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Title: Transferring biological specimens from formalin to alcohol

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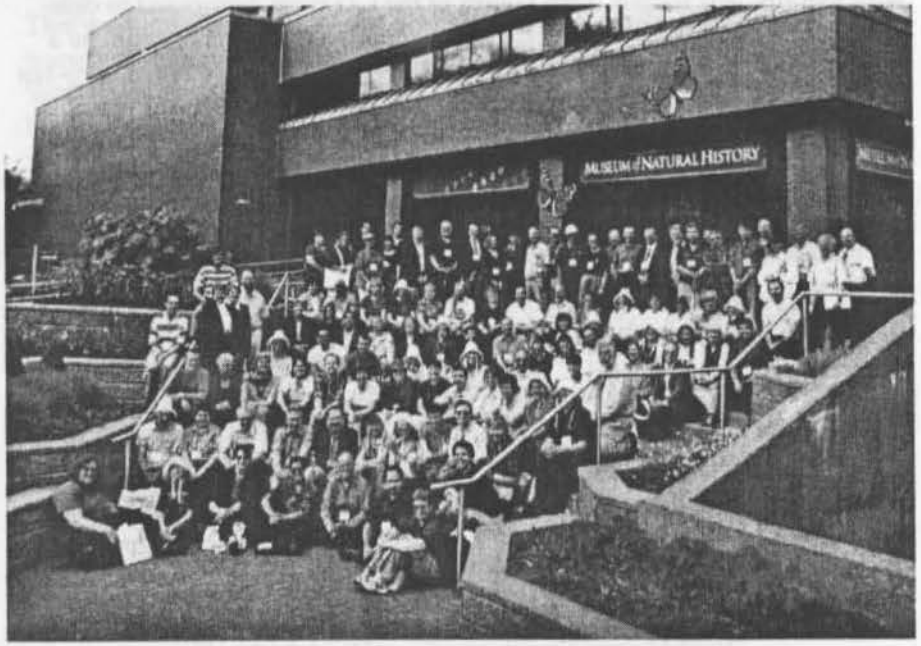
The bus ride back included a guided tour of Halifax with information ably imparted, by Alex Wilson, on the explosion of a munitions ship in 1917 which killed 1000 + people and we saw the Martello Tower built in 1790, in Point Pleasant Park.

Friday was spent at the Nova Scotia Museum of Natural History attending the Permits Workshop. This covered permits for agriculture, Health & Safety, CITES and cultural property. Much of the information was slanted toward the problems and legislation in Canada but the CITES information was of interest. The information presented at this workshop was produced as a bound volume with the disclaimer that the contents represented the opinions and experiences of the presenters and was given as guidelines only.

Thanks go to the co-chairs Iris Hardy and Alex Wilson for a stimulating and enjoyable conference. Next year the 16th SPNHC will be held at the California Academy of Sciences in San Francisco from 21st-26th June, 2001 (further information from Jean DeMouthe CAS, email: jde-



Transferring biological specimens from formalin to alcohol.

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In these days of greater Health & Safety awareness many curators are reviewing their fluid-preserved collections and transferring them from carcinogenic and dermatitic formalin to alcohol. Although this may seem straight-forward enough there are many traps and problems along the way.

First - are the specimens going to benefit from the transfer? Some will have been fixed and then preserved in special fluids, the nature of which is rarely recorded on the label (eg. formol glycerine), plant material may be preserved in 'Kew mixture' or a chlorophyll colour-preserving medium, transferring to alcohol will cause the chlorophyll to leach out (Moore, 1999).

Second- will the transfer improve DNA preservation? If the specimens have been fixed in 10% formalin (= 4% formaldehyde) then the DNA integrity will have been masked by the formaldehyde This reaction is non-reversible. Fresh specimens for molecular studies need to be stored in a minimum of 90% alcohol (Criscuolo, 1994).

Third- the transfer may seem to satisfy Health & Safety from the aspect of the personnel breathing in the fumes, but the transfer to alcohol brings in extra problems concerning flammability of the fluid. The added risk of faster evaporation (especially during Summer) means that more monitoring and topping up needs to be carried out. Keep in mind that as alcohol evaporates from a jar, the residual solution becomes dilute (Carter, 1995).

Fourth- most specimens will benefit from the change. Formalin requires buffering and does not fix lipids (only preserves them), alcohol dissolves lipids out and does not require buffering.

Fifth- wear surgical gloves - alcohol dehydrates the skin and can lead to dermatitic problems.

Sixth- check with your local Health & Safety Council, Water Board or County Council before tipping any diluted formalin down the drain. Small amounts (up to 5 litres per 24 hours, accompanied by ten times the equivalent volume of water) may be permitted - it is a useful bactericide but larger amounts may cause hazards to sewage personnel and might even neutralise settling tanks!

Order your IMS (Industrial Methylated Spirit - 74 OP - over proof) is the normal absolute alcohol, don't order ethanol as it is much more expensive. Check that you have a declaration of use from you local branch of HM Customs and Excise before you make the order. IMS comes in 2.5 litre bottles or 25 litre drums (cheaper pro rata) also ensure that you have the correct equipment for opening such drums (T key) and for storing them safely. Drums (especially) and bottles of IMS are potential fire hazards and require proper storage away from heat sources including sunlight. Securable metal bins are ideal or metal cupboards (for the bottles).

Technique

1: Find some empty (and clean!) 2.5 litre bottles and label them "Preserving IMS, 80% strength". Dilute your IMS to 80% (500ml of deionised water, tap-water will precipitate out any dissolved calcium salts), then add 2 litres of IMS). Most important - do this at least 24 hours before you start the process below as the mixing of these two fluids releases thousands of tiny air bubbles {dissolved in the water) and which will penetrate any immersed specimens and may cause them to float! 70-80% is ideal for preserving, full-strength can cause specimen embrittlement and be more volatile and flammable. Older deionisers can produce water ~with a low pH, check its pH (paper test strips, prior to use).
2: Make up baths of 20%, 40%, 60%, 80% IMS by diluting with deionised water. Again, this must be done at least 24 hours before any immersion can take place. If transferring fragile specimens, an additional 10% IMS bath is advisable.
3: Immerse your specimens in tap water until the smell of formalin disappears (but don't leave fragile specimens overnight or they may start to deteriorate).

4: Check inks on labels that are going to be immersed in alcohol for fastness and re-write labile-inked labels using Indian Ink or Pigma (archival ink) pens on suitable paper (Moore 1999).
5: Rinse specimens in deionised water and then transfer directly to the 10% or 20% IMS bath as appropriate.
6: Take the specimens up the IMS ladder to the 80% bath ensuring that specimens are left in each bath for 24-48 hours depending on, size and density. *[fish and other densely-muscled specimens may need longer to ensure penetration of the fluid]
7: Bottle the specimens in fresh 80% IMS. They should be the same size as before and should not have acquired any wrinkles or other signs of too-rapid dehydration. In some cases the colour may have been enhanced by the process but this may not last!
8: Check the specimens the next day, look for discolouration of the fluid - lipid leaching out? Leave it for a week or two checking frequently the depth of fluid colouration. When no further contaminant appears to be leaching out. renew the fluid. If any specimens are floating try to tease or gently squeeze out the trapped air. Small specimens will require vacuum treatment.
9: Vacuum treatment - specimens requiring vacuum treatment due to trapped air must be taken down the IMS ladder to deionised water. Any alcohol vapour in the vacuum line will damage the pump! After the trapped air has been released, bring the specimen up the ladder as before.

References

Carter, J. 1995. A short study into the changes in alcohol concentration due to evaporation. *Conservation News,* **56**: 24-25.
Criscuolo, G. 1994. Museum Spirit Collection and the preservation of DNA. *Conservation News,* 54: 39-40.
Moore S J, Fluid preservation, in *Care & Conservation of Natural History Collections,* Carter DJ & Walker, A. (eds). Butterworth & Heinemann, 1999.