

BALKAN DETOX LIFE

Operational capacities of referent national toxicological laboratories in the Balkans

BALKAN DETOX LIFE: STRENGTHENING NATIONAL
CAPACITIES TO FIGHT WILDLIFE POISONING AND RAISE
AWARENESS ABOUT THE PROBLEM ACROSS SEVEN
BALKAN COUNTRIES
(LIFE19 GIE/NL/001016)

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Subject and purpose of the document

This document is prepared within the framework of the BalkanDetox LIFE project (LIFE19 GIE/NL/001016) and relates to *Action B.10: Evaluation and improvement of operational capacities of national toxicological laboratories* and deliverable foreseen under this action: Analysis report of operational capacities of national referent toxicological laboratories for Albania, Bosnia and Herzegovina, Croatia, Greece, the Republic of North Macedonia, and Serbia.

This report is designed to provide clear overview on the capacities of existing referent national toxicological laboratories in the project countries to conduct forensic toxicological analysis necessary for investigation of wildlife poisoning incidents.

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About the project

The BalkanDetox LIFE project is a five-year endeavour with a €1.8 million budget, which aims to raise awareness and strengthen national capacities to fight the problem of wildlife poisoning across Albania, Bosnia & Herzegovina, Bulgaria, Croatia, Greece, the Republic of North Macedonia and Serbia. It received funding from the EU's LIFE Programme with a contribution of 1.004.792€ (54,82%) to the total project budget, and it is co-financed by the Vulture Conservation Foundation, the MAVA Foundation and Euronatur, as well as by the Whitley Fund for Nature and Environmental Protection and Energy Efficiency Fund for specific actions. Project partners are the Vulture Conservation Foundation as the coordinating beneficiary, and the Albanian Ornithological Society, Association BIOM, Bird Protection and Study Society of Serbia, Fund for Wild Flora and Fauna, Hellenic Ornithological Society, Macedonian Ecological Society, Ornitološko društvo NAŠE Ptice and the Protection and Preservation of Natural Environment in Albania as associated beneficiaries. Furthermore, this project is based on Spanish best practice experience and counts with the support from the Junta de Andalucía and the Spanish Ministry for the Ecological Transition and the Demographic Challenge.

Learn more at www.balkandetoxlife.eu



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Acronyms and abbreviations

AAS	Atomic absorption spectroscopy
AVS	Athens Veterinary Centre
CVI	Croatian Veterinary Institute
CSOs	Civil Society Organizations
GC	Gas chromatography
GC-MS	Gas chromatography - mass spectrometry
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma - mass spectrometry
ISUV	Food Safety and Veterinary Institute Albania
LC-MS	Liquid chromatography - mass spectrometry
LC-MS-MS	Liquid chromatography with tandem mass spectrometry
NPCC	National Poison Control Center Serbia
NSAID	Non-steroidal anti-inflammatory drug
PCBs	Polychlorinated biphenyls
TLC	Thin Layer Chromatography
VMP	Veterinary medical products

INTRODUCTION

Conduction of toxicological analysis is a crucial process in the investigation procedures of wildlife poisoning incidents. A positive toxicology screen is the only clear evidence that an animal has actually died as consequence of poisoning and is the only valid evidence for court trials of poisoning incidents. Therefore, toxicological laboratories have an irreplaceable role in the overall management of poisoning incidents and are vital for conservation efforts related to combating this type of environmental crime.

This report strives to assess the present operational capacities of existing national toxicological laboratories in the Balkan countries which are designated as referent institutions for conducting toxicological analysis on wild animals, and other laboratories which have potential capacities for this type of work. Conduction of forensic toxicological analysis has been recognized as one of the main gaps in the overall management of poisoning incidents in many Balkan countries. The lack of operational capacities (staff, equipment, expertise) of existing referent national laboratories is a key obstacle in combating the illegal practice of wildlife poisoning, as a positive toxicology screen is the only valid piece of evidence in court that an animal has indeed died from poisoning, that is that a criminal offence had been committed, and that a criminal investigation can be launched by the relevant authorities.

In order to collect the basic information about the current capacities of relevant laboratories in the Balkan countries a questionnaire (Annex) was prepared and distributed. Collected data was analyzed for each country and is represented in a separate chapter. It is important to point out that not all questionnaires distributed to laboratories were fully completed and were therefore excluded from the analysis of this report.

ALBANIA

Albania currently does not have a national laboratory which is designated by the relevant governmental authorities for conducting toxicological analysis on samples from wild animals. This can be attributed to the fact that poisoning of wild animals was not regarded as an illegal activity in Albania until the recent change in the relevant national legislation in 2019, when this specific type of environmental crime was officially banned. Therefore, favorable circumstances are now created in the country that would officially enable the establishment of a referent institution for conducting forensic toxicology on wildlife and thus facilitate legal proceedings of wildlife poisoning cases in court.

Two laboratories with sufficient potential capacities have been surveyed, operating within the of Faculty of Veterinary Medicine and Food Safety and Veterinary Institute in Tirana.

Faculty of Veterinary Medicine Tirana



➤ STAFF CAPACITIES

There are 6 staff members within the Faculty who are occasionally working on toxicological analysis and necropsies of presumably poisoned animals. The relevant staff comprises from two professors, which are specialized in infectious diseases of livestock, wildlife, and fish, and in veterinary toxicology, one laboratory specialist technician and three students of Veterinary Medicine.

These personnel capacities are perceived by the Faculty as being sufficient for performing the needed forensic necropsies and toxicological analysis on presumably poisoned wild animals in Albania. Additionally, the representatives from the laboratory did not specify whether their existing staff requires any additional specific training towards improving their work on this subject.

➤ EQUIPMENT CAPACITIES

No information was provided about the existing equipment that is being used for toxicological analysis in the laboratory of the Faculty, nor were any type of screening methods used for identifying pesticides and similar toxic compounds often used for

wildlife poisoning specified. On the other hand, it is specified that the lab would have a need for a gas chromatographer (GC) and some differential kits in order to have optimal capacities for conducting needed toxicological analysis. GC is an analytical technique used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present. These chemical components are usually organic molecules or gases. Gas chromatographs are frequently hyphenated to mass spectrometers (GC-MS) to enable the identification of the chemical components, and they represent the basic necessary analytical tool in toxicology.

Food Safety and Veterinary Institute



➤ STAFF CAPACITIES

Food Safety and Veterinary Institute of Albania (ISUV) has eight relevant staff members that could work on toxicological analysis and necropsies of presumably poisoned animals. Two staff members work on conducting necropsies (1 specialist and 1 technician), and six people work on toxicological analysis (4 technicians for the processing of the material and sampling, 1 person for analysis for pesticides, 1 person for analysis for heavy metals).

The existing personnel capacities as perceived by the ISUV as being sufficient for conducting the needed forensic necropsies and toxicological analysis on presumably poisoned wild animals in Albania. On the other hand, the representatives of the Institute believe that their personnel could benefit from additional specific training, especially training of newer staff members for conducting forensic necropsies as the current specialist is soon to retire.

➤ EQUIPMENT CAPACITIES

ISUV has sufficient capacities for conducting necessary toxicological analyses and are well equipped with analytical instruments that can be used to screen for presence of pesticides and similar compounds which are often used for wildlife poisoning. The following methods are used in ISUV for screening for presence of pesticides and other chemical compounds that can have a detrimental effect on animals:

1. LC-MS: Liquid chromatography (LC) - mass spectrometry (MS)

The coupling of MS with LC systems is attractive because liquid chromatography can separate delicate and complex natural mixtures, which chemical composition needs to be well established (e.g., biological fluids, environmental samples, and drugs). It is regarded as the leading analytical technique used in the field of bioanalysis and is specially involved in pharmacokinetic studies of pharmaceuticals, and the analysis of food, pesticides, and plant phenols.

II. *LC-MS-MS: Liquid chromatography with tandem mass spectrometry*

MS analyzers are useful in these analysis because of their shorter analysis time, and higher sensitivity and specificity compared to UV detectors commonly attached to HPLC systems. One major advantage is the use of tandem MS-MS, where the detector may be programmed to select certain ions to fragment. Both LC-MS and LC-MS-MS are used at ISUV for screening for pesticides.

III. *ICP-MS: Inductively coupled plasma (ICP) - mass spectrometry*

Widely used method because of its ability to detect metals and several non-metals in liquid samples at very low concentrations. It can detect different isotopes of the same element, which makes it a versatile tool in isotopic labeling. At ISUV it is used for identifying the presence of heavy metals in samples.

IV. *HPLC: High-performance liquid chromatography*

A widely used analytical technique for separating, identifying, and quantifying each component in a mixture. At ISUV this technique is mostly used for identifying VMPs and NSAID residues.

V. *GC-MS: Gas chromatography (GC) - mass spectrometry*

Frequently used to identify trace elements in materials that were previously thought to have disintegrated beyond identification. Like LC-MS, it allows analysis and detection even of tiny amounts of a substance. At ISUV it is commonly used for identifying organochlorines and PCBs.

Conclusions

Of the two relevant institutions in Albania whose operational capacities were analyzed, detailed information was only available for the Food Safety and Veterinary Institute. According to the available information it is evident that ISUV has sufficient both personnel and technical capacities to perform the forensic necropsies and toxicological analysis needed for investigation of wildlife poisoning incidents. Therefore, it is necessary to

launch an official initiative, in accordance with the relevant national legislation, to designate the ISUV to be the national referent institution in Albania for conducting forensic toxicological analysis on wild animals, which are an irreplaceable part of court trials for poisoning incidents.

Additional training for performing necropsies on wild animals, sampling, and preparation of samples for toxicological screening would be very beneficial for capacity building of the current staff at the ISUV.

BOSNIA AND HERZEGOVINA

Bosnia and Herzegovina currently does not have a national laboratory, in neither of the two constitutive entities (Republika Srpska and the Federation of Bosnia and Herzegovina), which is designated by the relevant governmental authorities for conducting toxicological analysis on samples from wild animals. Even though the use of poison and poison baits for the purpose of extirpating undesirable animals has been an illegal practice for more than 30 years, not a single institution in the country has been designated as a referent laboratory where samples of wild animals would be processed to confirm whether they died from poisoning or not, that is whether a criminal act was committed or not. On the other hand, the current legislation in place permits only reports from a referent national laboratory as valid evidence in court trials, while reports from private, commercial labs are not admissible as evidence. Additionally, according to the national legislation it is prohibited to transport samples from wild animals for analysis in other countries, which further complicates the situation and makes it practically impossible to confirm poisoning and that an illegal act has in fact been committed.

Laboratory within the Centre for Foodstuffs, Animal Feed Control and Environmental Protection, and the Laboratory for Toxicology, operating within the Faculty of Veterinary Medicine in Sarajevo, with sufficient potential capacities and interest for conducting forensic wildlife toxicology has been surveyed. Two additional laboratories in Bosnia and Herzegovina were contacted but expressed no interest to participate in the survey.

Faculty of Veterinary Medicine Sarajevo



➤ STAFF CAPACITIES

Currently there are no staff members from the two laboratories operating within the Faculty of Veterinary Medicine designated to work on wildlife toxicology. The current staff capacities are perceived to be insufficient, and that at least one additional employee would be needed to work with wild animals. Additionally, specific training in forensic wildlife toxicology is needed for existing staff members.

➤ EQUIPMENT CAPACITIES

Faculty of Veterinary Medicine of Sarajevo has several analytical instruments and methods that can be used for screening for pesticides and other toxic compounds commonly used for wildlife poisoning. For conducting toxicological analyzes on wildlife, representatives of the laboratories believe that it is necessary to provide standards and reagents that will be used for the preparation and analysis of samples.

I. HPLC: High-performance liquid chromatography

This technique is mostly used for identifying NSAIDs and other VMPs.

II. LC-MS: Liquid chromatography (LC) - mass spectrometry (MS)

III. LC-MS-MS: Liquid chromatography with tandem mass spectrometry

IV. GC-MS: Gas chromatography - mass spectrometry

V. AAS: Atomic absorption spectroscopy

A spectro-analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions. AAS is frequently used in pharmacology and toxicology research, and clinical analysis of metals.

Conclusions

Based on the information obtained from the Laboratory of Toxicology and Centre for Foodstuffs, Animal Feed Control and Environmental Protection it is evident that the Faculty of Veterinary Medicine has at their disposal sufficient equipment and technical capacities to perform forensic toxicological analysis needed for investigation of wildlife poisoning incidents. Therefore, it is necessary to launch an official initiative, in accordance with the relevant national legislation, to designate the Faculty of Veterinary Medicine to be the national referent institution in Bosnia and Herzegovina for conducting forensic toxicological analysis on wild animals, which are an irreplaceable part of court trials for poisoning incidents.

Additionally, specific training, regarding preparation of samples, selection of methods and screening procedures for substances most commonly used for illegal poisoning of animals is needed in order to increase the capacities of existing personnel.

CROATIA

There are several laboratories operating under different governmental institutions in Croatia which have potentially sufficient capacities to carry out forensic necropsies and toxicology screening on presumably poisoned wild animals. A total of five laboratories were surveyed about their operational capacities regarding wildlife toxicology for the purpose of this report. The most relevant laboratory for this would be the Laboratory for chemical-physical and toxicological expertise within the Forensic Science Centre “Ivan Vučetić” of the Ministry of Internal Affairs, who mostly participate in investigations of wildlife poisoning incidents and eventual court trials.

Institute for Public Health “Dr. Andrija Štampar”



The laboratory operating within the Department of Environmental Protection and Health Ecology of the Institute for Public Health “Dr. Andrija Štampar” is a public entity accredited



for performing toxicological analysis on presumably poisoned animals. It is also important to note that the Institute can perform toxicological analyses of samples received from third parties (any private individual or organization) commercially. The laboratory that operates within the institute has conducted toxicological analysis on presumably poisoned animals on 20 occasions during the last five years and has on several of them confirmed that pesticides or lead intoxication caused the fatal outcome.

➤ STAFF CAPACITIES

The laboratory currently employs 100 people, all of whom are working on toxicological analysis sampling or analyzing. It performs toxicological analysis with all kinds of samples, including water, food, feed for animals, waste, soil, air, and occasionally poisoned animals.

The current staff capacities are perceived to be sufficient for successful and timely completion of all required toxicological analysis of presumably poisoned animals that come into their lab. On the other hand, it is believed that additional personnel are needed for optimal coverage of required toxicological analysis of presumably poisoned animals. Also, additional training in forensic wildlife toxicology would be very beneficial for increasing the operational capacities of current staff members.

➤ EQUIPMENT CAPACITIES

The Institute for Public Health “Dr. Andrija Štampar” in Zagreb has several analytical instruments and methods that they use for screening for pesticides and other toxic compounds which commonly cause the death of domestic and wild animals.

I. LC-MS-MS: Liquid chromatography with tandem mass spectrometry

II. GC-MS-MS: Gas chromatography with tandem mass spectrometry

The second phase of mass fragmentation is added sometimes to try to quantitate low levels of target compounds in the presence of a high sample matrix background.

III. ICP-MS: Inductively coupled plasma - mass spectrometry

The representatives of the Institute believe that additional equipment is needed in order to achieve better results in toxicological screening, primarily QTRAP instruments, which would be coupled with LC-MS and GC-MS. QTRAP instruments provide additional and

unique MS and MS/MS scan functions to deliver both quantitative and qualitative information in a single triple quadrupole platform. QTRAP Technology is unique and based on a triple quadrupole mass analyzer configuration. It delivers equivalent or better data, and more of it, than you can capture on an ordinary triple quadrupole system.

Forensic Science Centre “Ivan Vučetić”



Laboratory for chemical-physical and toxicological expertise operates within the Forensic Science Centre “Ivan Vučetić” and is under jurisdiction of the Ministry of Internal Affairs of Croatia. It is one of the public entities accredited for performing forensic toxicological analysis on presumably poisoned domestic and wild animals. The laboratory that operates within the institute has conducted toxicological analysis on presumably poisoned animals on 125 samples during the last five years and has on numerous occasions confirmed that animals died as a consequence of poisoning. The following toxic compounds have been identified as the cause of death in poisoning incidents that occurred during this period: *Carbofuran, Metaldehyde, Bromadiolone, Chlorophacinone, Chlorpyrifos-methyl, Chlorpyrifos -ethyl, Methomyl, Zerbuthylazine, Metolachlor, Xylazin, Ketamine, NaOH, KOH, Cypermethrin, Acetamiprid, Brodifacoum, Aluminium phosphide, Imidacloprid, Omethoate, Dimethoate, 2,4D acid and 2,4DP acid*. The laboratory currently only conducts toxicological analysis upon official requests by the police, environmental inspectorate, and public prosecutors, but there is a possibility to perform analysis upon requests from third parties, such as Civil Society Organizations (CSOs).

➤ STAFF CAPACITIES

The laboratory currently has two staff members working on toxicological analysis of presumably poisoned animals. It is perceived that the current number of employees is sufficient to manage the current needs for forensic wildlife toxicology of their institution. However, additional personnel are needed for optimal coverage of required toxicological analysis of presumably poisoned animals.

Also, additional training in forensic wildlife toxicology would be very beneficial for increasing the operational capacities of current staff members, especially specialist training on LC-MS-MS and GC-MS-MS analysis.

➤ EQUIPMENT CAPACITIES

The laboratory for chemical-physical and toxicological expertise has the following analytical instruments at their disposal for performing forensic toxicological analysis on presumably poisoned animals:

- I. ***LC-MS: Liquid chromatography - mass spectrometry***
- II. ***HPLC: High-performance liquid chromatography***
- III. ***GC-MS: Gas chromatography - mass spectrometry***

Representatives from the Forensic Centre have highlighted the need for additional equipment, chiefly LC-MS-MS and GC-MS-MS instruments in order to be able to perform more precise analysis.

Faculty of Veterinary Medicine, University of Zagreb



Forensic laboratory operating within the Department of Forensic and State Veterinary Medicine of the Faculty of Veterinary Medicine in Zagreb is another accredited institution in Croatia for performing forensic toxicological analysis on presumably poisoned animals. The staff of the laboratory have performed nearly 30 animals during the course of the last five years, upon receiving official request by the relevant law enforcement agencies in the country. The following toxic compounds have been identified as the cause of death in some poisoning incidents analyzed: *Methomyl*, *Methiocarb*, *Hydroxy-carbofuran*, *carbofuran*, *Chlorfacinone* and *lead*. It is also important to note that the Institute can perform toxicological analyses of samples received from third parties (any private individual or organization) commercially.

➤ STAFF CAPACITIES

There are two employees in total working on toxicological analysis of presumably poisoned animals within the Department of Forensic and State Veterinary Medicine. The current staff capacities are perceived as insufficient, and probably two or more additional personnel is needed for optimal coverage of required toxicological analysis of presumably poisoned animals.

Considering the capacity and future development plan for the laboratory, additional training is needed in field of applied toxicology that include the analysis of the clinical and

dissection findings, use of sampling method from different occasions (crime scene/necropsy room) and implementation of basic analytical toxicology (thin layer chromatography – TLC).

➤ EQUIPMENT CAPACITIES

Unfortunately, no concrete information about analytical instruments and methods being used at the Faculty of Veterinary Medicine was provided. On the other side, it is perceived that the current capacities for performing toxicological analysis are sufficient.

Croatian Veterinary Institute



The Croatian Veterinary Institute (CVI) performs only forensic necropsies on presumably poisoned animals and prepares samples for toxicological analysis, which are usually conducted by the Forensic Science Centre “Ivan Vučetić”. During the last five years the CVI sampled a total of 585 organs from 191 wild birds for toxicological analysis. However, the Institute has its own analytical instruments which could be used for toxicological analysis of presumably poisoned wild animals. Currently, pesticide screening is being conducted only for analysis of food products and animal feed.

➤ STAFF CAPACITIES

According to the available information that we were able to obtain, the relevant staff of the CVI consists of five veterinary pathologists and two staff members working on toxicological analysis. General staff capacities are perceived as sufficient, although it was noted that having additional personnel in the toxicology department (histopathological, immunohistochemical and laboratory of molecular toxicopathology) would be beneficial.

Additionally, it is considered that it would be very beneficial for current staff to receive specific training regarding methodology of work in the field of bird toxicopathology (field work, laboratory work / dissection, organ sampling), processing evidence material (collection and storage), crime scene investigation and forensic entomology to increase their operational capacities relevant to processing cases with presumably poisoned animals.

➤ EQUIPMENT CAPACITIES

CVI uses the following types of screening methods and analytical instruments for identifying pesticides and similar toxic compounds, which are often used for wildlife poisoning:

- I. LC-MS-MS: Liquid chromatography with tandem mass spectrometry*
- II. GC-MS-MS: Gas chromatography with tandem mass spectrometry*

Conclusions

From the available data that we were able to obtain for the purpose of this report it is evident that the situation in Croatia regarding the possibility of conducting forensic toxicological analysis for processing wildlife poisoning incidents is very good, in comparison to other Balkan countries. Three of the four surveyed governmental institutions have very good technical capacities for accommodating the current need for performing forensic toxicological analysis on wild animals, while two laboratories already have significant experience and success in confirming poisoning as the cause of death in many wildlife poisoning incidents.

Apart from the Forensic Science Centre “Ivan Vučetić”, which works on most wildlife poisoning incidents that occur in Croatia and has an evident need for additional equipment (chiefly LC-MS-MS and GC-MS-MS instruments), other laboratories mostly have a need for specific training about processing wildlife poisoning incidents for their existing staff members in order to increase their capacities and achieve better results in their work.

If we compare the situation regarding the possibility of conducting forensic toxicological analysis for processing wildlife poisoning incidents in other target countries of this report, Croatia undoubtedly has the best capacities for this type of work. Therefore, it would be useful to consider the possibility of performing in Croatia these needed analyses for poisoning incident from other countries from the region in which there are currently no possibilities for this.

GREECE

According to the data that we were able to obtain from relevant governmental institutions in Greece, there is only one licensed laboratory in the country which is referent for performing forensic toxicological analysis for wildlife poisoning incidents and presenting relevant evidence in court procedures.

Athens Veterinary Centre



HELLENIC REPUBLIC
Ministry of Rural Development
and Food

The toxicological laboratory of Athens Veterinary Centre operates within the Department of Toxicology, Residues and Environmental Pollutants, General Directorate of Veterinary Medicine of the Ministry of Rural Development & Food. The toxicological laboratory of the Athens Veterinary Centre (AVC) has been performing forensic toxicological analysis on presumably poisoned animals for more than 20 years and has significant experience in this matter. In the last 5 years alone the AVC has proven poisoning to be the official cause of death in over 20 separate wildlife poisoning incidents. During this time period the following substance were confirmed to have caused death of both wild and domestic animals in Greece: *Methomyl*, *Potassium cyanide*, *Carbofuran*, *Cyproconazole*, *Metribuzin*, *Phorate*.

➤ STAFF CAPACITIES

Currently there is only one staff member within the AVC designated to work on toxicological analysis of presumably poisoned animals. One employee is clearly not sufficient to cover the annual needs for these forensic analysis as wildlife poisoning is a very common practice in Greece, and there are dozens, and dozens of potential poisoning events recorded every year. It is perceived that three to four additional employees are needed in order to cover the current workload of the AVC in forensic wildlife toxicology.

Additional practical training in laboratory examination and analysis of poisoning incidents of current staff of the AVC is also perceived as being very beneficial for strengthening of their current operational capacities.

➤ EQUIPMENT CAPACITIES

AVC uses the following types of screening methods and analytical instruments for identifying pesticides and similar toxic compounds, which are often used for wildlife poisoning:

I. HPLC: High-performance liquid chromatography

II. GC-MS: Gas chromatography - mass spectrometry

Representatives from the Athens Veterinary Centre believe that additional equipment is required in order to achieve a successful and timely completion of all required toxicological analysis of presumably poisoned animals that are delivered to them, specifically LC-MS or LC-MS-MS analytical systems.

Conclusions

The situation in Greece regarding the possibility of conducting forensic toxicological analysis for processing wildlife poisoning incidents is not very good, in comparison to other Balkan countries. On one hand the illegal poisoning of animals is very frequent in Greece, while on the other hand there is only one toxicological laboratory which is licensed by the government to conduct toxicological analysis, and to present their findings in court trials when necessary.

The current referent national laboratory does not have sufficient operational capacities in neither staff number nor equipment to cope with the actual needs in investigations of illegal poisoning incidents. Therefore, it is evident that significant improvements must be made towards capacity building of the current toxicological laboratory. Also, we recommend that efforts should be invested into researching the possibility of licensing additional laboratories in Greece in order to share the workload of the AVC and achieve a successful and timely completion of all required toxicological analysis of presumably poisoned animals.

NORTH MACEDONIA

There are currently no toxicological laboratories in North Macedonia which are designated by the relevant governmental authorities in the country to be referent institutions for performing toxicological analysis on presumably poisoned wild animals. Therefore, it is very difficult to confirm poisoning as the cause of death in potential poisoning incidents that occur in North Macedonia, which makes it even more difficult to reach a positive outcome in court trials. Forensic toxicological analysis were not performed in any potential poisoning incident that occurred in the country during the last ten years.

One toxicological laboratory with sufficient potential capacities and interest to expand their work area to include analysis of samples from wild animals was surveyed for the purpose of this report.

Faculty of Veterinary Medicine Skopje



The toxicological laboratory operating within the Faculty of Veterinary Medicine in Skopje has so far only conducted forensic necropsies and histopathology on animals suspected to have died of poisoning.

➤ STAFF CAPACITIES

The laboratory has four staff members working on toxicological analysis and currently only conducts analysis and screening for pesticides in food products and animal feed and does not conduct forensic toxicological analysis of presumably poisoned animals. The current staff is so far only experienced in performing forensic necropsies and there is a need for specific training for conducting toxicological analysis on wild animals, which include sample preparation (different matrices), extraction procedures and instrumental analysis of the samples. It is perceived by the representatives of the Faculty of Veterinary Medicine that the current number of employees is sufficient to manage the current needs for forensic wildlife toxicology of their institution if additionally trained in this matter.

➤ EQUIPMENT CAPACITIES

The toxicological laboratory of the Faculty of Veterinary Medicine in Skopje has the following analytical equipment for toxicological analysis at their disposal:

I. LC-MS-MS: Liquid chromatography with tandem mass spectrometry

II. GC-MS: Gas chromatography - mass spectrometry

Representatives from the laboratory have highlighted the need for additional equipment, chiefly Spectrophotometer, centrifuge, nitrogen evaporator, digester, vortex, balance instruments in order to be able to adequately perform toxicological analysis of presumably poisoned animals.

Conclusions

The clear lack of possibility for conducting forensic toxicological analysis on presumably poisoned animals makes it very difficult to tackle the illegal practice of wildlife poisoning in North Macedonia. The only toxicological laboratory that expressed interest in broadening their scope of work to include analysis of samples from wild animals operates within the Faculty of Veterinary Medicine in Skopje. Therefore, it is important to provide specific training for conducting forensic wildlife toxicology and invest efforts towards acquiring additional analytical equipment needed for the laboratory to have optimal capacities for performing necessary analysis.

Apart from building up the operational capacities of the laboratory, it is equally important to undertake an initiative to designate it as the referent national laboratory for forensic toxicology of presumably poisoned animals. This would solidify the long-term availability of the laboratory for providing invaluable evidence for court proceedings of wildlife poisoning incidents, and considerably increase the overall capacities for combating illegal wildlife poisoning as one of the biggest conservation threats for many species of avian scavengers in North Macedonia.

SERBIA

Based on information that we were able to obtain there are two toxicological laboratories in Serbia where wild animals suspected to have died from poisoning are sent for analysis, and whose findings are used for judicial proceedings of wildlife poisoning incidents. The National Poison Control Center (NPCC) of the Military Medical Academy in Belgrade is designated as the referent institution for forensic toxicology in the country. However, it was not possible to acquire any kind of information from the NPCC about the operational capacities of their laboratory, as none of our requests for information were answered.

The other relevant institution in the country for this type of work is the Scientific Veterinary Institute of Novi Sad, which is a public entity that conducts forensic necropsies and toxicological analysis of presumably poisoned animals, both for relevant institutions and commercially, for private citizens and organizations. So far, they have only been involved in those potential poisoning incidents that occurred in the north of the country, in Vojvodina province.

Scientific Veterinary Institute “Novi Sad”



During the last five years personnel of the Institute has performed analysis of 73 samples of poisoned animals. So far, analysis of samples from wild animals that were commissioned confirmed that poisoning was the cause of death in 14 separate incidents. *Carbofuran* was identified as the substance used for poisoning in most analyzed samples, but also lead poisoning was confirmed in one incident. The toxicological laboratory of the Institute apart from analyzing samples from poisoned animals also performs tests for monitoring the quality control of VMPs, food products and animal feed.

➤ STAFF CAPACITIES

The Scientific Veterinary Institute of Novi Sad has the following relevant staff members at their disposal: 5 epidemiologists, 2 pathologists, 4 analytical chemists and one veterinary toxicologist. It is perceived that the current staff capacities are sufficient to adequately manage the annual workload regarding forensic toxicological analysis of presumably poisoned animals and ensure timely completion of all required toxicological analysis. On the other side, it is noted that additional specific training in veterinary forensic pathology,

toxicological laboratory methods and interpretation of analytical data would be very beneficial for current staff members and increase their operational capacities.

➤ **EQUIPMENT CAPACITIES**

The toxicological laboratory of the Scientific Veterinary Institute has the following analytical equipment available for performing forensic toxicological analysis:

I. GC-MS: Gas chromatography - mass spectrometry

II. HPLC: High-performance liquid chromatography

III. UV-VIS: UV-visible spectrophotometry

A method routinely used in analytical chemistry for the quantitative determination of different analytes or sample, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. A UV/Vis spectrophotometer may also be used as a detector for HPLC.

IV. ICP-MS: Inductively coupled plasma - mass spectrometry

V. TLC: Thin Layer Chromatography

TLC is an affinity-based method used to separate compounds in a mixture. It is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis. TLC can be used to analyze virtually any substance class, including pesticides, steroids, alkaloids, lipids, nucleotides, glycosides, carbohydrates, and fatty acids.

Representatives from the lab have highlighted the need for additional equipment, chiefly LC-MS-MS and GC-MS-MS instruments in order to be able to increase their capacities to perform more precise forensic toxicological analysis.

Conclusions

From the available data that we were able to obtain for the purpose of this report it is evident that although the situation in Serbia regarding the possibility of conducting forensic toxicological analysis for processing wildlife poisoning incidents needs improvement, the existing accredited institutions have the basic operational capacities for this line of work. The Scientific Veterinary Institute of Novi Sad has been very active in

providing evidence for wildlife poisoning investigations during the last five years and has acquired significant experience in this line of work. Provision of specific training in performing forensic wildlife toxicology and additional analytical equipment would be very beneficial for the current capacities of the laboratory and improve its overall performance.

Apart from building up the operational capacities of the laboratory of the Scientific Veterinary Institute, it is equally important to undertake an initiative to designate it as the referent national laboratory for forensic toxicology of presumably poisoned animals, giving the fact that their engagement with this matter is much higher than NPCCs'. This would ensure the long-term availability of the laboratory for providing evidence for judicial proceedings of wildlife poisoning incidents and broaden the scope of their work to include samples from incidents that occur in other parts of Serbia as well. According to the information that we were able to obtain NPCC has not processed a potential poisoning incident for the past 15 years. Therefore, it would be very important to invest additional efforts to establish cooperation with this institution, assess their capacities and engagement with this topic in order to realize the realistic capacities for conducting forensic toxicological analysis that are available in Serbia.

Annex

Questionnaire for toxicological laboratories

1. GENERAL INFORMATION

Country:	
Institution/Toxicological laboratory	
Address:	
Telephone:	
E- mail:	
Webpage:	
Name and position of person providing the information:	
E-mail of the person providing the information:	

2. PERSONNEL CAPACITIES

Please specify how many personnel within your institution are working on toxicological analysis of presumably poisoned animals.

Do you believe that you institution has enough staff capacities for successful and timely completion of all required toxicological analysis of presumably poisoned animals?

Yes ☐

No ☐

- If the answer is NO, please specify in your honest opinion how many more additional personnel is needed for optimal coverage of required toxicological analysis of presumably poisoned animals.

Do you believe that personnel within your institution/laboratory require additional training or capacity building related to performing toxicological analysis on presumably poisoned animals?

Yes ☐

No ☐

- If the answer is YES, please specify to the best of your knowledge what type of training would be needed for personnel from your institution/laboratory.

3. EQUIPMENT CAPACITIES

Please specify what type of screening methods do you use for identifying pesticides and similar toxic compounds often used for wildlife poisoning.

- a)
- b)
- c)

Please specify what type of equipment does your laboratory/institution possesses for identifying pesticides and other toxic compounds.

- a)
- b)
- c)

Do you believe that your laboratory/institution requires additional equipment for toxicological analysis?

a) Yes ☐

b) No ☐

- If the answer is YES, please specify to the best of your knowledge what type of equipment would be needed for personnel from your institution/laboratory.

4. Are you aware of other laboratories/institutions in your country that can (are licensed to) perform toxicological analysis on presumably poisoned animals? If so, please provide information to the best of your knowledge in the table below.

Laboratory name	Type of laboratory (public, private)

5. Please specify how many toxicological analysis has your laboratory/institution conducted on presumably poisoned animals during the last 5 years.

6. Please specify what toxic compounds have you identified in samples from poisoned animals during the last 5 years.
