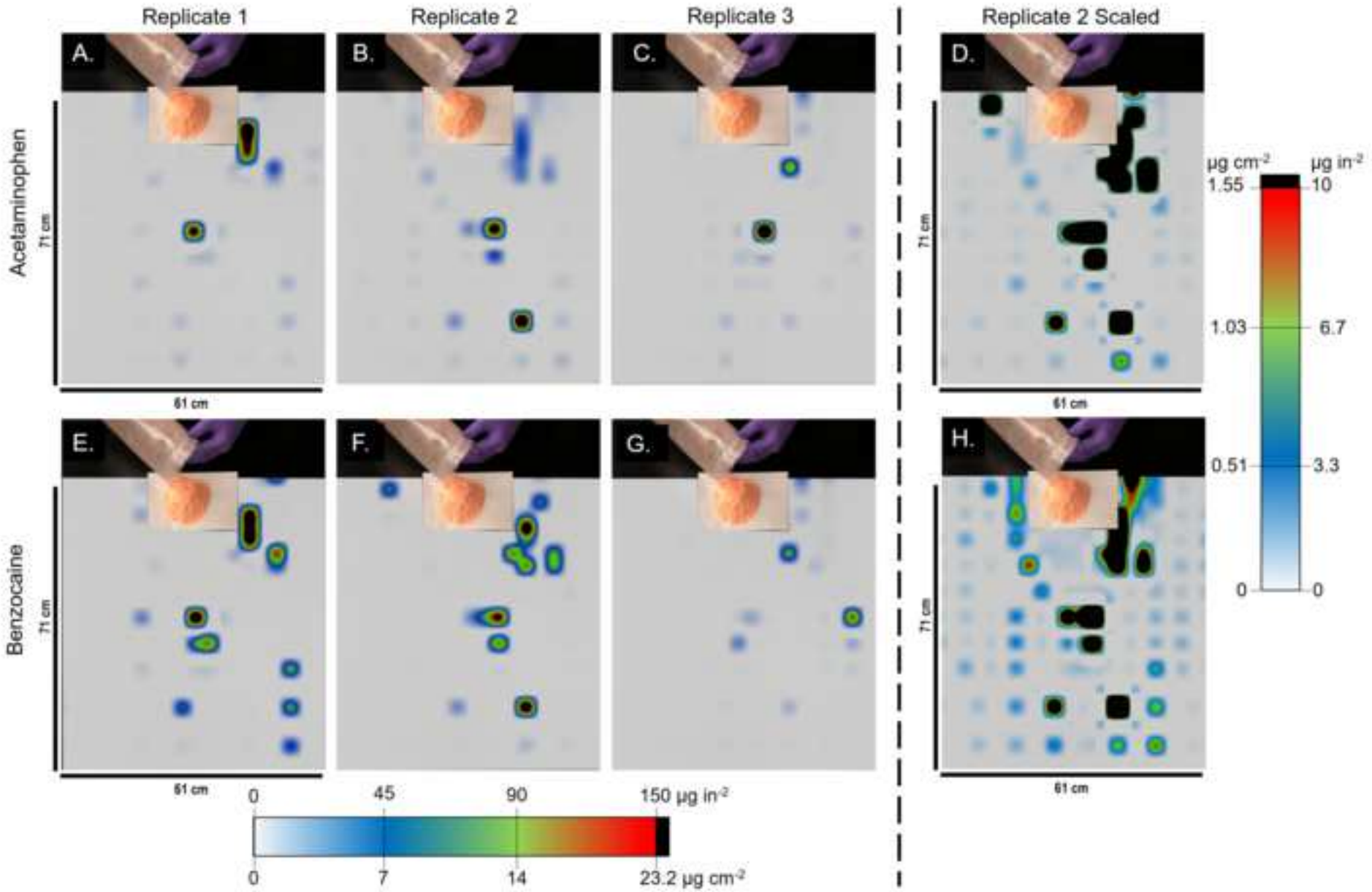
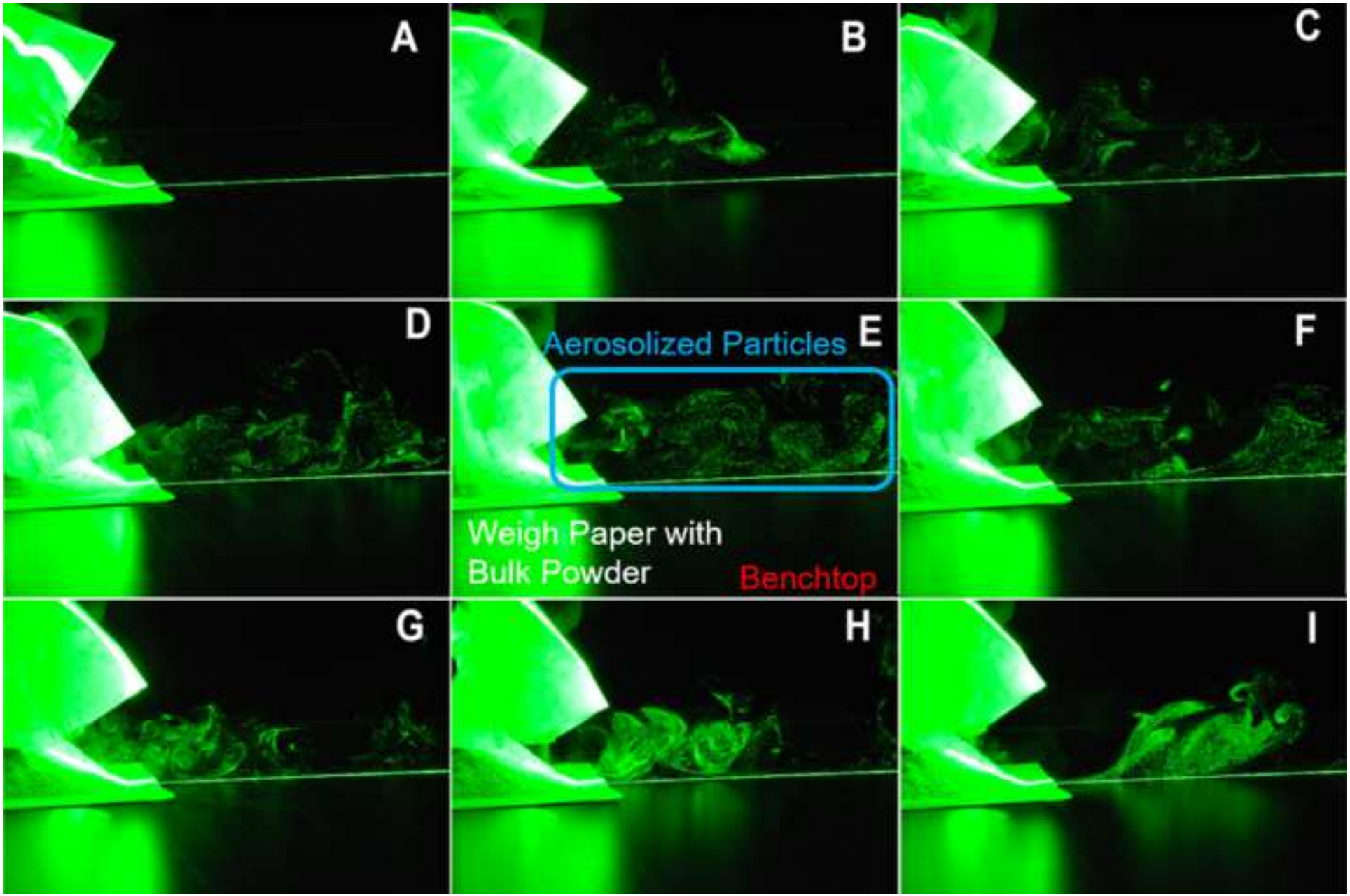


Figure 7



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Supplemental Video 1

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