College: L&S
Department/Unit/Discipline: Chemistry and Biology
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Introduction:

Previous experiments completed by Osada et al. displayed several chemical differences between rat and mouse urine samples, which stimulated different reactions of the rats or mice after smelling the urine samples. Similarly to these experiments, my mentor, a colleague from the University of Wisconsin-Whitewater Biology Department, has been monitoring different reactions of dogs to the smell of varying dog urine samples. It is our hope to identify the chemicals within these dog urine samples in order to aid her research on determining the reason for a dog's response. In order to do so, a similar method to the mouse and rat urine experiments that involves solid phase micro-extraction (SPME) will be used to analyze the volatile compounds in dog urine samples. It is likely that analogous compounds and chemicals in the previous rat and mouse experiments will be located within the dog urine samples.

SPME is a modern sample preparation method typically used for the isolation and pre-concentration of organic molecules from various conditions (Muller et al.). A micro-extraction, an extraction of a very small portion of analytes, is performed by exposing a silica fiber with a polymeric coating to a sample. In SPME a short piece of a fused silica fiber coated with a polymeric stationary phase is mounted in a device similar to a syringe. While being transported, stored, or manipulated, the fiber remains withdrawn into the needle portion of the device. During extraction or desorption of analytes, the fiber is exposed allowing the analytes present in a sample to divide into parts into or onto the coating depending on the type of analyte. Once equilibrium between the coating and the sample has been reached, the process will cease and longer extraction times will not result in larger amounts of analyte extracted. After completion of extraction, the fiber is retracted into the needle, and the device is transferred to a gas or liquid chromatograph for separation and determination.
For our specific experiment, we will be using a Thermo Scientific Trace Gas Chromatograph (GC) and a DSQ II Mass Spectrometer (MS) to determine the compounds in the dog urine samples. When using a GC, analytes are thermally desorbed from the fiber in an injector (Muller et al.). Manual operation of the device is simple according to Muller et al., and the fibers are reusable, which will allow for multiple tests using the same fiber after being properly cleaned.

Proposed Study and Methods:

In the rat and mouse experiments, samples were prepared for GC analysis by headspace solid-phase micro-extraction (HS-SPME) (Osada et al. 2009). In order to concentrate volatile chemicals from the urine, an SPME fiber (2 cm-50/30 µm DVB/Carboxen/PDMS StableFlex, Supelco) was inserted for 60 minutes into the headspace of a 4 ml vial with a Teflon septum (Supelco) containing 150 µl of rat urine, which was then saturated with NaCl, mildly heated to 37-40 °C, and constantly stirred. For this experiment's GC analysis, helium gas was used as the carrier, the column flow was 2.4 ml/min, and the oven temperature was maintained at 40 °C for five minutes, increased by 10 °C/min to 200 °C, and then elevated by 5 °C/min to 240 °C, while the injector temperature remained at a constant 230 °C (Osada et al. 2009). In addition, the rat and mouse experiments used identical column and oven conditions for the MS analysis as the GC analysis. Identification of structures representative peaks was assigned using both the NIST92 library and manual interpretation of mas spectra based on comparisons with reports in literature. An internal standard of 500 ng of 7-tridecanone (dissolved in methyl acetate) and commercial chemicals, such as 3,4-dehydro-exobrevicomin, 2-sec-butyl-4,5-dihydrothiazole, and 6-hydroxy-6methyl-3-heptanone were used as a hexane solution. Ideally, we plan to follow these basic guidelines to design our experiment after these studies. The only differences will include slight variations in instrumentation and the samples being tested.

Schedule:

In October and November, we plan on purchasing the needed fiber and becoming familiar with the procedure and new instrumentation by practicing with several blank solutions prior to testing with the urine samples. Testing of the dog urine samples will begin in late November and the beginning of December with GC and MS. Sample testing will end in March. The months of February and March will be designated for the analyses of the data received, compilation of the data and analyses, and transferal to my mentor's lab for further use and diagnosis. During March and April, a poster will be prepared to present at the NCUR conference or UW system conference.

Expected Significance:

My mentor has already found a correlation to the response of a dog after smelling certain urine samples and the position of the canine's tail when urinating. After analyzing these urine samples, differences in the chemicals found may lead to an additional reason for the canine's response to smelling the urine. The data received after analyzing the urine samples will be transferred to My mentor's lab in order to analyze any further relationships between the chemicals found in the urine samples and the reactions received by the canines.

R1: Good to include reference/citation. The complete bibliography list should be included in the designated section (separate from the body proposal).

R1: It is best to take ownership of the project so this is not just a project to aid in a mentor's research but rather, also a learning experience for the mentee who will try to contribute to the generation of new knowledge.
R3: Goal/objective stated in clear terms.

R1: Goal of research specified.

R1: SPME is only one part of the analysis process so this sentence is a bit misleading.

R3: I like the short description tying similar procedures done elsewhere, to what the project aims to do, and identify available resources on campus.

R1: This section includes quite a bit of experimental details. It is not a bad idea but for someone who is not an expert in the field, these details will not make much sense UNLESS the rationale behind such details are explicitly specified. It is a good idea to write for a range of readers with varying backgrounds/ experiences.

R1: Why do you need internal standards and why are these compounds chosen? If you include this level of details, you should explain them.

R3: Proposed activities are directly related to proposed objectives.

R1: How are these determined?

R1: The information in this section can be incorporated into the timeline table.
R3: Seems like a feasible project with the given timeline.

References:

Muller, L., Gorecki, T., and Pawliszyn, J. "Solid-phase Microextraction in Analysis of Pollutants in the Field." Encyclopedia of Analytical Chemistry, 1-16

Osada, Kazumi, Kashiwayanagi, Makoto and Izumi, Hiroshi "Profiles of Volatiles in Male Rat Urine: The Effect of Puberty on the Female Attraction." Oxford University Press, Chem. Senses 34 (2009) 713-721

Osada, Kazumi, Tashiro, Takuya, Mori, Kenji, and Izumi, Hiroshi "The Identification of Attractive Volatiles in Aged Male Mouse Urine." Oxford University Press, Chem. Senses 33 (2008) 815-823

Timeline (dates)	Goals and Objectives	Actions:	How Actions Support Goals and Objectives
Goal I: Literature Review			
September-October	First objective: Comprehensive background knowledge	Literature Reviews	Familiarize with other past experiments and outcomes
Goal II: Data collection			
November-March	First objective: Trial SPME experiments replicating previous work on volatile compounds in urine	Set up and perform extraction and analysis as in previous work, modify as necessary for our equipment and samples	validates method of analysis
	Second objective: Trial SPME experiments replicating previous work with rat and mouse urine	Use validated method to analyze dog urine samples	examines actual samples important to my mentor
Goal III: Analysis of data			
Time?	First objective: Determine volatile components of dog urine	Analyze mass spec data to determine volatile compounds	Determines volatile composition of samples
	What about correlating the mass spec data with the data on dogs' responses?		
Goal IV: Dissemination of results at UWW Undergrad. Research Day and NCUR/UW-System			
November-December	First objective: Apply to and be accepted at NCUR or UW System Conference	Write Abstract	Necessary for application to conference
March-April	Second objective: Present poster at conference	Prepare Poster	Necessary for conference presentation

R1: This stated objective is not consistent with the description under 'Action'. Most likely this should be 'dog' urine instead of 'rat and mouse' urine. Must be careful about careless errors that affect the comprehension of readers.

R2: This proposal is very well written. It is difficult to decipher who is original researcher, i.e. is this proposal written by the student or the mentor?

R4: I agree this is a thorough proposal with clear background, methodology and outcomes. A more explicit description of the impact of the study - to help with the dog urine research - would improve the proposal. How does the larger research project (the mentor research) impact our larger community? The timeline is realistic.

Evaluation:

1) Are project activities and outcomes connected to the stated goals and objectives? **Agree. However, there are gaps in timeline for work planned and the stated goals of the project in the Introduction.**

2) Project feasibility. How realistic and appropriate is the study for this student in the time available? **Appropriate, but there are inherent unknowns in this type of project since one cannot necessarily predict the level of technical difficulties. This can be partially compensated by including alternative methodology/approaches to answer the same research question but this proposal did not do that.**

3) Likelihood of project outcomes. Is the project likely to result in a data set, creative performance, art object, or academic project that can be presented and/or published? **Likely.**

R1: General comment: Proof reading a proposal before submission is important. Watch out for typos, formatting errors, etc, that interfere with the ability of evaluators to assess a submission.