

### Equipment

- **Isopropyl alcohol** - for immediate preservation
- **Leak proof vial(s)** - for transportation
- **Formalin** - for final preservation
- **Gloves** - for handling earthworms and chemicals

### Protocol

1. **Kill the earthworms in alcohol** (isopropyl – rubbing alcohol works great.)  
**Do Not Use ethyl alcohol as it will bleach the specimens.**
  - Get as much dirt off the earthworms as possible. Put the earthworms in the alcohol one at a time (rather than a whole handful all at once) so they don't get tangled up into a big mess of earthworms. After a few seconds, the earthworm will become anesthetized and relax into a mostly extended position.
  - Place the anesthetized earthworms into a vial. You can keep the earthworms in alcohol for up to 24 hours (keeping them cool and out of direct sunlight). But don't wait too long, after a day or so in alcohol they start to get mushy.
  
2. **Put the earthworms in formalin** within 24 hours.
  - Place the earthworms in a leak proof vial and cover them in Formalin (a cellular fixative that will prepare the specimens for long-term storage).
  - Once the earthworms have been in formalin for at least 24 hours, they can be placed back into alcohol (70-100% isopropyl, NOT ethyl which bleaches any pigmentation) for long-term storage. This allows you to re-use the formalin over and over again.

*CAUTION: Formalin is a dangerous chemical and safety precautions must be taken. It should be used only in a well ventilated area. It should not be breathed, swallowed or come in contact with bare skin or eyes. If external contact does occur wash the area with large amounts of water. If ingested contact a poison control center immediately. Formalin has been shown to cause cancer in laboratory animals.*



## Preservation Equipment, Protocol and Mailing Instructions



### Instructions for Mailing Earthworm Samples

#### **3. After you have preserved your worms for 24 hours in formalin:**

- Drain the formalin from vials containing earthworms\*\*
- Add enough Isopropyl alcohol to the vials to cover the worms when the vial is standing upright.
- Place the vials of worms in a large plastic bag (just in case one of them starts to leak).
- Place the plastic bag in a box and fill the rest of the box with packing material.
- Place the data sheets that correspond with the vials in a separate plastic bag and place in the box with the worms.

#### **4. Mail the datasheets and earthworms to:**

Ryan Hueffmeier  
Natural Resources Research Institute  
University of Minnesota  
5013 Miller Trunk Highway  
Duluth, MN 55811-1442

\*\*You can return specimens in the 10% formalin solution provided as it is what is called "10% formalin" is in fact, 3.7% or 4.0% formaldehyde (formaldehyde is a saturated solution of formaldehyde gas in water, measured by weight or volume concentration) and as such is unregulated for transport.

Graduate students at the University of Minnesota-Duluth will identify your worm samples to species and email or mail a list of what they found to the address you included on your data sheets. The identification process is time-consuming, so it may take a few months before you receive your results!