

2006-2007 ASD Grant Application

Name(s) Stephen Hoffmann

Department(s) or School(s) Chemistry and Environmental Studies

Title of Project

Heavy Metal Contamination in Grey Wolf Kidneys

Amount Requested \$2600

Your Email: shoffman@iwu.edu

- Will you use human beings as experimental subjects? Yes No
If yes, please submit the appropriate approval notice.
- Will you use animals as experimental subjects? Yes No
If yes, please submit the appropriate approval notice.
- If your proposal is funded, would you be willing for the Mellon Center to use it as an exemplary submission in the online *Handbook*? Yes No

Please complete the following checklist by placing a check mark against each item to insure that your application is complete. Incomplete and/or late applications will not be considered.

1. Project Summary (email to jhand@iwu.edu)
2. Have you requested IRB approval? Yes No NA
3. Proposal as per format described in handbook
4. A brief Vita
5. ASD grant budget page
6. Report of Previous ASD grants (File separately with the Associate Dean)
Yes NA

Stephen Hoffmann 3/26/07
Signature of Applicant and Date

Tina [Signature] (for Becky Reesner)
Signature of Chair or direct supervisor

Please note that a recommendation letter from a direct supervisor or chair is not required for ASD grants.

2. SUMMARY OF PROJECT

"Heavy Metal Contamination in Grey Wolf Kidneys"

In mammals, high levels of the heavy metals, such as mercury, lead, and cadmium, may cause neurotoxic effects. Despite their low natural abundance, emerging evidence indicates that industrial release of these metals followed by atmospheric transport can lead to their enhanced presence even in locations considered pristine, such as the Arctic. This work investigates the concentration of metals in the kidneys of Arctic and boreal grey wolves. Wolves are of particular interest because they represent the top carnivore of the terrestrial food chain, and therefore the bioaccumulation of metals in wolf kidneys may be an indication of broader contamination within the Arctic system. A remarkably large number of samples (~150) are already available at IWU, thanks to a collaborating project investigating pesticide residues in wolves. Data on metal levels in wolves is notably scarce in the literature, so this project should lead to significant interest and rapid publication.

3. SUMMARY OF PREVIOUS ASD GRANTS

I have received one previous ASD grant, in the 2005-06 year.

- a) Title: Organic contaminants in rivers: development of techniques to measure particulate and sediment partitioning.
- b) Date awarded: second review period of 2005-06. Amount awarded: \$3380.00
- c) End products have not yet been achieved, and the work from this grant is still ongoing. The primary work proposed in this grant should be complete by the fall of 2007.

Summary of accomplishments with this grant:

The subject of my 2005-06 ASD grant was the measurement of organic contaminants atrazine and estradiol in rivers. There are three main goals: (1) with my students, develop the expertise and laboratory protocols for the effective and reliable measurement of these compounds — this work is complete. (2) to determine the background levels and contamination patterns for several local rivers — this work is partially complete, and preliminary results were presented by a student at the 2006 JWPRC. (3) investigate the partitioning of these compounds to suspended particles and sediment — the planning and initial laboratory work is done for this goal, but the field work and actual measurement is not; it is scheduled for the summer of 2007.

4. NARRATIVE:

Brief context of the project:

The measurement of environmental contaminants in Arctic and boreal¹ carnivores, such as the grey wolf, represents important science for two reasons: (1) Arctic animals largely live far from local pollution sources, so evidence of increased contaminant levels represents a metric of global pollutant transport and integrated global contamination impact; and (2) carnivores are at the top of the food chain, where bioaccumulation² of compounds is most significant, and where levels integrate pollution sources across the entire ecosystem.

This proposed project is part of a larger collaboration with Professors Given Harper and Jeff Frick, who, along with their students, measure organochlorine pesticide contamination in wolves. The collaboration with several state and provincial wildlife management organizations throughout the Arctic and boreal forests have provided the group at IWU with an unusually large set of wolf kidney samples, which can be used for both heavy metal and pesticide analysis; I have joined the collaboration to lead the metal analysis work and interpretation.

4a. End-product of the project:

The work described in this proposal, both preliminary work already completed and proposed future work, represents the initiation of a second area of scholarly research in my laboratory, one that combines my experience in trace metal analysis with the opportunity for collaboration with colleagues at IWU who work on contamination of biota. It is an exciting area of research for me for several reasons: it crosses disciplinary boundaries between chemistry and biology, it combines my expertise in chemistry and environmental science, and the work has enormous potential for broad scholarly interest and publication. This interest largely stems from the fact that the important question of top predator trace metal contamination in the Arctic has simply not been answered. (Due to the difficulty in collecting tissue from Arctic carnivores, the number of papers in the literature is remarkably low.) This work directly and simply addresses that question.

I expect that the work laid out in this proposal would be suitable for publication in a high-impact journal; my primary target is *Science of the Total Environment*, which has emerged as the most common source for research in Arctic contamination. Potentially, *Archives of Environmental Contamination and Toxicology* is also an appropriate journal with a fast turn-around time, though it is less widely available and based more on basic measurement than on interpretation of patterns, processes, and trends. With the large sample set and current high-quality instrumentation available to me, I fully anticipate that this work will merit publication, potentially even by fall 2007. Dr. Given Harper is leading the collaborative project measuring pesticide levels in these same samples, but I expect that the extent of the metal data will merit an individual paper (i.e., the two target analytes, pesticides and trace metals, will not be combined into a single paper).

4b. Significance of the project

Most heavy metals, such as lead, mercury, and cadmium, are toxic to wildlife even at extremely low levels. While these metals are naturally occurring, high levels of contamination

¹ *boreal*: the boreal forest biome refers to the global northern temperate forest between about 45° and 65° N latitude; it includes the major forests of the northern US, Canada, Russia, and Scandinavia.

² *bioaccumulation*: the enhanced concentration of environmental contaminants in animals higher in the food chain.

(often orders of magnitude higher than background levels) usually indicate a local pollution source, such as a mine or ore smelting operation. However, metal level studies of the last 20 years have begun to show that elevated levels of metals may be found in some animals living at places far removed from local sources. Metals may be transported for thousands of miles in the atmosphere (in the case of mercury, as a gaseous atom, and in the case of other metals, attached to aerosol particles), where they can accumulate in the biotic food chain. Even though acute metal toxicity is unlikely in places far removed from local pollution sources (because contamination levels are not high enough), the lower levels may still lead to chronic toxicity in individuals or a population of animals.

Heavy metals, like many environmental contaminants, tend to concentrate in certain tissues of plants and animals, and many of them are not readily excreted or degraded by normal physiological processes. As those tissues are consumed by animals higher on the food chain, the metal contamination will be transferred. This transfer potentially continues for the lifetime of the carnivore, leading to a slow build-up of a significant life-long "body burden" of contamination. This process, called bioaccumulation, means that higher-level carnivores will have significantly higher levels of toxins (even several orders of magnitude higher) in certain tissues (including the kidney and the liver) than is present in the ambient environment, and often older carnivores will exhibit higher levels of contamination than younger individuals.

The proposed work here represents a unique opportunity to analyze the levels of several trace metals³ in the kidneys of Arctic and boreal wolves. This is exciting and scientifically interesting for several reasons:

- wolves are the top carnivores of the terrestrial food chain: analysis of wolves will therefore allow assessment of the overall impact of trace metals to this food chain, and assessment of the bioaccumulation factor of trace metals in this food chain.
- because wolves are the top predator, their populations are smaller than that of mammals lower on the chain (e.g., caribou, hares, elk), meaning they have been less sampled and analyzed in the literature.
- the wide geographic distribution of Arctic and boreal wolves (and the fact that the set of kidneys available at IWU encompass this wide distribution) leads to the likelihood that different populations will show different metal levels and patterns. The multi-element analysis allows the definition of "signature" patterns that may allow for the differentiation of populations, or the differentiation of primary contamination sources for different groups. Comparison of levels between different populations may also allow for the definition of what might be considered normal "background", or uncontaminated, levels of metals in wolf kidneys. This information is not, to date, readily available in the literature.

More generally, data about metal contamination in wolves is not as available as data about metal contamination in birds or in more common or more easily accessible mammal populations, a point which is particularly critical to the importance of this proposed work. Based on a complete search of the literature since 1990, there is a remarkably small number of papers with data that address trace or heavy metal levels in Arctic and boreal carnivores or other animals. Most of these papers have important differences from the work proposed here: as mentioned previously, work on caribou or other herbivores [1-6] is more common than work on

³ *Trace metals*: The phrase "trace metals" includes many of the heavy metals and other lighter metals; the key characteristic of a trace metal is its low concentration, generally on the order of one part per million by mass.

carnivores. Among carnivores, eagle and polar bear analyses [7-11] investigate an aquatic-based food chain rather than a terrestrial-based food chain (this has particularly important differences for mercury, because some processes of mercury in the environment occur at a disproportionate rate in the aquatic realm). Among terrestrial food chain-based carnivores, there are only two papers that analyze mink [5,12] and only two that analyze wolves [13,14].

The work of Shore, et al. [13] is limited by a small sample size, analysis only of wolf liver (which is likely different from wolf kidney), consideration of only two metals (mercury and lead), and use of an analytical technique with a relatively insensitive limit of detection. The work of Gamberg, et al. [14] is of high quality and analyzed several metals, but its conclusions were limited by geography (most wolves were from one area) and sample number (19 wolves). The available set of wolf kidneys for this proposed work is much larger: almost 150 wolves from at least three distinct geographical areas. The proposed analytical techniques described below should be able to analyze successfully at least ten metals with a very low limit of detection.

The basic analytical methodology of trace metal analysis involves three steps: representative sampling of the kidney, hot acid digestion (dissolving) of the kidney, and instrumental atomic analysis of the digestate. The details of the method differ slightly in each of the references cited above (for example, the mechanism, amount, and time of heat applied to the acid digestion step), but the basic scheme is universal. In preliminary tests during the spring of 2007, I have developed in my lab a safe and effective method for this digestion. All analysis steps need to be completed using "trace metal clean" techniques, or protocols designed to limit sample contamination from metal sources in the laboratory. From my previous work, I am extremely experienced with these techniques.

The third step, instrumental atomic analysis, generally takes one of three forms — these instruments are fundamental tools of analytical chemistry, and all are covered in detail in my Instrumental Analysis course. "Atomic absorption" is the simplest technique; in fact, it is regularly taught to chemistry students at IWU using instrumentation available in the department. However, the limit of detection⁴ for atomic absorption is relatively high — probably too high for wolf kidneys (based on results of Gamberg, et al. [14]). The two other methods, ICP-emission spectroscopy and ICP-mass spectrometry⁵ (or, ICP-ES and ICP-MS, respectively), show lower limits of detection, but are not available at IWU. However, I am spending a large portion of my current Junior Faculty Leave at the University of Wisconsin, where colleagues have graciously agreed to let me use these instruments. (I have been granted "Visiting Faculty" status in the lab, allowing me to use the instruments and be present in the building after hours).

For any of these instrumental methods, concentrations are determined based on comparison to known metal standards. Validation of the entire method is accomplished with the analysis of "standard reference materials," or SRMs, which are animal tissue samples whose metal levels have been measured and certified by governmental standard agencies like NIST.

I have more experience with ICP-ES and the instrument is more reliable than the ICP-MS, so I intend to analyze first with that method. However, ICP-MS has two advantages that make it worthy of an additional attempt: (1) limits of detection by ICP-MS are generally two to three orders of magnitude lower than ICP-ES — there is no question that they are low enough for

⁴ *limit of detection*: the lowest concentration that can be reliably detected as different from zero for a quantitative analytical method. Note that *lower* limits of detection are better.

⁵ *ICP*: stands for "inductively coupled plasma," which acts as the mechanism by which the sample is introduced into the instrument.

all metals, and (2) ICP-MS data includes information about different isotopes⁶ of the metals, which can further help in the identification of distinct populations with distinct contamination sources. An advantage of both ICP-ES and ICP-MS is that they are multi-element techniques, and all metals can be detected simultaneously. This allows for fast analysis (perhaps 25 samples can be analyzed in an afternoon's lab work), which allows for application of both methods in a timely manner. I anticipate that ICP-ES data would be sufficient and appropriate for publication (and, in fact, many papers use this method), but if I can derive useful data from the ICP-MS, the analysis and interpretation are likely to be even more robust.

The final methodological question is the selection of metals to analyze. The figure below shows the twelve metals I have chosen to investigate; these were chosen based on toxicity, likelihood of presence in wolf kidneys, and interest in the literature.

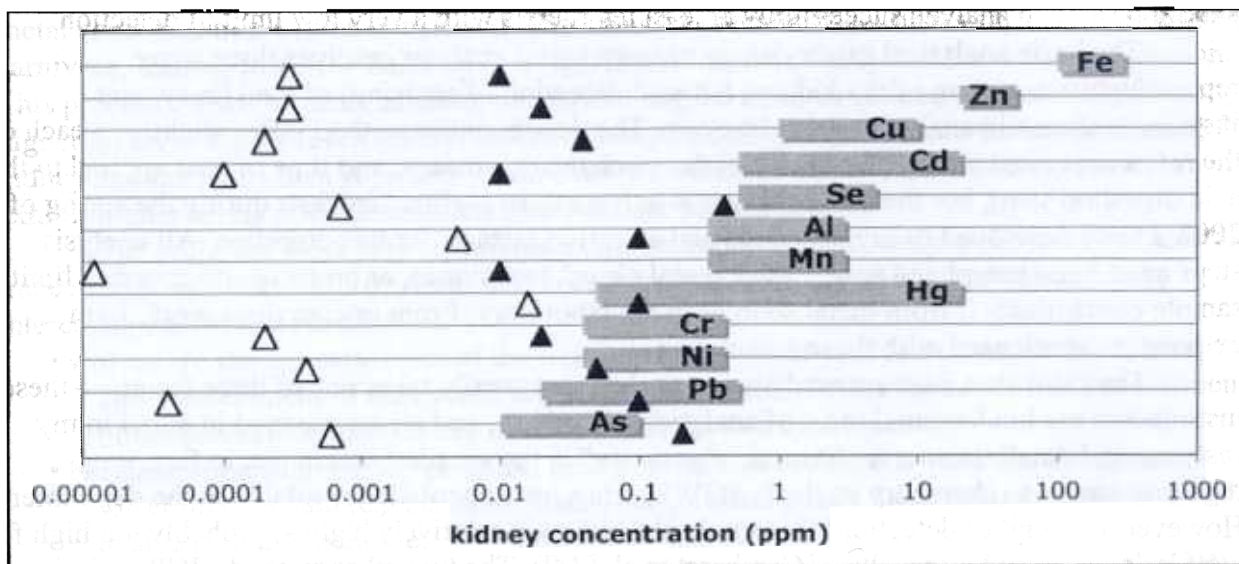


Figure: Analysis metals, expected concentrations, and limits of detection. For each metal, the gray bar represents the range of concentrations found in wild mammalian kidneys [1-14], and the triangles represent limits of detection, black for ICP-ES and white for ICP-MS [15]. In the cases where the grey bar is significantly to the right of the triangles, the method should be sufficiently sensitive. Chemical symbols: Fe = iron; Zn = zinc; Cu = copper; Cd = cadmium; Se = selenium; Al = aluminum; Mn = manganese; Hg = mercury; Cr = chromium; Ni = nickel; Pb = lead; As = arsenic. Note that the horizontal scale is logarithmic, not linear.

This proposal will have significant impacts on my personal professional development. First, it provides a way for me to tap into my experience with trace metals in water for my work at IWU. Metal levels are significantly lower in water than in mammal tissues, which makes trace metal *water* analysis too difficult to complete in my current lab (this is a primary reason why my other scholarly work, including my previous ASD grant, investigates organic compounds in water rather than metals). Second, the project has been designed as a relatively simple and stand-alone piece that can be completed and submitted for publication within a year – this will be a

⁶ *isotopes*: isotopes are two or more forms of a particular element that have different masses – because ICP-MS measures the mass of atoms, it can detect the different ratios of isotopes that may be present in different samples of a particular metal sample.

great benefit to me as I proceed toward the tenure decision. And finally, the project has already opened the door for future metals in biota projects — Given Harper and I have started discussing a collaboration with Pat Kramer the Raptor Center at the University of Minnesota to analyze metals (particularly lead isotopes) in eagles and other raptors of Minnesota that are experiencing acute lead poisoning. Using lead isotopes to determine sources is currently an intense topic of interest (for example, a similar project was recently the cover paper of an issue of *Environmental Science and Technology*, the top journal in my field [16]). Completion of this project on wolf kidneys (both for expertise in animal tissue analysis, expertise on ICP-MS, and collection of necessary plasticware and other materials) will help put me in a good situation to complete the eagle lead isotope project.

The granting of an ASD award will greatly increase speed of analysis and digestion: there are a large number of samples (which is one of the strengths of this proposed work and this opportunity), and parallel digestion of samples is possible if there is sufficient plasticware and other equipment. Without sufficient equipment, the work can be done, but it will be greatly slowed, wasting time and keeping me from other lines of inquiry. Without the ASD grant, this digestion work could be performed by a student, but the pedagogical benefits of repeating the same slow analysis for months are limited, and I am reluctant to ask a student to do that work, when their personal benefit would be so low.

4c. Justification of expenses:

The primary items requested in this application are equipment and consumables. As mentioned before, there are many ways to perform a digestion: after some trial studies using borrowed plasticware, I have determined that Teflon bombs⁷ are the best compromise between issues of safety, cost, cleanliness/contamination and throughput. The bombs noted in the budget are easily cleaned, are relatively inexpensive, and can be safely used under the digestion conditions that I have designed. Having 24 bombs in rotation could allow for the digestion of up to 40 samples a week.

My colleagues at UW have been generous in allowing me time on the instruments, but the analysis uses significant consumable supplies, notably argon gas, high-purity acid, and sample vials. Part of the budget request will allow me to replace the consumables that I use for this project.

4d. Proposed timetable for the project:

A major positive of this project is the relatively quick time schedule, and I have identified this work as one of the three major projects of the late spring and summer of my current Junior Faculty Leave. The development of the digestion method is already complete, the kidneys are currently available, and I will be making preliminary measurements with the ICP-ES during April 2007. If this application is successful, I plan to use the bombs to digest the kidneys during May and June, and analyze from June through August 2007. Data analysis and interpretation will be done during the early fall, with submission of a manuscript following shortly thereafter.

4e. Student assistance: Not applicable.

⁷ *bombs*: In chemistry, the term "bomb" refers to a sealed, constant-volume container, not an explosive.

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