NATIONAL PARK SERVICE
DEPARTMENT OF THE INTERIOR

Conserve O Gram

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Removing Wet Specimens From Long-Term Storage In Formalin

Since the nineteenth century, specimens of biological organisms have traditionally been stored in liquid preservatives, and over the years, many changes in techniques and preservatives have occurred. The chemical formaldehyde (CH_2O) plays an indispensable role in *fixing* the proteins in a fresh specimen in order to prepare it for wet preservation. Until recently, various solutions of formaldehyde have also been used as a preservative for long-term storage of specimens. The most common preservative used is formalin, a 10% solution of formaldehyde mixed in water[1] (i.e., 9 parts water and 1 part formaldehyde). However, it is now recognized that formalin, because of its acidity, makes a very poor long-term preservative for many specimens, resulting in the decalcification of bones, distortion of tissues, and acidic decomposition of specimens. Today, the recommended long-term preservative for maintaining a variety of plant, vertebrate, and invertebrate specimens is a 70%-75% solution of ethanol (also known as ethyl alcohol or C_2H_6O). Therefore curators of wet specimens stored in formalin need to consider transferring them into ethanol for long-term storage.

Some specialized collections, for example tissue samples, larval vertebrates, and some soft-bodied invertebrates, ***should not be transferred*** to ethanol. To prevent distortion, they require a higher water content than that provided by the recommended ethanol solution. Before any transfers are begun, consult with the Regional Curator on the advisability of the procedure for specific materials.

Test For Formaldehyde

If the type of preservative already in use is undocumented, and an ethanol preservative is desired, test the preservative in use for the presence of formaldehyde. Formaldehyde has a strong, acrid, pungent smell, usually noticeable in the work area, and a whiff test has traditionally been used to detect it. However, because of the toxicity of formaldehyde, it is best identified by the use of aldehyde test strips.[2] ***Note the safety precautions outlined on page 3.*** If formaldehyde is present, proceed with the transfer process.

Transfer Procedure

1. In a well ventilated work space, remove the specimen from the formaldehyde with forceps, using caution not to damage the specimen.

2. Immerse the specimen in distilled water and agitate gently for approximately one minute.

3. Test the original formalin preservative for acidity and record the pH in the specimen's accession or catalog folder. Use a pH meter if available; if not, use pH indicator test strips.[3] The meter is preferable because formaldehyde is a reducing agent that can change the color of the aniline dyes in the test strips.

   - If the pH tested is higher than 5.5, proceed to step 4.

- If the pH tested is less than 5.5, place the specimen in a solution of a weak base to buffer the formaldehyde present. A basic solution can be made using ***either*** 15 grams Ammonium Phosphate Dibasic [$(NH_4)_2HPO_4$] ***or*** 17 grams Potassium Phosphate [K_2HPO_4] dissolved in one liter of distilled water.

  Leave the specimens in the weak base for up to 6 hours, depending on the permeability of the specimens as discussed below.

4. *Step* transfer the specimens through a series of increasing concentrations of ethanol (40%, 60%, and 75% solutions), made by mixing ethanol and water in a ratio of parts given in the list below. Whether measured in liters or ounces does not matter, as long as the measure is consistent and by volume.

	Parts Distilled Water		Parts Ethanol		Ethanol Final Percent
Step 1:	3	plus	2	equals	40%
Step 2:	2	plus	3	equals	60%
Step 3:	1	plus	3	equals	75%

   The length of time the specimens are left in each step ethanol solution depends upon the size and permeability of the specimen. Insects will take a few hours in each step, frogs and small birds a day, and a raccoon or tortoise will require two to three days in each step. These estimates will vary depending on how *opened up* the specimens are by cuts made into the body cavity. The step ethanols can be used repeatedly for specimens from the same lot, but eventually will contain too much formaldehyde and wastes to be of use. This is particularly undesirable in the 75%, or last, step solution. The point at which the solution should be changed is determined by the presence of discoloration or particulates in the liquid, by a positive test with an aldahyde test strip, or by a check with a specific gravity meter for the percentage of alcohol content.

5. Place the specimen in 75% ethanol as a final storage solution.

   The old jars can be washed (in liquid soap and rinsed several times) and re-used, but make sure the lids still form a good seal. Monitor the specimens at least twice a year and ensure that bottles or lids are replaced whenever 1/3 or more of the volume has evaporated in less than six months. When lids are replaced, use polyethylene lids with polypropylene liners, or gasketed glass lids with spring clips. Replace all metal lids. Depending upon the condition of the old labels, it may be desirable to complete new labels (using NPS Form 10-500 to 10-512), either attached to the specimens with string or placed loose in the jar. Use a permanent, carbon based ink, ***never*** ballpoint or felt-tip pen. ***Never destroy the old label.*** If the prior label is damaged or cannot be maintained with the specimen, rinse it gently in clear water 3-4 times, blot, and then air dry. Sleeve the label in Mylar® or buffered paper, and file it in the specimen's accession or catalog folder along with an archival photocopy, if appropriate. One new label can be used for jars containing specimens that have the same collecting locality, date, and collector. However, for most larger vertebrates (mammals, birds, turtles, etc.), individual labels for each specimen are considered standard.

6. Record the entire transfer process in the specimen's accession or catalog folder. Place a photocopy of this *Conserve O Gram* in the file as well, indicating any changes in the actual procedures used.

Purchasing Ethanol

After a thorough inspection to determine the amount needed, obtain a supply of 95% or reagent ethanol that will meet your immediate needs, estimated refills, and new specimen needs for one year. Undenatured ethanol is preferred because it does not contain the impurities that denatured alcohol does. Denatured ethanol

contains impurities such as methanol, aviation fuel, and other fluids to make it toxic and undrinkable. However, because of its availablility from most chemical suppliers and its much lower cost, many field biologists use it as a preservative.

NOTE: The purchase of ethanol (or ethyl alcohol) that has not been denatured is subject to a special tax. If small quantities (less than about 10 gallons, or 20 proof gallons) are required annually, contact the State Liquor Board or State Liquor Control Commission for information on sources, such as liquor stores or chemical suppliers, and local regulations in force. The tax on such purchases will be included in the cost of the ethanol. If larger quantities are required within a year, contact the regional office of the Bureau of Alcohol, Tobacco, and Firearms to apply for an Industrial Alcohol Users Permit (ATF application form #5150.22 for ATF permit #5150.9). This permit allows the user to pay a flat annual tax of $250 and then purchase the necessary quantity of ethanol tax-free from the manufacturer.

Safety Precautions and Procedures

Obtain Material Safety Data Sheets for all sub-substances used in this transfer process and follow all safety precautions outlined. (See *Conserve O Gram* 2/1.) Note that formaldehyde is a known carcinogen and a sensitizer (a chemical that upon chronic exposure can cause an allergy to the substance to develop), and that ethanol is flammable. While transferring the specimens, wear safety glasses and an organic-vapor respirator. Wear gloves and an apron resistant to organic and solvent solutions, often made from neoprene, latex, or nitrile (all available from biological or chemical suppliers). Replace the gloves frequently to prevent seepage. Use both with adequate ventilation (using a laboratory fume hood if available), and observe appropriate fire safety precautions.

For health reasons, ensure that specimens that remain in formalin are retained in tightly-sealed containers to minimize vaporization of the preservative. Label these containers with the name of the preservative and the percentage if known (e.g., ***10% Formalin***); otherwise, label them ***Contains Formaldehyde in Solution.***

Store the flammable materials in a locked flammable storage cabinet (follow National Fire Protection Association Code 30). A log system recording the use of ethanol that has not been denatured is required to prevent unauthorized use. Label storage containers ***Flammable*** and ***Poison.*** After use, the step ethanols should be labeled ***Ethanol Solution Contaminated with Formaldehyde.***

Dispose of formalin and other chemicals in accordance with the park's hazardous waste disposal program. Contact the park or regional hazardous waste coordinator for guidance.

Notes

1. Formaldehyde is normally a gas. When it is purchased as a liquid, a 100% (or saturated) solution consists of 37% formaldehyde gas dissolved in water.

2. For example, E. M. Quant® Formaldehyde test strips are available from chemical suppliers such as VWR Scientific (cat. no. EM10036), (609) 467-2600, or Curtin Matheson Scientific, Inc., (cat. no. 246533), (800) 543-1661. Alternatively, test strips can be made on-site using procedures described in "A Spot Test to Distinguish Formalin from Alcohol Solutions," Robert Waller and Don E. McAllister, *Curator*, American Museum of Natural History, New York, NY. Vol. 30/3, 1987.

3. Both are available from chemical suppliers. The loan of a pH meter might also be arranged from park or regional scientists, or local high schools or universities.

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