# Methods for Sampling and Analysis to establish potential exposure of wildlife to persistent contaminants in remote areas

During the last three decades, numerous research efforts to identify the adverse effects, and the environmental fate of xenobiotics, have resulted in a large variety of field-methods for sampling and analysis of tissues from wildlife species. So far the majority of these research efforts have been conducted in areas which are relatively accessible and in close proximity to research facilities where scientific equipment to conserve and prepare samples is available. Ecotoxicological research in remote areas puts substantial constraints and limitations on the amount, and type, of equipment researchers are able to bring in the field. Ecotoxicological research in remote areas requires a creative and resourceful approach. Field sample collection, storage, and analyses need to be conducted in a manner that is practical, efficient, and reliable.

In this paper we review a number of techniques for sampling and analysis for persistent chemicals (e.g., halogenated compounds) which can be utilized in remote areas such as the Tibetan Plateau. We conducted this review especially for those researchers who are working to establish the ecology of the snow leopard (*Uncia uncia*) and other endangered species in the mountainous regions of central Asia.

## TARGET SPECIES AND COMPONENTS

As argued elsewhere (Hol et al., this volume), the presence of persistent chemicals in the Himalaya area may be of serious importance regarding the management of wildlife species, especially those in the higher trophic levels. Increasing use of chlorinated pesticides in countries surrounding the Tibetan Plateau necessitates the establishment of base-line data in different compartments of terrestrial and aquatic ecosystems. So far, no research has been conducted to establish the enviro-dynamics and the potential adverse effects of persistent chemicals on wildlife in this area. Several research projects have been conducted to study the ecology of ecosystems in this and other mountainous areas in central Asia. Other research projects are on-going. We submit that ecotoxicological research can be integrated in these projects, with minimal inconvenience or additional costs, provided that simple methods for obtaining samples are available.

In many studies, animals are captured or immobilized. Large mammals, such as ungulates and snow leopards, are immobilized in order to attach radio-collars to study behavioral aspects and distribution of the animals. Birds are captured and ringed in order to study migration. On various occasions, animals are found dead or moribund during field surveys or other research routines. These occasions offer excellent opportunities to obtain samples for analysis for contaminants. The contaminants of most interest are persistent chlorinated chemicals, such as organochlorine pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDFs) and polychlorinated dibenzodioxanes (PCDDs). With respect to collecting samples for analysis on persistent chemicals, there are some basic themes to sample collection and preservation that must be understood. Samples can generally sustain some period of time without refrigeration, since biodegradation of these components is slow or does not occur at all. On the other hand, sampling and storage of samples requires extreme care, since contaminant levels are generally low (nanogram/gram level), and careless sampling may introduce external contaminants to the sample, thereby precluding accurate analysis of material collected. Sampling materials and containers need to be inert (e.g., teflon bags or glass vials), free of potential contaminants, and must be rigorously cleaned after each sampling event to avoid cross-contamination between samples.

## SAMPLE COLLECTION FOR PERSISTENT CHEMICALS FROM CAPTURED ANIMALS

Sample collection from captured animals requires non-lethal methods, and non-invasive methods are preferred. In case invasive methods (e.g., blood collection) are used, it is necessary for the researcher to have sufficient familiarity and previous practice with the method to avoid injuries, trauma and excessive stress to the animal. Several types of samples can be collected for analysis of environmental contaminants: (a) blood, (b) hair, fur, nail clippings or feathers, (c) adipose tissue, and (d) excrements: faeces, urine or fecal-urate-samples.

### Blood

Sampling of blood is a valid method for the analysis of exposure to environmental contaminants, and only small volumes are required to obtain sufficiently low detection limits. Blood analysis has been successfully utilized in many studies to determine human exposure to persistent contaminants, such as organochlorine pesticides (Murphy et al. 1981, Bristol et al. 1982, Grasso et al. 1983, Mossling et al. 1985, Murphy and Harvey 1985, Violente et al. 1986, Wariishi et al. 1986, Pines et al. 1987, Krawinkel et al. 1989, De Jong 1991, Wolff et al. 1991, Bhatnagar et al. 1992, Nair et al. 1992, Lommel et al. 1992). In field studies blood samples are collected from birds to determine exposure to organochlorine pesticides (Court et al. 1990). Analysis of blood offers the possibility to detect relative short-term exposure to contaminants.

Although blood has been exploited as a tissue for the identification of exposure to contaminants, the methods for sampling and analysis are not standardized. A comprehensive review and comparison of the available methods has not been conducted. The amount of blood drawn for sample analysis varies between 5 and 30 ml in the studies reviewed. In some cases methods were used for whole blood, in other cases only the blood serum was utilized. As little as 0.5 ml of blood has been used for sample preparation and the resulting detection limits were acceptable (1 ng/ ml). This indicates that determination of persistent contaminants in blood can be utilized not only for large-bodied vertebrates but also can be used for small-bodied or young animals.

For larger animals, a sample volume of 5 to 10 ml whole blood is recommended. Ideally, the blood should be collected in a heparinized teflon container with screwtop and stored in a cooler, if conditions allow to do so. If possible, it is recommended to accurately determine volume or weight of the sample, since evaporation may reduce the volume during transport. Upon arrival at a field station, it is recommended that the samples are frozen at a minimum of -20$^{o}$C.

**Hair, Fur, Nail Clippings and Feathers**

Analysis of contaminants in hair, fur, nail clippings and feathers has been used to determine exposure to heavy metals (Airy 1983a, 1983b, Eaton and Ferrant 1982, Obrusnik and Paukert 1984, Matsubara and Machida 1985, Tavares et al. 1989, Agahian et al. 1990, Ahmed et al. 1990, Sree Khrisna Murti et al. 1990, Ahmed and Elmubarak 1991, Folin et al. 1991, Pfeiffer et al. 1991, Roelke et al. 1992, and many others). The utility of analysis and correlation with other organs to determine body burden of heavy metals is presently a topic of discussion (Sly-Peck and Joseph 1983, Matsubara and Machida 1985, Kievay et al. 1987, Chatt and Katz 1989, Lamand et al. 1990, Folin et al. 1991). Until this issue is resolved such analysis can only be used to identify exposure and to infer potential adverse impacts on the exposed individual. Although such information does not conclusively characterize the full scope of physiological consequences of contaminant exposure, the value of this information for management decisions should not be underestimated.

More recently, a variety of methods have been developed to establish the presence of controlled substances (also referred to as drugs of abuse) in human hair (Baumgartner et al. 1981, Smith and Pomposini 1981, Ishiyama et al. 1983, Suzuki et al. 1984, Balabanova and Hamoki 1987, Nakahara et al. 1990, Cone 1990, Cone et al. 1991, Goldberger et al. 1991, Harkley et al. 1991). Drugs of abuse belong to the category of moderately non-polar organic components. Most of these methods are extremely sensitive and can reveal the exposure history of the abuser by analyzing the deposition patterns in hair strands. Presently, results are published that indicate a utility for determination of exposure to other organic components such as ofloxacin (an antimicrobial agent) and to persistent contaminants. Implementation of these techniques into ecotoxicology field research looks promising (Miyazawa et al. 1991, Ohgami et al. 1991, Schramm et al. 1992). The possibility of using feathers of museum birds for the analysis of PCBs has been explored earlier by Jensen (Anonymous 1966). Miyazawa et al. (1991) demonstrate the possibility of determining exposure history of individuals to ofloxacin, using small samples of hair and a High Performance Liquid Chromatography (HPLC) analysis technique. Ohgami et al. (1991) report concentrations in hair of individuals exposed to PCBs which are approximately an order of magnitude higher than the corresponding concentrations in blood samples. Schramm et al. (1992) outline a method for determination of PCDDs and PCDFs in human hair.

Analysis of contaminants in hair has several advantages. The method is non-lethal and non-invasive and relatively simple to apply, samples do not need to be cooled or frozen, and the results offer insight in the exposure-patterns of the animals over an extended period of time. This knowledge may reveal diet-related exposure. Presently, the discussion on the utility of hair analysis seems to focus mostly

on the correlation between components found in hair and in other tissues, as to determine if hair analysis can replace tissue analysis. Roelke et al. (1992) have demonstrated a good correlation between concentrations of the organo-metal component methylmercury in hair and tissue of the Florida panther (*Felis concolor coryi*). Some variables that still must be resolved include the type (guard hair or undercoat) and location of hair collected, and optimal time of year for hair collection.

Samples of fur, hair, nail-clippings are recommended to have a weight between 5 and 20 grams. This amount would not be prohibitive for sample collection from larger animals. Samples are collected with a pair of scissors and stored in a teflon bag. Scissors are rinsed thoroughly with ethanol before use. Hair and/or fur is cut of as close to the skin as possible.

Sampling of feathers from birds as a non-lethal method is less useful. The collection of flight feathers may impair the bird's ability to fly. Therefore, feathers should be either contour or down feathers. For live birds, collection of blood samples is strongly recommended. Blood samples can be collected via jugular venipuncture on small-bodied birds or from the brachial vein on larger birds. Care must always be given to thoroughly clean (generally using an alcohol rinse) the skin and surrounding feathers prior to insertion of the needle so as not to introduce surface contaminants into the blood sample.

**Adipose Tissue**

Because most persistent chemicals are fat soluble (lipophilic), they are generally found in highest concentrations in adipose tissue. Concentrations in adipose tissue represent mid- to long-term exposure, depending on the lipophilic characteristics of the chemicals studied, as well as the persistence of the chemical and nutritional status of the organism. Sampling of subcutaneous fat is a method utilized successfully for larger marine mammals. Free-ranging terrestrial animals generally have little or no adipose tissue, and collecting an adipose tissue sample is traumatizing, complicated, and requires bringing a substantial amount of equipment in the field. This method is not recommended for living terrestrial animals.

**Excrement**

The presence of persistent chemicals in excrement represents recent exposure. Parent compounds of non-polar chemicals are usually found in feces, while more polar metabolites (i.e., more water-soluble) are excreted through urine. Collection of excrement is relatively simple, but the disadvantage of this method is that it is seldom known which animal produced the excrement, and it is sometimes difficult to determine the species. Sampling of excrement from captured animals is more complicated, but provides useful information on an individual. This method has been successfully used in the field for avian toxicology research. A method for the collection of fecal-urate samples from birds is described by Hol et al. (1992). Analysis of persistent chemicals in excrement may have some utility in specific research projects, but is less useful in studies to determine the effects of chronic exposure. Collection of excrement is not recommended if other tissues or hair, fur, nail clippings or feathers can be collected, and if the primary contaminants of concern are persistent organochlorine derivatives.

## SAMPLE COLLECTION FROM ANIMALS FOUND DEAD OR MORIBUND

Dead or moribund animals from endangered species are often collected for pathological examinations. If the condition of the carcass allows, the specimen should be transported to a laboratory facility where samples can be collected for analysis for toxic chemicals. In the United States the inclusion of contaminant analysis is a standard procedure in pathological examination. The analysis of liver, brain and adipose tissue for a variety of chemicals is a routine procedure in forensic and toxicologic research and standard methods are available from the United Fish and Wildlife Service Forensics laboratory in Ashland Oregon.

In case the carcass of the dead animal cannot be transported to a laboratory, it is still useful to collect tissue samples. If internal organs are still recognizable, subsamples (between 10 and 20 grams) of liver and or adipose tissue are generally sufficient to conduct analysis for persistent chemicals, to determine the lipid content of the sample and to determine the dry weight of the sample. Smaller animals (<100 g) can generally be collected as a whole. Samples are collected using surgical scissors and teflon tweezers

and stored in teflon containers or bags. Sampling equipment is carefully rinsed with methanol after the sample is collected to avoid cross-contamination. It is recommended to store the samples in airtight containers.

If the carcass has decayed, and internal organs are no longer recognizable, analysis of persistent chemicals is still possible and useful. In this case it is recommended to collect 10 to 20 grams of tissue from different areas of the carcass, to obtain a reasonable homogenous and representative sample of the whole body of the animal. Samples are collected using surgical scissors and teflon tweezers and stored in teflon containers or bags.

## DOCUMENTATION

Without proper documentation information derived from samples is useless. Every sample collected must contain the following details: date, time, name of collector, location, species, sample type and sample number. In addition, a sample log book is also recommended. In the log book samples can be tracked and additional pertinent information, such as characteristics of the setting in which samples were collected and handling procedures, can be recorded. A central coordinator should be assigned the task of tracking samples and ensuring that basic quality assurance and control procedures are followed.

## CONCLUSION

For larger mammals that are captured alive, collection of blood and fur, hair, or nail clippings is recommended for analysis of persistent chemicals. Since there is little or no previous experience with respect to sampling and analysis of tissues from animals of the mountainous regions of Central Asia, systematic determination of the correlation between blood and hair concentrations of persistent chemicals will help determine the utility of hair analysis for several species and different chemical compounds. Such an approach will be very helpful to simplify future projects. Blood samples and some feathers should be collected from birds captured alive. Birds must be sufficiently large to allow drawing of about 1 ml of blood. This will be the case for most birds. As a rule of thumb, birds can sustain a loss of approximately 0.1-0.5% of body weight in blood without undue stress.

It is not recommended to sample blood or fur from small mammals. Blood sampling from small mammals is meticulous and difficult and will only yield small volumes of blood. In some cases the animals are traumatized, or do not survive the treatment. In order to collect a sufficient amount of fur, a substantial part of the fur has to be removed, which may lead to hypothermia. Adipose tissue, liver tissue or brain tissue should be collected from animals found dead in the field if the internal organs are still recognizable. Tissue samples from various parts of the animal can be collected if the organ structure of the carcass is beyond recognition as a result of advanced decomposition. Smaller animals (<100 g, or anything fitting a sampling container) found dead in the field should be collected intact.

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