Société Herpétologique de France

# HYGIENE PROTOCOL FOR AMPHIBIAN DISEASE CONTROL IN THE FIELD

## TECHNICAL SHEET

Health protocol . version 02.2022

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**Société Herpétologique de France**

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# PREFACE

This sheet has been designed to provide educational and technical information in the field of the study of reptiles and amphibians.

The technical sheet "Hygiene protocol for the control of amphibian diseases in the field" is part of the collection of sheets available on the internal site of the SHF (lashf.org/fiches-techniques/).

**The Société Herpétologique de France**

The Société Herpétologique de France (SHF) is an association law 1901 founded in March 1971. It has been approved for environmental protection since February 28, 1978, approval renewed on December 31, 2012. The SHF aims to:

- facilitate relations between French-speaking herpetologists,
- raise awareness of Reptiles, Amphibians and their role in natural balances,
- contribute to a better knowledge of French fauna and its distribution,
- protect different species and their environment, to improve the breeding conditions of Reptiles and Amphibians, in particular for scientific purposes.

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This document was produced by Claude Miaud (EPHE, Montpellier).

*Document citation*

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# CONTEXT

Amphibians are exposed in their natural environment to numerous organisms which can be more or less pathogenic.
For twenty years, massive deaths of amphibians have been observed in France, caused by pathogens (fungi and viruses) that may have been introduced into the natural environment through human activities. In this context, people who can access and/or work in aquatic environments, likely to introduce or disseminate these pathogens, must integrate into their activities a **standard protocol of precautionary health measures**, to be implemented during their field campaigns. If this protocol is targeted on the pathogens responsible for mortality in amphibians, the precautions will also make it possible to limit the dissemination of invasive plant or animal species.

**This document must allow to:**

- Prevent or reduce the risk of transfer of pathogenic organisms within and between populations of amphibians in the wild.

- Allow identification and bring about an appropriate procedure when discovering dead or diseased amphibians in the wild.

**Which reader for this document?**

This protocol is proposed for all environmental professionals, researchers, space managers, professional and amateur naturalists, students, etc., (hereinafter called field operators) who frequent aquatic environments and in particular carry out observations and/or amphibian studies.
This protocol should in particular be disseminated to professionals and amateurs who regularly contact and handle amphibians in the field. This protocol is an update of the hygiene protocol proposed by the RMC Water Agency (Miaud, 2014) taking into account advances in knowledge of amphibian diseases. Its application must become a rule for any action that requires the handling of amphibians in the field.

# FIELD IMPLEMENTATION

## General principles

Field operators who work in aquatic environments (with or without amphibians) often visit several aquatic sites during the same field campaign. It is therefore necessary to define the limits between the sites frequented and to take measures to limit the spread of potential pathogens:

**1.** If the intervention is carried out on sites **where the presence of the pathogen is suspected** (observation of amphibian mortalities, presence of exotic species, etc.) **or proven**, it is imperative to rigorously apply the hygiene protocol.

**2.** If several aquatic sites must be visited during the same field campaign, **disinfect the equipment between each site** (see below). When working on a large body of water (marsh, river, large lake, etc.), regularly disinfect the equipment.

**3.** Before any outing in the field, it is essential to ensure that all the equipment that will be used (boots, waders, landing net, etc.) has been properly disinfected at the end of the last field campaign in which it was been used. If in doubt, disinfect it.

**4.** There are several effective disinfectant products commercially available to eliminate chytrids and ranaviruses (70% alcohol, bleach). Nevertheless, for reasons of effectiveness on most infectious agents (bacteria, viruses and fungi), and respect for the environment, we recommend the **use of Virkon® or F10®.** The release of these disinfectants into the environment should be limited.
The manufacturer recommends the disposal of Virkon® through the waste water networks.
**Before use, read the instructions for use provided by the manufacturer** (safety data sheet No. 130000014173).

**5.** When handling amphibians, **it is recommended to use non-powdered disposable gloves, or previously moistened bare hands**. Whenever possible, captured individuals should be kept individually ("one bag = one amphibian") in zip lock plastic bags, plastic boxes, etc. in order to limit contact and the risk of pathogen transmission between animals.

# DEFINITION OF A SITE

The objective of the hygiene protocol is to limit the risks of transmission of pathogens, and a high frequency of decontamination of materials contributes to this limitation.

It is obviously necessary to take into account the operational aspect and the appropriation of the protocol by the field operators: a compromise between "effort" of disinfection and "risk" of contamination must be found, and it is based on the definition of the limits of the frequented sites.

**If the presence of a pathogen is known at one or more sites, they should be visited, preferably at the end of the field session.**

**In the event of surveys in nearby aquatic sites** (archipelago of ponds, marshes, rivers, etc.) **in the same region, the equipment will be disinfected**, for example after half a day in the field.

**In the event of prospecting between distant regions** (change of watershed, etc.), **the equipment must be disinfected when the field operators leave the region.**

# IMPLEMENTATION OF THE DISINFECTION PROTOCOL*

**1. Prepare a 1% solution of Virkon® in a sprayer.** The product becomes ineffective when the pink colour disappears. We recommend preparing a new solution during each field campaign. The solution can be prepared in the field using water from a river or pond (Virkon® is available in a powder sachet or tablet). For the F10, there are ready-to-use formulas in a sprayer. Otherwise, prepare the solution following the manufacturer's dilution recommendation. Prefer the F10FC version, which does not require rinsing.

**2.** 1. When leaving a site and before going to a new one (see point 2 - implementation in the field), **clean the equipment** (boots, waders, landing net, etc.) **using a brush** to remove sludge and debris, and using water from the site just visited.

**3. Spray the disinfectant solution on all the equipment that has been in contact with water and leave to act for 5 minutes before reuse** (preferably until the equipment is dry). Small equipment that has been in contact with amphibians (scales, scissors, etc.) can be disinfected by immersion in the disinfectant or with wipes impregnated with 70% alcohol. Do not flush the equipment to prevent the disinfectant from being introduced into the environment. If necessary, the equipment can be rinsed on return from the field (with evacuation into the waste water network).

**4. Spray disinfectant on the soles of your boots or walking shoes** before leaving the site.

**5. Store the disinfected equipment in disposable plastic bags then in a storage bin** in the vehicle.

**6. Disinfect your hands** with wipes impregnated with 70% alcohol or a hydroalcoholic solution.

**7.** When returning from the field, **place all disposable equipment (gloves, bags, etc.) in a garbage bag** before throwing it away. **Field clothing can be disinfected regularly by machine washing at 60°C.**

Transmission of pathogens via road vehicles is unlikely. The spread of ranavirus via the hull of boats (kayaks) has been shown. If a machine (vehicle or boat) has travelled in aquatic sites populated by amphibians, a disinfection procedure (spray of disinfectant) can be applied.

# LIST OF MATERIALS NEEDED

- Brush;
- Sprayer;
- Virkon® (tablets) or F10 (available in particular in veterinary surgeries);
- Powder-free disposable gloves (for preparing the Virkon® solution and when handling amphibians);
- Wipes impregnated with 70° alcohol or hydro-alcoholic solution (available in supermarkets and pharmacies);
- Disposable plastic bags of different sizes (to be thrown away at the end of each field campaign);
- Plastic storage bins (remaining in the vehicle and regularly disinfected).

If you run out of Virkon® or F10 during your field campaign, and the product is not available locally, you can replace it with 70° alcohol or bleach (4%) in spraying and taking care of the precautions for use for the operators and not to put in contact with the aquatic environment before its evaporation.

# OBSERVATION OF SICK OR DEAD AMPHIBIANS*

## TO BEHAVE

Knowledge about amphibian diseases goes through the collection of data in the field. Sick or dead amphibians (except in the case of mortalities attributed to an obvious cause such as predation or roadkill) should be collected following a standard protocol:

### Clinical signs in sick or dying amphibians

There are no specific clinical signs of chytrid or ranavirus infections. We can list signs that may be involved in these infections:

On the general appearance:

- Dorsal epidermis darkened, stained;
- Pinkish, reddish dorsal epidermis - Skin lesions (wounds); Swelling of the limbs (posterior);
- Emaciated appearance;
- Eye infection;
- Bleeding (eyes, nostrils, etc.).

On behaviours:

- Lethargic movements of the limbs (hind);
- Absence of escape behaviour;
- Abnormal swimming (adults and tadpoles);
- Daytime exposure for nocturnal or discreet species;
- Weak or no reaction if touched.

### Collection and storage protocol

The use of disposable gloves is highly recommended when handling dead or diseased amphibians. Animals must be kept and/or stored in individual containers (e.g. plastic Ziplock bags). Dead individuals should be kept as cool as possible during the field season, then frozen.

Dead animals can also be fixed in the field with alcohol at 70° (min).

If possible, open the animal and place it in a container with a volume at least equal to 10 times the volume of the specimen.

If many specimens are collected, some may be fixed and others frozen. The containers used must inform about the date of sampling, the place, the date and the identity of the sampler (and if possible his contact details).

*Adapted from NSW National Parks and Wildlife Service (2001). Hygiene protocol for the control of disease in frogs. Information Circular Number 6. NSW NPWS, Hurstville NSW

## Thanks

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All of the field operators who contribute to knowledge about these diseases through their vigilance and their samples in the field.

## References

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