Biological preserved specimens

Posted by Anonymous on Fri, 2020-02-28 16:11

Biological preserved specimens: What are the rules for storing and/or disposing of biological preserved specimens?

Since Science ASSIST has received a number of questions, which all relate to this topic, we will cover all the issues in this one question.

Q1: We have specimens that have been preserved in formaldehyde, which are used for demonstration purposes only. What are the legal requirements for keeping these specimens on campus? How should they be maintained and stored?

Q2: We have many jars of biology specimens (beetles, frogs etc) with an unknown liquid in them. Some of the liquid has evaporated. My teacher is worried that it may be in the environment in her classroom. Is this dangerous and can we top up with formaldehyde?

Q3: There seems to be a collection of half-empty specimen jars in my Lab classroom and we are not sure of the liquid used to "preserve" the specimens. How can specimens in

an unknown solution be safely and legally disposed of?

Q4: Calf Foetus: A student has brought in a calf foetus stored in methylated spirits. Can you suggest what to transfer it into for preservation?

Q5: Preserved specimens: We have some preserved baby sharks for which the solution needs topping up. We don't know what solution is currently in the glass container. What is the correct solution to top the container up with?

Q6: Preserving sheep brains without formalin. We no longer use formalin (formaldehyde) for tissue specimens. Which reagents or products do you recommend for fixing and preserving sheep brains? We've had mediocre results with alcohols, and freezing did not work.

Voting:
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7
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Senior Secondary
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Laboratory Technicians

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Answer by labsupport on question Biological preserved specimens

Submitted by on 28 February 2020

Answer reviewed 23rd January 2023

Traditionally, solutions of formaldehyde[1] were used for preserving biological specimens. Many old existing preserved biological specimens in schools contain formaldehyde and or other hazardous solutions such as formalin, which is usually a saturated solution of formaldehyde at 37%.

Schools are not prohibited from having biological specimens in formaldehyde, unless it is banned by their school jurisdiction or sector. If your school has specimens in formaldehyde, it is important to be aware of the hazards and ensure that suitable controls are in place to reduce your risk of exposure, as there are risks associated with exposure to formaldehyde.[2] It is a known carcinogen, toxic, a severe sensitiser and skin irritant. If the specimens in formaldehyde are in well-sealed jars that are inspected regularly, the risk of exposure is low. Preserved biological specimens may now be purchased in less hazardous solutions.

Storage advice for preserved biological specimens:

- Store specimens in screw capped containers that are non-reactive to the storage fluid. The seal should prevent evaporation of the storage solution, and metal lids should be avoided as they may corrode and eventually leak.
- Use Parafilm to provide an additional seal.
- Affix a label indicating the storage solution that the specimen is in.
- Check the condition of all containers and use safe procedures (referring to specific SDSs, wearing appropriate PPE and working in a fume cupboard or well-ventilated area), to either replace compromised lids or place the contents in another container for storage or waste disposal.
- Regularly monitor for fluid levels, compromised containers and defective lids etc.
- Store under conditions to prevent deterioration of the specimen (and solution) i.e., a cool dry place with good ventilation, low-light levels and out of direct sunlight.

Evaporation of the storage solution may indicate that the seal on the jars is not adequate. If the specimens are stored in the classroom and the teacher is in the classroom all day every day, then they are more at risk of exposure to hazardous fumes than students who would probably only be in the classroom for a few lessons per week. The only way to determine if workplace exposure standards have been exceeded is to undertake air monitoring for formaldehyde. The most effective control to reduce the risk of exposure is to remove or eliminate the hazardous chemical.

If your specimens are in good condition: you may wish to keep them. A range of strategies could be used to minimise that risk of exposure such as:

- Move them to another location, which is well ventilated.
- Transfer them (using safe procedures) to suitable jars which have an air-tight seal
- Transfer them (using safe procedures and a stepped process to avoid osmotic issues) into a less hazardous (and known) storage solution.
- Seek further help from a local museum, which may have expertise in this area

If your specimens are in poor condition: then it may be best to arrange for disposal of the specimen and the solution, by a licenced chemical waste contractor. Consideration could be given to finding an alternative, such as specimens embedded in resin, which are very resilient to student handling.

If you do not know what the storage solution is: then you should not top up the jars, as you may be combining incompatible chemicals resulting in the generation of new substances that have unknown properties and unknown hazards, creating a potentially violent chemical reaction or damage to the specimen.

Note: Science ASSIST strongly advises against formaldehyde (methanal) being used in a school science setting. It is a Category 1 (known) human carcinogen, has acute toxicity, and is a Category 2 drug precursor. See references for further information. Formaldehyde is not included in the Science ASSIST List of recommended chemicals[3] because of its acute health hazards, and because it is not regarded as essential or important to the science curriculum.

Disposal of biological preserved specimens:

The best way to dispose of biological preserved specimens is to arrange for disposal 'as is' from a licenced chemical waste contractor.

- If the specimens are in unknown liquids, sometimes these may even be in old food jars, it is not worth the risk or the time and effort to try to combine them into larger jars. We recommend leaving the specimens in the jars that they are in.
- Specimens in unknown liquids should be labelled as being in an unknown solution.
- Store the specimens in an appropriate well-ventilated area, until such time as they are disposed of.

It is a good idea to combine a disposal of these with any other chemicals that need disposal. Science ASSIST recommends that you:

- audit your chemical store for any unwanted chemicals
- check on the integrity of the chemical containers and the condition of the chemicals to see if any have deteriorated/ been degraded
- see if your disposal can be combined with a whole school waste disposal or with schools in your geographical area.

Handling unknown chemical solutions:

It is not recommended to top up unknown chemical solutions of preserved specimens for several reasons.

1. You may be combining incompatible chemicals, which may generate new substances that have unknown properties and unknown hazards.
2. The stability of the chemical is not known and you may create a violent chemical reaction or dangerous substance.
3. The concentration of components of the original preserving fluid may have altered due to the evaporative loss.
4. The solution that is produced may damage the specimen.
5. The solution may discolour or become cloudy not allowing the specimen to be viewed.

'Managing risks of hazardous chemicals in the workplace – Model Code of Practice',[4] states:

> Section 2.1: Identifying hazards

> “The first step in managing risks involves identifying all the chemicals that are used, handled, stored or generated at your workplace…”
>
> Section 2.3: Labels
>
> “If the contents of the container are not known, this should be clearly marked on the container, for example, 'Caution - do not use: unknown substance'. Such a container should be stored in isolation until its contents can be identified and, if it is then found to be hazardous, the container is appropriately labelled. If the contents cannot be identified, they should be disposed of in accordance with relevant local waste management requirements.”
>
> Section 4.2: Specific Control Measures: Keeping Hazardous Chemicals Stable and Transfer of Hazardous Chemicals.
>
> “This section includes information on key control measures that should be considered when managing risks from hazardous chemicals in the workplace.”

The chemicals used to fix and preserve specimens can be hazardous and dangerous and may include chemicals such as formalin, 70% ethanol and a range of other chemicals.[5] Science ASSIST recommends you treat all unknown chemicals as hazardous and conduct a site-specific risk assessment to assess and control the risks. You will need to determine how to safely handle and dispose of the solution. We have developed a Risk Assessment template for schools to use, see Risk Assessment Template.[6] You will also need to make sure that any new preservatives you use are approved for use in your jurisdiction and educational sector. Local guidelines for the disposal of waste fixative and preservative chemicals should be followed.

Replacing the old preserving solution:

If a specimen is considered to be in a condition worth saving, it is recommended to go through the procedure of decanting, followed by rinsing, then replacement with new preservative solution. You will need to take precautions to avoid exposure by contact with skin and eyes, inhalation, and ingestion. It is recommended to work in a fume cupboard and wear appropriate PPE. Collect the unknown chemical in a suitable container for chemical disposal. Care must be taken to avoid damaging the specimen in the process.

When working with preserved specimens it is important to:

- refer to the specific SDS for the new chemicals being used;
- wear appropriate PPE (i.e., safety glasses, gloves, laboratory coat, closed-in shoes);
- work in a fume cupboard or well-ventilated area.

A simple method for replacing your old preserving solution is set out below:

1. Working in the fume hood or well-ventilated area, decant the solution into a glass container which can be properly sealed to avoid evaporative loss. Label as 'Caution - Do Not Use - Unknown Substance (possible fixative or preservative)' for disposal via a chemical waste contractor. Whilst waiting for pickup, store and segregate the waste chemicals safely in approved store rooms or chemical storage cabinets.
2. Wash the specimen several times by soaking for 30 minutes in tap water to remove the old preservative. Collect the washings and store and dispose of as above.
3. Take the specimen through several increasing concentrations of the new fresh preservative (e.g., 1 day in each of the following concentrations 30%, 50% and 70%) to avoid any osmotic issues. Always handle one specimen at a time.

4. Place the specimen into the final preservative solution in a clean glass container that has a tight-fitting lid to prevent any evaporation. Use either 70% ethanol or one of the safer alternatives (see below). The use of Parafilm or silicone sealant can be used to provide a good seal.
5. Label the container to state the type of specimen, type and date of preservative. Most museums put the jar label inside the jar, not on the outside or on the lid. This will lessen the likelihood of the specimen and label being separated. It is important to use paper intended for long-term preservation in fluids.[7] There are a several papers that are suitable. Resistall paper was commonly used by museums for labelling but over time, the acidic nature of the paper causes acid to migrate into the preservation fluids and alter the pH of the solutions. Synthetic papers have been introduced to natural history fluid collections because of their known chemical resistance to solvents and durability. Archival polymers like polyester and polyethylene have also been used because they are durable and chemically inert.

A soft lead pencil can be used to write on the paper and there are certain archival quality inks or ink pens that can be used as well. They are resistant to fading and smearing, and are insoluble in the preservative solution. Suitable inks and ink pens can be found in some art or office supply stores and museum supply companies. It is recommended to allow the ink to completely dry before placing the label into the storage solution. Ordinary ballpoint pens should not be used for labelling as they generally dissolve in most preservative solutions. See the Science ASSIST School science suppliers [8] list for local museum supply companies for similar products.

6. Store under conditions to prevent any deterioration i.e., a cool dry place in low light levels and out of direct sunlight.

Fixation and preservation methods:

When preparing specimens for preservation they are generally put through a multi-stage process.

1. Fixation to prevent autolysis and microbial breakdown.
2. Water wash to remove excess fixative.
3. Preservation for long-term storage.

Fixation: Traditionally, marine vertebrates are fixed in a solution of formalin, usually a 10% solution made by combining 1 part formalin with 9 parts water. This is still the best fixative in use today. Formalin is toxic, carcinogenic, highly irritating, acts as a potent sensitizer and should be handled with great care in a fume cupboard or well-ventilated area.

Long Term Storage: 70% ethanol has been the method of choice for long-term preservation. Ethanol is highly flammable, prone to rapid evaporation and is a skin irritant. It is also possible to preserve fish specimens for a long time in formalin. Some reports indicate that the formalin is required to be buffered, as the high acidity is able to render some specimens brittle and transparent.[9,10]

Alternatives:

There are some less-hazardous alternatives to these traditional fixatives and preservatives. However, some are untried for long-term storage and may not protect the specimen adequately.

Glycerol[11] (synonyms: glycerin, glycerine) is a safe and reliable alternative to ethanol. Glycerol has low-toxicity, a flash point at 160°C, preserves and revives colour. And, if the specimen is transferred through baths of increasing concentration, it will not shrink the specimen. The Australian Museum[12]uses either 100% glycerol or 70% ethanol for preservation of its fish specimens.

Another alternative is 2-phenoxyethanol[13] (synonyms: phenoxetol, phenoxytol) which is non-flammable compared with 70% ethanol and is less volatile and of lower toxicity when compared with formaldehyde.[14] Below is a recipe for the storage solution taken from an archived article on the rtg.wa.edu.au website.[15]

10mL	phenoxytol (2-phenoxyethanol)
50mL	glycerol
940mL	distilled water

Fresh specimens must be properly preserved before storage by preserving animal specimens in 10% formalin for 3 weeks, washing well to remove the formalin, then transferring to the storage solution. Specimens that have already been preserved, may be transferred to the storage solution after washing in water. With phenoxytol solutions, specimens do not need to be completely immersed - although for presentation, it is preferred. Using this chemical removed the requirement in WA, to store specimens that were preserved in 70% ethanol, in a flammable liquids cabinet.

It is important to check that the preservative you choose is approved for use in your jurisdiction and educational sector.

Preserving sheep brains:

Fresh brain tissue is delicate, very soft, and can be easily damaged, even when being gently handled. Specimens are generally fixed to prevent tissue breakdown and to render them firm to allow for easy dissection. For long-term storage, they are kept in preservative solutions.

You will need to make sure that any fixative or preservatives are approved for use in your jurisdiction and educational sector and are disposed of appropriately following local guidelines.

Below is an outline of some methods to try. As they require the use of a needle and syringe, we recommend you check with your school jurisdiction for any regulations on the use of needles.

Note: These preservative solutions should not be handled by students.

Chemical Methods:

(Note: % v/v is used for concentrations of solutions of liquids and is calculated as [(volume solute)/(volume of final solution)] x 100%. For example, 80% v/v means that 100 mL of solution contains 80 mL of the solute.)

Method 1: 25% glycerol in 80% v/v ethanol:

The Tasmanian Museum[16] uses this method for preservation of its soft-bodied animal specimens.

1. First prepare an 80% v/v ethanol solution: for 100 mL, measure 84 mL ethanol (95%) and make up to 100 mL with distilled water.
2. For 100 mL glycerol/ethanol solution, measure 25 mL glycerol and make up to 100 mL with the 80% ethanol solution (or combine 1 part glycerol with 3 parts ethanol (80%)).
3. Using a 10 mL syringe and 21 g x 38 mm needle, inject the solution into the brain tissue in several places—in both the left and right cerebral hemispheres. Injection of the brain helps penetrate the inner tissues before immersion in the preservative solution.
4. Immerse the brain in the solution for storage in a clean glass container that has a tight-fitting lid to prevent any evaporation. The use of Parafilm or some silicone sealant can be used to provide a good seal.
5. Store under conditions to prevent any deterioration i.e. a cool dry place with low-light levels and out of direct sunlight. As the solution is flammable, it should be stored in the flammable liquids cabinet.
6. Leave for 2–3 days to fix the tissue.
7. After 3 days, pour off the solution and refresh with a new batch.
8. Leave for at least one month before use for dissection or as a display specimen.
9. Collect any waste solution into a glass waste container, which can be properly sealed to avoid evaporative loss. Label appropriately and dispose via a chemical waste contractor.

It is beneficial to use at least 2–3 fresh changes of the solution. The more changes the better for fixing (retaining colour) and rendering the tissue firm and rubbery for dissection purposes.

Using the ratio of 25% glycerol in 80% ethanol has the benefit of no real shrinkage; the glycerol makes the brain tissue pliable and it is relatively safe to use. Glycerol has low toxicity and helps in preserving and reviving colour.

Method 2: 75% ethanol in 95% ethyl acetate solution: (a variation on the Ethanol/Glycerol method)

1. First prepare a 95% ethyl acetate solution: for 100 mL, measure 95 mL ethyl acetate and make up to 100 mL with distilled water.
2. For 100 mL of ethanol/ethyl acetate solution, measure 80 mL of ethanol (95%) and make up to 100 mL with the 95% ethyl acetate solution (or combine 4 parts ethanol (95%) with 1 part of the 95% ethyl acetate solution).
3. Using a 10 mL syringe and 21 g x 38 mm needle carefully inject the solution deep into the brain tissue in several places—in both the left and right cerebral hemispheres, then leave the specimen in the solution for fixation for around 48 hours. This assists in hardening and reduces shrinkage.
4. Drain and then preserve in the ethanol/glycerol mixture described above in Method 1. Store in a flammable liquids cabinet.
5. Leave for at least one month before use for dissection or as a display specimen.
6. Dispose of waste ethanol/ethyl acetate solution and ethanol/glycerol solution as in Method 1.

The idea behind this is to “set” the tissue before preserving while the glycerol keeps it from getting too hard. If the brain tissue still ends up too soft, then before putting into the preserving solution, place in a solution of 20 drops of ethylene glycol to 100 ml of ethanol/ethyl acetate for 24 hrs.

Collect any waste ethylene glycol/ethanol/ethyl acetate solution into a glass container, which can be properly sealed to avoid evaporative loss. Label appropriately for disposal via a chemical waste contractor. Ethyl acetate is used as a fixative and is classified as hazardous and flammable. It is a colourless liquid and has a characteristic sweet smell. Ethylene glycol assists with hardening tissue. It is classified as hazardous and is toxic if ingested.

Method 3: 75% v/v Ethanol Solution:

1. For 100 mL of solution: measure 80 mL of ethanol (95%) and make up to 100 mL with distilled water, (or combine 4 parts ethanol (95%) with 1 part distilled water). Note: do not use denatured alcohol as a preservative. Some denaturants can have adverse effects on specimens.
2. Using a 10 mL syringe and 21 g x 38 mm needle carefully inject the ethanol solution deep into the brain tissue in several places—in both the left and right cerebral hemispheres.
3. Immerse the brain in the ethanol solution for storage in a clean glass container that has a tight-fitting lid to prevent any evaporation. The use of Parafilm or some silicone sealant can be used to provide a good seal.
4. Leave for 2–3 days to fix the tissue.
5. After 3 days, pour off the solution and refresh with a new batch.
6. Leave for at least one month before use for dissection or as a display specimen.
7. Store under conditions to prevent any deterioration i.e., a cool dry place with low-light levels and out of direct sunlight. Store in a flammable liquids cabinet.
8. Collect any waste solution into a glass waste container, which can be properly sealed to avoid evaporative loss. Label appropriately and dispose via a chemical waste contractor.

This is a good fixative and preservative that will not overly dehydrate the tissue. Concentrations higher than this are not recommended as they can excessively dehydrate the tissue.

Note: While waiting for pick up, store and segregate waste chemicals safely in approved store rooms or chemical storage cabinets.

Method 4: 2-phenoxyethanol:

Another alternative is 2-phenoxyethanol (synonyms: phenoxetol, phenoxytol), which is non-flammable compared with 70% ethanol, is less volatile and of lower toxicity when compared with formaldehyde.

Safety:

When working with preserved specimens it is important to:

- work in a fume cupboard or well-ventilated area;
- wear appropriate PPE (i.e., safety glasses, nitrile gloves, laboratory coat, closed-in shoes);
- refer to the specific SDS for the chemicals being used and prepare a site-specific risk assessment;
- rinse preserved specimens with running water or preferably soak overnight in water before using for dissection;
- store all flammable preserving solutions in a flammable-liquids cabinet;
- dispose of all waste solutions via a chemical waste contractor;
- following dissection, all of the brain tissue should be wrapped in newspaper, double bagged and frozen for later disposal via a waste contractor;
- attach and dispose of syringe needles safely. Attach **sheathed needles** only to the syringe and **never re-sheath needles** as this is how most needle-stick injuries occur.

Dispose of used syringe needles in an approved sharps container—positioned at the point of use. Seal and dispose of a full sharps container at a sharps collection facility, sharps disposal bin via a State Health

recommended facility or a facility recommended by your local council. More information on sharps and disposal can be found on the Science ASSIST website Sharps container disposal.

Alternatives:

1) Purchase of preserved specimens

Purchasing preserved brains from a reputable biological supplier is another option available to you. Specimens are initially fixed with a formaldehyde solution, then displaced with water and lastly preserved with a glycol solution, producing a low-fume specimen, which will not decay over time. No refrigeration is required.

Safety Note–Specimens should be washed in water before use and the dissection should be performed in a well-ventilated laboratory.

Wear appropriate personal protective equipment (PPE).

Obtain and read the safety data sheet (SDS) from the supplier and prepare a site-specific risk assessment.

2) Freeze-dried specimens

Freeze-dried brains. They are preserved without chemicals or fluids, can be used for external study and dissection. The specimens are rehydrated with dilute (20%) alcohol solution and need to be rinsed before use. This is also a technique taxidermists use to preserve specimens. However, this is an expensive procedure and would only be cost effective with a bulk order of specimens.

Contact your local museum or taxidermist for more information.

3) Rubber/plastic models

Rubber/plastic brain models to examine the brain. These can be sourced from biological suppliers.

4) Freezing

Fresh offal is always best for dissection purposes, but if you want to store fresh sheep brains in the freezer for later dissection, they are best dissected semi-frozen to avoid the mush factor! Freezing can cause loss of integrity, colour and some shrinkage. The formation of ice crystals can also damage the tissue.

Using a 0.9% saline solution may assist the cells and tissue structure of the brain to maintain their normal state during the freezing process.

Soak the brain tissue overnight in the 0.9% saline solution and inject parts of the brain tissue (described in Method 1) before freezing separately in sealed zip-lock bags.

Preserving a calf foetus:

Source of animal:

We recommend you check with your school jurisdiction for regulations regarding the use of dead animals or animal body parts that may not have been sourced from a certified abattoir, butcher or science supply company. More information on this can be found on the Science ASSIST website link: Dissection materials.

Preservative solutions for foetal tissue:

Foetal tissue is very delicate and is usually preserved in a weak formalin solution (5%), or 75% to 80% ethanol containing 25% glycerol to maintain colour and softness of specimens. Normally preparation includes injection

of preservative into the flesh in several places and into the body cavities to avoid decay of the internal structures.

Recommended Solutions:

The following are recommended as suitable and safe long-term preservative solutions. Depending on the size of the foetus, it may require the use of a needle and syringe. We recommend you check with your school jurisdiction for any regulations on the use of needles. Detailed methods for all three solutions are provided in the previous section, as well as instructions for transferring from one preservative to another.

- 25% glycerol in 80% v/v ethanol
- 75% v/v Ethanol Solution
- 2-phenoxyethanol

Detailed method for replacing the methylated spirits with new preservative solution:

There is not a lot of difference between methylated spirits and ethanol, except that it makes the specimen quite brittle and it may have rendered the skin of the foetus transparent, since alcohol destroys most colours almost immediately.

Before transferring the calf foetus to a new preservative solution, it is recommended that the work be done in a fume cupboard with appropriate PPE. You should also not pour any preservative chemicals down the sink and at all times, care must be taken to avoid damaging the specimen in the process.

1. In the fume cupboard or well-ventilated area, decant the methylated spirit solution into a glass container, which can be properly sealed to avoid evaporative loss. Label as 'Caution Flammable liquid - Do Not Use - for disposal via a chemical waste contractor. Store and segregate the waste chemicals safely in approved store rooms or chemical storage cabinets prior to disposal.
2. Wash the calf foetus several times by soaking for 30 minutes in tap water to remove the old methylated spirits solution.
3. Place the foetus into the final preservative solution in a clean glass container that has a tight-fitting lid to prevent any evaporation. Use either 25% glycerol in 80% v/v ethanol or one of the alternatives. The use of Parafilm or some silicone sealant can be used to provide a good seal.
4. Label the container to state type of specimen, type and date of preservative, using paper intended for long-term preservation in fluids. As mentioned above, put the jar label inside the jar, not on the outside or on the lid.

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