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Field Data Collection Protocols for NIJ Grant The Impact of Drugs on Human Decomposition and the Postmortem Interval: Insect, Scavenger and Microbial Evidence

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Hayden S. McKee-Zech[1], Erin A. Patrick[1], Allison R. Mason[2], Katha Hoeland[3,4], Charity Owings[5], Sarah T Schwing[1], Thomas Delgado[1], Amanda May[3,4], Mary C. Davis[1], Jennifer Debruyn[6], Shawn Campagna[3,4], Dawnie W Steadman[1]

[1]Department of Anthropology, Forensic Anthropology Center, University of Tennessee - Knoxville;
[2]Department of Microbiology, University of Tennessee - Knoxville;
[3]Department of Chemistry, University of Tennessee - Knoxville;
[4]Biological and Small Molecule Mass Spectrometry Core (BSMMSC); [5]University of Tennessee;
[6]Department of Biosystems Engineering and Soil Science, University of Tennessee - Knoxville

Allison R. Mason: Current Affiliation: University of Michigan;
Katha Hoeland: Current Affiliation:;
Thomas Delgado: Current Affiliation:;

Hayden S. McKee-Zech
Department of Anthropology, University of Tennessee - Knoxvi...

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We use this protocol and it's working

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Disclaimer

Deals with sensitive topics including but not limited to human decomposition and death.

Abstract

The following is the data collection protocols for the National Institute of Justice funded grant "The Impact of Drugs on Human Decomposition and the Postmortem Interval: Insect, Scavenger and Microbial Evidence" (DOJ-NIJ-2018-DU-BX-0180). These protocols include those for anthropology, soil sciences, and chemistry field sampling events. This project was designed to examine the range of effects on decomposition of common drugs ingested by human donors through prime decomposers (e.g., scavengers, insects and microbes).

Attachments

PDF **Insect collection fo...** 1.6MB

PDF **Scavenging collectio...** 1.4MB

PDF **Fluid collection for...** 724KB

DOCX **Toxicology Project F...** 26KB

DOCX **Insect labels Tox St...** 13KB

XLSX **Tox Chain of Custody...** 9KB

PDF **Protocol.io term for...** 241KB

Image Attribution

Logo owned by the University of Tenessee-Knoxville's Forensic Anthropology Center

Guidelines

In cases of extreme weather, data collection at the outdoor Anthropology Research Facility was suspended for safety measures of the researchers.

Materials

Human remains with enrollment criteria:

1. Known date of death
2. Obtainable blood serum
3. Known medical information including drugs used, medical conditions, treatments 7-30 days prior to death
4. No outward trauma.

Human remains were acquired through the UTK Body Donation Program at the University of Tennessee - Knoxville's Forensic Anthropology Center.

Consent for participation in decomposition research, including thermal trauma, was granted by individuals or next of kin prior to enrollment in the research study.

Intake:

- Camera
- Printed Forms
- Blood Card
- 20cc syringe
- Cryovial
- Tabletop centrifuge
- Liquid Nitrogen
- 7 oz Whirlpak bags
- Skinfold Calipers

Soil Sampling:

- Flags for marking control sites (1 per donor, only for placement/initial time point)
- Sharpie
- Corers (10cc syringes with the tips cut off, stored in clean plastic bag) (2 per donor)
- 7 oz Whirlpak bags (2 per donor)
- 4 oz Whirlpak bags (2 per donor)
- Small kitchen scale for weighing samples
- Tweezers
- Spoon, spatula or scoop for dividing soil
- EtOH and papertowels for sterilizing tweezers and spatula between samples
- Dewar/Cryopod with liquid Nitrogen
- PPE (gloves, shoe covers, pants, sleeves)

Decomposition Fluid Collection Protocol:

- PPE: Gloves, pants, shoe covers, and gowns
- 30cc sterile luer-lock syringes (BD REF: 302832) (1 per sampling location)
- Scissors (1)
- 4oz whirlpak bags (2 per donor)

- 2mL sterile cryovials (4 per donor)
- 50 ml sterile falcon tubes (1 per donor)
- Dewar with liquid nitrogen (1)

Anthropology Observations:

- Tiny Tag (German logger)
- Game Camera
- Printed observation forms
- Clipboard
- Pen
- Camera with SD cards

Safety warnings

The materials handled in these protocols are potentially hazardous and Blood Borne Pathogen (BBP) precautions should be employed. This work was performed in a Biohazard Safety Level (BSL)-2 laboratory.

Ethics statement

Individuals who register to donate their bodies prior to their death or legal next-of-kin who donate their loved one's body after their death to the Forensic Anthropology Center (FAC) at the University of Tennessee, Knoxville consent to their participation in decomposition research at the Anthropology Research Facility. More information about the body donation program can be found at the FAC **website**. The University of Tennessee, Knoxville, Human Research Protections Program (HRPP) reviewed this project and determined that research with deceased human donors is exempt under 45 CFR 46.101. As this submitted protocol is laboratory based, personal information of participating donors was not deemed necessary and thus, is not included here.

Before start

Definitions:
Donor is an deceased individual who has donated their body to the Forensic Anthropology Center's body donation program at the University of Tennessee-Knoxville.

Soil sampling in advance:

1. Prepare 10cc syringes (Norm-ject Henke-ject REF: 19E16C8) to use as disposable corers (2 per donor): wearing gloves and using a sterile syringe, cut the end off of a 10cc syringe. Store prepped syringe corer in a clean plastic bag. This creates a 15 cm diameter soil corer.
2. Fill dewer/CryoPod with liquid N

Decomposition fluid sampling in advance:

1. In advance:
2. Label a 4 oz. whirlpak bag with donor and date information (2 per donor).
3. Label 4 2mL cryovials with donor and date information (4 per donor). (Bring extra sterile 2mL cryovials and a sharpie in case there is extra fluid).
4. Fill dewar/CryoPod with liquid N

Enrollment Criteria

1 Human remains with enrollment criteria:
1. Known date of death
2. Obtainable blood serum
3. Known medical information including drugs used, medical conditions, treatments 7-30 days prior to death
4. No outward trauma

Intake

2 Photographs - A standard sequence of photographs should be taken at intake for each enrolled donor, including: an overhead view of the exact condition in which the donor arrived, an overhead view with clothing and coverings removed, overhead views of the donor's body in overlapping thirds (head to nipples, shoulders to mid-thigh, hips to toes), frontal and lateral photos of the head, photos of hands and feet to record presence or absence of digits, and any distinguishing marks (scars, tattoos, discoloration, medical devices, etc.).

3 Anthropometric data- Cadaver stature (centimeters) and weight (ideally, kilograms) should be documented at intake for each donor. Body fat percentage should be estimated using calipers for a skin fold test. Loose skin at the top of the iliac crest and the base of the rib cage should be pinched together for measurements. Calipers should measure the thickness at the base of the skin pinch. The measurement at each site should be repeated until the same measurement is obtained twice.

4 Aorta (central) and femoral (peripheral) blood sticks should be performed. Approximately 4 mL of each blood type should be collected.

4.1 The samples are split, with half of each sample stored as whole blood and the other half spun into serum (see next section).

1 cryovial - Central - whole blood 1 mL stored at -80 °C

1 cryovial - Femoral - whole blood 1 mL stored at -80 °C

3 cryovials - Central - serum 200 µL each stored at -80 °C

3 cryovials - Femoral - serum 200 µL each stored at -80 °C

Total of 8 cryovials per donor

Serum Preparation

5 Draw whole blood into tube(s) containing no anticoagulant. Draw approximately 2 ½ times the volume needed for use.

6 Incubate in an upright position at room temperature for 00:45:00 (no longer than 60 min) to allow clotting. 45m

7 Centrifuge for 00:15:00 at 2000 rcf, Room temperature 15m

8 The resulting supernatant is designated serum. Carefully aspirate the supernatant (serum) at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells.

Note

Use a clean pipette for each tube.

9 Inspect serum for turbidity

9.1 Turbid samples should be centrifuged for 00:15:00 at 2000 rcf, Room temperature again and aspirated again to remove remaining insoluble matter. 15m

10 Aliquot into triplicate samples 200 µL or 1 mL serum into 3 cryovials with a minimum for a single replicate ~100 uL.

Note

1) Ensure that the cryovials are adequately labeled with the relevant information.
2) It is important to avoid freeze-thaw cycles.
3) If possible in addition to serum we want a single cryovial of whole blood to also be stored at -80°C.

11 Store cryovials of serum at -80 °C

Placement

12 Climate monitor- A Tiny Tag data logger should be placed in the field near each donor, approximately 1 meter off the ground and within 1 meter of the donor. Localized, hourly recordings of temperature and relative humidity must be collected to allow for the calculation of accumulated degree hours (ADH).

13 Game Camera- Mount or secure a wildlife trail camera with the focus encompassing the entirety of the subject to the best of your ability. Settings should include 3 burst images to be taken at 1 second intervals when movement is detected. Regularly check game cameras to ensure batteries are charged and the memory SD card is not full.

14 Control site- Identify a site approximately one meter away from the donor placement site. Control site should be located uphill if donor resides on a slope, and should have soil available for regular sampling. Soil samples will be taken at the same ADH intervals as the donor soils for a baseline comparison throughout decomposition.

15 Placement photographs - all photographs include a North arrow when possible to provide spatial orientation.

	Location
	Detail photos - areas of interes t
	Lower third of the body (hips, l egs, feet)
	Genitalia
	Upper third of the body (head, neck, chest)
	Head striaght on
	Identification Number
	L Axilla
	L elbow
	L Foot
	L hand
	L Hip
	L Knee
	L Profile of head
	L Torso Ground Body Interface
	Oblique of body
	Overview
	R Axilla
	R Elbow

	Location
	R Foot
	R hand
	R Hip
	R Knee
	R Profile of head
	R Torso Ground Body Interface
	Middle third of the body (torso)
	Weather Station - same photo of the trail at every observation - to document evironmetal conditions

List of photographs to be taken at every daily observation.

Field Soil Sampling Procedure

16 Soil Sample collection- Initial collection occurs at placement (0 ADH). Subsequent samples should be collected at time stamps of 100, 250, 500, 750, 1000, 2000 ADH, and then every 500 ADH until unenrollment. At each sampling timepoint, soils should be collected from control sites and within decomposition-impacted areas. PPE (Gloves, pants, shoe covers) should be worn during all steps of the soil sampling protocol.

17 At placement: Identify a control site for each donor. Should be 1 – 1.5 m uphill (if on slope) from the donor, and not in another decomp zone.

17.1 Mark the control site with a flag labelled with donor number and “control”.

18 Label one 7 oz bag and one 4 oz whirlpak bag with donor number, date, and "donor".

19 Using a clean corer, collect approximately 5-8 soil cores from multiple locations around the body, avoiding areas that have already been sampled. If there is a visible decomposition island (dark soils), collect from these soils.

19.1 To take one soil sample: Identify the location of the soil sample and gently remove any large debris (leaves, twigs, etc.). Remove the sterile syringe (with the tip cut off) from its covering and remove the plunger from the syringe. Press the end of the corer into soil vertically to a depth of ~5 cm. Carefully reinsert the plunger to push soil cores into a 7 oz whirlpak bag (one bag per donor). Repeat this process until at least 35 g (use kitchen scale to check) of soil have

been collected (~5-8 cores). Dispose of the corer. If the corer breaks or becomes damaged due to hard, dry soil, rocks, etc., use a new sterile corer.

20 Homogenize sample by hand: gently press the bag between your thumb and index/pointer fingers to 'mix' the soil and break up individual soil cores. Pour the mixed soil into a large sterile weigh boat and remove any roots, rocks, hair, maggots or other foreign (non-soil) material using sterile tweezers as best as possible.

21 Using a sterile spatula or scoop, take a 20 g subsample of soil and place in a 4 oz whirlpak bag labeled "DNA/Enzymes", donor number, date, and sample type (donor or control). Next take a 5 g subsample of soil and place in a 4 oz whirlpak bag labeled "Met", donor number, date, and sample type (donor or control). There should be at least 10 g of soil left in the 7 oz whirlpak. Close bags.

22 Put both 4 oz whirlpak bags into dewar/CryoPod™ Carrier of liquid N, ensuring they are submerged. The 7 oz whirlpak bags can be transported at ambient temperature, after which they should be stored at 4° C.

23 Label one 7 oz bag and one 4 oz bag with donor number, date, and "control". At control site (1 to 1.5 m uphill, marked with a flag), remove large debris to expose the soil surface and use a clean corer to repeat steps 18-22.

24 Repeat steps 18-22 with additional donors, using a new corer for each donor. Dispose of corers and PPE in autoclave bags.

25 Transport samples from field to lab. Samples in 4 oz bags, labeled "DNA" or "MET" are stored in a -80°C freezer until analysis while 7 oz bags are stored in the 4° C fridge.

Note

Laboratory Processing:
Basic soil chemistry (7oz whirlpak bag unfrozen soil)
Soil moisture analysis:
1. Weigh an aluminum weigh dish and record weight
2. Weigh 2 x 2 g soil into dish and record weight
3. Dry in 104°C oven until completely dry (at least 72 hours)
4. Obtain and record final weight.
5. Archive dried soils.

pH and EC
1. Weigh 3 g into a 50ml Falcon tube.
2. Add 6 ml dH2O
3. Vortex 5-15 sec, measure EC.
4. Let settle (approximately 5 min), measure pH.

Additional analysis (4oz whirlpak bag frozen soil)
1. DNA extraction
2. Metabolite extraction
3. Enzymes assays

Field 2x Daily observations (AM + PM)

26 Photographs

20m

	Location
	Weather Station - same photo of trail every observation - to document environmental conditions.
	Middle third of the body (torso)
	R Torso Ground Body Interface
	R Profile of head
	R Knee
	R Hip
	R hand
	R Foot
	R Elbow
	R Axilla
	Overview
	Oblique of body
	L Torso Ground Body Interface
	L Profile of head
	L Knee
	L Hip
	L hand
	L Foot
	L elbow
	L Axilla
	Upper third of the body (head, neck, chest)
	Head striaght on
	Genitalia
	Lower third of the body (hips, legs, feet)
	Detail photos - areas of interest
	Identification Number

List of photographs to be taken at every daily observation.

27 Record arthropod documentation, photographs and field notes of arthropod activity at each observation time. All samples must contain a label inside of the collection vial with the following information: date, time, case number, sample number, and collector's initials.

27.1 Arthropod Observation Form
This includes adult arthropod arrival, presence of immature arthropods (e.g., eggs, larvae, pupae), formation of larval aggregations, and larval dispersal behavior. Absence of arthropods will also be documented. See attached form.

27.2 Arthropod Collection

28 Scavenger Form

29 Decomposition Fluid Form

29.1 Decomposition Fluid Collection

30 Tiny Tag Temperature log reading

31 Game Camera

Field Entomology Sampling

1m

32 Conduct visual observations and note the presence or absence of various arthropod life stages on
the data collection form.

33 Do five aerial sweeps with a butterfly net to collect flying arthropods.

33.1 Place captured flying arthropods in 70% EtOH in 20 mL scintillation vials with appropriate labels.

34 Using forceps or another collection device (e.g., plastic spoon, spatula), collect immature arthropods from at least three locations and document on collection form.

34.1 Collected larvae will be divided into two samples for either metabolic analysis or morphological species identification, with each sample containing roughly 50 individuals.

34.2 Larvae intended for species identification will be killed via parboiling. An electric kettle containing distilled water will be allowed to reach boiling point (100 °C). Water will then be decanted into each 20mL glass scintillation vial containing live larvae and allowed to incubate for 00:01:00 before being strained to remove water. Larvae will be placed back into their respective vials, which will be filled with 70 - 85% ethanol for indefinite preservation. 1m

34.3 For metabolic analysis: NEED TO BE FROZEN IN LIQUID NITROGEN in 1-2 ml cryo vials

Field Collection of Decomposition Fluid

35 In the field, observe donor to determine if there is fluid visibly pooling around the body. Record observations in field notes. If there is enough (~15 ml of collectable fluid), proceed with sampling. If not, note “not enough fluid”.

36 At each sampling, decomposition fluid samples will be collected for both DNA extraction and untargeted metabolomics, therefore the fluid sample will be aliquoted into respective 4 oz bags. Begin by labelling 2 4 oz whirlpak bags with donor number, date, analysis type (DNA or MET), and location of collection.

37 Open a sterile 30cc luer-lock syringe and ensure the plunger is fully depressed. Gently place the tip into the pooling fluid, holding the syringe at ~ 45 degree angle and ensuring the tip is not pressing into the soil.

38 Slowly pull the plunger to draw fluid into the syringe. If the fluid is viscous and will not draw into the syringe, cut the tip back with sterile scissors to enlarge the tip.

39 Clean the syringe tip by wiping with a clean paper towel. Dispense fluid into 50 ml falcon tube. Take at least 15 ml of fluid if possible. Cap and gently invert the 50 mL tube 5 times to mix the fluid.

Note

Do NOT shake the tube.

40 Label 2mL cyrovials (at least 4) with donor number, date, sample type (DNA or MET), and location of collection (2 will be labeled DNA and 2 MET). Use a clean syringe to aliquot 1.75 mL of fluid into each 2mL cryovial to leave room for expansion when frozen.

41 Screw the cap on the cryovials and place in the corresponding labelled 4oz whirlpak bag. All cryovials from one donor and analysis type should go into one bag.

42 Seal the whirlpak bag and place it in liquid nitrogen immediately.

43 If there are other locations with adequate fluid pooling, repeat steps 36-43 for that donor.

44 Repeat steps 36-43 for each donor.

45 Store collected decomposition fluid samples at -80 °C

Note

Samples will be used for:
1. DNA Extraction
2. Metabolomics
3. Enzyme Assays

PM Daily Observations

46 Photographs

47 Insect Form

48 Scavenger Form

49 Decomposition Fluid Form

50 Tiny Tag Temperature log reading

Unenrollment Criteria

51 Unenrollment Criteria - The donor's abdomen should cease to release fluid, with most tissues desiccated, marking the end of the active decomposition phase.