In general three important fields of applications can be discerned: identification of chemicals in products characteristic for a specific animal or plant species; discrimination of populations based on relative (trace) elemental concentrations partly reflecting local soil conditions to determine the provenance of disputed species and the comparison of materials that are of importance in these investigations.

GC-MS is used mainly to identify (extracts of) endangered animal and plant species in products, particularly in Traditional Chinese Medicines (TCM): musk from the musk deer, identified by the characteristic chemical compound muscone; components extracted from the bark of Prunus africana, containing compounds such as α-sitosterol, esters of ursolic, oleanolic and ferulic acid; whale oil, based on the fatty acid composition and bear bile, based on the cholic acid composition.

Chemical compounds that are unsuitable for GC can often be separated by **HPLC** and **TLC**. Again this technique is used mainly to identify (extracts from) endangered species in products, particularly in Traditional Chinese Medicines: Panax ginseng, based on the ginsenoside pattern and bear bile, based on the cholic acid composition. Not only can these techniques distinguish between bile from bears and from other species, but they can even differentiate between wild bears and bears raised in captivity

XRF can be used to identify a wide spectrum of chemical elements in various materials, often at low concentrations. The technique has been used to demonstrate that: the calcium content of Traditional Chinese Medicines suspected of containing ground tiger bones was very low, which is inconsistent with the presence of tiger bones, in certain Traditional Chinese Medicines the concentrations of arsenic and mercury are so high that they would seriously damage the health of anyone taking them and the chemical composition of suspected counterfeit bird rings slightly differed from the composition of the original rings.

ICPMS allows a broad spectrum of chemical elements to be identified at extremely low concentrations in a variety of materials. The technique is highly suitable for determining the composition of elements in plant and animal material. The underlying principle is 'you are what you eat' and uses the fact that the geographical variation in the composition of the soil where plants and animals live is to a certain extent reflected in the elemental composition of the plants and animals living there. In the USA the similar Thermal Ionisation Mass Spectrometry (**TIMS**) technique is already used in forensic investigations. In one example the isotope ratios of strontium (87Sr/86Sr) in confiscated deer antlers were used to discriminate between two possible geographical locations they could come from. Laser ablation (LA) ICPMS is used in South Africa in forensic analyses of stolen ivory, comparing it with ivory from different areas. A final application of ICPMS is the identification of industrially produced materials by their trace element contents. One such possibility is distinguishing between genuine and fake aluminium bird rings as mentioned above. The ICPMS technique is much better at identifying such differences than the XRF technique.

IRMS is used to determine to a very high level of accuracy a limited number of isotope ratios, particularly the stable isotopes of H, C, N, O and S. In the last decade it has been discussed eg whether different elephant populations in Africa can be discriminated by the ratios of C, N and Sr isotopes in bone tissue and ivory. One of the groups also used lead isotopes. The results appeared at first so successful that the researchers argued that the method could be widely used to identify the provenance of individual tusks. Another research group demonstrated later however that the specific isotope variation in the bones of elephants from Amboseli Park in Kenya was so large that it covered most of the variation found within Africa as a whole.

Capillary electrophoresis (**CE**) separates a mixture of proteins into its different components. By coupling CE or LC with MS, proteins can be separated and information about the structure can be obtained as well. Both techniques are used to identify animal species from blood stains and blood mixtures (α- and β-haemoglobin chains). These haemoglobin chains are presently also analysed using MALDI MS.

IEF is another technique for analysing proteins. In 2001 methods were developed to differentiate between caviar and the roe of other fish species. Results of a comparison of various methods of analysing haemoglobin from different deer species showed that the IEF results are consistent with those from HPLC. The IEF method characterises the intact tetrameric haemoglobin molecule; the HPLC method separates the α- and β-haemoglobin chains.

The importance of MS methods in this field is clear from the above, both for identification of organic components as well as for (trace) elementary composition analysis and comparison. Sometimes various forensic methods are available to answer a forensic question. To strengthen conclusions a combination of independent forensic techniques can also be used. At FSS, e.g., a combination of morphological and DNA methods is being used to further objectivate the Shahtoosh and Pashima wool classification.

Reference: [1] Forensic methods for criminal investigations related to endangered species, Marion A. Stelling and Gerard J.Q. van der Peijl, Netherlands Forensic Institute Report, December 2001

Keywords: Wildlife Forensics, Analytical Chemistry, MS

WIL-FOM-02
Identification of Rhino Products Using DNA and SEM EDX

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Introduction: The trade in endangered species for supposed medicinal reasons is a continuing and real threat to the survival of many animal species. This problem is no more acute than in the trade of Rhino material in the markets in the Far East. All the five extant species of Rhino (White, Black, Indian, Sumatra and Java) are on the CITES list of endangered species, and are all perilously close to extinction in the wild. This paper examines the use of two techniques, DNA profiling and SEM EDX, in the analysis of unknown seized material to determine the presence and body part of suspected Rhino material. The cytochrome B gene has become one of the best studied DNA loci for species identification, having been used extensively in taxonomic studies. The complete cytochrome B gene is approximately 1,140 bp in size, which may not always be amplified from degraded seized material. A partial fragment of the DNA locus is 402 bp, which has proved of value in previous species identifications. This part of the cytochrome B gene was used to identify a number of samples from unknown species, but suspected of being rhino. A further test, using SEM EDX was used to determine whether the samples were of bone or horn origin.

Materials & Methods: DNA: Samples of known materials were provided by the Taiwan Council of Agriculture. Unknown powdered material, of which there were six in the trial, came from seizures. The six unknown samples were suspected of being Rhino in origin. An additional sample from Holstein Cow was analysed as an out group. DNA was extracted from all the samples using a standard commercial method. Amplification of the 402 bp fragment of the cytochrome b gene was performed using the primers L14696 and H15197. The PCR products were directly sequenced and aligned to using PileUp and GCG computer programmes.

Materials & Methods: SEM EDX: Samples of known and unknown Rhino bone and horn were mounted on SEM stubs using double sided carbon adhesive tape. They were coated in carbon prior to elemental analysis.

Results: The unknown samples were fully sequenced and compared to the EMBL database. Four of the six samples aligned to the Black Rhino (Diceros bicornus). The greatest genetic distance between one of these unknown samples and Black Rhino was 0.0333.

The other two samples aligned to the White Rhino (Ceratotherium simum) with the genetic distance being 0.0176. The Holstein Cow sample showed a genetic distance of 0.2295 when compared to the closest previously unknown sample. The results confirmed that the unknown samples were from Rhino, with four being of Black Rhino and two being of White Rhino. The results do not determine from which part of the animal the powdered material originated; probably either horn or bone. Elemental analysis using SEM EDX could differentiate material high in sulphur (horn) from material high in calcium (bone). The results indicated that all the six samples were more likely to be from horn than bone. High power microscopy was also able to produce images of scale patterns found in horn, thus again supporting the material coming from horn and not bone. The DNA sequence from the cytochrome B gene was used to compare all the five extant species of Rhino. The DNA data support the Java and Indian Rhino being closely related. The White and Black Rhino are closely related, both being from Africa. The Sumatra Rhino is in between the two groupings, surprisingly more related to the African Rhino than the Java Rhino.

Conclusion: The use of a partial DNA sequence from the cytochrome B gene has been found to be highly effective at the identification of animal species. The DNA locus could differentiate samples originating from any of the five extant species of Rhino. The combination of elemental analysis and SEM allows the body part from where the sample was taken to be determined.

Keywords: Rhino, DNA, Cytochrome B

WIL-FOM-03
Source Location of African Elephant Ivory and Rhinoceros Horn by Stable Isotope Ratio Analysis

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We have explored the use of stable isotope ratio measurements for the purpose of tracing African elephant ivory and rhinoceros horn to the wildlife refuges from which they were obtained. This procedure is based on differences in plant cover, rainfall and geological history. Three different isotope ratios are usually sufficient to identify a source. Savanna grasses follow the C_4 photosynthetic pathway, producing plant material with $^{13}C/^{12}C$ ratios ($\delta^{13}C$ values, relative to PDB) of about –12.5 ‰ (per mil), while trees and shrubs follow the C_3 pathway and produce foliage with $\delta^{13}C$ values of about –26.5‰; dense forests have plants with $\delta^{13}C$ values more negative than -22‰. Nitrogen isotopes ratios are related to rainfall or water stress: animals from areas with rainfall >400mm/year have $\delta^{15}N$ values of about 5-10‰, while the values are higher in arid regions. These isotope ratios are recorded in the tissues of herbivores, with known fractionations during the synthesis of different tissues. The age of the geological substrate can be determined by stable isotope ratios in which the parent is radioactive: we have used $^{87}Sr/^{86}Sr$, but various isotope ratios of, e.g., neodymium and lead are also effective.

For elephants we have compared the isotope ratios for 20 refuges in 10 countries (van der Merwe *et al.*1990) and we continue to add to this database. Since elephants prefer to browse, their $\delta^{13}C$ values track the availability of trees and shrubs in their feeding range, while the nitrogen and strontium isotope ratios are patterned as predicted.

For rhinos, source location is less complicated, due to the scarcity of rhinos in Africa. The $\delta^{13}C$ values of rhino horn distinguish browsing black rhinos from grazing white rhinos (which are essentially confined to South Africa), while a combination of nitrogen, strontium and lead isotopes can identify the source (Hall-Martin *et al.*1993). Trace element data have also been successfully integrated with isotopic ratios in exploratory studies.

It is possible, in theory, to determine the source of ANY wildlife product with the right combination of isotopic and trace element data, but an extensive database is required to do so. It is easier to confirm whether a wildlife product comes from a specified source, e.g., the stockpile of ivory in Kruger National Park, South Africa, which is about to be sold with CITES approval. It is also possible to use "isotopic tagging" to monitor such shipments and the items that are made from the raw material (Kruger 1996).

Finally, where only specimens from captive breeding programs may be legally sold, e.g., various species of birds, their diet can be chosen to have an isotopic label that is significantly different from natural populations in the wild; similarly, cultivated endangered plants (e.g., cycads) can also be traced (using the technique for animals) or labeled through fertilizer or water of a specific isotopic or trace element composition.

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Keywords: stable isotopes, ivory, rhino horn

WIL-FOM-04
Ivory Sourcing Using Stable Isotopes

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Stable isotopes indicate aspects of diet and of water sources. Ratios of carbon and oxygen isotopes in ivory ($^{13}C/^{12}C$ and $^{18}O/^{16}O$, respectively) differ significantly between different parts of Africa, and even within different African countries. In particular, the $^{13}C/^{12}C$ ratios of savanna elephants differs from that of forest elephants, so that ivory from the forest elephants of central Africa differs from those from east or southern Africa. This difference is due primarily to differences of $^{13}C/^{12}C$ ratios within C3 plants. Regionally in eastern and southern Africa, elephants from certain regions incorporate C4 plants into their diet, allowing further differentiation. The total range in d13C in dentine from ivory, including hippopotamus ivory, is from +1 to -19 permil. The $^{18}O/^{16}O$ ratio of ivory is determined by the $^{18}O/^{16}O$ ratio of local precipitation, which varies somewhat across the continent. The total range in d18O of African ivory is from +3 to -7 permil. Thus, crossplots of $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios can often differentiate elephant ivory source regions. This method has promise to be used in conjunction with other methods to help determine possible source regions of confiscated ivory. In this presentation, we discuss differences between ivory samples from East Africa and those from Central Africa.

Keywords: elephants, isotopes, diet

WIL-FOM-05
Natural Stable Isotope Variation as a Tool to Trace Back the Origin of Organic Material

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Our environment is the sum of atoms that differ in their composition of neutrons and protons. This results in different chemical