European Raptor Biomonitoring Facility
Advice Hub

How to collect and submit samples

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HOW TO COLLECT AND SUBMIT SAMPLES

This chapter will focus on the main aspects of collecting samples for chemical analysis:

- How to collect samples
- How to collect specimen contextual data
- How to submit samples
- How to package, label and send samples

HOW TO COLLECT SAMPLES

Raptors are widely used as sentinel species in raptor biomonitoring programs. A major current challenge is to facilitate large-scale biomonitoring by coordinating contaminant monitoring activities and by building capacity across countries. This requires sharing, dissemination and adoption of best practices. A recently published schematic sampling protocol for contaminant monitoring in raptors provides guidance on sample collection with a view to increasing sampling capacity across countries, ensuring appropriate quality of samples and facilitating harmonization of procedures to maximize the reliability, comparability and interoperability of data. The protocol can be used by professionals and volunteers as a standard guide to ensure harmonized sampling methods for contaminant monitoring in raptors. For detailed guidelines visit the schematic sampling protocol provided as Supplementary Material at:

Espín et al. 2021. (https://doi.org/10.1007/s13280-020-01341-9).

Figure 1 – A preview of the main menu of the schematic sampling protocol (Espín et al. 2021)

In this scheme it is possible to find detailed information on protocols for collecting different sample matrices during active sampling (i.e., blood, plasma/serum, deserted/addled eggs, feathers, preen oil, regurgitate pellets/prey remains) and passive monitoring (i.e., internal tissues from carcasses, gastric content, feathers, preen oil/gland). The reader can click on the sample type and is redirected to the specific protocol which offers further hyperlinks to additional information, photographs and videos. Some general guidelines are also provided regarding sampling and ethical permits, personal safety and wildlife health, animal welfare, labelling samples, and guidance to avoid contamination and to record basic data. Information on the volume/mass of sample needed for contaminant monitoring, suitable container types to conserve the samples and conditions required for transportation and storage are also provided.

USEFUL REFERENCES & VIDEOS

Espín et al. 2014. Sampling and contaminant monitoring protocol for raptors. Research Networking Programme – EURAPMON (Research and monitoring for and with raptors in Europe). European Science Foundation.

Espín et al. 2014. Short summary (available in 9 languages): Sampling and contaminant monitoring protocol for raptors. Research Networking Programme – EURAPMON (Research and monitoring for and with raptors in Europe). European Science Foundation.

Espín, S., Andevski, J., Duke, G., Eulaers, I., Gómez-Ramírez, P., Hallgrimsson, G. T., ... & García-Fernández, A. J. (2021). A schematic sampling protocol for contaminant monitoring in raptors. Ambio, 50(1), 95-100.

Collection of raptor blood samples (video): click here

Obtention of plasma and serum (video): click here

Measuring eggs and eggshells (video): click here

Necropsy of Eagle owl Bubo bubo (video): click here

How to pluck body feathers from living raptors (video): click here

COLLECTING SPECIMEN CONTEXTUAL DATA

The contextual data related to a specimen and related sample(s) provide essential information for the better interpretation of contaminant data in raptor samples. This data may be classified as essential, when the sample is not valuable if this data is not provided, and in other valuable data depending on the objective of the study. Essential contextual data includes the raptor species, type of matrix, unique ID code, location and year. Other valuable data (which may be essential depending on the aim of the study) may include e.g., coordinates, age, sex, specimen conditions, cause of death, ring number, body measurements, name of collector etc. For additional information on specimen contextual data, examples and justification see the **Recording sheet at the end of this section (Table 1)**.

TABLE 1– Recording sheet for collecting contextual data associated with each specimen sampled

CONTEXTUAL DATA	EXAMPLE	JUSTIFICATION
	Essential data[a]	
Year	Year	Basic data. Identification of sample collection date and different exposure with time
Descriptive location	Village, municipality, district, country	Basic data. Identification of sample collection location and different exposure depending on the area
Raptor species	Subspecies also identified if possible	Basic data. Use Latin name if possible to avoid misinterpretation

[a] *Essential data: Sample is not valuable if this data is not provided*

CONTEXTUAL DATA	EXAMPLE	JUSTIFICATION
Matrix sample	Tissue, organ, blood, pellets, eggs, shed feathers, bones	Essential for analyses (all matrices are not suitable for all compounds analysis)
Unique ID label	Combine country + collector + (species + year) + id + sample matrix	Essential for unique identification of each sample, always label sample containers
Other valuable contextual data[b]		
Date	Day/month/year	Identification of sample collection date and different exposure with time, differences depending on the season, etc.
Coordinates	X and Y coordinates obtained by GPS or Google maps (if possible/permitted)	Important to investigate potential sources of contamination and other nest data
Name of the collector	ID/Anonymous	Full name, institution if possible. Refer for additional information, collaboration, etc.
Age	Nestling / Adult. Euring age codes (1,2,3,4)	Potential differences in pathways and metabolism with age, potential accumulation of contaminants with age. For certain types of contaminants, it may be essential.
Sex	Female, male, unknown	Potential differences between male and females, particularly during breeding season for some compounds. For certain types of contaminants, it may be essential.
Specimen condition (alive bird)	Injured, healthy, emaciated	Body condition can affect contaminant levels in tissues
Specimen condition (dead bird)	If dead mention fresh or decomposed	Post-mortem decomposition and cause of death can both potentially affect tissue contaminant concentrations
Ring number (if ringed)	Ring number with Ringing Centre Code and country (ringing season	Important for identification. Ringed birds can provide additional data on

b Other valuable data: depending on the objective of the study, some data within this section may be essential.

CONTEXTUAL DATA	EXAMPLE	JUSTIFICATION
	or found dead ringed)	survival, breeding performance, etc.
Type of feather	Primary (and number), secondary, tail, breast, back, rump, nestling down	Important for some contaminants (e.g. metals, organochlorines; potential differences between feather types and moult pattern)
Morph type/ plumage pattern	Dark/ light morph	It could be interesting in some highly polymorphic species such as Tawny owl (different morphs are physiologically different and might differ in contaminant susceptibility)
Cause of death	For dead specimens, cause of death if known	
Photos	Photo of the individual, carcass, nestlings, injuries, etc.	Important for carcasses in the field and for necropsy (it can help identify cause of death) - Note that the identification (label) should be included in all photos
Body measurements	Wing length, tarsus length, weight	May be useful to estimate body condition index, may be relevant for sex/age
Egg measurements	Length, width and weight (eggshell thickness and mass). Identity (ring number) of breeding female if possible	Egg morphometrics can be used to detect shell thinning, an early warning biomarker that occurs at exposures far below those that cause direct impacts on reproduction
Diet composition	Record diet remains in nest, pellets, stomach content	Interesting to understand diet for some contaminant exposure (e.g. proportion of rodents for SGARs)
Individual reproductive performance	Clutch size, number of hatched chicks and number of fledglings, hatching success, breeding success, etc.	Interesting to relate some contaminants with breeding performance (multiple visits may be needed - disturbance)
Blood slide	Endoparasites, cell count	Interesting to detect changes in blood coagulation caused by rodenticides
Bleeding area	Part of the body for taking blood sample	Not useful for contaminant interpretation. Animal welfare

CONTEXTUAL DATA	EXAMPLE	JUSTIFICATION
Reporting unexpected observations	Morphological/behavioural abnormalities (aggressiveness), eggs	May be useful for interpreting contaminant concentrations. Abnormal behaviour could be associated to some chemical exposure
Environmental contextual data		
Land use	Land use and practices	Useful to record management relevant to contaminant sources
Known contamination sources in the area	Industry, agriculture, hunting sites, contaminated water course	Useful to record potential contaminant sources
Bait (specific for poisoning events)	Collecting remain baits, especially when poison is observed at the exterior	May be useful for interpreting contaminant concentrations
Entomofauna (specific for poisoning events)	Insects that consume the carcass and soil below the carcass	Useful to estimate time/cause of death

USEFUL REFERENCES

See guidelines for contextual data collection from Michel et al. Guidelines for contextual data collection for contaminant studies on Peregrines and other falcons. Research Networking Programme – EURAPMON.

- See a review of constraints and solutions for collecting raptor samples and contextual data for a European Raptor Biomonitoring Facility at:
- Dulsat-Masvidal et al. 2021. A review of constraints and solutions for collecting raptor samples and contextual data for a European Raptor Biomonitoring Facility. Science of the Total Environment 793: 148599. https://doi.org/10.1016/j.scitotenv.2021.148599
- See protocol to **classify the stages of carcass decomposition** and estimate the time of death at:
- Valverde et al. 2020. Protocol to classify the stages of carcass decomposition and estimate the time of death in small-size raptors. European Journal of Wildlife Research 66: 93. https://doi.org/10.1007/s10344-020-01429-3

HOW TO SUBMIT SAMPLES FOR ANALYSIS

Contaminant monitoring has some requirements regarding the volume or mass of sample needed for contaminant analyses, the most suitable container type to conserve the samples, prevent contamination of the sample, and the necessary conditions (i.e., temperature and time) required for transportation and storage. In addition, regulation exists regarding the packaging and labelling to transport biological substances. This section tries to provide guidance and the main documents on these issues.

Volume/Mass of sample, type of container and **transport conditions** required for contaminant monitoring in different matrices: See Table 2 from Espín et al. 2021.

Sample identification: All samples must be labelled. Label the individual sample containers prior or immediately after the sample is collected. Each sample should be identifiable from a unique code (this code will be the same that appears in the sampling report). A short and self-explanatory identification system that is easy to implement in the field should be used. For further details see Espín et al. 2021 and Espín et al. 2014.

Detailed information on **packaging, labelling, paperwork** and **legal considerations**: click here.

TABLE 2 – Volume/Mass of sample, type of container and transport conditions required for contaminant monitoring in different matrices

(from Espín et al., 2021). (Abbreviations and notes can be found at the end of the section)

		Matrix[a]	Blood[b]	Plasma/serum[b]	Feathers[c]	Eggs[d]	Liver[e]	Kidney[e]	Brain[e]	Bone[e]	Muscle[e]	Fat[e]	Preen oil	Regurgitated pellets / Prey remains
Metals (Pb/Hg)	Volume/Mass (range)		0.1-0.25 ml (~1 ml when using AAS)	NA	ca. 0.1-0.2 g (min. 0.02 g when using DMA for Hg and 0.1 g when using ICP-MS); BF: 5-10 units; TF/WF: 1-2 units	0.2-0.5 g ww (min. 0.02 g when using DMA for Hg, 0.1 g when using ICP-MS, ~3g when using AAS); whole egg if possible	0.2-0.5 g ww (min. 0.02 g when using DMA for Hg, 0.1 g when using ICP-MS, ~3g when using AAS)	0.2-0.5 g ww (min. 0.02 g when using DMA for Hg, 0.1 g when using ICP-MS, ~3g when using AAS)	0.2-0.5 g ww (min. 0.02 g when using DMA for Hg, 0.1 g when using ICP-MS, ~3g when using AAS)	0.2-0.5 g ww (~3g when using AAS)	0.2-0.5 g ww	0.2-0.5 g ww (min. 0.02 g when using DMA for Hg, 0.1 g when using ICP-MS, ~3g when using AAS)	MI	What you find in the field
	Type of container		PP tubes (metal free)	NA	Sealed plastic bag / Envelope	PP jar (metal free)	PP jar (metal free)	PP jar (metal free)	PP jar (metal free)	PP jar (metal free)	PP jar (metal free)	PP jar (metal free)	MI	Sealed plastic bag
	Transport conditions	Temperature	Cold blocks	NA	Ambient temperature/Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	MI	Ambient temperature/Cold blocks
		Time	ca. 24 h	NA	Indef	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	MI	Indef (ca. 24 h for prey remains)
	Storage conditions	Temperature	−20°C	NA	Ambient temperature/ −20°C [1] (preferrably in darkness)	−20°C	−20°C	−20°C	−20°C	−20°C	−20°C	−20°C	MI	Ambient temperature/ −20°C (prey remains)
		Time	Indef	NA	Indef	Indef	Indef	Indef	Indef	Indef	Indef	Indef	MI	Indef
Agrochemicals	Volume/Mass (range)		1-2 ml	0.2 ml	0.2-0.5 g; BF: 5-10 units; TF/WF: 1-2 units	0.2-3 g; whole egg	0.2-3 g	0.2-3 g	0.2-3 g	NA	0.2-3 g	0.2-3 g	0.01-0.1 g	For prey remains different tissues could be analysed (see other columns)
	Type of container		PP tubes	PP tubes	Plastic sealed bag / Envelope	PP jar	PP jar	PP jar	PP jar	NA	PP jar	PP jar	PP jar	
	Transport conditions	Temperature	Cold blocks	Cold blocks	Ambient temperature / Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	NA	Cold blocks	Cold blocks	Cold blocks	
		Time	ca. 24 h	ca. 24 h	Indef	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	NA	ca. 24 h	ca. 24 h	ca. 24 h	
	Storage conditions	Temperature	−20°C	−20°C	Ambient temperature/ −20°C [1] (preferrably in darkness)	−20°C	−20°C	−20°C	−20°C	NA	−20°C	−20°C	−20°C	
		Time	See note [4]	See note [4]	See note [4]	See note [4]	See note [4]	See note [4]	See note [4]	NA	See note [4]	See note [4]	See note [4]	

TABLE 3– Volume/Mass of sample, type of container and transport conditions required for contaminant monitoring in different matrices (from Espín et al., 2021) - continued

		Matrix[a]	Blood[b]	Plasma/serum[b]	Feathers[c]	Eggs[d]	Liver[e]	Kidney[e]	Brain[e]	Bone[e]	Muscle[e]	Fat[e]	Preen oil	Regurgitated pellets / Prey remains
Pharmaceuticals	Volume/Mass (range)		P-NC	0.1-0.25 ml	1 g	2 g	2 g	2 g	2 g	NA	2 g	2 g	MI	For prey remains different tissues could be analysed (see other columns)
	Type of container		P-NC	PP tubes[2]	Sealed plastic bag / Envelope[2]	PP jar[2]	PP jar[2]	PP jar[2]	PP jar[2]	NA	PP jar[2]	PP jar[2]	MI	
	Transport conditions	Temperature	P-NC	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	NA	Cold blocks	Cold blocks	MI	
		Time	P-NC	< 24 h	< 24 h	< 24 h	< 24 h	< 24 h	< 24 h	NA	< 24 h	< 24 h	MI	
	Storage conditions	Temperature	P-NC	−20°C/−80°C[3]	−20°C/−80°C[2] (preferrably in darkness)	−20°C	−20°C	−20°C	−20°C	NA	−20°C	−20°C	MI	
		Time	P-NC	See note[4]	See note[4]	See note[4]	See note[4]	See note[4]	See note[4]	NA	See note[4]	See note[4]	MI	
Rodenticides	Volume/Mass (range)		1 ml	1 ml	NA	0.5-2 g	0.5-2 g	MI	NA	NA	MI	NA	NA	Plastic sealed bag for pellets. For prey remains different tissues could be analysed (see other columns)
	Type of container		PP tubes	PP tubes	NA	PP jar	PP jar	MI	NA	NA	MI	NA	NA	
	Transport conditions	Temperature	Cold blocks	Cold blocks	NA	Cold blocks	Cold blocks	MI	NA	NA	MI	NA	NA	
		Time	< 24 h	< 24 h	NA	< 24 h	< 24 h	MI	NA	NA	MI	NA	NA	
	Storage conditions	Temperature	−20°C	−20°C	NA	−20°C	−20°C	MI	NA	NA	MI	NA	NA	
		Time	See note[5]	See note[5]	NA	See note[5]	See note[5]	MI	NA	NA	MI	NA	NA	
Perfluorinated compounds	Volume/Mass (range)		0.2-1 ml	min. 0.2 ml	ca. 0.1-1 g	0.5-1 g	ca. 1 g	ca. 1 g	ca. 1 g	MI	ca. 1 g	0.5-1 g	0.01-0.1 g	ca. 1 g
	Type of container		PP tubes	PP tubes	Sealed plastic bag / Envelope	PP jar	PP jar	PP jar	PP jar	MI	PP jar	PP jar	PP jar	PP jar
	Transport conditions	Temperature	Cold blocks (<4°C)	Cold blocks	Ambient temperature/Cold blocks	Cold blocks	Cold blocks	Cold blocks	Cold blocks	MI	Cold blocks	Cold blocks	Cold blocks	Cold blocks
		Time	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h	MI	ca. 24 h	ca. 24 h	ca. 24 h	ca. 24 h
	Storage conditions	Temperature	−20°C	−20°C	Ambient temperature/ −20°C[1] (preferrably in darkness)	−20°C	−20°C	−20°C	−20°C	MI	−20°C	−20°C	−20°C	−20°C
		Time	Indef	Indef	Indef	Indef	Indef	Indef	Indef	MI	Indef	Indef	Indef	Indef

[a] *Please note that these are general guidelines. Take advice from the laboratory undertaking the chemical analysis.*

[b] *Volume criteria: A general rule is that the collection weight should not exceed 2% of the body weight of the animal in any 14-day period, or 1% at any one time. Values provided in the table are volume/mass ranges generally needed in the Toxicology lab for analysis, but it will depend on the technique used.*

[c] *From live birds, plucked contour body feathers (e.g. back/breast feathers) are preferred. Moulted feathers, chick down feathers and feathers from museum specimens are also useful. Consideration should be given to possible external contamination of museum feathers, e.g. due to conservation treatments.*

[d] *This protocol does only deal with non-destructive sampling, thus it only refers to deserted or addled eggs.*

[e] *Internal tissues collected during necropsies.*

[1] *For feathers when they are wet or have tissue/blood attached to them, they need to be cleaned/dried or they need to be stored in a freezer and not room temperature (as then this may lead to further decay)*

[2] *Pharmaceuticals is a broad group, and plastic containers may contain some compounds (e.g. UV filters), this should be considered or part of the plastic container analysed.*

Take advice from the laboratory undertaking the analysis.

[3] −80°C recommended for some drugs and for long storage periods (> 3 months), take advice from the laboratory undertaking the analysis.

[4] Agrochemicals and pharmaceuticals are broad groups. Some are not easy to break down (e.g. PCBs and most chlorinated pesticides has been found to be stable for at least one year at −20°C) but others may be rapidly degraded over time (e.g. significant degradation of some antibiotics after 2-24 weeks depending on the compound and tissue type when conserved at −20°C; O'Brien et al. 1981; Vanderkop et al. 1989; or metamidophos insecticide after 60-90 days in liver samples conserved at −20°C; MacLachlan et al. 2003). −80°C would be to recommend for long-time banking of soft tissues.

[5] Few studies have been done investigating the stability of rodenticides in frozen samples (eg. bromadiolone concentrations decreased 6-41% in whole blood samples stored at −20°C after 83-201 days; Vindenes et al., 2008). −80°C would be to recommend for long-time banking of soft tissues.

BF:Body feathers, TF/WF:Tail feathers/Wing feathers, ww: wet weight, PP:Polypropylene, Indef: Indefinitely (consider dessication), NA:Not applicable (sample type not useful for that group of compounds), MI: more information is needed, P-NC: possible but not the sample of choice

Example of containers: polypropylene (PP) tubes, PP jar and sealed (to avoid freezer burn) plastic bags.

Although PP containers are recommended in general, glass could be considered if practical (note that some compounds such as flame retardants and plasticisers could be in plastics and there could be potential contamination, so take advice from the laboratory and use field blanks when possible). Containers may be specifically precleaned for some contaminants (e.g. POPs, metals, perfluorinated; ask the laboratory).

USEFUL REFERENCES

World Health Organization. (2019). Guidance on regulations for the transport of infectious substances 2019–2020: applicable from 1 January 2019 (No. WHO/WHE/CPI/2019.20). World Health Organization

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PACKAGING, LABELLING, PAPERWORK & LEGAL CONSIDERATIONS

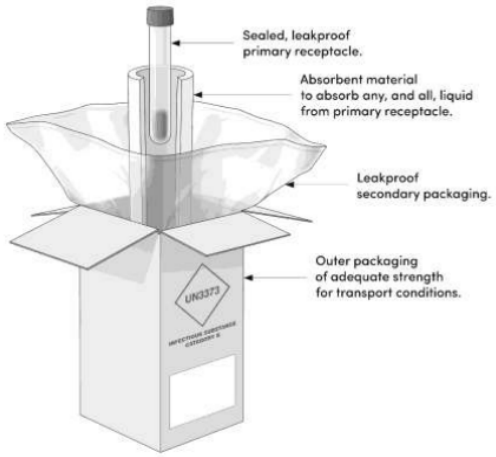
Check the **Guidance on regulations for the Transport of Infectious Substances (World Health Organization; WHO, 2019)**: click here to download the document in English, Spanish or French. A brief summary is presented here (but the whole guidance should be checked):

- Infectious substances are sub-classified as **Category B** when they **contain biological agents capable of causing infection in humans or animals**, but NOT meeting the criteria for Category A; that is, the consequences of an infection are not considered severely disabling or life-threatening.
- The UN number and proper shipping name for most shipments of Category B infectious substances is **UN 3373, Biological substance, Category B**.
- When a package of infectious substances is moved between the point of origin and its destination, it can be subject to movement, vibrations, changes of temperature, humidity and pressure. It is therefore essential that the packaging used to contain infectious substances during transport is of good quality and is strong enough to withstand the various possible challenges on the way. Hence, infectious substances must be contained in a **triple-layer packaging system,** where redundant layers of packaging and sufficient amounts of absorbent material can be used to control leakages or breaches of the container.
- **Triple-layer packaging system:** a primary receptacle; a second, watertight and leak-proof or sift-proof packaging to enclose and protect the primary receptacle; and a third, outer layer of packaging that is used to protect the secondary packaging from physical damage while in transit.

- **Examples of basic triple packaging materials**. Source: Modified from illustration created for the 4th edition of the WHO Laboratory Biosafety Manual (WHO, 2019 at: https://www.who.int/publications/i/item/9789240011311).
- **Packing instruction P650** (category B infectious substance requirements)

In addition to the basic triple packaging system, stipulations outlined in P650 include the following: for surface transport, either the secondary or outer packaging must be rigid (a rigid outer packaging is always required for air transport). The complete triple package must be capable of passing a 1.2 m drop test, to prove that it is of an appropriate strength and quality. Either the primary receptacle or the secondary packaging must be capable of withstanding an internal pressure of 95 kPa (0.95 bar). This must be tested using an appropriate methodology that is based on the receptacle or packaging type being used (e.g. internal hydraulic or pneumatic pressure gauges, or external vacuum testing).

- A coolant (also known as a refrigerant) is a substance that is used to maintain a cool temperature around the dangerous goods, to preserve their integrity until they reach their final destination. The coolant must be placed between the secondary packaging and outer packaging, or in an over-pack used to transport multiple packages together.
- Once the correct packaging materials have been assembled, they must be properly marked and labelled to provide information about the contents of the package, the nature of the hazard and the packaging standards that have been applied. All marks and labels must be placed in such a way that they are clearly visible and not covered by any other label or mark.
- The following marks must be provided on the outer package of all infectious substances: the shipper's name and address; the receiver's name and address; the UN number of the infectious substance (UN 3373), followed by the proper shipping name of the substance (Biological substance, Category B); and when a coolant (e.g., dry ice) is used, the UN number and the proper shipping name of the coolant, followed by the words "AS COOLANT" is required. In addition, the net quantity of coolant present should be given.
- At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm.



2 Example of triple packaging materials that may be used to comply with P650 for Category B infectious substances.

Source: Illustration created for the 4th edition of the WHO Laboratory Biosafety Manual (WHO, 2019).

3 Example of the UN number

FIGURES AND CHARTS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