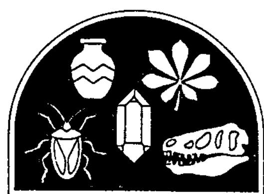
Collection Forum



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AN EXPLORATORY ASSESSMENT OF THE STATE OF A FLUID-PRESERVED HERPETOLOGICAL COLLECTION

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Abstract.—The methodology and results of a pilot assessment of the herpetology collection at the University of Kansas Natural History Museum are presented. We evaluated 139 characteristics describing the composition and state of the containers, fluids, labels, and specimens in 110 samples. Simple univariate descriptive statistics provided a preliminary assessment of the usefulness of the results for each characteristic, as it was determined. Four characteristics (preservative concentration, container volume, preservative fluid plus specimen volume, and specimen volume) were found most important, since they allowed determination of the effect of topping procedures on final preservative concentrations. Characteristics that assessed the state of preservation of the specimens themselves are clearly important, unfortunately, those characteristics remain subjective and, consequently, are probably not highly replicable over time or between collections. Characteristics that assessed the preservative fluid are objective and replicable but the relevance of most fluid characteristics to long-term collection preservation is not yet clear. The results of this study provide a view of the means and dispersions of a wide range of characteristics of samples in a fluid-preserved collection.

INTRODUCTION

For most museum collections, the effects of the storage environment on deterioration modes and rates are understood, at least qualitatively, and increasingly quantitatively, through studies that correlate measured environmental factors with observed modes and rates of deterioration (e.g., Waller 1992, Erhardt and Mecklenburg 1994, Michalski 1994). Generally, this correlation is first observed in an anecdotal fashion in collections. Subsequently, structured surveys might clarify the causes and effects, and controlled aging experiments quantify rates of deterioration.

In fluid-preserved collections, the preservative fluid is the immediate environment for the specimen. At present, little is known about how measurable properties of the preservative fluid relate to modes and rates of deterioration of specimens. This situation results from not knowing which sample properties should be measured and not knowing how to characterize and document the state of specimen preservation. Cato (1990) published the first systematic study of the condition of a fluid-preserved collection, measuring ten, and evaluating six, characteristics of a fluid-preserved mammal collection. Other previous projects assessing fluid-preserved collections (Waller and McAllister 1987, Von Endt 1994, Palmer 1996, Thede 1996, Pickering 1997) evaluated different, limited sets of characteristics. The present work greatly extends the diversity of characteristics considered within a single study.

In 1988, the Assessment Subcommittee of the Society for the Preservation of Natural History Collections Conservation Committee (SPNHC-CC) was directed to address the general lack of knowledge about assessing the state of fluid-preserved collections. The subcommittee launched a project to establish means for characterizing the fluid and the specimen components of fluid-preserved collec-

tions (SPNHC-CC Assessment Subcommittee 1990). The project described here is a pilot study designed to address this objective of the subcommittee. Many of the members of that subcommittee (listed in Acknowledgements) contributed to our list of characteristics to measure and to finding the best means to measure them. Many other SPNHC members contributed to evaluating some proposed characteristics at a workshop at the Fifth Annual Meeting of SPNHC in Chicago, 1990. The final set of characteristics measured in this pilot assessment was determined by the authors immediately before the study. Characteristics used were intentionally kept general to cover a diversity of container types, preservative fluids, and taxonomic groups but we expect customization will be required for application to some other collections.

Our expectation of this pilot assessment project was not to produce data that would be of immediate practical value. Instead, our intent was to take a first step in learning how we might study the state of a fluid-preserved collection from a broad, epidemiological perspective. To accomplish this we evaluated an assessment method proposed by the subcommittee, and refined by the authors, on the herpetology collection at the University of Kansas Natural History Museum (Simmons and Waller 1994). The survey work was completed during the period October 1990 to February 1991 by one of the authors (JES). The time required for setting protocols for measuring characteristics was several person-months although this work will not have to be repeated, obtaining the data required one person-month, and evaluation of data is ongoing.

Data are assessed using simple univariate methods to determine the apparent usefulness of characteristics, as determined, with respect to resolution, systematic errors, and cost and time requirements. Data for each characteristic were also evaluated as a function of order of measurement although this is reported only where significant deviations or variations with measurement sequence were noted. A subsequent paper will explore multivariate relations among characteristics.

The objectives of this paper are to:

- Describe what characteristics were used and how they were measured.
- Evaluate each characteristic and its method of measurement with respect to:
  - Cost—do measurements require expensive equipment or materials?
  - Time—are measurements time-consuming to make?
  - Priority—do we consider the data for a characteristic sufficiently resolved, and its measurement sufficiently repeatable, to provide meaningful information and to justify the cost and time to obtain?
- Document the mean and variability of data for each characteristic.

Collection Description

At the time of the assessment, the herpetological collection contained 209,000 cataloged specimens of reptiles and amphibians. The collection held specimens of 43 percent of the known taxa of reptiles and amphibians. The collection ranked fifth in size in the United States and is an important national and international resource.

Specimens are assigned to jars based on identification and geographic area and subject to available space in existing jars. The majority of specimens are whole animals preserved in 70 percent volume/volume (%v/v) ethanol. Amphibian lar-

Table 1. Descriptors of cost (per hundred) for equipment and materials and time (per single measure) to perform and record measurements.

Term	Time	Key	Term	Cost	Key
fast	<5 sec	1	inexpensive	<$1	1
moderately fast	5–30 sec	2	moderately inexpensive	$1–10	2
moderately time-consuming	30 s–2 min	3	moderately expensive	$10–100	3
time-consuming	2–5 min	4	expensive	$100–1000	4
very time-consuming	>5 min	5	very expensive	>$1000	5

vae and eggs are preserved in 10 percent formalin (3.7%w/v formaldehyde) buffered with borax at a concentration of about one tablespoon per quart of solution. Both ethanol and formalin solutions were prepared with tap water. Analyses of the tap water are not available but a white precipitate was noted when mixing water with ethanol. Prior to the mid-1980s discolored or oily fluids were replaced when encountered. The collection also contained about 3,500 dry and 3,500 cleared and stained preparations that were not assessed as part of this project.

The fluid-preserved specimens were stored on wooden shelving in a building that lacked special climate controls for the collection. Until shortly before the assessment, the overhead fluorescent lights were not UV-filtered. Thermohygrograph records show that over a 12-month period, including the time of the assessment, temperature in the collection areas ranged from 16.7 to 24.4°C ($\bar{x}$ = 19.7°C). Daily temperature fluctuations were rarely greater than two degrees. The relative humidity ranged from 28 to 60 percent ($\bar{x}$ = 56%). Temperature and relative humidity levels are thought to have been similar since the collection entered the building in 1964.

Light intensity varied from 538 lux on the uppermost shelves (nearest the lights) to 22 lux on the lowest shelves. Since the early 1980s, lights were on only when the collection room was occupied by staff. Exposure rate since then is estimated at less than 20 hours per week. Prior to that time, the rate of light exposure would have been considerably higher, perhaps 80 hours per week.

Method

Evaluating Characteristics

Obtaining measurements and recording data should not be unduly expensive or time-consuming. This latter consideration becomes increasingly important as large numbers of measurements are required for large sample sets. Measurements of characteristics are described, both in terms of equipment and material costs, and in terms of time to perform and record. Costs are estimates, based on the assumptions that all required equipment must be purchased specifically for the assessment project and that approximately 100 samples will be assessed. Table 1 provides a key to descriptive terms for time and cost used in subsequent sections and summarized in the Time and Cost columns of Appendix 1.

Characteristics should provide information that is useful for depicting the state of the collection with respect to accrued deterioration or predicted rates of deterioration or both. Unfortunately, in some cases, the usefulness of information will not be evident until the information is analyzed in detail or combined for analysis with data from many collections. Consequently, in this paper, we consider a char-

acteristic useful if data for it are well resolved and its measurement thought to be sufficiently repeatable. These factors are combined to give our sense of priority (Appendix 1).

Sampling Strategy

We used a stratified random strategy (Manly 1992) to select between 50 and 100 specimen containers from each of the ethanol and formalin collections. From a randomly selected starting position, and following the systematic ordering of shelves, every third shelf in the ethanol collection and every second shelf in the formalin collection were selected. Next, we chose a container by random draw, from the selected shelf, provided the shelf was not empty. If the container held multiple specimens then determination of specimen preservation characteristics was based on a single specimen selected by random draw.

Characteristics

Appendix 1 provides a list of characteristics in the order they were recorded during the survey. These characteristics were selected based on work of the SPNHC-CC Assessment Subcommittee with modifications based on what standards and test strips could be obtained or produced at the time of the assessment. The numbers in the first column of Appendix 1 are used below as an index number to each characteristic.

Physical Location

Five characteristics (1, 2, 3, 5, and 6) describe the physical location of a sample container. One additional characteristic (4, reason for omission) was used only to indicate why a sample container was not available in the location identified by the sample selection process.

Sample Identification

Four characteristics (7, 8, 10, and 12) identified the container. Of these, the first and last (lowest and highest) catalog numbers were used in this study. Scientific binomen (12) was not recorded at the time of the assessment. In this collection, individual specimens are assigned unique catalog numbers and are assigned to containers based on species and geographic origin. Containers in this collection were not numbered, hence, characteristic 7, "container number," was not used. The date of collection of the specimen identified by the first catalog number (characteristic 9) was used to provide our best estimate of the age of the container.

Container and Lid

Four characteristics (13 to 16) described the type of container and lid and the manufacturer based on Simmons (1995). Container types are shown in Table 2. Ease of opening (17) was intended to identify loose, or very difficult to open, closures. It was based on a key of five written descriptors (Table 3). Characteristics 18 to 20 described the material comprising the liner, gasket, and sealant. In this assessment, we assigned the codes "RO" for old-style red "rubber" gaskets or "BR" for black "neoprene" gaskets.

Table 2. Codes used to record container types (13).

Code	Container description	Container volume, mL
MA	bail top, half-pint	265
MB	bail top, pint, regular	500
MC	bail top, pint, tall	390
MD	bail top, pint, squat	555
ME	bail top, quart, regular	955
MR	bail top, quart, wide-mouth	1010
MG	bail top, half-gallon	1845
TB	bail top, two-liter "Triumphe"	2230
GA	two-piece plastic lid, gallon	3680
ST	screw-top, 8 ounce	285

Fluid Quantity and Description

Seven characteristics (21 to 27) provided a visual assessment of the fluid before physically disrupting fluid, specimens, or precipitate within the container. The assessment included simple observations, ruler measurements, and estimations. Characteristic 21 indicated whether the type of preservative fluid was indicated on the container. Characteristics 22 and 23 were measurements of fluid and container height and 24 was an estimate of the amount of specimen material exposed above the surface of the fluid as a percent of total specimen volume.

Standards were prepared to provide consistent estimates for characteristics 25 (oils), and 27 (precipitated material). Oil standards were prepared using weighed quantities, in a four-step logarithmic series (0.020, 0.093, 0.431, and 2.000 g), of oil added to 25 mL distilled water contained in two ounce, 35 mm ID, jars. Vacuum pump oil was selected because of its apparent similarity to typical specimen oils when mixed with an aqueous solution. Precipitated material standards were prepared using weighed quantities, in a four step logarithmic series (0.020, 0.093, 0.431, and 2.000 g), of 1.0 μm alpha alumina polishing compound added to 25 mL 1.0 M sodium chloride solution contained in two ounce, 35 mm ID, jars. Both of these series of standards could be duplicated in other laboratories but may require adjustment of proportions depending on the size and shape of the container holding the standard. Standards were made in a jar size that differed from all samples to avoid any systematic bias that might result from having the format of the standards match some, but not all, of the samples.

Table 3. Descriptors used to record the ease of opening a container (17).

Key	Descriptor
1	lid is virtually loose
2	lid can be easily loosened using fingers along the edge
3	considerable effort is required but lid can be opened without using a tool
4	a tool is required to open the container but with the use of an appropriate tool, the container is not difficult to open
5	the container is exceedingly difficult to open even with the aid of an appropriate tool

Table 4. Descriptors used to characterize condition related to labels (37, 39, 41, 45, 47, 49, and 52).

Key	Label substrate or attachment (37, 45, and 52)	Data or print media (39, 41, 47, and 49)
1	excellent	excellent
2	aged but satisfactory	aged but satisfactory
3	deteriorating: should replace	faded: should replace
4	very weak: urgent	very faded: urgent
5	beyond help	beyond help

Fluid Properties

Four chemical properties (28 to 31) were measured before the fluid was disturbed by removal of the specimens. pH was measured with a Cole Parmer N-05658-05 electrode and oxidation-reduction potential (ORP) was measured with a Fisher 13-620-115 electrode which employed a silver/silver chloride in 4 M KCl reference. Temperature was recorded as displayed on the Cole Parmer N-05996-80 Digital Field pH meter. Measurements were repeated later using a Hach 44300-01 electrode for pH and a 44480-01 electrode (also with a silver/silver chloride in 4 M KCl reference) for ORP. Conductivity was measured with a Hach portable conductivity meter.

Immediately after removing the specimens from the container, the height of the fluid was measured again (32). This allowed later calculation of the volume of preservative fluid (92) and of specimen material (93). Volume of preservative fluid (33) was only used where pouring the fluid into a measuring container volume was more convenient than removing specimens.

Label Materials and Condition

Twenty characteristics (34 to 53) were related to description and condition assessment of container labels and of attached labels or tags and their method of attachment. The characteristic "standard form printing" (38) referred to preprinted information or guidelines for formatting data. The characteristic "data" (40) referred to information recorded on the label when, or after, the label was first put in use. Keys used for condition assessment of label materials, media, and attachments are given in Table 4. The condition of the specimen at the point of attachment was judged to match one of three descriptions: 0—excellent, no affect; 1—deformation only; and 2—cuts, breaks, or severe abrasion.

Container History

Four characteristics (54, 56, 58, and 60) described the container-specimen-fluid history. Four additional characteristics (55, 57, 59, and 61) recorded estimates of the uncertainty (± one, five, or ten years), of these historical data.

Fluid Chemistry

Ten characteristics (62 to 71) related to the composition of the solution, concentration of the principle component (i.e., alcohol or formalin), and measurement of titratable acidity. The first (62) was the response of the solution to a formalin test strip (Waller and McAllister 1987). The second (63) recorded the nominal composition of the solution, e.g., ethanol or formalin. Density (64) and temper-

ature (65) were measured with a Parr model DMA 35 digital density meter. An electronic meter of this sort enables measurements of smaller samples than a hydrometer can measure. The results of titrations of 10.0 mL subsamples, with 0.100N sodium hydroxide (NaOH), to the thymolphthalein endpoint (66) and to the phenolphthalein endpoint (70) were used to calculate titratable acidity with thymolphthalein and with phenolphthalein (67 and 71), respectively, as indicators. Characteristics 68 and 69 show the results of titrations of 10.0 mL subsamples with 0.100N hydrochloric acid (HCl) and back titration with 0.100N NaOH, respectively, after addition of sodium sulfite. Those results were used to calculate formaldehyde concentration (see METHODS—*Calculated Values*).

Color and Turbidity

Three sets of standards were prepared for fluid color comparisons (72 to 74) and one set for turbidity estimates (78). Three additional characteristics (75 to 77) provided a measure of the ease of use of each of the three color comparison standards.

Color comparison standards were prepared by dilution to provide nine concentration steps spanning four orders of magnitude of concentration. Stock solutions were 1.00 g iodine per litre of 75%v/v ethanol, 2.00 M potassium chromate, and 0.400 M potassium dichromate. These solutions and concentration ranges were chosen to provide colors and intensities of colors thought to be similar to those found in collections. For each sample, the ease of comparison against the standards was ranked as 1 (easiest) through 3 (most difficult). Turbidity was measured by comparison with a series of standards. These standards were prepared by dilution of a 0.500 gL^{-1} suspension of deagglomerated alpha alumina. Standards 1 through 9 contained 0.50, 1.50, 2.50, . . . , 8.50 g suspension made up to 10.00 g total by addition of distilled water. Turbidity standards were shaken before each use to ensure an even suspension. Color and turbidity standards were kept in 16 mL screw-top test tubes. A 10 mL fluid sample was placed in the same size test tube for making comparisons. Care was taken to not disturb precipitated material in the sample container before drawing the fluid sample for turbidity determination.

Chemical Spot Tests

A variety of test strips was employed for determining trace impurities (characteristics 79 to 89). Total hardness (combined Ca^{2+} and Mg^{2+}; 79) was measured using the Aquadur® commercial test strip. A medical test strip for urinalysis, Ames N-Multistix™, provided measures of multiple characteristics (80 to 88) on each single strip, although our primary interest was in dissolved protein (81). A sodium sulfide strip was prepared with the intention of indicating any dissolved metals that might form dark insoluble precipitates with sulfide. In particular, the presence of mercury might be indicated with this test. No preliminary experiments were done to determine the detection limits of these strips.

Calculated Values

Several characteristics were obtained by calculations based on previous measurements or through look-up tables. Volumes of specimen and fluid (91) and container (95) were taken from a table of volume versus fluid height prepared

specifically for this study for each major jar type in the collection. Volume of preservative fluid (92), if not measured directly in characteristic 33, was also taken from the table using the measurement of height of fluid without specimens (32). Volume of specimen material (93) was the difference of characteristic 91 minus 92. Ratios (90, 94, and 96) are simple divisions of the appropriate values. Residue on evaporation (98) was calculated as a weight percent based on the weight remaining when a 10 g sample of minimally disturbed fluid was evaporated to dryness at room temperature in an aluminum-weighing dish. Formaldehyde concentration (99) was calculated from titration results according to the procedure for the sodium sulfite method (Walker 1964). Formalin concentration (100) is simply the formaldehyde concentration expressed in the more familiar form of volume percent formalin. The ethanol concentration (97) was found by looking in a table of ethanol concentration as a function of density and temperature (Revenue Canada 1980).

Specimen Identification

Two characteristics, catalog number and species name (102, 104) pertained to the individual specimen within a container that was selected for assessing the state of specimen preservation. Characteristic 105 provided a count or an estimate of the total number of specimens within the container. Characteristic 106 indicated the type of specimen (fish, amphibian, or reptile). Characteristic 107 indicated whether the specimen was larval or juvenile, or adult or subadult.

Specimen Preservation

Fourteen characteristics (within the group 108 to 139) provided measures of specific aspects of preservation quality. These were appraised using five-step keys designed to cover the range from ideal preservation (response = 0) to complete failure (reponse = 4). The keys for each of these characteristics are given in Table 5. Associated with each of these were measures of observer confidence based upon 90 percent certainty that the value recorded for the characteristic was thought to be correct, was within ± one choice, or ± two choices, of the true value. Other characteristics recorded whether the specimen had skin or scales (120), exhibited ecdysis (123), and whether specimen parts were observed to be missing or dissolving (138). Characteristic 139 provided a general assessment of preservation quality.

Results and Discussion

Descriptive statistics for characteristics, together with time, cost, and priority evaluations are summarized in Appendix 1.

Sampling Strategy

The sampling strategy identified 53 containers from 62 shelves in the ethanol collection and 57 containers from 58 shelves in the formalin collection.

Physical Location

Shelves were selected without a general bias for higher- or lower-numbered shelves. In the formalin collection, however, even-numbered shelves were selected more often than odd-numbered shelves. This bias was a result of the sampling

Table 5. Descriptors used to characterize specific aspects of the specimen's state of preservation (108–139).

Key	Descriptor
ASPECT OF PRESERVATION ASSESSED	
Parameter	
CONSISTENCY OF PRESERVATION QUALITY	
Extent of areas that are distinctly less well preserved than other areas (*e.g.* soft areas).	
0	none
1	1–4%
2	5–19%
3	20–50%
4	>50%
CONDITION OF MUSCLE TISSUE	
Loss of firmness	
0	intact and as firm as recently fixed material, or more firm
1	intact but slightly soft
2	unmistakably soft
3	very limp—flacid
4	disintegrating
Embrittlement	
0	firmness equal to or less than that of freshly fixed material
1	slightly too firm
2	very firm, unmistakably increased firmness
3	nearly brittle
4	brittle, crumbles under slight pressure
Elasticity - Apply modest pressure for three seconds to a major muscle group using a rounded eraser on the end of a pencil. Estimate the extent of recovery to original form after the pressure has been released for five seconds.	
0	81–100% regain
1	61–80% regain
2	41–60% regain
3	21–40% regain
4	0–20% regain
Flexibility of whole body or of major limbs—With modest force, specimen flexes:	
0	as much as expected for a freshly fixed specimen
1	61–80% as much as expected; slightly stiff
2	41–60% as much as expected; unmistakably stiff
3	21–40% as much as expected; very stiff
4	0–20% as much as expected; extremely stiff
VISUAL ASPECTS OF THE SPECIMEN	
Apparent condition of specimen at time of death	
0	excellent
1	slight natural (parasite/predator) damage
2	natural or capture damage to 1–4% of specimen
3	natural or capture damage to 5–25% of specimen
4	damage to more than 25% of specimen, roadkill or partial specimen

Table 5. Continued.

ASPECT OF PRESERVATION ASSESSED **Parameter** Key	Descriptor
Loss of skin or scales	
	Considering the apparent condition of the specimen at time of death, estimate post-mortem skin or scale loss.
0	none
1	<5%
2	5–20%
3	20–50%
4	>50%
Loss of patterns	
0	patterns are clearly evident
1	patterns are slightly faded
2	patterns are unmistakably faded
3	patterns are severely faded
4	patterns that should exist are absent
Bleaching of specimen	
0	specimen does not appear bleached
1	specimen appears slightly bleached
2	specimen is unmistakably bleached
3	specimen is severely bleached, not crumbly
4	specimen is completely bleached, pulpy, crumbly
Darkening of specimen	
0	specimen does not appear darkened
1	specimen appears slightly darkened
2	specimen is unmistakably darkened
3	specimen is severely darkened, "preservative brown"
4	specimen is completely dark, "preservative black"
Clearing of tissue	
0	tissues show no evidence of clearing
1	tissues appear to be slightly cleared
2	tissues show unmistakable signs of clearing
3	tissues are extensively cleared
4	tissues are completely cleared
FORM AND COMPLETENESS OF THE SPECIMEN	
Extent of contraction of the specimen	
0	specimen appears full, no shrinkage
1	specimen appears slightly shrunken, slight wrinkling of skin
2	specimen is unmistakably shrunken
3	specimen is severely contracted, looks like a plump prune
4	specimen is very severely contracted, looks like a dry raisin

Table 5. Continued.

ASPECT OF PRESERVATION ASSESSED / **Parameter** / Key	Descriptor
Extent of bloating of the specimen	
0	specimen does not appear bloated
1	slight bloating evident on close examination
2	unmistakably but not severely bloated
3	severely bloated, some proportional measurements might be affected
4	very severely bloated, most proportional measurements would be affected
Condition of abdomen and internal organs	
0	abdomen is not cut
1	abdomen is cut, all organs are in place and recognizable
2	abdomen is cut, organs are partially out of place but are in specimen container and are recognizable
3	abdomen is cut, some organs are missing from specimen container or are disintegrating and are not recognizable
4	abdomen is cut, most organs are missing from specimen container or are disintegrating and are not recognizable
If descriptor 3 or 4 was chosen for the above category organs were:	
	dissolving
	missing
GENERAL ASSESSMENT OF PRESERVATION QUALITY	
0	excellent
1	usable without difficulty for most purposes
2	useful with some difficulty for most purposes
3	unsuitable for some normal specimen uses, e.g. teaching, taxonomic studies, etc.
4	useless

strategy of selecting every second shelf where each shelf set contained an even number of shelves. We do not consider this systematic bias a significant problem since we do not anticipate using the shelf number data for anything other than relative height with respect to nearness to ceiling lights, visibility to casual observation, etc.

In total, ten shelf locations selected for sampling did not contain samples available for study (4). In seven instances the shelf was empty, in two instances the specimens in the container were on loan, and in one instance the jar contained only small eggs. The distribution of samples on shelves was even from left to right (6) but systematically overrepresented the front of the shelves (5). This was thought to fairly represent the real distribution of samples on the shelves where shelf spaces at the front are filled before those at the back.

All five physical location characteristics are inexpensive and fast to obtain. They indicate that the sampling strategy provided samples that fairly represent the physical distribution of containers in the collection.

Sample Identification

Catalog numbers, or lowest numbers in the case of multiple specimens per container, appeared randomly distributed for the ethanol collection. Higher (more

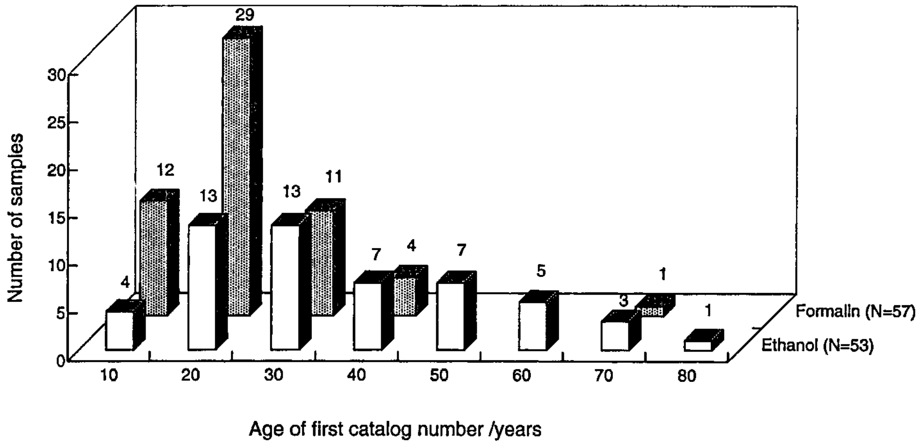

Figure 1. Specimen ages as inferred from the earliest date of collection or accession date of first catalog number.

recent) numbers appeared more frequently in the formalin collection. This is thought to reflect the fact that the formalin collection has grown more rapidly in recent years. Overall, numbers higher than 125,000 were more represented than lower, earlier numbers. These distributions reflect a shift in collection development to a greater emphasis on collecting amphibians (including larvae and eggs) and to an increased geographic diversity of specimens, beginning about 1967, approximately at catalog number 125,000 (Fig. 1).

With the exception of genus and species names, these characteristics are inexpensive and fast to obtain and can provide an indication of sampling bias with respect to catalog numbers. Scientific names were not recorded at the time of the assessment since they can be obtained from catalog information.

Container and Lid

Several of the characteristics that describe containers and lids were not used or were invariant within a collection. For example, no sealant was found on any of the containers and all containers in the formalin collection were the same container type. Nonetheless, we recommend including these characteristics in a general survey form. We did not include characteristics describing the condition of lids and sealant, though that may be desirable for some collections. The manufacturer of containers and lids was frequently not known. In subsequent assessments, it may be preferable to combine manufacturer and container or lid type in a single field.

For both collections the data for ease of opening (17) were approximately normally distributed (Fig. 2). In both the ethanol and formalin collections, regression analysis showed a statistically insignificant (one-sample t-test, $P = 0.05$) trend to be more difficult to open as assessment proceeded. Hence, although variations between assessors will result in offsets to the data, the descriptors (Table 3) are thought to be well defined. In summary, all characteristics related to the container and lid are inexpensive and fast to obtain and provide useful information.

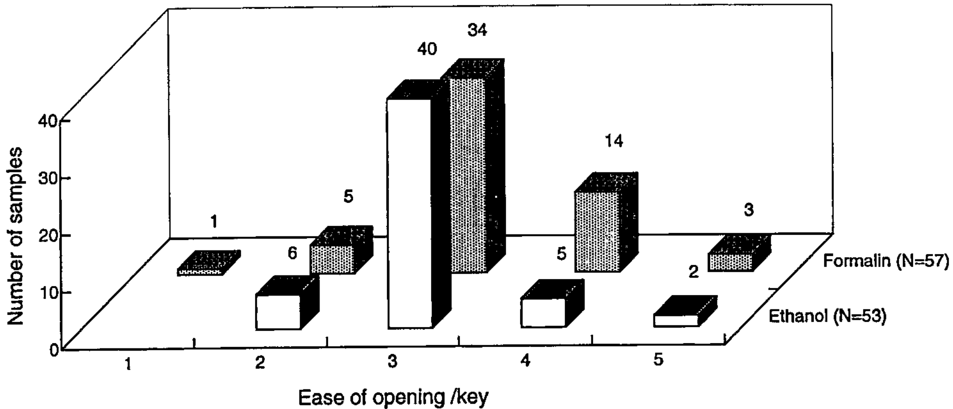

Figure 2. Ease of opening container (see Table 3 for key).

Fluid Quantity and Description

All containers in the formalin collection were identified with the word "formalin" on the labels. Any containers without the word "formalin" on the label are assumed to contain ethanol. Consequently, the characteristic "fluid preservative identified" (21) was positive for all samples. Fluid height divided by the container height (90) provides an easily determined measure of fluid levels throughout the collection (Figure 3). Because maintaining preservative fluid level is an important aspect of basic collection maintenance, this information provides a measure of the standard for ongoing maintenance of a collection.

Approximately 15 percent of samples from the ethanol collection and five percent of samples from the formalin collection had some specimen material exposed above the preservative fluid (24). In all cases, less than 5%v/v of the specimen material was exposed.

Only six of 53 samples in the ethanol collection and three of 57 samples in the formalin collection contained sufficient oil to be recorded as greater than the first oil standard (0.8 gL^{-1} oil in water). No samples were judged greater than

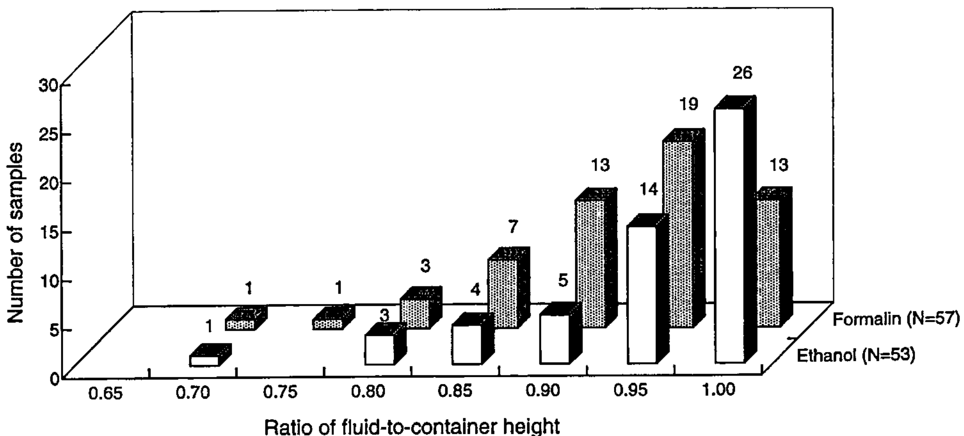

Figure 3. Ratios of height of preservative fluid to height of containers.

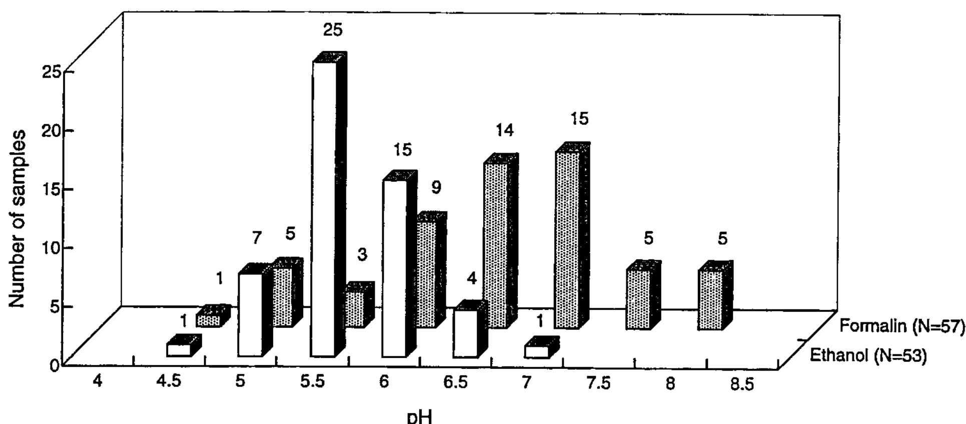

Figure 4. Distributions of pH levels in ethanol and formalin collections from initial pH measurements.

our second oil standard (3.7 gL^{-1} oil in water). Similarly, only two of 53 samples in the ethanol collection and two of 57 samples in the formalin collection contained sufficient precipitated material to be recorded as greater than our first precipitate standard (0.8 gL^{-1} alumina in 1 M NaCl). No samples had sufficient precipitated material to match our second precipitate standard (3.7 gL^{-1} alumina in 1 M NaCl). For this collection, both the oil in water and the precipitated material standards covered too great a range of concentrations to resolve the small differences found in samples. Still, it was difficult to visually discern differences in our standards. If greater resolution of these data is needed to provide useful results then an alternate, perhaps instrumental, method of measurement will be required. No samples in either collection exhibited a continuous oil layer.

All of these characteristics were fast and inexpensive to determine and provide well resolved and repeatable data.

Fluid Properties

Initial review of results for pH and ORP (28 and 30), as a function of order of measurement, led us to suspect the results. We subsequently remeasured the pH in ethanol and remeasured both pH and ORP in formalin. Second measurements were taken with a dispensing electrolyte electrode (Hach) which is thought to give more reliable results, particularly in alcohol-water solutions. Unfortunately, retopping of fluid levels after the initial study makes it difficult to separate real differences in pH from uncertainties and inaccuracies in the measurements. Data in Appendix 1 are from the first set of measurements. There is a need for further work to determine the repeatability of pH measurements in the preservative fluid. Until that is done, it is probably wise to consider individual pH measurements no more replicable than $\pm$ one pH unit. The mean pH of samples was similar for both the ethanol collection ($\bar{x}$ = 6.38) and the formalin collection ($\bar{x}$ = 6.32) but varied more widely in the formalin collection (Fig. 4). Unfortunately, a simple, singular interpretation of a pH reading in an ethanol-water solution is not possible. Constants for acid-base equilibria change by varying extents for different chemical species. A solution with pH = 6 at 70%v/v ethanol may or

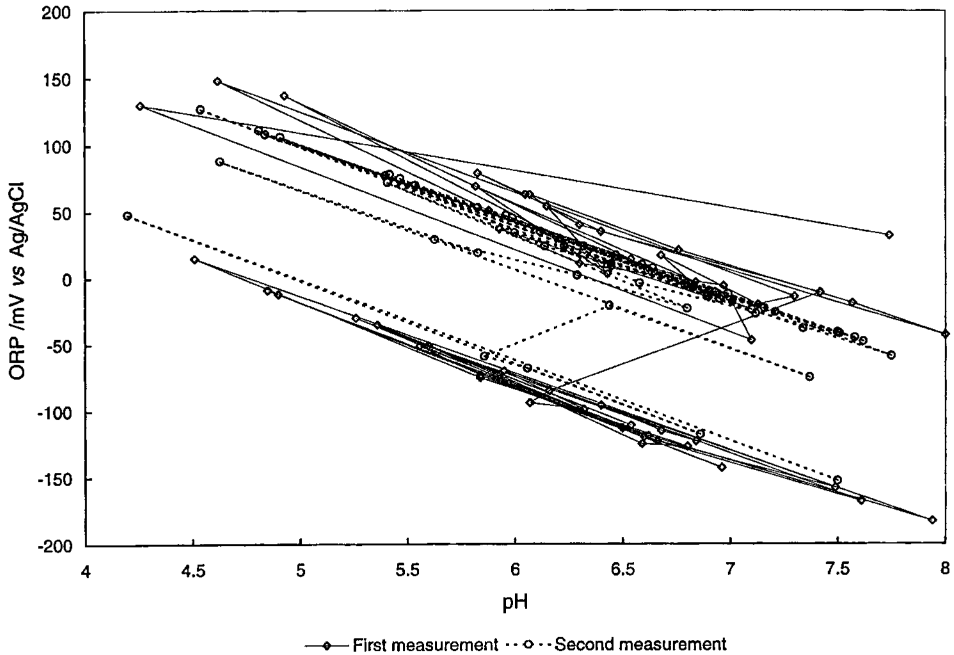

Figure 5. Oxidation-reduction potential as a function of pH and order of measurement of formalin samples.

may not be more acidic than a solution with pH = 7 at 0%v/v ethanol. Notwithstanding, from the perspective of the glass-sensing electrode—meter system, a solution giving a reading of pH = 6 appears one unit (one order of magnitude) more acidic than a solution giving a reading of pH = 7. The measurement of pH is moderately time-consuming and expensive but provides information thought to be useful for characterizing the state of a collection.

ORP measurements from both the ethanol and the formalin collection, including the remeasurement of the formalin samples, indicate that order of measurement greatly influenced the values obtained. This is evident in Figure 5 where the lower linear cluster of data points includes the last 27 measurements of the first set of measurements and the first five measurements of the second set. We suspect that the depression of measured ORP by approximately 120 mV may have been caused by contamination of the platinum wire electrode. These measurements were expensive and time-consuming to obtain and did not provide useful data. It appears that ORP in preservative fluids can not be reliably measured with a platinum wire electrode.

Conductivity provides a measure of total dissolved ionic chemical species. The distribution of conductivity (Fig. 6) indicates much higher and more variable levels of conductivity in the formalin collection as compared to the ethanol collection. The outlying very high conductivity samples in the formalin collection must have particularly high dissolved salt concentrations. Conductivity measurements are expensive but fast to obtain. They provide well-resolved and replicable data.

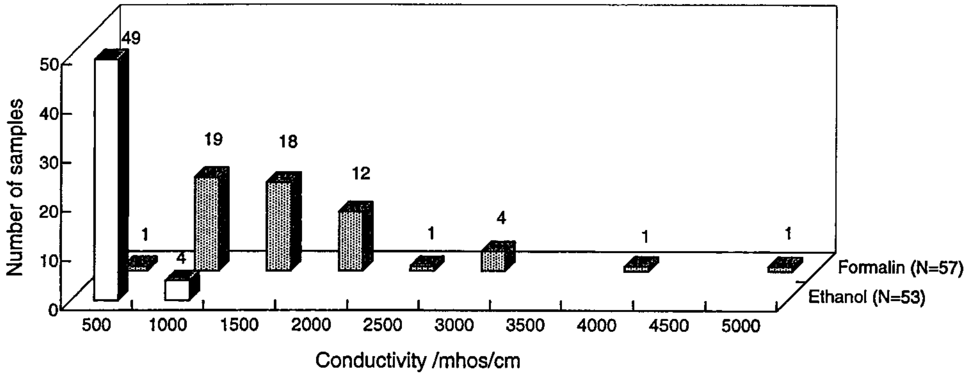

Figure 6. Distributions of conductivity readings in ethanol and formalin collections.

Label Materials and Condition

Labels and the print media and data were all in good condition (Appendix 1). Only condition keys 1 and 2, out of the five available (Table 4), were recorded. Consequently, data for these characteristics will be of little use in multivariate analyses of data for only these collections. Nevertheless, it is thought that the descriptions of condition used in these keys are appropriate, both in terms of range and resolution, and would not be altered in a repeat study.

Attached labels were only encountered in samples from the ethanol collection. In this collection, two types of attachment are used, either a synthetic filament (polyester) or a cellulose thread (crochet thread: white, size 10, 100 percent mercerized cotton). The data for the condition of the attachment material and the specimen at the point of attachment are shown in Figure 7. Of the two attachment materials, synthetic filament appears more aged (28 percent of synthetic vs. six percent of cellulose threads), and is causing more damage to specimens (36 percent of synthetic vs. 12 percent of cellulose threads exhibited cuts, breaks or abrasion at point of attachment), than cellulose thread.

All label material and condition characteristics were inexpensive and fast to record and provide information thought to be useful for characterizing the state of a collection.

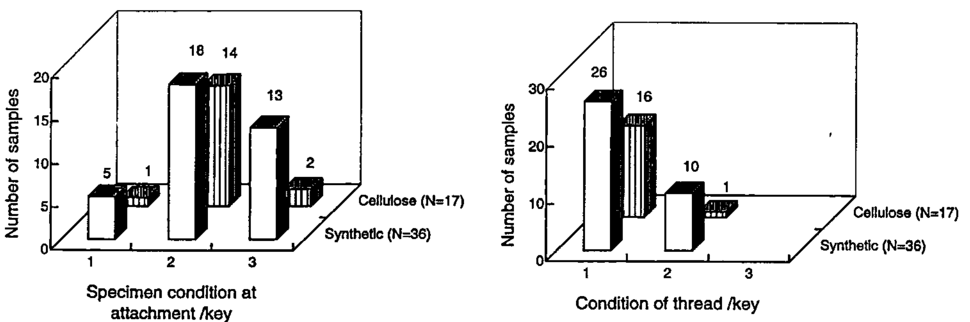

Figure 7. Distributions of condition of specimen at attachment (see text) and condition of thread (Table 4).

Container History

The year that the container was put into service was generally unknown. That response (54), and the response describing its uncertainty (55), were only recorded for the first six ethanol samples selected. The year that the specimen entered the container (56) was inferred from the catalog number. It could more readily be derived from the recorded catalog number in conjunction with catalog number and date information. Consequently, this response was not useful. There was also a high level of uncertainty regarding characteristics 58 (year fluid last changed) and 60 (year fluid last topped). Unless these activities were known to have occurred in the past few years, they could only be estimated with great uncertainty. We do not believe that these historical characteristics provide useful information and we recommend their use only for a collection that maintains container-specific records.

Fluid Chemistry

The formalin test strip (62) was not used in this assessment since it is redundant to the quantitative determination of formaldehyde concentration. All containers in the ethanol collection were considered nominally ethanol while all in the formalin collection were considered nominally formalin (63). Density and temperature of density (64 and 65), although time-consuming and very expensive to determine, provide the measure of ethanol concentration and, hence, are considered essential.

Titration to the thymolphthalein endpoint (66; pH 9.4–10.6, Weast 1976) is accomplished with little extra investment in time since neutralization to the thymolphthalein color-transition pH is an essential step in the formaldehyde titration. A second titration to the phenolphthalein endpoint (70; pH 8.2–10.0, Weast 1976) was done as this was thought to provide a better measure of acidity and a means of calculating buffer capacity. One of the formalin samples resulted in being an extreme outlier (sample F1, 40 $meqL^{-1}$ $pHunit^{-1}$) and was excluded from subsequent data analyses.

The ethanol collection had both a higher mean and greater range of titratable acidity than the formalin collection (Appendix 1). Unfortunately, after conducting this assessment, we learned that differences in the influence of ethanol and formalin concentrations on the behavior of indicators make it difficult to interpret the significance of these results. For example, the color transition for phenolphthalein shifts from pH 8.2 at 0%v/v ethanol to pH 10.7 at 70%v/v ethanol. Conversely, the color transition is shifted to a significantly lower pH in the presence of formalin. Titratable acidity is thought to be an important characteristic that could be measured with accuracy. In the future, titrations should be performed on a sample added to a minimum of, perhaps, five parts of pure water (adjusted to the transition pH) to one part of sample. This would serve to reduce the effect of the preservative on the indicator but the best protocol for measuring titratable acidity in samples of varied alcohol and formalin concentrations needs to be established. The data collected in this assessment have some value for comparison within the ethanol collection. However, meaningful quantitative comparisons between the ethanol and formalin collections can not be made.

Color and Turbidity

Among the color standards, iodine (72) provided the best resolution of color because all except two of the formalin samples showed a color greater than the

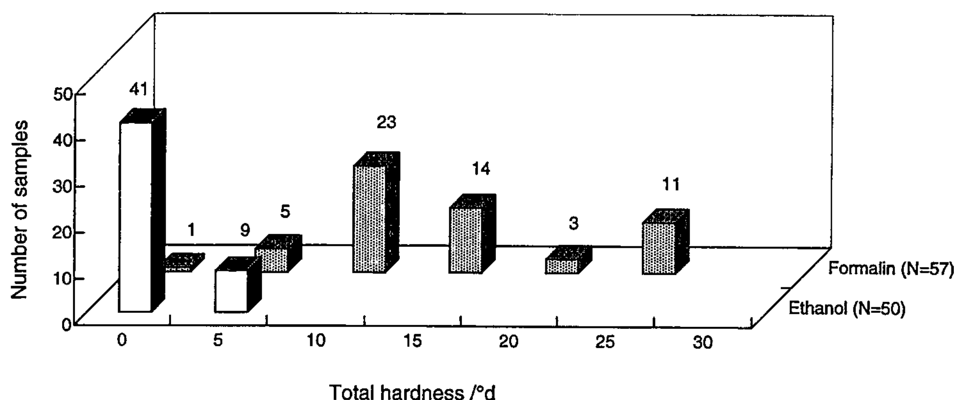

Figure 8. Distributions of total hardness in ethanol and formalin collections.

lowest standard. Iodine was also ranked the easiest to use (75) of the three standards. The chromate standards were, in most instances, the most difficult to use (76). More than half of the samples were judged less colored than the lowest concentration chromate standard. Chromate standards are not recommended for future studies. The dichromate standards were intermediate to the other two both in ease of use (77) and in ability to resolve differences in slightly colored solutions. In future studies, a lower concentration range of dichromate standards, perhaps starting at 0.040 M potassium dichromate, might be useful. It should be noted that some months after the assessment was completed, the iodine standards were observed to have decolorized. There are several causes of instability of iodine solutions (Skoog and West 1976). The rate of decolorization is not known but must be determined before the use of iodine standards can be recommended for further studies.

The method of turbidity measurement (78) provided fair resolution of moderately turbid samples but did not resolve differences among the less turbid samples. Nearly half (43/110 or 39 percent) of the samples were less turbid than the lowest standard. Still, differences in turbidity among these relatively clear solutions were difficult to see. A different method, sighting along a longer column of solution, or employing an instrumental method, will be required to improve resolution. In general, both the ethanol and the formalin samples showed remarkably similar ranges and distributions of turbidity levels.

When standard preparation time is included, comparisons of color and turbidity with standards are time-consuming and expensive. Color data were better resolved than turbidity data. Color and turbidity measurements may eventually prove useful but their significance to preservation of specimens is not clear. Instruments for measurement of color and turbidity are becoming progressively less expensive and, if available, would provide better results with less effort.

Chemical Spot Tests

Variations in hardness (total dissolved calcium and magnesium; 79) appear to be well resolved in the data from the formalin collection (Fig. 8). Samples from the ethanol collection all had relatively low levels of hardness. This result is consistent with the general lowering of solubilities of inorganic salts in solutions

of increasing alcohol concentration. These data are considered well resolved and repeatable although the accuracy of the response in alcohol-rich solutions has yet to be verified. If hardness proves to be an important characteristic, then a titration method of analysis might be preferred as it would provide more precise and accurate data for hardness in both ethanol and formalin solutions.

The pH values from the test strip (80) were generally lower than those obtained instrumentally (28). The mean pH in the ethanol collection was 5.5 according to the test strips compared with 6.4 from the electrode-based readings. In the formalin collection, the mean pH was 5.7 according to the test strips compared with 6.3 from the electrode-based readings. The instrumental readings also exhibit a greater range of pH values (Appendix 1), particularly in the ethanol collection. Although there are technical difficulties involved with both test strip and instrumental measurements, we consider the instrumental readings to be more accurate.

Protein concentrations (81) were well resolved for the ethanol samples with none of the samples showing a zero concentration. Only one formalin sample indicated more than a trace amount of dissolved protein and the majority (43/57) showed a zero concentration. This difference in protein concentration distributions seems reasonable in light of the protein-fixing properties of formaldehyde. It is noteworthy that the ethanol collection shows a range of dissolved protein concentrations despite those samples having been initially fixed in formalin. Unfortunately, the test strips determine protein through measurement of protein-error shift in a pH indicator and both ethanol and formalin were found to cause pH indicator shifts (see titratable acidity discussion in RESULTS—*Fluid Chemistry*). Consequently, systematic tests will need to be conducted to test the reliability of this type of test in preservative fluids.

The responses of urinalysis test strip sections for ketone (82), glucose (84), and blood (85) were negative for all samples, and urobilinogen (87) section showed a constant null response. Therefore, these characteristics are not considered useful. The test for nitrite (86) indicated a positive result for 2/53 ethanol samples and 15/57 formalin samples. The test for bilirubin (83, a constituent of bile) showed a positive result for just 3/52 ethanol samples and 1/57 formalin samples. The presence of a gastric system chemical in a preservative fluid supports the hypothesis of Von Endt (1994) that stomach contents influence the fatty acid concentration distributions in fluids. The specific gravity sections of the urinalysis test strip (88) are based on changes in an indicator material in relation to ionic concentration. Responses were distributed over ranges that were nearly exclusive between the formalin and ethanol samples. These specific gravity responses did not correlate with either density or conductivity and their meaning and significance are not understood.

All samples gave negative responses to the sodium sulfide test strips (89). Their use in future assessments is recommended only if the specimens are suspected of having been treated with mercury.

Test strips tend to be moderately fast to use and moderately expensive. Only those that indicated hardness, protein, bilirubin, and nitrite appear to give well-resolved results. Still, their usefulness as indicators of past deterioration or potential rate of deterioration is not yet known.

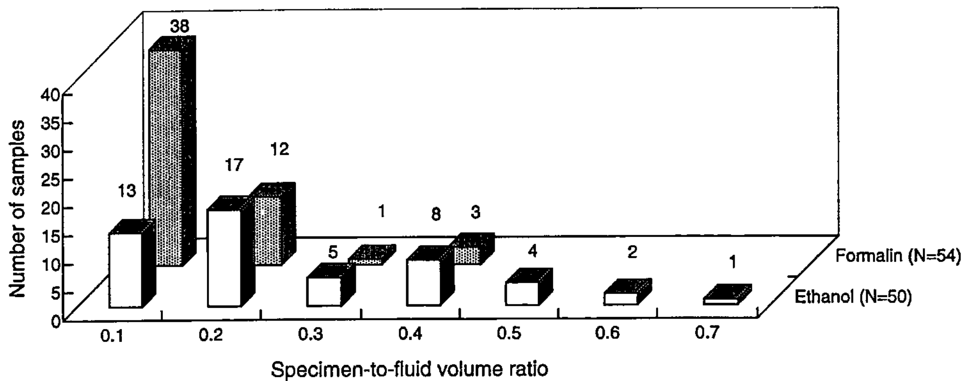

Figure 9. Distributions of ratios of specimen volume to preservative fluid volume in ethanol and formalin collections.

Calculated Values

The ratio of specimen to fluid volume (94) is thought to be an important characteristic in governing the extent of fluid-specimen interactions. The ethanol collection had a broader distribution of those ratios (Fig. 9) and a higher mean specimen to fluid ratio than the formalin collection, 0.21 vs. 0.07, respectively.

Residue on evaporation (98) was similar in mean (ethanol = 0.27 and formalin = 0.31% w/w) and distribution for both collections (Fig. 10). The highest residues on evaporation were found in the formalin collection. Since assessments were not conducted on samples of stock solutions, the amount of residue inherent in solutions can not be distinguished from that derived from specimens. Future assessments should evaluate samples of stock solutions, preferably samples drawn from different batches.

Formalin concentration (100) in the ethanol collection samples is shown in Figure 11. All samples contained at least a small amount of residual formalin. The distribution of formalin concentrations is similar to that found in the Canadian Museum of Nature's isopropanol-based fish collection (Waller and McAllister 1987). We plotted the residual formalin concentration in ethanol-based specimens as a function of age of the first catalog number (Fig. 12). Two of the three oldest samples (63 and 71 years) have residual formalin concentrations nearly ten times

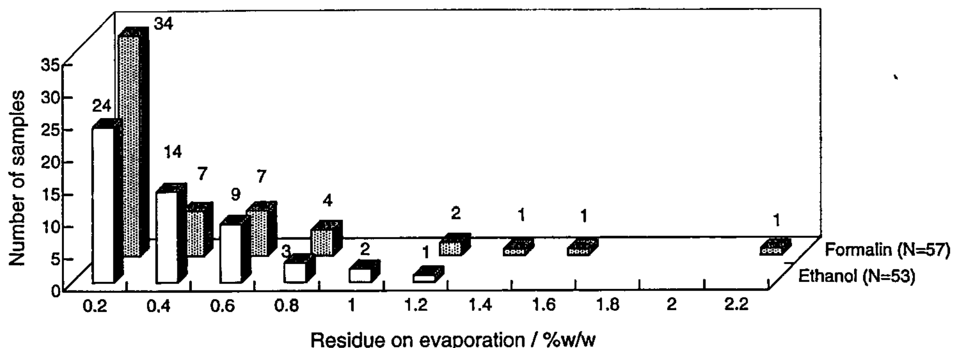

Figure 10. Distributions of residues on evaporation in ethanol and formalin collections.

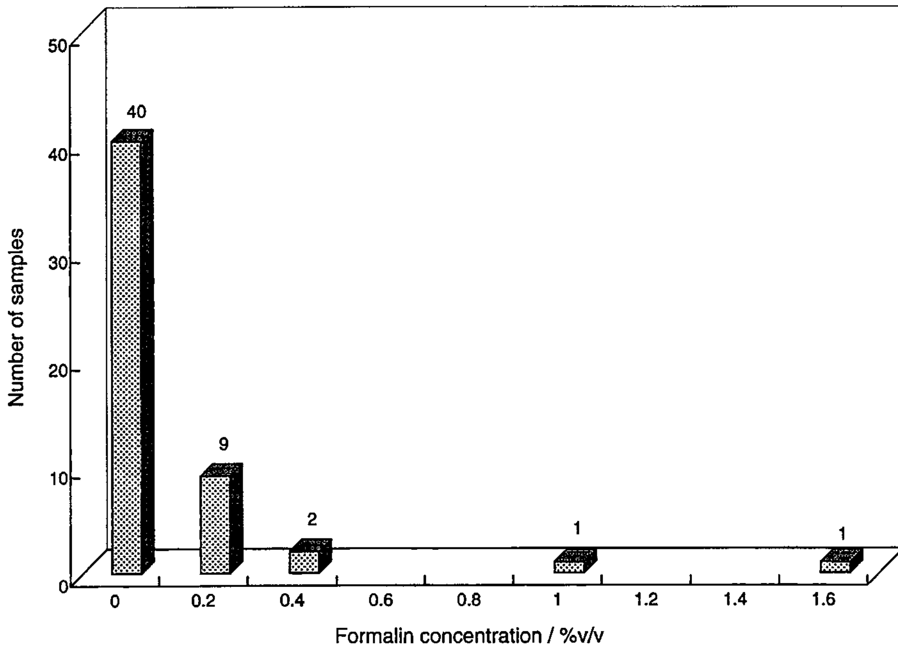

Figure 11. Distribution of formalin concentrations (%v/v) in the ethanol collection (N = 53).

the mean and more than double the next highest value. These outlying values suggest that a less intensive "washing" scheme was followed when specimens were transferred to an ethanol solution following fixation. The linear regression line, fit to all but the outlying data points, shows a statistically significant (one-sided t-test, $P = 0.001$) trend for older specimens to have lower residual formalin concentrations. This trend could be a function of formaldehyde oxidation over time, a gradual consumption of formaldehyde by further reaction with specimens, dilution from occasional partial or complete changes of preservative solution within a container, a gradual change in "washing" practices over time, or a combination of these factors.

Levels of residual formalin in ethanol collections are important for several reasons. First, residual formaldehyde can reduce the quality of recoverable DNA from fluid-preserved specimens (Tuross 1999). Second, residual formaldehyde can oxidize over time contributing to acidification (Walker 1964) although Carter (1997) has speculated that such oxidation might not be significant at normal collection temperatures. However, our data (Fig. 13) show a statistically significant trend (one-sided t-test, $P = 0.001$) for titratable acidity (phenolphthalein endpoint) to increase with increasing age of the first catalog number. The antipathetic trends of formalin concentration and acidity are consistent with oxidation of formaldehyde being a significant process. In addition, health hazards associated with formaldehyde, through both skin contact and inhalation, must be considered for alcohol collections holding formalin-fixed specimens. In contrast, a certain level of residual formalin may contribute to improved preservation of some specimen characteristics such as gross morphology (Von Endt 2000) and pigmentation. Research is required to determine the risks and benefits associated with specific ranges of

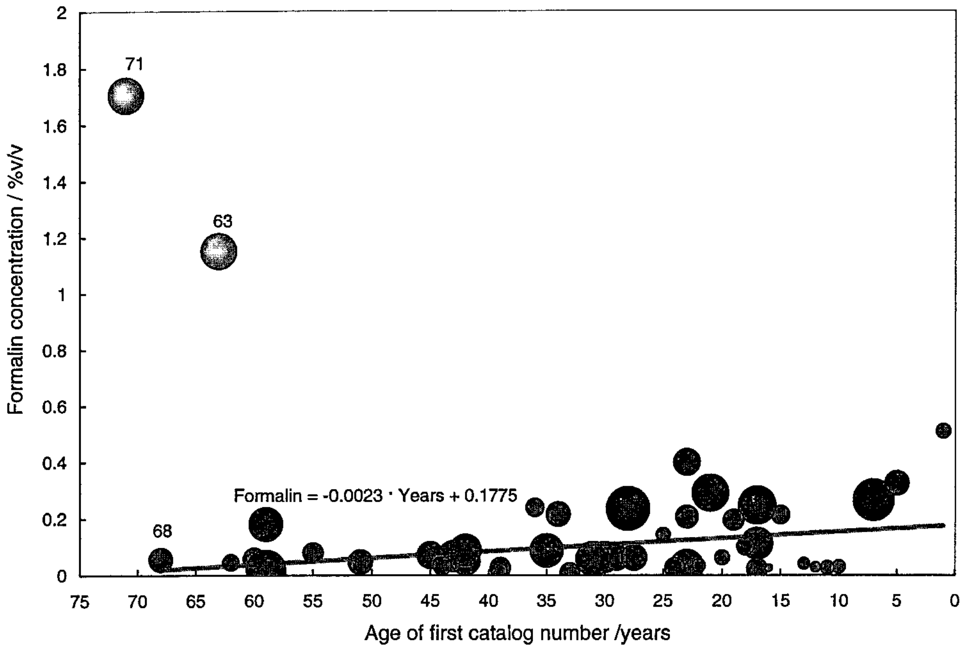

Figure 12. Formalin concentrations (%v/v) in the ethanol collection as a function of age of the first catalog number. Area of bubbles is proportional to specimen-to-fluid volume ratio.

residual formalin concentration as well as the effectiveness of practices for "washing" specimens between formalin fixation and alcohol preservation. It is interesting to note that the slope of the least squares fit in Figure 12 indicates that a sample with the extrapolated mean starting concentration of 0.1775%v/v formalin will reach zero percent formalin in 77 years. Assuming all samples start with a residual formalin concentration of less than 0.4%v/v, all residual formalin will be lost in about 175 years.

The ethanol concentration (97) distribution for the ethanol collection ("current" distribution in Figure 14a) was approximately normally distributed about a mean value of 59.3%v/v (median = 59.8%v/v). These data are comparable to those published by Cato (1990) and Palmer (1996) for mammal collections and Pickering (1997) for a general zoological collection. The mean is about 10%v/v lower than the nominal 70%v/v target but we expect this is typical of alcohol-preserved collections with a mean age of at least several decades. The principal cause of this lowered ethanol concentration is the preferential evaporation of ethanol compared with water, due to the higher vapor pressure of ethanol. The practice of topping jars with standard 70%v/v ethanol contributes to this problem. The concentration of vapor evaporating from a 70%v/v solution is about equivalent to the concentration of an 85%v/v ethanol solution (Waller and Strang 1996). Consequently, topping with a solution that is less than about 85%v/v ethanol will not restore solutions to their original concentration. As with formalin, consistent errors in preparing stock solution (i.e., being weaker than the nominal concentration) may also be a factor. Palmer (1996) determined that incorrect preparation of stock solutions was a significant factor in lowering the mean ethanol concentration in

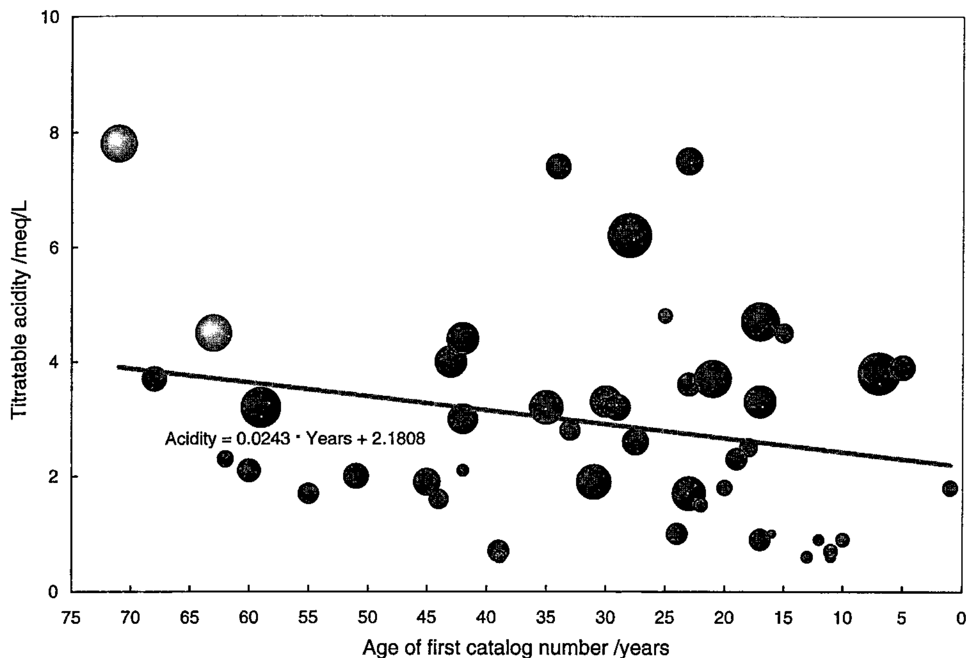

Figure 13. Titratable acidity, to phenolphthalein endpoint, in the ethanol collection as a function of age. Area of bubbles is proportional to specimen-to-fluid volume ratio.

a fish collection. Implications of how these data can be used to determine the best preservative concentration for a topping solution are discussed below (see Practical Applications of the Data).

Formalin concentration (100) in the formalin collection samples is shown in the "current" distribution in Figure 14b. Nearly all (54/57) formalin concentrations were higher than the nominal 10%v/v intended for the collection. The mean (13.05%v/v, Appendix 1) is 3%v/v higher than the target (nominal) concentration. We believe this to be primarily a result of concentration of the (less volatile than water) formaldehyde as evaporation proceeds. Consistent errors in preparing stock solution (i.e., to be stronger than the nominal concentration) may also contribute to higher concentrations. A practice of continuing to top these containers with the nominal 10%v/v formalin allows concentrations to climb over time.

Only one specimen in the formalin collection (sample F27) had a density measurement lower than one (0.959K gL^{-1}) corresponding to an ethanol concentration equivalent to 31%v/v. Except for that one sample, alcohol concentrations could not be determined from density measurements in the formalin collection. Nevertheless, density is a solution property that is easy to measure accurately, provides well-resolved and repeatable data, and identifies samples that contain significant concentrations of alcohol and its measurement is recommended.

All calculated values provide useful information and, with the exception of residue on evaporation (98), are fast and inexpensive to calculate once the necessary measurements and titrations are complete. Although residue on evaporation is time-consuming and very expensive to determine if a balance must be pur-

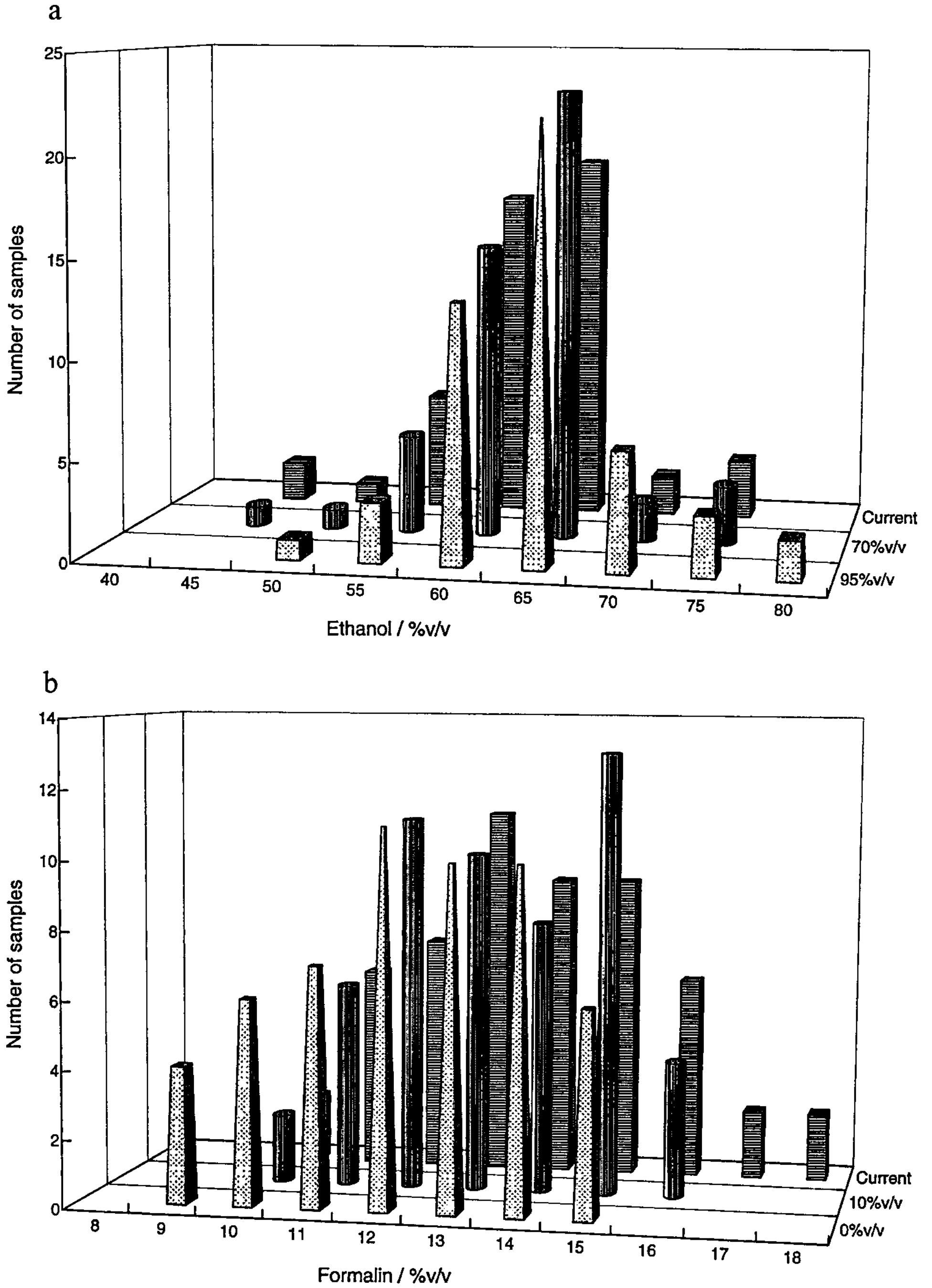

Figure 14. Predicted distribution of preservative concentration levels in the (a) ethanol (N = 50) and (b) formalin (N = 54) collections after topping with different concentrations of stock solutions.

chased, it provides an accurate determination of total nonvolatile impurities in the preservative fluid, which may be an important characteristic.

Specimen Identification

Data distributions for "type of specimen" (106) and "adult or juvenile" (107) are thought to fairly represent the distributions in the collections as a whole. With the exception of "Genus and species" (104), these characteristics were all fast and inexpensive to record and provide potentially useful information. "Genus and species" was not recorded during the survey but that information can be retrieved through the catalog number.

Specimen Preservation

Data on thirteen of the fourteen specific aspects of preservation quality, and the general assessment of preservation quality (139) are shown in Figure 15 (a and b). The characteristic "clearing of tissue" (130) is not shown due to insufficient data. The characteristic was only reported for a single sample in the ethanol collection and for eight samples from the formalin collection. In most instances where a response was given for clearing of tissue, the judgement of confidence associated with the response was "cannot assess." We conclude that no changes in the state of these amphibian and reptile specimens could be correlated with the descriptors used for this characteristic (Table 5).

With the exception of clearing of tissue, all other preservation quality characteristics could be readily assessed for samples from the ethanol collection. The mean of the judgements of confidence was 0.08 or less. Judgement of preservation quality characteristics was more difficult in the formalin collection (Figure 15b). The mean of judgements of confidence ranged from 0.09 for condition of abdomen/organs to 1.19 for extent of loss of skin or scales (35 responses for 57 samples) and 1.41 for elasticity (20 responses for 57 samples). Much of the increased difficulty in assessing preservation quality characteristics in the formalin collection is attributed to problems in discerning the condition of the small-sized egg, larval, or juvenile specimens in that collection.

All of the preservation quality characteristics were fast and inexpensive to record and, with the exception of clearing of tissues, yielded well-distributed results with the descriptors used.

Practical Applications of the Data

This pilot assessment project was not designed to produce data of immediate practical value. Instead, our intent was to take a step in learning how we might study the state of a fluid-preserved collection from a broad, epidemiological perspective. Our principle goal, therefore, was to present a profile showing the means, ranges, and natures of distributions of a diversity of charactersitics for containers, fluids, and specimens. Still, some practical results were found while considering the data. Here we present two such results ranging from very simple, in the case of selecting attachment thread material, to quite complex in the case of selecting concentrations for collection-wide topping solutions.

The simplest example was the association of cuts, breaks or severe abrasion of specimens with the use of a synthetic filament to attach labels to specimens. The data (see Results and Discussion—*Label Materials and Condition*) indicate that

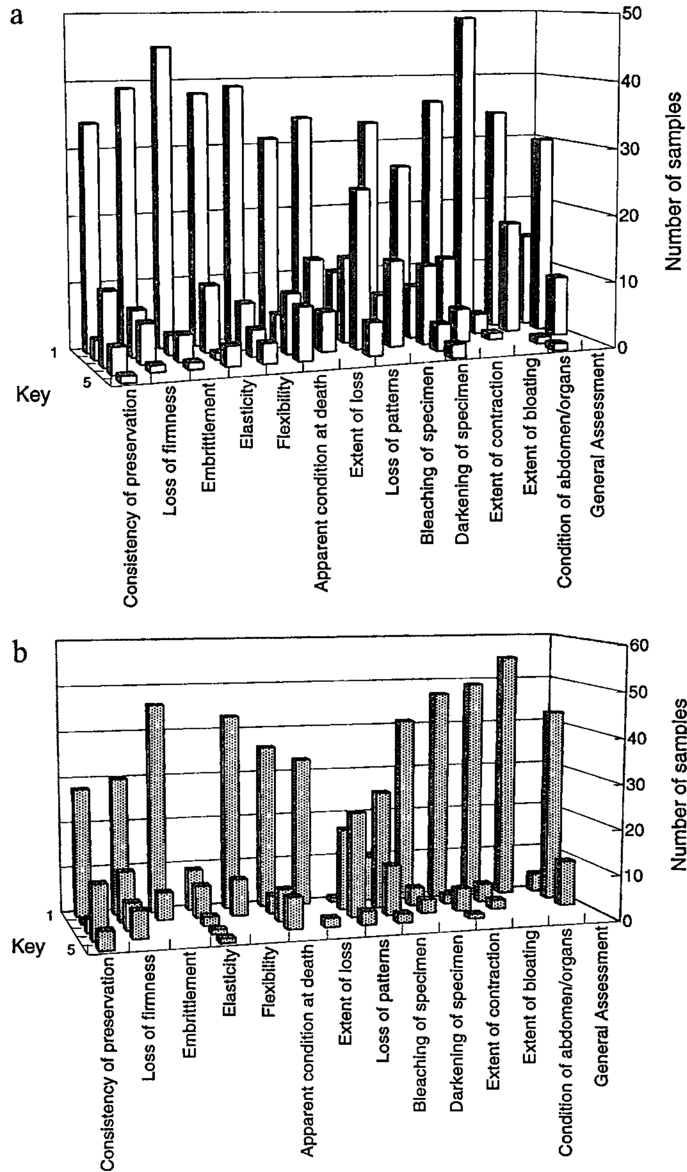

Figure 15. Distribution of data describing state of specimen preservation in the (a) ethanol and (b) formalin collection. See Table 5 for keys to characteristics and Appendix 1 for statistics.

for attaching labels to specimens, cellulose thread is less damaging to specimens and more durable than synthetic filament. This should lead to the use of cellulose thread as the standard method of attaching labels to fluid-preserved specimens.

A second practical application allows us to use data to predict the effect of different replacement fluid concentrations on preservative concentration distributions thereby enabling us to recommend an optimal concentration of topping fluid for use throughout a collection. This approach is warranted when measurement and correction of the concentration of each sample are impractical.

Table 6. Statistics describing ethanol and formalin concentration distributions before and after topping with different concentrations of stock solution.

	Ethanol collection			Formalin collection		
	Current	70% v/v	95% v/v	Current	10% v/v	0% v/v
Mean	59.3	60.0	61.7	13.2	12.8	11.8
Std. Deviation	6.23	5.46	5.70	1.96	1.65	1.80
Minimum	40.1	44.0	47.3	9.7	9.7	8.5
Maximum	74.1	73.7	77.7	17.8	15.6	14.9
Range	34.0	29.7	30.3	8.1	5.9	6.4

Using data for concentration of preservative (97 or 100), and for volumes of specimen (93), solution (92), and container (95), and a known concentration of topping fluid, we developed the following formula to predict expected preservative concentration after topping:

$$C_{final} \approx \frac{V_{solution} \times C_{solution} + V_{specimen} \times F_{specimen} \times C_{solution} + (V_{container} - V_{solution}) \times C_{topping}}{V_{container} - (1 - F_{specimen}) \times V_{specimen}}$$

where:

C_{final} = final concentration in %v/v
$C_{topping}$ = concentration of topping solution in %v/v
$C_{solution}$ = concentration of preservative solution in container in %v/v
$F_{specimen}$ = ratio of total specimen volume containing preservative fluid that will exchange with the surrounding fluid
$V_{container}$ = volume of the specimen container in mL
$V_{solution}$ = volume of preservative solution in mL
$V_{specimen}$ = volume of the specimen material in mL

The expected concentration distributions in the collection after topping the jars with two concentrations of topping solution are shown in Figure 14 (a and b) with summary statistics presented in Table 6. These values were calculated assuming 90 percent of the volume of the specimen is occupied by exchangeable solution. Since the mean ratio of specimen-to-fluid volumes in both collections was not large, the results varied only slightly with changes in the assumption of how much specimen volume contained exchangeable fluid. This was true for both extremes in assumptions, that the specimen contains no exchangeable preservative fluid and that the entire specimen volume was occupied by exchangeable preservative fluid. The effect of solution shrinkage on dissolution after mixing was not accounted for in arriving at these expected concentrations. The effect of this shrinkage on final concentration can be shown to be less than 1%v/v.

For the ethanol collection, topping with a solution of 70%v/v ethanol makes little difference to the initial mean concentration of 59.3%v/v. Even using a 95%v/v solution for topping only raises the mean ethanol concentration by 2.4 to 61.7%v/v. The highest expected final concentration among these samples, after topping with 95%v/v ethanol was about 78%v/v. Consequently, the risk of adding a solution of ethanol that is too concentrated in some collection jars appears small relative to the benefit of increasing the overall concentration of ethanol. These data support the use of 95%v/v ethanol as a topping solution, for this collection,

at this time. This conclusion applies to this collection at this time and is not a general recommendation.

For the formalin collection, using pure water (0%v/v formalin) brings the mean concentration the closest to the nominal 10%v/v at which the collection is intended to be maintained. The use of water would significantly lower the minimum concentration from 9.7 to 8.5%v/v formalin. If this is considered an acceptable minimum level, then the use of pure water for a topping solution is advised, for this collection, at this time and again, this is not a general recommendation.

It is also possible to use a spreadsheet goal-seeking, or equation-solving, function to determine the optimum concentration to be used for topping a collection. For these collections, the (theoretically) optimum concentration for topping solutions was less than zero percent for formalin and greater than 100 percent for ethanol. Because this is technically impossible, we believe that the addition of pure water for this formalin collection, and stock 95%v/v ethanol for the ethanol collection, would make better topping solutions, at this time, than the nominal-concentration preservative solutions.

Summary and Conclusions

A pilot assessment of the University of Kansas Museum of Natural History Herpetological collections has been described. The 139 characteristics have been assessed based on expense and ease of measurement and the resolution and expected repeatability of the data they provided.

A baseline dataset describing 139 characteristics of an ethanol- and a formalin-preserved collection has been established. The broader goal for this work was to determine to what extent broad-based epidemiological studies of fluid-preserved collections might contribute to our knowledge of deterioration processes and requirements for preservation. This question will be addressed in a subsequent paper dealing with the multivariate analysis of these data.

An important finding is that data for most characteristics showed continuous distributions with the occurrence of outlying data being relatively rare. This suggests that collection preparation and maintenance materials and procedures have been consistently applied in this collection. The validity of this interpretation is supported by the fact that outlying values for residual formalin concentration in the ethanol collection could be attributed to former, no longer employed, collection care methods (e.g., Fig. 12).

In general, more deviations from ideal preservation were noted for specimens than for labels and label data. This is due, in part, to the practice of replacing tags and labels when any significant deterioration is noted. Consequently, loss of collection utility due to specimen degradation is a more serious risk than loss of data due to label or inscription degradation. Specimen degradation was noted among samples whose fluid properties were not outlying values. This indicates that failure to maintain an optimal state of specimen preservation is not merely a result of occasional lapses or failures in collection management practices. It is a characteristic of current and traditional collection management practices. This is a conclusion of fundamental importance as it points to fundamental problems with the system of fluid preservation that cannot be rectified by improvements in the application of current practices. Multivariate analyses of these data might be expected to provide some insight into relations between fluid and state of preser-

vation characteristics. This will be the subject of a forthcoming paper dealing with this data set. Those results, in turn, will provide direction for focussed laboratory studies of subsets of characteristics.

Based on our assessment of the resolution and repeatability of measurements, and consideration of time and cost to determine, we recommend a minimum of four characteristics be included in any assessment of a fluid-preserved collection. These are preservative concentration, container volume, preservative fluid plus specimen volume, and specimen volume. Using these characteristics, one can determine the effect of topping procedures on preservative concentration. As shown in the priority column of Appendix 1, we conclude that an additional 71 characteristics have high priority for inclusion in future assessments, 48 characteristics have secondary priority while the remaining 16 characteristics have low priority. Some characteristics will need to be revised for dealing with other preservative fluids (e.g., glycerin or phenoxetol) or with other taxonomic groups (e.g., invertebrates).

Data collected on the condition of the labels, including printing or inscriptions and means of label attachment, are both useful and repeatable. In this study, for example, they led to the conclusion that cotton thread should be used instead of synthetic filament. Characteristics for assessment of the state of preservation of the specimens themselves are clearly an important part of an assessment as they are closely related to the goal of collection preservation. Unfortunately, measurements of these characteristics remain subjective and will exhibit limited repeatability over time or between collections. Measurements of characteristics that assess the preservative fluid are objective and repeatable but the relevance of any of them to long-term collection preservation is not yet clear.

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Appendix 1. List of characteristics, units of measurement, and results obtained, including number of responses, minimum, maximum, range, mean, and standard deviation (Std. Dev.) and time, cost, and priority estimates. Priority is the authors' consideration of the overall importance of including that characteristic in future assessments.

No.	Characteristics	Units	Time	Cost
1	Shelf set number	integer	1	1
2	Shelf number	integer	1	1
3	Number of shelves	integer	1	1
4	Reason for omission	key, 1–4 (explanations)	1	1
5	Front to back	key, 1–5	1	1
6	Left to right	key, 1–5	1	1
7	Container number	number	1	1
8	Catalog number (first)	number	1	1
9	Age of first catalog number	years	2	1
10	Catalog number, last	number	1	1
11	Age of last catalog number	years	2	1
12	Genus and species	binomen	2	1
13	Container type	letter code	1	1
14	Manufacturer (container)	proper name	1	1
15	Lid material	key, 1–9, 0 (list of materials)	1	1
16	Manufacturer (lid)	proper name	1	1
17	Ease of opening	key, 1–5 (descriptions; Table 4)	1	1
18	Cap liner	key, 1–3 0 (list of materials)	1	1
19	Gasket	key, 1–7, 0 (list of materials)	1	1
20	Sealant	key, 1–7, 0 (list of materials)	1	1
21	Fluid preservative identified	no—0, yes—1	1	1
22	Fluid height	mm	1	1
23	Container height	mm	1	1
24	Material above fluid	percent ratio of total specimen(s)	1	1
25	Oils	key, 0–4 (standards)	1	1
26	Continuity of oil layer	no—0, yes—1	1	1
27	Precipitated material	key, 0–4 (standards)	1	1
28	pH	pH units	3	4
29	Temperature	°C	1	1
30	Oxidation reduction potential	mV vs. Ag/AgCl	4	4
31	Conductivity	mhos/cm	1	4
32	Height of fluid without specimens	mm	1	1
33	Volume of preservative fluid	mL	1	1
34	Label(s) not attached	key, 0–1 (absent/present)	1	1
35	Current format	key, 0–1 (no/yes)	1	1
36	Material	key 1–4, 0 (list of materials)	1	1
37	Condition	key 1–5, 0 (descriptions)	1	1
38	Standard form printing	key, 0–1 (absent/present)	1	1
39	Condition	key 1–5, 0 (descriptions)	1	1
40	Data	key 1–5, 0 (list)	1	1
41	Condition	key 1–5, 0 (descriptions)	1	1
42	Label(s) attached	key, 0–1 (absent/present)	1	1
43	Current format	key, 0–1 (no/yes)	1	1
44	Material	key 1–4, 0 (list of materials)	1	1
45	Condition	key 1–5, 0 (descriptions)	1	1
46	Standard form printing	key, 0–1 (absent/present)	1	1
47	Condition	key 1–5, 0 (descriptions)	1	1
48	Data	key 1–5, 0 (list)	1	1
49	Condition	key 1–5, 0 (descriptions)	1	1
50	Attachment, current format?	key, 0–1 (no/yes)	1	1
51	Material	key 1–4, 0 (list of materials)	1	1
52	Cond. of attachment material	key 1–5, 0 (descriptions)	1	1
53	Condition at attachment	key 1–3 (descriptions)	1	1
54	Year container put in service	year	1	1
55	Confidence	± 1, 5, or 10 years	1	1
56	Year specimen entered container	year	1	1
57	Confidence	± 1, 5, or 10 years	1	1
58	Year fluid last changed	year	1	1
59	Confidence	± 1, 5, or 10 years	1	1
60	Year fluid last topped	year	1	1
61	Confidence	± 1, 5, or 10 years	1	1
62	Formalin test strip	response, 0–1 (negative/positive)	1	1
63	Nominal solution composition	key 1–3, 0 (list)	1	1

Appendix 1. Extended.

			Ethanol collection				
No.	Priority	Responses[1]	Minimum	Maximum	Range	Mean	Std. Dev.
1	1	62	1	184	183		
2	1	61	1	10	9		
3	3	62	8	10	2		
4	3	9					
5	2	53	1	5	4	1.47	0.97
6	3	53	1	5	4	2.92	1.43
7	2	na					
8	1	53	1,643	216,192	214,549	110,266	71,116
9	1	53	1	71	70	31.2	17.5
10	2	53	1,648	216,192	214,544	136,509	64,862
11	2	53	1	70	69	23.7	14.9
12	3	nr					
13	1	53					
14	2	nd					
15	1	53					
16	2	nd					
17	2	53	2	5	3	3.06	0.60
18	1	na					
19	1	41					
20	1	na					
21	2	53	1	1	0	1	0
22	1	53	79	241	162	152	49
23	1	53	121	254	133	171	50
24	1	53	0	0.025	0.025	0.0021	0.0067
25	2	53	0	1	1	0.11	0.32
26	2	53	0	0	0	0	0
27	2	53	0	1	1	0.04	0.19
28	1	53	5.19	7.55	2.36	6.38	0.45
29	2	53	18.7	23.6	4.9	21.1	1.0
30	3	53	−95	226	321	−7	65
31	2	53	38	920	882	206	202
32	1	53					
33	1	nd					
34	1	53	1	1	0	1	0
35	1	53	1	1	0	1	0
36	1	53	1	1	0	1	0
37	1	53	1	2	1	1.13	0.34
38	1	53	1	1	0	1	0
39	1	53	1	2	1	1.06	0.23
40	1	53	2	2	0	2	0
41	1	53	1	2	1	1.28	0.45
42	1	53	1	1	0	1	0
43	1	53	1	1	0	1	0
44	1	53	1	1	0	1	0
45	1	53	1	2	1	1.06	0.23
46	1	53	1	1	0	1	0
47	1	53	1	2	1	1.17	0.38
48	1	na					
49	1	na					
50	1	53	1	1	0	1	0
51	1	53	2	4	2	3.36	0.94
52	1	53	1	2	1	1.21	0.41
53	1	53	1	3	2	2.17	0.61
54	2	6	1,973	1,987	14	1,983	5.5
55	2	6	1	10	9	6	3.5
56	2	17	1,973	1,989	16	1,985	3.8
57	2	17	1	10	9	4.8	3.5
58	2	37	1,980	1,990	10	1,987	2.2
59	2	37	1	10	9	3.5	3.4
60	2	38	1,986	1,990	4	1,988	1.0
61	2	38	1	5	4	2.2	1.8
62	3	nd					
63	2	53	1	1	0	1	0

Appendix 1. Extended.

		Formalin collection				
No.	Re-sponses	Minimum	Maximum	Range	Mean	Std. Dev.
1	58	187	199	12		
2	58	1	10	9		
3	58	4	10	6		
4	1					
5	57	1	5	4	2.54	1.44
6	57	1	5	4	2.98	1.53
7	na					
8	57	6,000	216,836	210,836	153,550	50,437
9	57	2	65	63	17.8	10.1
10	57	58,200	216,836	158,636	157,814	43,806
11	57	2	31	29	16.7	7.7
12	nr					
13	nr					
14	nd					
15	57					
16	nd					
17	57	1	5	4	3.23	0.76
18	57	0	3	3	2.72	0.56
19	na					
20	na					
21	57	1	1	0	1	0
22	57	85	133	48	113	11
23	57	126	136	10	133	4
24	57	0	0.025	0.025	0.0005	0.0033
25	57	0	1	1	0.05	0.23
26	57	0	0	0	0	0
27	57	0	1	1	0.04	0.19
28	57	4.26	8	3.74	6.32	0.85
29	57	16.6	26.1	9.5	21.0	1.5
30	57	−183	148	331	−31	79
31	57	460	5,000	4,540	1,471	842
32	55					
33	2	114	157	43	136	30
34	57	1	1	0	1	0
35	57	1	1	0	1	0
36	57	1	1	0	1	0
37	57	1	1	0	1	0
38	57	1	1	0	1	0
39	57	1	1	0	1	0
40	57	2	2	0	2	0
41	57	1	3	2	1.44	0.57
42	57	0	1	1	0.02	0.13
43	1	1	1	0	1	
44	2	1	1	0	1	0
45	1	1	1	0	1	
46	2	1	1	0	1	0
47	1	1	1	0	1	
48	na					
49	na					
50	1					
51	1	4	4	0	4	
52	1	1	1	0	1	
53	nr					
54	nd					
55	nd					
56	57	1,960	1,990	30	1,977	8.3
57	57	1	5	4	2.2	1.8
58	57	1,960	1,990	30	1,977	8.4
59	57	1	10	9	2.2	2.1
60	57	1,984	1,990	6	1,985	1.3
61	57	1	5	4	4.4	1.5
62	nd					
63	57	2	2	0	2	0

Appendix 1. Continued.

No.	Characteristics	Units	Time	Cost
64	Density	gmL^{-1}	4	5
65	Temperature	°C	1	1
66	Titration to thymolphthalein	mL NaOH	4	4
67	Titratable acidity, thymol	$meqL^{-1}$	4	4
68	Total 0.100N HCl added	mL HCL	4	4
69	0.100N NaOH added as titrant	mL NaOH	4	4
70	Titration to phenolphthalein	mL NaOH	4	4
71	Titratable acidity, phenol	$meqL^{-1}$	4	4
72	Color/Iodine	key, 0–9 (standards)	4	4
73	Color/Chromate	key, 0–9 (standards)	4	4
74	Color/Dichromate	key, 0–9 (standards)	4	4
75	Ease/Iodine	key, 1–3 (descriptions)	1	1
76	Ease/Chromate	key, 1–3 (descriptions)	1	1
77	Ease/Dichromate	key, 1–3 (descriptions)	1	1
78	Turbidity	key, 0–9 (standards)	4	4
79	Total hardness	°d (6 steps of 5°d)	2	3
80	pH	pH units (7 steps of 0.5)	2	3
81	Protein	gL^{-1} (variable steps)	2	3
82	Ketone	$mmolL^{-1}$ (variable steps)	2	3
83	Bilirubin	negative/+/++/+++	2	3
84	Glucose	concentration (variable steps)	2	3
85	Blood	concentration (variable steps)	2	3
86	Nitrite	negative/trace/positive	2	3
87	Urobilinogen	Erhlich unit dL^{-1} (variable steps)	2	3
88	Specific gravity	(7 steps of 0.005)	2	3
89	Sodium sulphide test strip	negative/positive	2	3
90	Fluid to container height	ratio	1	1
91	Volume of specimen and fluid	mL	3	2
92	Volume of preservative fluid	mL	3	2
93	Volume of specimen material	mL	3	2
94	Specimen to fluid volume	ratio	1	1
95	Container volume	mL	1	1
96	Contents to container volume	ratio	3	1
97	Concentration of ethanol	percent by volume	2	2
98	Residue	percent by weight	5	4
99	Formaldehyde concentration	gL^{-1}	5	4
100	Formalin concentration	percent by volume	5	4
101	Buffer capacity	$meqL^{-1}$ pH $unit^{-1}$	1	1
102	Catalog number	number	1	1
103	Age of catalog number	years	2	1
104	Genus and species	binomen	2	1
105	Number of specimens	key, 1–5 (1,2,3–5,6–10,>10)	1	1
106	Type of specimen	key (fish, amphibian, reptile)	1	1
107	Adult	key, 0–1 (no/yes)	1	1
108	Consistency of preservation	key, 0–4 (descriptions)	1	1
109	Confidence	key, 0,1,2 (level of confidence)	1	1
110	Loss of firmness	key, 0–4 (descriptions)	1	1
111	Confidence	key, 0,1,2 (level of confidence)	1	1
112	Embrittlement	key, 0–4 (descriptions)	1	1
113	Confidence	key, 0,1,2 (level of confidence)	1	1
114	Elasticity	key, 0–4 (descriptions)	1	1
115	Confidence	key, 0,1,2 (level of confidence)	1	1
116	Flexibility	key, 0–4 (descriptions)	1	1
117	Confidence	key, 0,1,2 (level of confidence)	1	1
118	Apparent condition at death	key, 0–4 (descriptions)	1	1
119	Confidence	key, 0,1,2 (level of confidence)	1	1
120	Skin or scales	key (1 = skin, 2 = scales)	1	1
121	Extent of loss	key, 0–4 (descriptions)	1	1
122	Confidence	key, 0,1,2 (level of confidence)	1	1
123	Ecdysis at death	key (no = 0, yes = 1)	1	1
124	Loss of patterns	key, 0–4 (descriptions)	1	1
125	Confidence	key, 0,1,2 (level of confidence)	1	1
126	Bleaching of specimen	key, 0–4 (descriptions)	1	1
127	Confidence	key, 0,1,2 (level of confidence)	1	1
128	Darkening of specimen	key, 0–4 (descriptions)	1	1
129	Confidence	key, 0,1,2 (level of confidence)	1	1

Appendix 1. Continued Extended.

			Ethanol collection				
No.	Priority	Responses	Minimum	Maximum	Range	Mean	Std. Dev.
64	1	53	0.873	0.947	0.074	0.909	0.013
65	1	53	18.7	23.6	4.9	21.06	1.01
66	1	53	0.05	1.07	1.02	0.33	0.22
67	1	53	0.5	10.7	10.2	3.3	2.2
68	1	53	1	23	22	2.6	3.7
69	1	53	0.06	1.11	1.05	0.50	0.26
70	2	53	0.04	1.04	1	0.29	0.21
71	2	53	0.4	10.4	10	2.9	2.1
72	2	53	1	4	3	2.87	0.59
73	2	53	0	1	1	0.26	0.45
74	2	53	0	2	2	0.79	0.63
75	2	53	1	3	2	1.17	0.47
76	2	53	1	3	2	2.79	0.53
77	2	53	1	3	2	2.02	0.46
78	2	53	0	2	2	0.77	0.61
79	1	50	0	5	5	0.85	1.86
80	3	53	5	6	1	5.53	0.50
81	2	52	30	2,000	1,970	726	785
82	3	53	0	0	0	0	0
83	3	52	0	1	1	0.06	0.24
84	3	53	0	0	0	0	0
85	3	53	0	0	0	0	0
86	3	53	0	1	1	0.04	0.19
87	3	53	0.1	0.1	0	0.10	0.00
88	3	53	1.01	1.033	0.023	1.025	0.003
89	3	49	0	0	0	0	0
90	1	53	0.63	0.99	0.36	0.89	0.080
91	1	53	220	3,620	3,400	1,252	1,312
92	1	53	195	3,280	3,085	1,000	1,037
93	1	53	0	1,340	1,340	248	331
94	1	53	0.02	0.62	0.60	0.207	0.152
95	1	53	265	3,680	3,415	1,311	1,355
96	1	53	0.70	1.00	0.30	0.94	0.06
97	1	53	40.1	74.1	34.0	59.3	6.1
98	1	53	0	1.16	1.16	0.27	0.29
99	1	53	0.03	6.81	6.78	0.63	1.12
100	1	53	0.01	1.70	1.70	0.16	0.28
101	2	53	0.13	2.25	2.12	0.72	0.46
102	1	53	1,643	216,192	214,549	123,902	67,498
103	1	53	1	71	70	26.8	14.9
104	3	nr					
105	1	53	0	10	10	3.40	1.82
106	1	53	2	3	1	2.58	0.50
107	1	53	0	1	1	0.98	0.14
108	1	53	0	4	4	0.77	1.14
109	2	50	0	0	0	0	0
110	1	53	0	3	3	0.42	0.77
111	2	53	0	0	0	0	0
112	1	53	0	3	3	0.26	0.68
113	2	51	0	0	0	0	0
114	1	53	0	3	3	0.40	0.79
115	2	52	0	1	1	0.02	0.14
116	1	53	0	3	3	0.45	0.87
117	2	52	0	1	1	0.02	0.14
118	1	53	0	3	3	0.89	1.17
119	2	50	0	0	0	0	0
120	1	53	1	2	1	1.58	0.50
121	1	53	0	2	2	0.47	0.70
122	2	48	0	1	1	0.02	0.14
123	1	53	0	1	1	0.15	0.34
124	1	52	0	3	3	1.46	0.92
125	2	53	0	2	2	0.08	0.33
126	1	53	0	2	2	0.62	0.86
127	2	51	0	0	0	0	0
128	1	52	0	4	4	1	1.19
129	2	53	0	2	2	0.08	0.33

Appendix 1. Continued Extended.

	Formalin collection					
No.	Re-sponses	Minimum	Maximum	Range	Mean	Std. Dev.
64	57	0.959	1.019	0.06	1.010	0.008
65	57	16.6	26.1	9.5	21.03	1.47
66	56	0.03	0.74	0.71	0.11	0.12
67	56	0.3	7.4	7.1	1.1	1.2
68	57	20	25	5	21.0	2.0
69	57	0.44	7.58	7.14	3.6	1.9
70	57	0	0.5	0.5	0.05	0.06
71	57	0	5	5	0.5	0.6
72	57	0	4	4	2.68	0.71
73	57	0	2	2	0.09	0.34
74	57	0	2	2	0.35	0.52
75	57	1	3	2	1.04	0.26
76	57	2	3	1	2.95	0.23
77	57	1	2	1	1.98	0.13
78	54	0	3	3	0.61	0.68
79	57	0	25	25	12.32	6.49
80	57	5	8	3	5.69	0.80
81	57	0	30	30	0.75	3.97
82	57	0	0	0	0	0
83	57	0	1	1	0.02	0.13
84	57	0	0	0	0	0
85	57	0	0	0	0	0
86	57	0	1	1	0.26	0.44
87	56	0.1	0.1	0	0.10	0.00
88	57	1	1.01	0.01	1.004	0.002
89	52	0	0	0	0	0
90	57	0.63	0.99	0.35	0.85	0.075
91	57	195	285	90	258	22
92	57	185	280	95	243	26
93	57	0	65	65	15	18
94	57	0	0.316	0.316	0.068	0.087
95	57	285	285	0	285	0
96	57	0.68	1	0.32	0.91	0.08
97	nd					
98	56	0	2.14	2.14	0.31	0.44
99	57	38.62	71.17	32.55	52.19	7.77
100	57	9.65	17.79	8.14	13.05	1.94
101	57	0	1.77	1.77	0.32	0.33
102	57	58,199	216,836	158,637	157,819	43,804
103	57	2	31	29	16.8	7.6
104	nr					
105	57	1	10	9	3.88	1.70
106	57	2	2	0	2	0
107	57	−1	1	2	−0.02	0.23
108	57	0	4	4	1.23	1.42
109	55	0	2	2	0.64	0.75
110	57	0	3	3	0.77	1.01
111	55	0	2	2	0.16	0.54
112	57	0	1	1	0.12	0.31
113	55	0	2	2	0.15	0.52
114	57	0	4	4	0.90	0.65
115	54	0	2	2	1.41	0.81
116	57	0	1	1	0.16	0.35
117	56	0	2	2	0.25	0.64
118	57	0	3	3	0.72	1.09
119	55	0	2	2	0.18	0.51
120	57	0	1	1	0.96	0.19
121	57	0	3	3	0.17	0.55
122	54	0	2	2	1.19	0.78
123	46	0	0	0	0	0
124	57	0	3	3	1.62	0.58
125	55	0	2	2	0.55	0.83
126	57	0	3	3	1.10	0.71
127	55	0	2	2	0.47	0.77
128	57	0	2	2	0.21	0.50
129	56	0	2	2	0.36	0.75

Appendix 1. Continued.

No.	Characteristics	Units	Time	Cost
130	Clearing of tissue	key, 0–4 (descriptions)	1	1
131	Confidence	key, 0,1,2 (level of confidence)	1	1
132	Extent of contraction	key, 0–4 (descriptions)	1	1
133	Confidence	key, 0,1,2 (level of confidence)	1	1
134	Extent of bloating	key, 0–4 (descriptions)	1	1
135	Confidence	key, 0,1,2 (level of confidence)	1	1
136	Condition of abdomen/organs	key, 0–4 (descriptions)	1	1
137	Confidence	key, 0,1,2 (level of confidence)	1	1
138	Organs are dissolving/missing	key (dissolving = 1, missing = 2)	1	1
139	General assessment	key, 0–4 (descriptions)	1	1

[1] Responses: na = not applicable, nr = not recorded, nd = not determined.

Appendix 1. Continued Extended.

			Ethanol collection				
No.	Priority	Responses	Minimum	Maximum	Range	Mean	Std. Dev.
130	2	1	0	0	0	0	
131	2	53	0	2	2	1.96	0.27
132	1	53	0	2	2	0.42	0.66
133	2	53	0	0	0	0	0
134	1	53	0	2	2	0.09	0.35
135	2	53	0	2	2	0.04	0.27
136	1	53	0	4	4	0.45	0.77
137	2	51	0	0	0	0	0
138	1	2	1	1	0	1	0
139	1	53	0	2	2	0.91	0.66

Appendix 1. Continued Extended.

	Formalin collection					
No.	Responses	Minimum	Maximum	Range	Mean	Std. Dev.
130	8	0	2	2	0.63	0.92
131	52	0	2	2	1.85	0.50
132	57	0	3	3	0.27	0.69
133	56	0	3	3	0.14	0.55
134	57	0	2	2	0.15	0.44
135	54	0	2	2	0.11	0.42
136	57	0	0	0	0	0
137	54	0	2	2	0.09	0.40
138	0	0	0	0	0	
139	57	0	2	2	1.11	0.49

THE THEORETICAL BASES OF COLLECTIONS MANAGEMENT

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Abstract.—A collection may be managed as individual elements or as a set of elements. Collection management can be depicted graphically with order shown on the x-axis, collection growth on the y-axis, and conservation on the z-axis. Individual collection elements, or the entire set, can be graphed as points in xyz-space. The management of the collection can be evaluated by analyzing the location and form of the cluster of points p (x, y, z). This analysis may be done in conjunction with other methods of collection management evaluation, such as the Collection Health Index. The cost of management of a collection is equivalent to the cost of reducing the expression of entropy in the collection, which is accomplished by controlling the agents of deterioration (direct physical forces; theft; vandalism, displacement and curatorial neglect; fire; water; pests; contaminants and pollutants; light and radiation; incorrect temperature; and incorrect relative humidity). These are easiest to control at the microenvironment level, and microenvironments are best controlled using the principles of the theory of enclosures. Collections should be managed with an understanding of entropy, enclosure theory, and preventive conservation theory to direct the investment of resources.

Resumen.—Los especimenes en una colección son manejados como elementos individuales, pero la colección entera es manejada como un grupo. El manejo de las colecciones puede ser explicado de manera gráfica con orden sobre el eje x, el crecimiento de la colección sobre el eje y, y la conservación de los especímenes sobre el eje z. La ubicación de los elementos individuales (ejemplares) en una gráfica permiten evaluar y predecir el estado en que se encuentra la colección. Mucho del costo del manejo de una colección es equivalente al costo de reducir la expressión de la entropía en la colección y, esto va acompañado del control que se esté realizando sobre los agentes de deterioro de la colección (fuerzas físicas directas, ladrones, vándalos, fuego, agua, pestes, contaminación, radiación, temperaturas y, humedades relativas incorrectas). Los agentes de deterioro están orientados a controlar los niveles del microambiente y, los microambientes se controlan usando los principios de la teoría de envolturas. El control de la tasa de la entropía en el cuidado y manejo de colecciones de historia natural es parte del conservación preventiva. Las colecciones deben ser manejadas con el fin de mantener un equilibrio entre el uso corriente y su preservación para el uso futuro, para ello se deben aplicar las teorías de la entropía, envolturas y, conservación preventiva y de esta manera redireccionar la inversión de los recursos.

The history of preservation is much older than the history of museums. For example, mummies were prepared at least 7,800 years ago in Peru and 5,000 years ago in Egypt (Brier 1998). It is estimated that at present there are approximately 2,500,000,000 natural history specimens and objects (Duckworth et al. 1993) in some 6,500 collections (Mares 1993). Worldwide, the ratio of collection care workers to natural history collection objects averages 1:200,000. However, there are relatively few specimens in natural history museums collected before 1850, and the oldest specimens in collections are often not in a good state of preservation (Hawks 1990). All of the causes of collection deterioration—organic, inorganic, and organizational—are expressions of entropy. Given the quantity and age of the specimens and objects in collections, and the low number of trained

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professionals to care for them, how can collection management resources be best directed to prolong the useful life of collections?

A *collection* is a set of related *elements* (*cf.* Pearce 1992). The elements may be horseshoes or horseshoe crabs, but in all cases, for the set C, with elements (a, b, c, . . . , z), we can state that the elements are members of the set C by use of the symbol ∈, thus

$$(a, b, c \ldots, z) \in C.$$

The specimens or objects in a collection may be managed as individual elements (a, b, c, . . . , z), but the entire collection is also managed as a set. One of the characteristics of a collection of elements that distinguishes it from other assemblages of elements is that a collection has some sort of order (Pearce 1992). *Entropy* is the quantitative measure of the degree of disorder (lack of order) in a system (Arnheim 1971). Historically, the most fundamental aspect of collection management has been establishing and maintaining order among the elements of the set that comprises the collection, thus reducing entropy. The management of a collection can be depicted graphically, as show in Figure 1A, with the x-axis as the *order axis.* This is the traditional view of collection management—resist an increase in entropy.

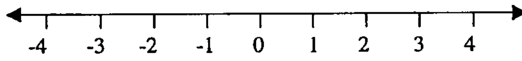
The extremes of the order axis are very chaotic situations and very organized situations. In a properly ordered collection, each element has an appropriate physical location within the organizational structure. This physical location is determined by the systematic organization of the collection and the type of specimen or object it is (e.g., a dry specimen, a fluid-preserved specimen, or a frozen tissue sample in a collection that is arranged in alphabetical order by scientific name). The physical location for the element is called a *cell* (R. Waller, pers. comm. 1987). The cell is a particular, special and unique place for the element in the collection. To the extreme left on the order axis (Fig. 1A), the collection is disorganized—there is more than one element per cell, there are elements that are not in their cells, or there are elements in the collection that do not have cells. To the extreme right of the order axis the collection is highly organized, with each element located in its respective cell. The elements to the extreme left are unusable due to their disorder. The elements on the extreme right are unusable due to their order—the elements are in an order that cannot be maintained, an order that is very expensive to maintain, or the use of the elements drastically changes their position on the order axis.

The mid-point on the x-axis is the state in which each element of the collection is in its appropriate cell, in a usable order. Elements to the left of the midpoint show increasing entropy because one or more elements are not in their cells. Elements to the right of the midpoint show decreasing entropy, resulting from some further ordering or organization of the elements in their cells. *Disorder* may be defined simply as the absence of order (Feibleman 1968); while *order* may be defined as "a similarity among disparate elements" (Feibleman 1968:3) or a recognizable pattern. Defined this way, order "has the limitation that it cannot be total" (Feibleman 1968:4). The mid-point of the x-axis is thus set at the point where disorder among elements may be said to end and order among elements be said to begin, as the order axis extends in both directions to infinity.

The second consideration for collection management is the *collection growth*

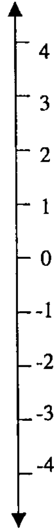
A

Disorder Order



B

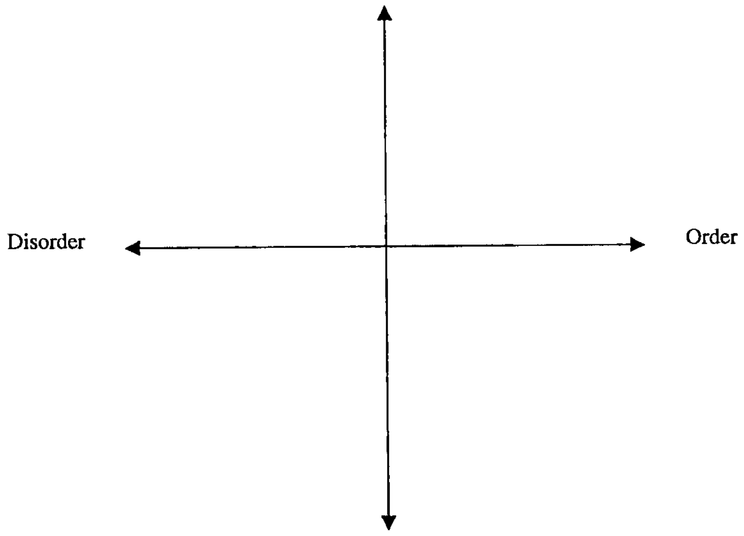
Collection is growing (number of elements is increasing)



Collection is depleting (number of elements is decreasing)

C

Collection is growing (number of elements is increasing)



Collection is depleting (number of elements is decreasing)

Figure 1. Graphic representation of the management of collections. A. Order (x-axis); B. Collection growth (y-axis); C. Collection Management.

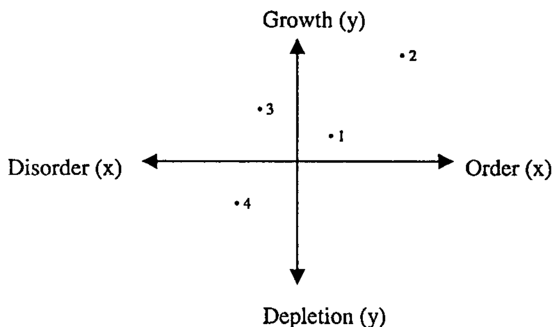


Figure 2. Graphical representation of collection situations. 1. p (1, 1), a stable and ordered collection; 2. p (4, 3), an ideal collection—growing but remaining in order; 3. p (−1, 2), a collection that is growing but falling into disorder; 4. p (−2, −2), a collection that is becoming disordered and decreasing in size.

axis, depicted as the y-axis (Fig. 1B). The collection may be growing (with the addition of new elements) or depleting (with the loss of elements due to custodial neglect, consumptive use, or deaccessioning).

Depletion of a collection is not always easy to detect, as it may occur simultaneously with collection growth, and thus may be masked by the growth. Significant collection depletion has occurred in many museums, particularly in the oldest institutions. Comparisons of museum catalogs from the 1600s and 1700s with specimens surviving in collections today indicate that a significant loss of specimens has occurred in these collections (Impey and MacGregor 1985, Murray 2000, Whitehead 1970, 1971). Most collection depletion is due to the limitations of preservation technology. Many of the standard techniques and chemicals used in collection preparation and care are actually detrimental to the useful life span of the collection elements (Hawks 1990, Williams 1999, Williams and Hawks 1987). This problem is often not recognized because collection deterioration is a much longer-term phenomenon than the working life of a curator. Most processes of collection deterioration are very slow (Rose and Hawks 1995, Waller 1995)—during a working life of 30 or 40 years, a curator may not notice the gradual deterioration of the individual elements of the collection. There is a lack of long-term studies of preservation and deterioration as well as a lack of accelerated aging studies of natural history collections (Duckworth et al. 1993, Hawks 1990, Williams 1999).

The mid-point for the y-axis is defined as the point of *stasis,* at which the collection is neither growing nor depleting (Fig. 1B).

The order axis (x-axis) and growth axis (y-axis) combined present a more complete representation of collection management (Fig. 1C). The location of an element (an object or a specimen) or a set (the collection) can be determined relative to these two axes. A point p (x, y) may represent a single element of the set (C) or the generalized location of the set (C) (Fig. 2). A better representation of the collection results when the set (C) is plotted as a cluster of points, each of which represents an individual element in the collection.

The third function of collection management is depicted on the z-axis, or the *conservation axis,* which describes the state of preservation of the collection elements (Fig. 3A). The mid-point on the z-axis is defined as the condition of a

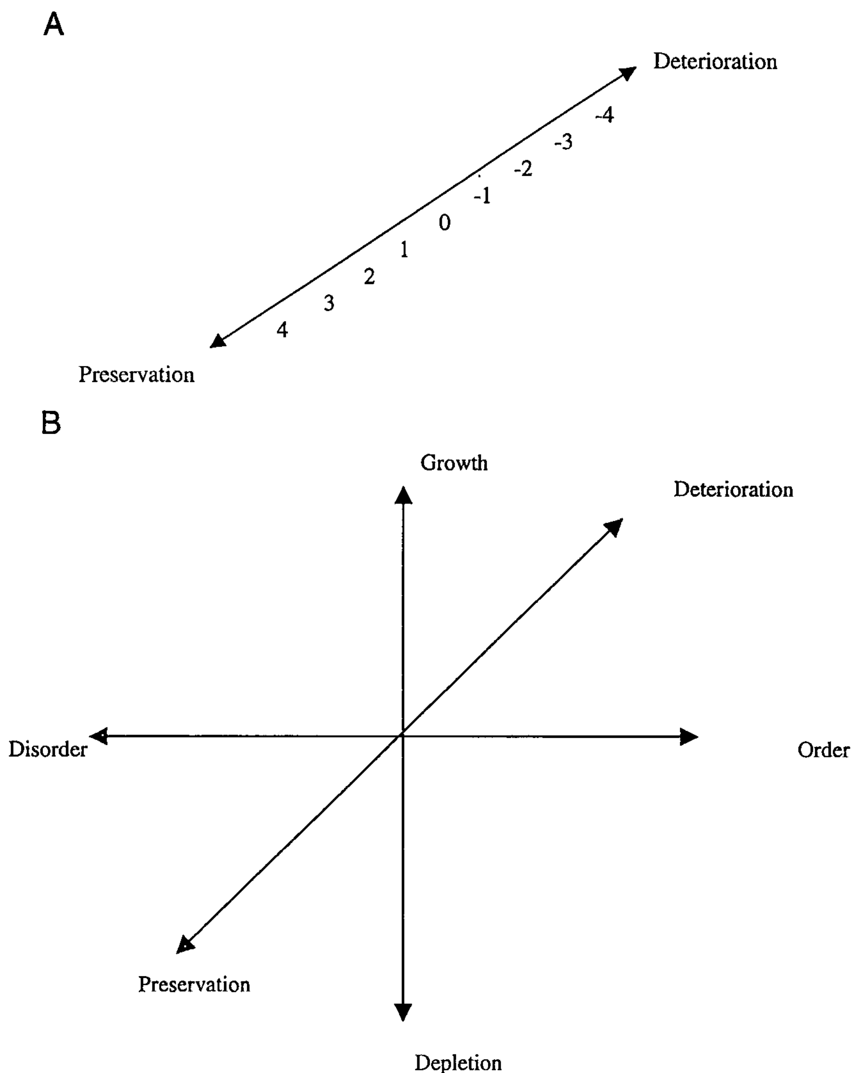


Figure 3. Graphic representation of collection management. A. The conservation axis (z-axis); B. The complete graphic of collection management.

specimen upon its receipt in the collection. Positive conservation reflects any stabilization or improvement in the conservation status of the element (such as rehousing in archival materials). Negative conservation is the result of deterioration of the element from its condition when received (such as a cellulose chain undergoing scission due to acid hydrolysis).

When these three axes are combined, they present a graphical representation of three considerations of collection management: (1) order, (2) growth, and (3) preservation (Fig. 3B). The three axes intersect at their points of stasis, or balance, such that the intersection of the axes is described as p (0, 0, 0). In actuality, the points representing the collection elements are seldom clustered at the intersection of the three axes. Collection elements are always distributed unevenly. When the

collection is graphed as a set of points (each point corresponding to a collection element) forming a three-dimensional cluster in xyz-space, the shape and position of the cluster of elements making up the set (C) describes the collection as a whole.

The Measure of Order and Disorder: Entropy

William Thompson (Lord Kelvin) proposed the concept of entropy when he defined the second law of thermodynamics in 1853 (Kuntz 1968, Lightman 2000). The word *entropy* was introduced in 1865 by Rudolph Clausius (Lightman 2000). Entropy (S) is a measure of change (d), for example, the tendency of a closed system to move from a complex state to a more simple state. Entropy may be a reversible change (e) with an input of energy into a system from the outside (d_eS), as when the efforts of a collection care worker restores order to disordered collection elements; or an irreversible change (i) which occurs without an input of energy from the outside (d_iS), such as the deterioration of a fossil from pyrite disease. A reversible change in entropy is a *non-spontaneous change* (pushed by an outside force); irreversible entropy is a *spontaneous change* (Prigogine 1994). Systems of the reversible (d_eS) type can either increase or decrease in entropy (Lightman 2000), depending on the input of energy from outside the immediate entropy system. Left to itself, any spontaneous natural process proceeds in only one direction, driven by an increase in entropy, thus, a spontaneous change in nature, such as the deterioration of an organic compound, results in a change to a state of greater disorder (greater entropy).

In information theory, "... order is described as the carrier of information because information is defined as the opposite of entropy, and entropy is a measure of disorder. To transmit information means to induce order" (Arnheim 1971: 15). In considering collection management, we may derive a corollary from this concept—the facility with which information that can be obtained from a collection is inversely correlated to the degree of disorder (entropy) in the collection. Furthermore, we know that entropy is a function (f) of the number of different ways (m) in which an ordered state can be realized (Fast 1962). Thus, entropy may be quantified as $S = f(m)$. There are many ways to order a collection, but not all of them are equally useful. The preferred method for ordering the collection is the way that produces the value for S closest to zero. This is the order that allows the elements to be retrieved from and returned to their cells with the lowest investment of resources. Minimizing or maximizing the complexity of order (such that $S < 0$ or $S > 0$) decreases the information that can be obtained from the set (the collection). Maintaining proper order (such that $S \approx 0$) increases the information obtainable from the set.

Entropy is also a probability statement about a system, because the entropy of a system increases as the number of possible states increases (Lightman 2000). In the case of collections, the number of possible states (m) may be equal to the number of cells in which the collection elements may be located, or the number of ways in which the specimens may be arranged within those cells. Entropy thus increases with collection size (the more cells there are in a collection, the more elements there are to be displaced from their cells, and the more possible incorrect arrangements of the elements in the cells), such that larger collections have more entropy than smaller collections. Probability theory predicts that the amount of

resources (energy) necessary to reduce the rate of entropy increases geometrically as collection size increases arithmetically, an important consideration for the allocation of collection care resources.

A collection contains many elements. Each individual element may start to increase in entropy at random, and continue until it is eventually deaccessioned from the collection. As stated by Waller, "the state of disorder in a collection is a summation of a bunch of little disorders in the collection" (R. Waller, pers. comm. 1987). To evaluate the status of a collection, it is necessary to consider the degree of disorder of all of the elements of the set. This can be done by determining the placement of the individual elements of a collection in xyz-space. Evaluation of the location and shape of the cluster of collection elements allows predictive statements to be derived about the status and the future of the collection, serves as a guide in setting collection care goals, and helps determine the costs of collection management. For example, in an irreversible (d_iS) system (e.g., organic specimens), the inevitable increase in entropy means that the shape of the collection cluster will, over time, inexorably drift toward negative values on the z-axis. The goal of collection management is to slow this transition as much as possible, while preventing the collection cluster from entering the negative spaces of the x-axis and y-axis. In this model of collection management, the individual elements (1) will inevitably and irreversibly drift into the negative region of the z-axis; and (2) will be subject to drift into the negative regions of the x-axis and y-axis in a dynamic, but reversible fashion.

Much of the *cost of management* of a collection is the cost of reducing the rate of entropy in the collection. It is probable that "zero" entropy is a price that no collection can afford. The lowest possible entropy requires the highest costs to achieve. In fact, some amount of entropy indicates that the collection is thriving, or is being used. Considering these aspects, the question becomes this: what is an acceptable level of entropy in a collection? An acceptable level of entropy is when (1) there is order in the collection (which is to say, each element has its own cell); (2) growth does not exceed the ability to assign elements to cells; (3) the loss of elements is minimized; (4) each element can be found with a minimum of effort; (5) no single element is displaced from its cell for a prolonged period of time; and (6) element deterioration is slowed as much as possible. In a well-managed collection, the cluster of elements of the set in xyz-space rises and falls about the x-axis and y-axis, while remaining relatively stable about the z-axis. A certain level of order can be maintained in a system over the long term, yet some amount of entropy will always be present. The objective is to maintain a manageable level of entropy, indicating that the collection is used but that this use does not adversely affect the order of the elements, the preservation of the elements, or the growth of the collection. However, given an acceptable level of entropy in a collection, the cost of controlling entropy increases with the growth of the collection because $S = f(m)$.

Control of the Rate of Entropy

Some changes in entropy can be prevented (d_eS), others can only be slowed (d_iS). In either case, it is important to understand the forces of disorder and deterioration that affect collections. In preventive conservation theory, these have been grouped into categories as the *agents of deterioration*: direct physical forces,

theft, vandalism and displacement, curatorial neglect, fire, water, pests, pollutants and contaminants, light and radiation, incorrect temperature, and incorrect relative humidity (Costain 1994, Michalski 1994a, Rose and Hawks 1995, Waller 1995).

Direct physical forces.—These are cumulative or catastrophic forces of irreversible entropy (d_iS). Waller (1995) delineated a series of states of these physical forces acting on collections, characterizing them as mild/gradual, severe or catastrophic. He characterized their occurrence as constant, sporadic, or rare.

Theft, vandalism, displacement, and curatorial neglect.—These are human activities that affect collections by disordering and damaging specimens. Because these forces are theoretically avoidable with proper allocation of resources, they are characterized as reversible (d_eS) entropy.

Fire.—Fire causes fundamental, irreversible (d_iS) chemical and physical changes in specimens or objects that are heated or burned. Fire has secondary effects in that collection elements that are not burned may still need to be cleaned because of their exposure to the fire, or they may have been damaged by the fire suppression system.

Water.—Exposure to water produces both reversible (d_eS) and irreversible (d_iS) dimensional changes and other damage in most natural history collection elements.

Pests.—With the use of integrated pest management, pests should be a controllable (d_eS) system. The damage caused by pests, however, is permanent (d_iS).

Pollutants and contaminants.—These are the results of changes in entropy in other systems that, in turn, affect the collections. These changes may be reversible (d_eS) (e.g., inert dust) or irreversible (d_iS) (e.g., sulfur dioxide, SO_2). Most contaminants produce permanent changes in collections.

Light and radiation.—All forms of damaging radiation are cumulative in specimens and therefore are irreversible (d_iS) factors. Most radiation damage is preventable (e.g., by blocking ultraviolet exposure).

Incorrect temperature.—Temperature may be incorrect if it is too high, if it is too low, and when it fluctuates excessively. Heat generally accelerates chemical processes (an increase in temperature means an increase in molecular movement); heat and cold both cause dimensional changes in the structure of specimens and objects, which result in stress damage. Incorrect temperatures are an expression of irreversible (d_iS) entropy.

Incorrect relative humidity.—Relative humidity is incorrect if it is too high, if it is too low, and when it fluctuates excessively. Incorrect relative humidity may be expressed as either irreversible (d_iS) or reversible (d_eS) entropy.

Entropy and Collection Storage

Collection elements react continuously with even very small changes in their environment. All collection elements (and the media used in documentation) are susceptible to deterioration over time; this deterioration will be accelerated if collection storage presents incorrect environmental conditions. Preventive conservation theory predicts that the best storage environment for collections and documentation is the most stable storage environment (within an appropriate range of temperature and relative humidity). Preventive conservation theory also predicts that it is most cost-effective to achieve a correct storage environment if the same type of materials are stored in the same storage area—for example, dry

preparations in one storage environment, specimens in fluid in another. This is generally referred to as like-with-like storage.

Entropy and the Theory of Enclosures

It is easier to control collection storage conditions at the microenvironment level than the macroenvironment level (Weintraub and Wolf 1995), and microenvironments are easier to control using the principles of the theory of enclosures. The theory of enclosures was derived as a theoretical model by Michalski (1994b), based on leakage rates, and further developed by Rose and Hawks (1995) and Waller (1995). The basis of the theory is that the more enclosures that are provided around a collection element, the lower the rate of environmental (air) interchange, therefore the more stable the internal environmental conditions and the better protected the element is from the agents of deterioration. According to the theory of enclosures, each container that encloses an element (an element in a box, the box in a drawer, the drawer inside a cabinet, which is inside a room, inside a building) forms a protective barrier around the specimen. The better the integrity of each enclosure, the better the protection of the specimen from the agents of deterioration (including fluctuations in the storage environment). The theory predicts that enclosed storage furniture provides significantly more protection for collections than open storage furniture, thus in terms of collection care resource allocation, it is usually better to use an inadequate enclosure than no enclosure at all. For example, it is better to put curtains on shelving than to use open shelving. In general, some enclosure is almost always better than none. According to Michalski (1994b), leakage from an enclosure may be expressed as mass flow, volume flow, or exchange rate. Michalski concluded that "exchange rate best represents the notion of leakage as an inherent quality of an enclosure" (1994b:170). Leakage from enclosures is a measure of the change in entropy, and thus reflects the effectiveness of the storage system. An obvious corollary is that collection care resources should be directed towards improving enclosures beginning with those that enclose the most collection elements.

A small effort, such as the erection of a barrier to block an agent of deterioration, may significantly slow the rate of entropy because the progress of entropy is an energy function (R. Waller, pers. comm. 1987). The better the barrier, the more entropy can be slowed. In collection management, good collection handling procedures themselves can be good barriers. The establishment of a barrier requires an input of directed energy, but the amount of energy required may be very small compared to the resultant reduction in entropy. For example, the closure may be replaced on a leaking container of fluid preserved specimens, or the lighting on a taxidermy mount may be equipped with an ultraviolet filter. Undirected (random) energy will not result in the slowing of entropy, but rather in its increase. Thus, undirected or random energy inputs (such as elevated collection storage temperatures, or fire in the collection) should be avoided; but directed energy should be focused on establishing barriers to the increase of entropy in the collection.

Assigning Values to Measures of Entropy

When the individual elements of a collection are assigned coordinates in xyz-space, the resultant shape that is formed and its location describes the collection,

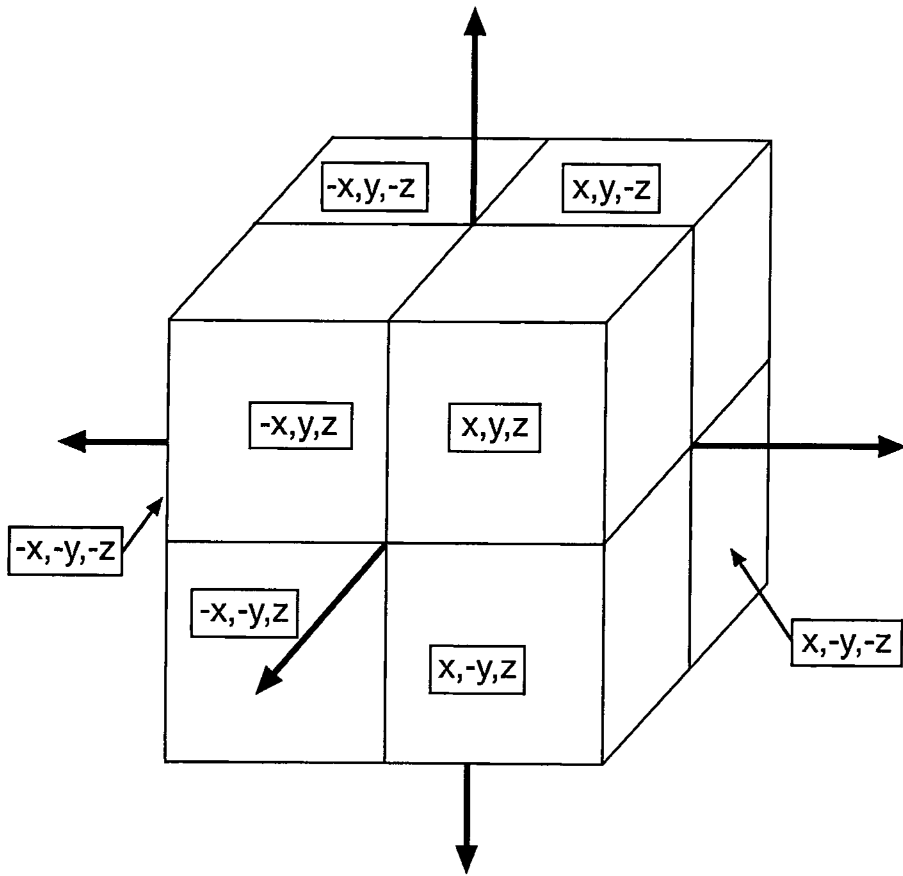


Figure 4. Octant characteristics of x,y,z-space: (octant) (x, y, z), (characteristics) order, growth, preservation; (x, −y, z), order, depletion, preservation; (x, −y, −z), order, depletion, deterioration; (x, y, −z), order, growth, deterioration; (−x, y, −z), disorder, growth, deterioration; (−x, y, z), disorder, growth, preservation; (−x, −y, z), disorder, depletion, preservation; (−x, −y, −z), disorder, depletion, deterioration.

enables predictions to be made concerning the future status of the collection, and provides a basis for decisions regarding the most effective allocation of collection care resources. Values on the three axes can be assigned to collection elements either on an individual basis, a relative basis, or a combination of the two. For example, values on the x-axis (order) could be either an actual count of the number of elements not in their cells, or a relative number expressing the degree of disorder in the collection relative to the degree of order. Values on the y-axis (growth) could be the actual count of elements lost and added to the collection, or a number expressing the ratio of elements lost to elements added. Values on the z-axis (conservation) could be the actual count of elements deteriorating or stabilized, or a number that reflects the relative deterioration or stabilization of elements of the collection. The position of the collection elements in xyz-space can be interpreted in conjunction with other collection assessment methods such as the Collection Health Index (McGinley 1993, Williams et al. 1996). In general terms, the characteristics of each octant of xyz-space are shown in Figure 4.

Conclusions

All natural history collections are managed—some well, some poorly, some by design, others by neglect. Collection management was once seen as a fairly simple concept—bring order to chaos, and then maintain the order. Because of this perception, who is in charge of the collections is frequently the result of historical accident rather than design (Simmons 1993). With the growth in size and complexity of natural history collections, and the tremendous increase in our knowledge of how to care for these collections, the current trend in natural history museums is to employ professional collection managers. In the past, the collections were under the direct care of researchers usually titled "curator" (Ford and Simmons 1997). However, scientists do not have the time or the specialized technical knowledge to care for collections. The body of knowledge necessary to care for a collection—preventive conservation, materials science, storage environment, integrated pest management, collection management policies—is outside of the training and knowledge base of traditional curators (Simmons 1993). It is not reasonable to expect researchers to manage and care for collections. If collections are to be preserved for future use, the care and management of the collections has to be carried out at a professional level by persons trained in the theory and techniques of collection management to maintain an equilibrium between current use and preservation for future use (Cato et al. 1996, Williams and Cato 1995). We must apply our knowledge of entropy, enclosure theory, and preventive conservation theory to direct the investment of resources to maximize the effectiveness of collection care.

Acknowledgments

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USE OF TEMPERATURE TO CONTROL AN INFESTATION OF BISCUIT OR DRUGSTORE BEETLE *STEGOBIUM PANICEUM* (L.) (COLEOPTERA: ANOBIDAE) IN A LARGE ECONOMIC BOTANY COLLECTION

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Abstract.—An infestation of biscuit or drugstore beetle, *Stegobium paniceum*, was discovered in the Economic Botany Collection at Kew. Large numbers of adult insects were found in the collection and a wide range of specimens were infested in one area. Severely attacked specimens were starch-rich, such as *Manihot*, and many other roots, fruits and seeds. The options for control of *Stegobium* included fumigation and freezing. Because the collection storage area was air conditioned, we decided to lower the temperature to 17°C to prevent the insects breeding. Beetle numbers immediately dropped, but the following year there was a further problem with emerging adults. An additional chiller unit was installed that enabled the temperature to be reduced to 13°C. The population of insects again declined and, apart from isolated occurrences in enclosed jars, no live adults have been seen. Insect traps have been used to monitor wandering adults and attractant lures were effective in increasing trap catch when temperatures were above 17°C. The *Stegobium* infestation was successfully controlled for four years without the high labour costs that would have been incurred by moving objects for treatment. The collection is currently safe from attack as long as temperatures remain low.

The Economic Botany Collections were begun by William Hooker in 1847. Today the collections include more than 32,000 timber samples and over 44,000 specimens of economic plants, spanning the whole spectrum of uses, including; food, forage, fuel, fibres, medicines, poisons, dyes, gums, and resins. All specimens are now stored in plant family order in an open shelf, compactor system, in an air-conditioned, purpose-built storage space in the Sir Joseph Banks Building, completed in 1988. Items were computer catalogued, repackaged, then removed systematically from the old museum buildings, and from the date of completion of the Banks Building were gradually unloaded into the racking system in the storage space. The move was completed in 1994.

During the cataloguing of the Collections prior to their installation in the Banks Building, several isolated specimens were found to contain large numbers of dead biscuit beetles (*Stegobium paniceum*). These items such as sesame seeds (*Sesamum orientale*) and root starch of cassava (*Manihot esculenta*), were discarded or frozen before storage in the Banks Building. In the new storage space, the first appearance of live biscuit beetles was noted in summer 1993, but adult beetles first appeared in large numbers in summer 1994.

Biscuit beetles are found in many countries where they are pests of a wide range of food stuffs including cereals, biscuits, dried meat, beans and seeds (Aitkin 1975). It is also called the drugstore beetle because of its ability to breed in substances such as paprika, turmeric and other drugs and spices, which are poisonous to other species. This ability to breed in dried plant material has made it a particular problem in herbarium collections where it can cause serious damage to dried plant specimens (Croat 1978). More recently, there have been problems

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with *Stegobium* attacking freeze-dried animal specimens (Carter and Walker 1999). Low numbers of biscuit beetles are regularly found on traps in the main herbarium at Kew (Harvey 2001).

The length of the life cycle is determined by the nutritional value of the food and the ambient temperature. *Stegobium* is more tolerant of low humidities than many other pests and will survive in food with a moisture content of 10 percent. Lefkovitch (1967) stated that given a high carbohydrate diet, the life cycle can be completed in as little as six weeks at 30°C. At the lower limit of development of 17.5°C, it may take a year to complete the cycle. At all the temperatures studied, humidities above 50 percent relative humidity reduced mortality and encouraged faster development.

Many stored-product insects, such as biscuit beetle, which have a relatively short adult life, produce pheromones to attract mates. The main component of the pheromone produced by *Stegobium* females is stegobinone, 2,3-dihydro-2,3,5-trimethyl-6-(1′-methyl-2′-oxobutyl)-4H-pyran-4-one (Kuwahara et al. 1975). A further component called stegobiol [2,3-dihydro-2,3,5-trimethyl-6-(1′-methyl-2′-hydroxybutyl)-4H-pyran-4-one] was identified by Kodama et al. (1987). These two chemicals are extremely difficult to synthesize, because they each exist in eight stereoisomers, only one of which is attractive to insects. Because of this difficulty, a biscuit beetle lure was produced by Fuji Flavours based on 2,3-dihydro-2,3,5-trimethyl-6(1′-methyl-2′-butenyl)-4H-pyran-4-one, a mimic called Stegobiene and used in the Fuji 87 traps. The furniture beetle, *Anobium punctatum,* also produces stegobinone (White and Birch 1987). The pure pheromone was successfully synthesised in quantity in 1996 and is used in the AgriSense Anobid trap. There is evidence that these Anobid lures will also attract *Stegobium* males (Pinniger et al. 1998).

Distribution and Monitoring of Infestation

The pattern of infestation and the responses to it are complex. Appendix 1 summarises the chronology of the infestation and the action taken. Adult biscuit beetles first appeared in large numbers in the new store during the summer 1994. This was due to the introduction into the building of some untreated wheat samples mounted on large cardboard sheets, which were later found to be heavily infested. The beetles became very common and large numbers were found near the windows along the balcony of the collections space. Spot checks of the collection on the shelves revealed isolated infestations in a very wide range of specimens, easily identified by bore holes left by the damaging larval stages and adult *Stegobium.*

The store room was fitted with air conditioning that enabled the temperature to be controlled to within 1°C of the set point. However, it had been set to run at about 20°C which was not sufficiently low to prevent breeding by *Stegobium* (Lefkovitch 1967). We therefore decided to run the storeroom at a temperature of 17°–18°C for one year. This temperature was not sufficiently low to kill adult beetles or larvae but should have prevented them from breeding or completing their life cycle. Monitoring of temperature and relative humidity began in October 1994. An additional chiller unit was installed in July 1995 to further reduce temperatures to 13°C.

Fuji 87 adhesive traps were placed in each run of shelves to monitor wandering

Stegobium adults. Ten traps were with Stegobiene lures and ten without to assess the performance of the lures. These were found to be helpful indicators of beetle activity and so after the first year, an additional 20 Fuji traps with lures were used. To control any wandering insects, the floor of the storage space was sprayed with the encapsulated formulation of the insecticide chlorpyriphos (Empire 20) in October 1995. Floors, shelves and cupboard entrances were cleaned regularly to remove insect corpses. In addition, a freezer was installed in the curation bay to treat all infested items and all incoming specimens, the procedure being to bag specimens and expose them for 72 hr at −30°C. This follows the guideline of the lethal boundary proposed by Strang (1992) of three days at −30°C. A longer exposure is required if the object to be frozen is protected by layers of insulating material.

Observations

Appendix 2 lists the plant species that have been attacked by *Stegobium*. The collection is arranged on the shelves according to the system of Bentham and Hooker (1876) and therefore consecutive Family numbers are in very close proximity. Specimens particularly severely attacked were starch-rich samples such as cassava (*Manihot*), and many root samples such as; rhubarb (*Rheum*), buckwheat (*Fagopyrum*), mandrake (*Mandragora*) and *Ferula*. Also attacked were fruits of chestnut (*Castanea*), poppy (*Papaver*) and nutmeg (*Myristica*). Seeds of castor oil (*Ricinus*) and other poisonous plants such as monkhood (*Aconitum*) were damaged. *Stegobium* is known to attack the poisonous roots of *Strychnos* and *Atropa belladonna* and, although the *Strychnos* in the collections has not been attacked, some insects were found in the *Atropa*. Inflorescences of safflower (*Carthamus*) and pitcher plant (*Nepenthes*) were damaged and samples of *Salvia, Lonicera, Eryngium,* and some starchy Gramineae were also infested. Specimens on top, middle and lower shelves were all affected. The only case of apparent cross-infestation was in chestnut (*Castanea sativa*) and castor oil (*Ricinus communis*) where the compactor units are face to face when the system is closed.

Almost immediately after the temperature was lowered, the number of beetles found dwindled and then they stopped appearing altogether. Monitoring continued and after the first few weeks, when a total of 12 beetles were trapped on ten pheromone traps, and three on ten non-pheromone traps, no more were found. Isolated infested items discovered during routine checks in the following months were frozen, although the size of the collections and lack of manpower limited the scope of these checks.

The temperature remained between 13° and 17°C day and night between October 1994 and October 1995 apart from one day when the temperature reached 18–19°C due to an undiagnosed fault. Control of relative humidity was more problematic with occasional peaks of 75 percent but with a general level of around 50 to 60 percent.

In April 1995, a new outbreak occurred with new adult beetles emerging. Two pheromone traps in the Gramineae (general grasses section) and *Gentiana* caught beetles: ten on one trap and six on the other. Non-baited traps in the vicinity were empty. Near each trap a severely infested item was found, in one case on the next shelf up, in the other two shelves below the trap. The adult beetles were much

more sluggish than in 1994, and they were found in significant numbers on the floor of the building and on the lower walls, but not near the windows.

Preliminary results from 20 additional traps with lures over one weekend showed that most traps were empty but five beetles were trapped on each of two traps, indicating a nearby problem. *Stillingia* roots (Euphorbiaceae) in two boxes beside one trap were heavily infested and the other trap helped to locate a problem in *Vinca* roots (Apocynaceae) on the opposite shelves. Insects were also found on the shelf where there were no traps, near *Angelica* (Umbelliferae).

The installation of the additional chiller unit was successful in reducing the temperature to about 14°C in the summer and 12 to 13°C in the winter. There were a few occasions when it rose to 15°C and one day it rose to 17°C when there was a fault in the system. This further cooling of the storeroom produced an immediate decrease in the numbers of insects found. Relative humidity continued at around 60 percent RH and it was discovered that the system could not cope with lowering temperature and humidity at the same time. Regular checks were made to ensure that there were no localised higher humidities in dead spaces on shelves, which would encourage mould growth.

Since October 1995, when exposed areas of the floor of the storeroom was sprayed with the encapsulated formulation of the insecticide chlorpyriphos (Empire 20), no insects have been found near the light sources in the room, nor have they been found on the walls or floors.

One or two adult carpet beetles, *Anthrenus verbasci,* were found by the windows and cast larval skins of *Anthrenus* on the floor. There were numbers of spiders and larger ground beetles (Carabidae) which had come into the building, and the carpet beetle larvae were probably feeding on the corpses of these as *Anthrenus* have not, to date, been found anywhere near specimens. Removal of insect bodies and the treatment with chlorpyriphos have controlled the *Anthrenus* infestation.

Only on two occasions in 1996 were live beetles found and these were in enclosed boxes where large numbers of beetles had been breeding. A large number of dead beetles and two very sluggish live adults were found in closed containers of *Artocarpus* sp. (Moraceae), root starch and also in *Taraxacum* sp. (Compositae). The dead beetles were removed, specimens frozen to −30°C for 72 hours, and a few very damaged specimens de-accessioned and discarded.

No live beetles were found on traps or shelves in 1997. The air conditioning system maintained very low temperatures of 11 to 12°C in the latter months of 1996, but there were repeated problems with RH control, which rose from around 55 percent, to about 65 percent and occasionally higher.

When the positions of the infestations were marked on a plan of the storage units, it clearly showed that the problem was concentrated almost exclusively at one end of the compactor shelving system. The heaviest infestations occurred mainly in two rows near the divide in the shelving. This is also shown by the Family numbers of the infested specimens listed in Appendix 2. A re-investigation of those species where problems had been found in the past was carried out in February 1997, and some severe local infestations were discovered in seeds of *Chenopodium quinoa* and *Plantago ovata.* The affected specimens were all in secured jars, while boxed specimens on the shelves nearby were all clear.

Low temperatures have been maintained in the storeroom and no live insects

were found in 1998. The only live *Stegobium* found in the entire storage facility in 1999 were in a sample of Chinese biscuits. No live beetles were found in 2000.

Discussion

In the past, a number of approaches have been tried in different herbaria. Merrill (1948) described an innovative technique of using sacrificial bundles of plant specimens distributed around the herbarium. These were used to lure insects that would then be killed by fumigating the specimens. This method was deemed successful when combined with the use of large quantities of naphthalene and paradichlorobenzene as repellents. Croat (1978) summarises the two basic approaches to pest control—establishing "sterile entry" and "permanent fumigation." Many of the fumigant chemicals formerly used, such as carbon disulphide and carbon tetrachloride, are no longer used because of the hazards caused by their toxicity. Hall (1988) describes the use of freezing to kill incoming pests and also the need to control infestation sources both inside and outside the herbarium. The use of microwaves to disinfest specimens is discussed but this is "not entirely without risk to specimens." Strang (1999) also considers that microwave heating can cause locally high temperatures which may cause damage to specimens and that convection heating is preferable. Hall (1988) also reviews the safety and use of a wide range of residual insecticides and fumigant gases with useful comments on their hazards and effectiveness in a herbarium context. His final section on "Integrated control" is one of the first publications to list for herbaria the need to consider trapping, environmental conditions, exclusion, quarantine and, building and cabinet design. Strang (1999) continues this theme with a very useful assessment of the priorities for Integrated Pest Management (IPM) and guidelines for implementing IPM in herbaria.

Many museums and herbaria now use low temperatures of −18°C to −32°C to control pests in specimens (Carter and Walker 1999), but few have considered lowering the temperature of the storage facility. The novel approach of using very low ambient winter temperatures experienced in Wisconsin, of −18°C and below, to kill infestation in a herbarium is described by Miller and Rajer (1994). Although effective, the timing of treatment with this method is not predictable and it can only be applied in certain years in countries with very low winter temperatures. Barringer (1999) gives one of the few published accounts of using low temperatures as part of a preventive strategy for preventing infestation in the Brooklyn Botanic Garden Herbarium. This herbarium, opened in 1991, was designed with heating, ventilation and air conditioning capable of running the facility at 15° to 18°C. The author concludes that treatment of previously infested collections and the controlled environment in the collections area eliminated most insect pests in just over two years.

The history of the *Stegobium* infestation in the Economic Botany Collection and the choice and efficacy of the action taken can serve as a useful example of the modern approach to integrated pest management. The initial infestation probably could have been eliminated and prevented from persisting in the new storage facility by treating all specimens at −30°C. However, the logistics of handling and cost of treating all objects would have been prohibitive. The discovery of infestation initiated action to determine the extent of the problem and to assess the available control options. The results of the trapping programme showed that

the traps were effective in catching *Stegobium* when temperatures were above 18°C. Once the temperatures in the storeroom dropped to below 15°C, the activity of the adult beetles was so reduced that they were unlikely to be wandering and caught on the sticky traps. Similarly, the attractant lures appeared to be effective at higher temperatures but were not so attractive when the insects were less mobile.

The use of methyl bromide gas to fumigate the entire storage building was considered but the combined factors of chemical contamination of objects, staff risk, and cost ruled out this option. Carbon dioxide could have been used to treat batches of infested material in separate enclosures but it was not possible to use this gas to treat the collection *in situ* in the storage facility. When published information on the temperature limits on the life history of *Stegobium* were examined, it seemed as if a temperature of 17°C would be sufficient to prevent completion of the life cycle. We therefore decided to adopt a regime of dropping the storage facility temperature to this level. The reduction in temperature to 15–17°C had an immediate effect on the population and the level of activity crashed. A local emergence of adult beetles in 1995 probably represented the final delayed development from those larvae which were already a substantial size in the autumn of 1994 when the temperature was first lowered. However, it became clear that although the adults were rapidly dying, a significant number of larvae were still surviving in some of the containers. A re-examination of published information (Lefkovitch 1967) showed that some of the data were misleading and that there was evidence that some larvae could survive for long periods at 17°C. We therefore decided to lower the temperature in the store to 12 to 14°C and this has undoubtedly caused a substantial reduction in the population to very low levels. Despite the low temperatures, a few surviving larvae have still been found in some specimens. It is possible that some beetles were inadvertantly put into the jars when the specimens were transferred in 1994 and the developing infestation may have built up its own microclimate within the jar producing a higher temperature and humidity than the surrounding environment. This theory was reinforced by the discovery of a few live larvae in the *Plantago*. Subsequent incubation of these jars at normal room temperatures of about 20°C produced an emergence of adult beetles a few months later. It is not known whether these adults were able to mate and lay eggs to continue the infestation cycle.

The unexpected survival may also indicate that some the published information (Lefkovitch 1967) on development of *Stegobium* may be incorrect. This is perhaps not so surprising when one realises that all the published information is based upon studies of a strain of *Stegobium* that had been kept at a constant 25°C in laboratory culture for many years. A wild strain, such as that found at Kew, which has been subjected to a less benign environment, may well be far more tolerant of cold conditions (Solomon and Adamson 1955).

Our records of which plant species were attacked, confirm that *Stegobium* can feed on a very wide range of species. Some of these contain chemicals such as alkaloids and strychnine that would be poisonous to many other insect pests. It also confirms that the most successful infestations and the greatest damage were to plants with a high starch content such as *Manihot*.

One problem which was encountered was the difficulty of keeping relative humidity at a safe level. The air handling equipment was not originally designed

to run the environment at such low temperatures and occasional peaks above 70 percent gave cause for concern that mould might develop on unprotected objects. Regular checks were made of unbagged objects in areas where they might be exposed to damp dead spaces on shelves. The environmental conditions were checked with portable thermohygromers but it would be better if permanent data logging points could be installed to provide a continuous record.

The overall conclusion of the case study is that the manipulation of temperature has successfully controlled the *Stegobium* infestation and prevented further significant damage to the Economic Botany Collections. This has been achieved without the high labour costs that would have been incurred by moving objects for a mass off-site treatment. It has also been achieved without any chemical contamination of the collection and without risk to staff. Had the collection been treated with methyl bromide, carbon dioxide, or freezing, it may well have killed all the insects in the objects which were treated but would have conferred no protection against re-infestation from insects once they were returned to the storage facility. The low temperature regime has resulted in additional costs caused by the increased power consumption and there is also some inconvenience to staff who have to work in an uncomfortably cool environment. The advantages and disadvantages of such a technique have to be carefully considered depending upon priorities, budgets and environment. We are confident that the economic botany collection is currently safe from attack by *Stegobium* and will remain safe as long as temperatures are maintained at this low level.

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APPENDIX 1. CHRONOLOGY OF DISCOVERY OF INFESTATION AND THE ACTION TAKEN

Date	Event
August 1993	Adult *Stegobium* beetles first found by windows in the storeroom.
July 1994	Large numbers of adults are found by windows and on walls. Spot checks revealed some heavily infested specimens in one area, all specimens with a starch/poison content. A few adult *Anthrenus* carpet beetles are found near windows (none found in botanical specimens).
October 1994	Temperature of the storeroom was reduced to 16 to 17°C and monitoring started on a daily basis. Humidity levels rose to about 60 percent RH. Regular cleaning of the storeroom started to clear out debris or dead insects on which pests might feed.
October 1994	Fuji 87 traps are placed in the storeroom, some with attractant lures. Large numbers of adult *Stegobium* are found on shelves. Beetles are more widespread, some very heavy infestations found in specimens.
April 1995	A new outbreak of *Stegobium* detected in Gramineae and *Gentiana.*
July 1995	Traps are placed at regular intervals in shelves. Tiny tag monitors are placed in four specimen containers to check localized temperatures and to see if microclimates were created.
July 1995	Recommendations revised to lower average temperatures to 11 to 13°C. An additional chiller unit is installed to keep temperature at this level. Humidity still high at 60 percent RH.
August 1995	Vast drop in numbers of insects and activity is observed. No catches in traps and no wandering adults near windows. Some isolated discoveries of live beetles in sealed jars.
October 1995	Storeroom floor is sprayed with encapsulated chlorpyriphos (Empire 20).
July 1996	Attractant lures are renewed in Fuji traps. No *Stegobium* are caught. Two live adults and a few live larvae are found in boxed specimens.
February 1997	A localised *Stegobium* infestation found in *Plantago* and *Chenopodium.*
May 1997	No pests caught on traps.
May 1998	No pests found.
May 1999	No insects in traps, a few live beetles found in Chinese biscuits.
May 2000	No pests found.

Appendix 2. Species in the collection that have been attacked by *Stegobium.*

Family number[1]	Family	Species	Affected parts
1.01	Ranunculaceae	*Aconitum* sp.	roots
4.01	Magnoliaceae	*Magnolia* sp.	fruits
6.00	Menispermaceae	*Jateorhiza palmata*	roots
7.01	Berberidaceae	*Berberis* sp.	fruits
10.00	Papaveraceae	*Papaver somniferum*	fruits
70.02	Trapaceae	*Trapa* sp.	fruits
80.00	Umbelliferae	*Angelica ursina*	roots
		Conium maculatum	seeds
		Eryngium sp.	roots
		Ferula sp.	roots
83.01	Caprifoliaceae	*Lonicera* sp.	roots
88.00	Compositae	*Arctium majus*	compressed leaves
		Carthamnus tinctorius	inflorescences
		Taraxacum sp.	roots
106.00	Apocynaceae	*Vinca major*	roots
109.01	Gentianaceae	*Gentiana lutea*	roots
114.00	Solanaceae	*Atropa belladonna*	roots
		Lycium chinense	fruits
		Mandragora officinalis	roots
125.01	Verbenaceae	*Lippia deserticola*	herb
		Vitex sp.	fruits
126.00	Labiatae	*Collinsonia canadensis*	roots
		Mentha piperata	compressed leaves
		Salvia pratensis	roots
		Scutellaria baicalensis	roots
127.00	Plantaginaceae	*Plantago ovata*	seeds
		Plantago palmata	roots
131.01	Chenopodiaceae	*Beta vulgaris*	root pulp
		Chenopodium quinoa	seeds
134.00	Polygonaceae	*Fagopyrum esculentum*	roots
		Fagopyrum tataricum	roots
		Polygonum tinctorium	roots
		Rheum officinale	roots
		Rheum palmatum	roots
		Rheum tataricum	roots
		Rumex vesicarius	roots
136.00	Nepenthaceae	*Nepenthes* sp.	inflorescence
141.00	Myristicaceae	*Myristica fragrans*	fruits
147.00	Eleagnaceae	*Eleagnus* sp.	fruits
151.01	Euphorbiacae	*Manihot esculenta*	roots
		Manihot utilissima	roots
		Ricinodendron sp.	seeds
		Ricinus communis	seeds
		Stillingia sylvatica	roots
153.05	Moraceae	*Artocarpus communis*	fruits
		Treculia africana	seeds
159.03	Fagaceae	*Castanea sativa*	fruits
191.00	Araceae	*Symplocarpus foetidus*	roots
200.00	Gramineae	Undetermined	seed cake

[1] System devised by Bentham and Hooker (1876).

OH NO! ETHNOBOTANY. THE SAFE HANDLING AND STORAGE OF HAZARDOUS ETHNOBOTANICAL ARTIFACTS

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Abstract.—*Oh No! Ethnobotany,* a hazard communication training program that addresses health and safety issues inherent in the handling and storage of hazardous ethnobotanical artifacts, was designed, developed and prototyped at the Science Museum of Minnesota. The program looks beyond the wide range of residual toxic chemicals present from the treatment of an ethnobotanical artifact to specifically address concerns raised by toxic chemicals inherent in the object itself. This paper provides an outline of the *Oh No! Ethnobotany* program, including a list of hazardous ethnobotanical artifacts present in collections. It includes a description of policies and procedures for hazard communication, labels and other forms of warning, accessibility of information, ethnobotany material safety data sheets (EMSDS) and training.

INTRODUCTION

Collections management technicians literally uncovered health and safety concerns raised by the storage and handling of hazardous ethnobotanical artifacts during the unpacking phase of the 1999 collections move at the Science Museum of Minnesota. After unpacking the South American collection, Kubiatowicz complained of uncharacteristic cognitive dysfunction that included forgetfulness and minor misjudgment. The temporary inconvenience and distraction of being "slightly off" was problematic enough to warrant concern and further investigation into its probable causes. Aside from the certainty of residual toxic chemicals applied to the artifacts to help preserve them, the question of possible toxic chemicals produced by the object itself was raised.

Given the nature of the South American collection, including various roots, vines, seeds, curare-tipped darts and other artifacts made from unknown plant materials, Kubiatowicz suspected that she had been exposed to hazardous ethnobotanical material. Artifacts at the Science Museum of Minnesota are identified in storage with an accession number and may or may not be tagged with a culture group or other identifying information. For example, some curare-tipped darts and arrows are clearly identified with a warning, yet many with visually notable residues are not. Identifying information is found in accession files located in the records room or on the collections database (ARGUS). The lack of identifying information in storage is problematic for both staff and visiting researchers, who cannot be expected to identify phytochemical and toxicological properties based only on visual inspection and accession number.

We performed a drawer-by-drawer survey of ethnobotanical artifacts in the collection, noting the accession number and location of vines, roots and other suspected ethnobotanical material (Table 1). Preliminary research raised immediate concerns about the barbasco vine (*Tephrosia toxicofera*). Consultation with a local ethnopharmacognicist revealed that breathing the dust of the barbasco vine,

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Table 1. Survey of suspected hazardous ethnobotanical artifacts.

Description	Location	Accession #
achiote seeds	AC47.D1006	60-96
arrow, poison?	AC46.D1458	X
	AC46.D1708	X
	AC48.D515	A80:5:3
	Rack	A71:6:62
	AC50.D598	54-1317
	AC50.D598	A66:10:14
	AC50.D598	No # (7)
	AC50.D1826	A76:2:604
	AC27.D1456	A76:2:1133
	AC10.D259	A86:17:2 a-r
	AC10.D259	A86:17:3 a-l
	AC10.D260	A86:17:8 a-g
	AC29.D148	1-558
	AC32.D171	A67:9:177
ayahuasca branch	AC47.D1817	58-150 a,b
banana tree blossom	AC09.D219	A76:2:1017
barbasco (rotenone) weir	AOS6.S5	A71:9:125
root	AC47.D585	58-151
vine	AC48.D541	A71:9:102
bean (red) necklace	AC48D.515	A76:2:554
betel nut	AC16.D356	A67:9:252
	AC16.D356	A67:9:152
cassavi	AC49.D835	A71:6:52
cinchona vine	AC48.D535	58-152
cinnamon	AC27.D103	A64:12:10
clavohuasca vine	AC45.D1347	58-149
clove boat	AC27.D999	A95:6:1
coca gourd	AC49.D835	A71:6:29
container with ?powder	AC47.D1820	A61:3:25
curare pot	AOS30.S3	A71:9:2
curare tipped darts, arrows	AC50.D593	A67:3:1-5
	AC50.D1826	A76:2:604
	AC48.D534	A76:565
	AC48.D537	58-146
	AC49.D1335A	A71:6:55
	C48.D1823	A71:9:100
	AC48.D534	A76:2:563
	AC48.D534	A76:2:564
	AC49.D587	A76:2:605
	AC48.D534	A76:2:606
	AC48.D567a	A83:15:4
	AC48.D513	58-126
	AC49.D1825	A71:6:56
	AC50.D1828	A71:9:62 a-e
dried medicines	AC0.D219	A76:2:987
drugs, unknown	AC19.D403	A63:24:229
gourd with seeds inside	AC8.D225	A76:2:888
gourd with tea leaves	AC47.D1006	A64:6:100
guayduros & Job's tears & ?	AC60.D1506	A97:3:50
necklace	AC60.D1506	A97:3:44
	AC60.D1506	A97:3:51
guayduros necklace	AC48.D515	A76:2:555
seed hat	AC47.D518	A61:3:4

Table 1. Continued.

Description	Location	Accession #
Indian tobacco		
Job's tears, eye of the deer necklace	AC60.D1506	A97:3:53
Job's tears seed necklace	AC29.D168	A73:1:41
kava root	AC41.D1447	A76:2:1210
leaves	AC48.D1264	A83:15:7
leaves, crushed	AC16.D356	A67:9:253
leaf	AC51.D673	A63:24:190
lichen	AC9.D239	A76:2:985
mahogany button	X	A89:5:5
drill	X	A97:3:13
belt	X	A63:30:462
pod	AC09	A76:2:969
seeds	AC09	A76:2:1015
opium paraphernalia	AC20.D467	A88:1:116
		A88:1:117
		A88:1:118
		A88:1:119
		A88:1:120
		A88:1:121
		A88:1:122
		A88:1:123
palm base leaf	AC49.D1539	A71:9:82
	AC51.D839	1-392
papyrus leaves	AC16.D350	A67:9:155
	AC16.D357	A67:9:156
pearl millet	AC08.D213	A76:2:851
peyote buttons	AC101.D47	A76:2:504
precatory pea necklace	AC60.D1506	A97:3:52
root doll	AC48.D513	58-129
rubber	AC29.D126	A73:1:52
	AC49.D514	A71:6:24
seed ornaments	AC50.D1826	A66:7:113
	AC50.D565	A71:9:47
	AC29.D168	A73:1:40
	AC09.D219	A76:2:1008
	AC48.D515	A76:2:552
	AC48.D516	A76:2:556
	AC48.D515	A76:2:557
	AC48.D515	A76:2:559
	AC49.D496	A71:6:47
	AC48.D516	A76:2:550
	AC48.D515	A76:2:553
	AC09.D219	A76:2:1025
	AC48.D541	A71:9:59
seed pods	AC51.D839	1-393
seed rattle	AC09.D229	A76:2:941
	AC07.D246	A76:2:680
	AC48.D531	A76:2:543
	AC48.D531	A76:2:544
	AC48.D531	A76:2:545
	AC48.D515	A76:2:546
	AC48.D531	A76:2:548
	AC48.D531	A76:2:542
	AC49.D577	A76:2:547

Table 1. Continued.

Description	Location	Accession #
soap bark	AC29.D306	A67:9:186
spear, poison?	AC50.D598	59-13
tobacco cigar	AC27.D413	A67:9:142
plug	AC49.D496	A71:6:39
cigars	AC50.D595	A71:9:55 a–c
	AC62.D712	A77:1:59
	AC103	A77:18:121
	AC103	A77:18:178
	AC103	A77:18:180
	AC103	A77:18:197
	AC103	A77:18:213
	AC103	A77:18:217
	AC81.D876	A70:12:3
	AC81.D8acAC	A70:12:2
	81.D876	A70:12:1
	AC103	A77:18:119
	AC103	A77:18:179
	AC101.D9	60-91
	AC73.D1465	A70:12:34
	AC103	A77:18:236
tucum nut & seed necklace	X	A71:9:43
	AC0.D532a	A71:9:58
	AC46.D1446	A71:9:32 a,b
yoco	AC46.D1446	A71:9:138

a potent metabolic poison containing rotenone, could have caused the problems experienced. Rotenone is a natural substance contained in the stems and roots of certain tropical plants, such as the jewel vine or flame tree (*Derris* spp.), lacepod (*Lonchocarpus* spp.) or hoary pea (*Tephrosia* spp.). Used as a fish poison, it works by inhibiting a biochemical process at the cellular level making it impossible for fish to use oxygen in the release of energy needed for body processes (ODFW 2002). In humans, rotenone produces symptoms similar to cyanide poisoning. Acute poisoning causes nausea, vomiting, gastric pain, muscle tremors, incoordination, convulsions, and stupor (Merck 1996, EPA 1993, Shimkim and Anderson 1936).

Staff members, visiting researchers, interns and volunteers who work with natural history collections have both a need and a right to know the physical and chemical hazards inherent in the materials to which they are exposed when working (OSHA 1998). They also need to know what protective measures are available. We initiated a program that not only focuses on issues of worker health and safety but also brings increased awareness of safe handling and storage needs to the fore. This program attempts to identify *Oh No! Ethnobotany* material and provide museum staff and visiting researchers with the information they need.

Oh No! Ethnobotany

Oh No! Ethnobotany refers not only to hazardous ethnobotanical artifacts but also to a hazard communication-training program that focuses on establishing workplace policies and procedures that address safe handling and storage. "Eth-

nobotany" is defined as both the ethnobotanical material or object itself, and also the study of how and why people use and conceptualize plants in their local environments. The two questions most asked are (1) how and in what ways people use nature and (2) how and in what ways people view nature. Ethnobotanists gather data mainly from living peoples in hopes of gathering a view of their past existence as well as an understanding of present uses of plants for ceremony, ritual, clothing, food, medicine, construction materials and tools (Salmón 1999).

Although ethnobotany is an academic field of study, it maintains a multidisciplinary character in both theory and methods. In the botanical tradition, the theoretical distinctions are clear. Plants and plant uses are the focus, although ecological patterns, plant dispersals, resource utilization and horticultural and agricultural patterns have become popular avenues of study among botanists (Castetter and Underhill 1935, Densmore 1928, Gilmore 1919, Shreve and Wiggins 1964, Whiting 1939). Several theoretical avenues are used by ethnobotanists of the "New Synthesis," the holistic merger of all methods and theories (Ford 1978). Their research is often conducted combining botanical theories with those from anthropology, as well as from other fields including linguistics, pharmacology, musicology, toxicology, architecture, conservation biology and many others.

The *Oh No! Ethnobotany* project is a pilot program that can potentially help museums and other natural history collectors provide safer places for both work and research. When museums have information about the chemical composition of hazardous ethnobotanical objects in collections, steps can be taken to reduce exposures and intoxications; promote understanding of ethnobotany, pharmacology and toxicology; and establish safer work practices. These efforts will help prevent the occurrence of work and research related illnesses and injuries caused by hazardous ethnobotanical artifacts.

Some parts of *Oh No! Ethnobotany* are technical, but the basic concept is simple. In fact, the components reflect and build upon what the Science Museum of Minnesota has been doing for years. The collections manager and conservator are already in compliance with museum standards and provisions for safe object handling and storage (Alberta Museums Assoc. 1990, ICM 1990, SPNHC 1994). By implementing *Oh No! Ethnobotany,* existing practices are enhanced to meet new policy recommendations. These recommendations encourage the effective transmission of warnings regarding both physical hazards (such as stability and reactivity) and health hazards (such as irritation, lung damage and cancer).

Museums that use hazardous ethnobotanical artifacts in their collections need a program to ensure that information is provided to exposed staff members and visiting researchers. "Use" means to mount, handle, research, exhibit or transfer. This is an intentionally broad scope and includes any situation where hazardous ethnobotanical artifacts are present in such a way that staff or visiting researchers may be exposed under normal conditions of use or in a foreseeable emergency.

Oh No! Ethnobotany is a four-part, straightforward, communication-training program that includes written hazard communications; ethnobotany material safety data sheets (EMSDS); labels and other forms of warning; and training.

Written Hazard Communications

The written plan describes how *Oh No! Ethnobotany* is implemented and includes a list of hazardous or suspected hazardous ethnobotanical artifacts present

in collections. The plan also describes policies and procedures for hazard communication, labels and other forms of warning, accessibility of information, ethnobotany material safety data sheets (EMSDS) and training.

Initially, a drawer-by-drawer survey of ethnobotanical artifacts judged to be potentially hazardous on the basis of preknowledge, identifying information and subjective opinion resulted in an inventory of objects for which further investigation was warranted. The broadest possible perspective was taken. *Oh No! Ethnobotany* seeks to identify hazardous materials in all physical forms—liquids, solids, gases, vapors, dusts, fumes and mists—whether they are contained or not. The hazardous nature of each individual object and the potential for exposure are factors that determine safe handling and storage procedures (e.g., whether an object is placed in a darkened, covered mount). Preparing the list imparted some idea of the scope of the program required to meet the needs of the museum in addressing the hazards associated with ethnobotanical objects. We focused on twelve of the listed objects (Table 2) to develop and test the *Oh No! Ethnobotany* program.

The success of *Oh No! Ethnobotany,* like any safety and health program, depends upon commitment at every level of the organization. This is particularly true for hazard communication, where success requires a change in behavior. Management's role and support in setting goals, providing feedback to motivate use of the knowledge gained, and offering incentives are all critical pieces in ensuring a successful program (OSHA 2001). *Oh No! Ethnobotany* incorporates important elements such as these in a framework designed to achieve success.

Ethnobotany Material Safety Data Sheets

In consultation with Museum Safety Manager Mike Frigon, ethnobotany material safety data sheets (EMSDS) were designed to provide specific information about listed hazardous ethnobotanical artifacts (see Appendix).

Ethnobotany material safety data sheets (EMSDS) are modeled after material safety data sheets (MSDS), well-known industry-wide chemical information sheets. Employers are required by OSHA to post a MSDS for each chemical used or stored in the workplace. Ethnobotany material safety data sheets, similar in concept, build upon the familiarity with and acceptance of the MSDS. However, the EMSDS is distinctive in its application to the museum situation and other natural history collections. It fills a unique need that specifically addresses the use of hazardous ethnobotanical objects.

The EMSDS takes into account 2001 Occupational Safety and Health Administration (OSHA) regulated specific information requirements. Each EMSDS is developed using available, relevant and scientific health hazard/toxicity data, including 50% lethal dose (LD_{50}) when known. The LD_{50} is the amount of a solid or liquid material, given in one dose, which causes the death of 50 percent of a group of test animals. It is one way to express the short-term poisoning potential (acute toxicity) of a material. In general, the smaller the LD_{50} value, the more toxic the chemical is.

Several references were used to ascertain the primary chemical agents for each ethnobotanical artifact (e.g., Amdur et al. 1991, Goodman and Gilman 1970, Merck 1996). Duke (1998), Sigma-Aldrich (2002); TOXLINE, TOXNET and the Hazardous Substances Data Bank (HSDB) (National Library of Medicine 2002);

Table 2. Survey of hazardous ethnobotanical artifacts.

Description	Primary agent	Cultural use	Action	Location	Accession #
ayahuasca branch	harmaline, harmine	ceremonial, entheogen, medicinal, narcotic	central nervous system (CNS) stimulant, monoamine oxide inhibitor*	AC47.D1817	58-150
barbasco root	rotenone	fish poison, insecticide, medicinal, pesticide	powerful mitochondria electron transport inhibitor*	AC47.D585	A71:9:125
betel nut betel leaf	arecoline chavicol	intoxicant, medicinal, parasiticide, religious, stimulant, vermifuge	CNS stimulant**	AC16.D356	A67:9:252
cinchona vine	quinine	medicinal, muscle relaxant	potassium channel blocker, CNS suppressor, seems to interfere with function of plasmodial DNA.***	AC48.D535	58-152
clavohuasca vine	eugenol	aphrodisiac, medicinal, stimulant	unknown	AC45.D1347	58-149
curare tipped darts	d-tubocurarine, tubocurarine	medicinal, poison	produces muscle paralysis**	AC50.D593	A67:3:1-5
Indian tobacco	lobeline	ceremonial, religious, medicinal	CNS depressant and stimulant, ganglionic stimulating agent***		
kava root	kavalactones	ceremonial, medicinal, sedative	analgesic, mind-mood changing***	AC41.D1447	A76:2:1210
peyote buttons	mescaline	medicinal, narcotic, religious	psychomimetic,** mind-mood changing***	AC101.D47	A76:2:504
precatory pea necklace	abine, abrin	jewelry, medicinal, poison	ribosome inactivating protein, toxic lectin**	AC60.D1506	A97:3:52
tobacco cigar	nicotine, pyridine	ceremonial, medicinal, poison, religious	CNS depressant and stimulant, ganglionic stimulating agent, mind-mood changing***	AC27.D413	A67:9:142
yoco	caffeine	medicinal, stimulant	CNS stimulant,* mind-mood changing***	AC46.D1446	A71:9:138

* Merck (1996).
** Amdur et al. (1991).
*** Goodman and Gilman (1970).

Table 3. Approximate LD_{50}s of representative chemical agents.

Agent	LD_{50} (mg/kg)*	Source**/subject	Toxicity class***
eugenol	1930.[2]	orl rat	slightly toxic
chavicol	1230.[1]	orl rat	slightly toxic
pyridine	891.[1]	orl rat	slightly toxic
quinine	294.[2]	unr man	moderately toxic
harmine	200.[2]	ivn mouse	moderately toxic
caffeine	192.[2]	orl man	moderately toxic
rotenone	143.[2]	orl man	moderately toxic
mescaline	132.[1]	orl mouse	moderately toxic
harmaline	120.[2]	scu rat	moderately toxic
arecoline	100.[1]	scu mouse	moderately toxic
lobeline	40.[1]	ipr mouse	highly toxic
nicotine	30.[1]	unr man	highly toxic
tubocurarine	28.[1]	orl rat	highly toxic
abrin	0.00004[3]	orl mouse	extremely toxic

* LD_{50} values from Duke 1998[1]; Sigma-Aldrich 2002[2]; Patocka 2001[3] is the dosage (mg/kg body weight) causing death in 50% of test population.
** ivn = intravenous; ipr = intraperitoneal; orl = oral; scu = subcutaneous; unr = unreported.
*** Hodge and Sterner Scale.

and peer-reviewed literature (Patocka 2001) provided data about each primary chemical agent's properties and toxicity. Toxicity ratings based on published LD_{50} values were assigned using a common toxicity scale, the Hodge and Sterner Scale of Toxicity (CCOHS 1999, Hodge and Sterner 1949) (Table 3).

The EMSDS presents information and toxicity ratings based on extensive research. This information includes common and scientific names; collection information, accession number and storage location; effects of exposure; phytochemical and toxicological properties; first-aid measures; labeling, safe handling and storage recommendation; stability; description including "how it works" and "traditional recipes"; worldwide uses; disposal methods; disclaimers and reference/research notes. Storage recommendations include specific instructions that address the design and fabrication of mounts that take into account the object's chemical properties, toxicity, reactivity and stability.

Hardcopy EMSDS, including a glossary of terms, are kept in conspicuous *Oh No! Ethnobotany* three-ring binders located in the storage vault, the collections workroom and the ethnology lab. They are readily accessible to staff, volunteers, interns and researchers during their work shifts. It is recommended that information acquired about hazardous ethnobotanical artifacts contained in the EMSDS also be added to accession files as well as to on-line databases.

Generally speaking, workers using hazardous ethnobotanical artifacts will primarily be concerned with EMSDS information regarding hazardous effects and recommended protective measures. However, because the EMSDS is designed to elicit interest and be easy to read, it acts as a useful tool to provide internal motivation that will increase knowledge about specific objects and ethnobotany in general. Knowledge, in turn, will increase the value of each object and the general collection as a whole. It is important to write and maintain an EMSDS for every identified hazardous ethnobotanical artifact inventoried.

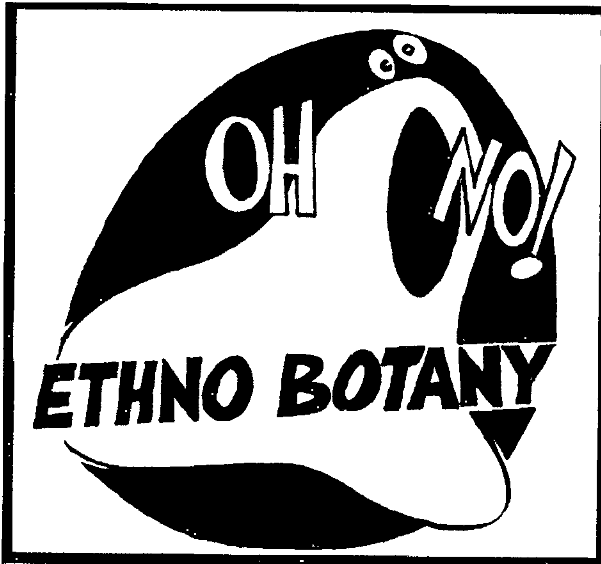

Figure 1. Storage cabinet label.

LABELS AND OTHER FORMS OF WARNING

The Environmental Health Education Center at the University of Maryland Medical School (1997) reported that a fully harmonized hazard communication system must take into account comprehensibility, readability and other human factors regarding the use of labels and safety data sheets. It found that warnings containing a pictorial (pictograph), color, or an icon elicited significantly faster response times among subjects than warnings without them. Additionally, it found the shape of a label enhanced the conspicuousness of a warning, even if the targeted user was not actively searching for safety information. The paper reported that one of the key variables on warning effectiveness is where the information is placed.

Oh No! Ethnobotany integrates many of the key findings and recommendations of this research through the use of evocative pictographs that are designed to draw attention to the location of hazardous ethnobotanical artifacts and to the existence of an EMSDS. The pictographs effectively elicit interest and build upon familiar iconography associated with themes of poison, hazard, caution and fright. The *Oh No! Ethnobotany* pictographs include three separate designs by artist Verne Anderson, a museum volunteer.

Using the *Oh No! Ethnobotany* pictographs, a three-tier labeling system was designed. Labels, printed on magnetic sheets and cut to shape, are placed conspicuously on the storage cabinet door and on the cabinet drawer containing the hazardous ethnobotanical artifact. Additionally, an object safety label is affixed with double sticky adhesive tape to the object mount itself. First, the storage cabinet label is intended to draw attention to the existence of hazardous ethnobotanical artifacts within the cabinet (Fig. 1). A label on the outside of the cabinet drawer (Fig. 2) further identifies the object with a signal word that designates a level of hazardousness determined through review and analysis of LD_{50} values.

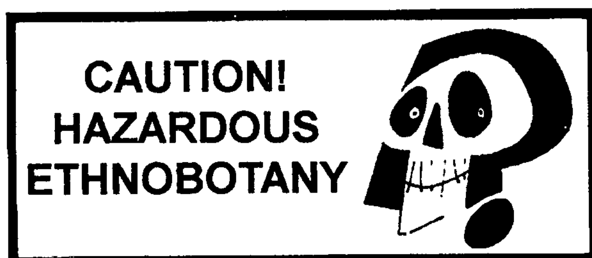

Figure 2. Cabinet drawer label.

"Deadly Poison" indicates an imminently hazardous situation which, if extreme caution in storage and handling is not taken, will result in death or serious injury. This signal word is limited to the most extreme situations involving extremely or highly toxic ethnobotanical artifacts, for example, curare tipped darts and precatory pea (*Abrus precatorius*) necklaces. "Poison" indicates a potentially hazardous situation which, if caution in storage and handling is not taken, could result in death or serious injury. This signal word includes moderately toxic ethnobotanical artifacts, for example, the barbasco vine (*Tephrosia toxicofera*). "Hazardous" indicates a potentially hazardous situation which, if caution in storage and handling is not taken, may result in minor or moderate injury. This signal word includes moderately to slightly toxic ethnobotanical artifacts, for example, the clavohuasca vine (*Tynnanthus panurensis*) and ayahuasca vine (*Banisteria caapi*). Finally, a safety label placed on the object mount adds statements of the hazard, a toxicity rating and scale, and a directive to refer to an EMSDS (Fig. 3).

Each hazardous ethnobotanical artifact is tagged or labeled with its identity and appropriate hazard warnings. If the object is transferred from a labeled mount/location to another mount/location, that mount/location is labeled. The "identity" is any specific term that appears on the label, the EMSDS, and the list of hazardous ethnobotanical artifacts, and thus links these three sources of information. The identity may be a common or scientific name (e.g., precatory pea or *Abrus precatorius*) or an ethnographic catalogue number (e.g., A97:3:52). The hazard warning is a brief statement of the hazardous effects of the ethnobotanical object (e.g., "ribosome-inactivating protein" or "toxic and fatal if thoroughly masticated"). Labels frequently contain other information, such as precautionary measures (e.g., "do not expose to light" or "do not breathe dust").

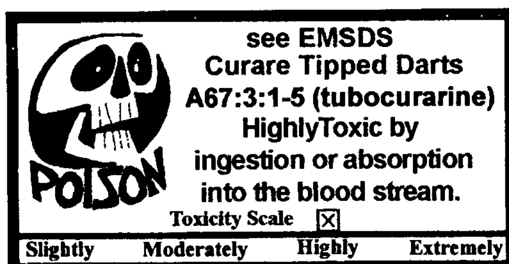

Figure 3. Object mount safety label.

Training

Staff members, interns, volunteers and visiting researchers who may be exposed to hazardous ethnobotanical artifacts are given information and training prior to initial assignment with a hazardous ethnographic object and whenever the hazard changes. *Oh No! Ethnobotany* accomplishes hazard communication and training through a process that includes several tools for transferring information and influencing behavior. Laughery and Brelsford (1993) noted that "numerous studies have shown the greater the a priori perception of hazardousness, the more likely people will look for and read a warning, and the more likely they will comply by taking safety precautions." Training employs a combination of pictographs, signal words, labels, EMSDS, computerbased instruction and cognitive apprenticeship (Shute, Gluck and Lajoie 2000).

Computer based training in the form of a multimedia presentation provides a series of instructional segments that give an overview of the *Oh No! Ethnobotany* program. This is followed by an attempt to anchor instruction within the work situation where the acquisition of knowledge and skills become embedded in the social and functional context of their use. During cognitive apprenticeship, "master" staff members share their knowledge of the collection and experiences with new staff, volunteers, interns and visiting researchers. Often sharing information takes the form of storytelling and oral tradition which serves to address issues of cultural sensitivity, attract interest and enhance engagement.

Conclusion

The underlying purpose of *Oh No! Ethnobotany* is to reduce the incidence of illnesses and injuries due to exposure to hazardous ethnobotanical material. This can be accomplished by modifying behavior through the provision of hazard information and information about protective measures. For *Oh No! Ethnobotany* to work, staff and visiting researchers must understand the nature of hazardous ethnobotanical artifacts within natural history collections. The procedures established regarding labeling and other forms of warning, EMSDS, storage, handling, and training all work to reduce the risks of exposure. Furthermore, knowledge and understanding of phytochemistry, pharmacology and toxicology increase, and proper object handling practices are enhanced.

The most important aspect of *Oh No! Ethnobotany* is to ensure that staff and visiting researchers are aware that they are exposed to hazardous ethnobotanical artifacts, that they know how to understand labels and ethnobotany material safety data sheets and that, as a consequence of learning this information, they are following appropriate protective measures. It is hoped that this program will enthuse and incite museums and natural history collectors to implement *Oh No! Ethnobotany* or construct similar programs that work to enhance health and safety for staff, volunteers, interns and researchers.

Acknowledgments

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Associate Editor: Suzanne B. McLaren

Appendix

ETHNOBOTANY MATERIAL SAFETY DATA SHEET

Name: PRECATORY PEA NECKLACE:

Family.—Fabaceae; Leguminosae

Scientific names.—L. *Abrus precatorius*; F. *Rhynchosia precatoria*

Common names/Synonyms.—*Abrus abrus* (L.) W. Wight, *Glycine abrus* (L.), Crab's eye, pois rouge, jerquerity, jequirity bean, prayer beans, precatory pea or bean, rosary beans, rosary pea, rosarypea, tento muido

Part(s) used.—Root, leaf, seed

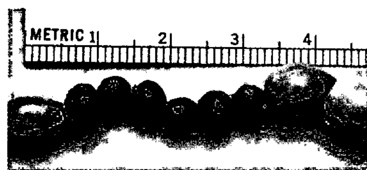
Collection Information/Storage Location:

Culture.—Mexico

Year and location collected.—Lacandon

Accession number.—A97:3:52

SMM location.—AC60.D1506



A97:3:52
Precatory pea and Job's tears

Health Hazard Identification:

Precatory Pea seeds are EXTREMELY TOXIC by ingestion. (TOXNET 2001). Kingsbury (1964) states that a toxicity dose is about 0.00015% of a human subject's weight. A single well-masticated seed can kill an adult. Effects of exposure depend upon present levels of bio-activity inherent in the plant material itself. Abrin causes large-scale disruption in lymphoid tissues, with apoptotic cell death. If the seed is swallowed without damage to the seed coat poisoning is unlikely and the seed will tend to pass without incident. In cases where the seed coat is chewed or opened (as in drilling to make jewelry), toxic signs and/or death are likely. There may also be added health risks resulting from exposure to residues of toxic chemicals used to treat the artifact. The seeds in this necklace have NOT been laboratory tested for bio-activity. Use precautions. [DATA for abrin is similar to that for ricin, the main constituent of the castor bean plant (*Ricinus communus*): ORL HUMAN LD <5 mg/kg; A TASTE (<7 DROPS) FOR 70 kg PERSON (150 lb), IPR MOUSE LD_{50} =0.02 mg/kg] Sources: TOXNET 2002, Patrocka 2002, Duke 2000.

Important constituents of precatory pea.—Include abrin*, glycoside called abric, abrine* (not to be confused with the albuminous substance abrin).

Known actions.—Agglutinin*, ribosome-inactivating protein, toxic lectin**, hemagglutinin, toxalbumin* (*Merck 1996, **Amdur et al. 1991).

The precatory pea contains insidious proteins called lectins that can cause red blood cells to clump together (agglutinate) and may stimulate abnormal cell division in B and T-lymphocytes. A well-masticated precatory pea can kill (Kingsbury 1964) by destroying an important component of the protein synthesizing machinery of cells, the ribosome (Merck 1996). It works as a slow poison, eventually causing a total body collapse as necessary proteins are not replaced (Amdur et al. 1991).

Toxicological Information:

Acute effects.—Severity of an exposure is often dependent on the degree of mastication of seeds. Toxic and fatal if thoroughly masticated; abdominal pains; cecum, red and inflamed; cerebral edema; death; diarrhea; gastric mucosa, purple; nausea; stomach, gray mucous; vomiting.

First-Aid Measures:

- In case of contact, immediately flush eyes or skin with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash contaminated clothing before reuse.

- Assure adequate flushing of the eyes by separating the eyelids with fingers.
- If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
- If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

Handling and Storage:

- Wear appropriate NIOSH/MSHA respirator, chemical-resistant gloves, safety goggles, other protective clothing.
- Safety shower and eye bath.
- Use chemical fume hood.
- Do not breathe dust.
- Do not get in eyes, on skin, on clothing.
- Avoid prolonged or repeated exposure.
- Wash thoroughly after handling.
- Keep inside tightly closed labeled mount, which clearly and properly identifies it as Extremely Toxic by ingestion. Deadly Poison.
- Store in a cool dry place.
- Post ethnobotany material safety data sheet (EMSDS) in storage.

Stability and Reactivity:

Incompatibilities.—Strong oxidizing agents. Sensitive to light. Sensitive to air.

Hazardous combustion or decomposition products.—Toxic fumes of: carbon monoxide, carbon dioxide.

Description/History of Use:

This plant is a small, climbing tropical vine native to tropical climates and certain areas of southern Florida. The 1 to 1½ inch seedpod contains several seeds that are bright red with a black spot. The castor bean plant (*Ricinus communis*) and the rosary pea plant (*Abrus precatorius*), are the two deadliest plants in the world. Because of their remarkably uniform weight of 1/10th of a gram, goldsmiths of East Asia used the seeds of *Abrus precatorius* as standard weights for weighing gold and silver. According to Armstrong (1998) the famous Koh-i-noor diamond of India, now one of the British crown jewels was reportedly weighed using seeds of *Abrus precatorius.*

Traditional method of use.—In healing the leaves and roots are used in teas, tinctures and salves.

Medicinal uses.—General: abortion, ache (head), aphrodisiac, bite (snake), bladder, boil, CNS sedative, cancer, chest, cold, colic, conjunctivitis, contraceptive, convulsion, cough, diarrhea, diuretic, emetic, expectorant, fever, freckles, gastritis, heart, hookworm, insomnia, jaundice, kidney, laxative, malaria, night-blindness, purgative, rheumatism, sedative, skin, throat, venereal

Other uses.—Deadly poison, refrigerant, sweetener, soap, vermifuge

Disposal Methods:

Dissolve or mix with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe Federal, state, and local environmental regulations.

Other Information:

—The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. For additional information contact Rose Kubiatowicz, kubi0029@umn.edu, Science Museum of Minnesota, 120 W. Kellogg Blvd., Saint Paul, Minnesota 55102.

—Photos: 2001 Tim Ready.

—Consultants: Mike Frigon, M.S., Safety Manager, Science Museum of Minnesota; Gretchen Anderson, Conservator, Science Museum of Minnesota.

—See Industry *Material Safety Data Sheet (MSDS): 'abrine'* for further information.

—The North American butterfly Ceraunus Blue (*Hemiargus ceraunus* [Fabricius]) uses the rosary pea (*Abrus precatorius*) as caterpillar host.

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EFFECTS OF VARIOUS FUMIGANTS, THERMAL METHODS AND CARBON DIOXIDE TREATMENT ON DNA EXTRACTION AND AMPLIFICATION: A CASE STUDY ON FREEZE-DRIED MUSHROOM AND FREEZE-DRIED MUSCLE SPECIMENS

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Abstract.—The effects of several treatments for pest eradication on natural specimen DNA were examined using freeze-dried mushroom and freeze-dried muscle. The data clearly showed that the fumigants methyl bromide, methyl bromide/ethylene oxide mixed gas, ethylene oxide, propylene oxide and methyl iodide, all caused significant degradation of specimen DNA, even with a single fumigation. Subsequent steps at amplification of certain DNA fragments by PCR (polymerase chain reaction) were difficult in these samples. There were more problems in amplifying larger DNA fragments than smaller fragments. The main cause for the decreased efficiency of PCR was thought to be the degradation of template DNA. On the other hand, thermal methods (heating and low temperature), 60 percent carbon dioxide treatment, and fumigation by sulfuryl fluoride seemed scarcely to affect the DNA molecules of the specimens, allowing subsequent PCR to be successfully performed. Direct sequencing of the PCR products was also performed to check for the possibility of modifications to the DNA sequences. When the PCR products were amplified to the sufficient amount for direct sequencing, the DNA sequences were normal in our examination of a few gene regions.

INTRODUCTION

Much emphasis has been placed on visible damage to collections, such as mechanical damage or color changes, caused by some treatments. But invisible damage to macromolecules such as DNA is also critical to the preservation of natural history collections in light of the increasing use of DNA methodologies.

While DNA analysis of natural specimens, especially for taxonomic studies, has become increasingly common, in the course of recent trials, some conservators have become concerned about the possibility that some fumigants may adversely affect DNA molecules of natural specimens. Saito (2002) showed that when insect muscle was fumigated with methyl bromide/ethylene oxide mixed gas, the efficiency of DNA amplification by PCR (polymerase chain reaction) was significantly diminished in the fumigated samples, compared to untreated samples. Akiyama (2001) suggested that fumigation with methyl bromide/ethylene oxide mixed gas occasionally decreased the amount of intact DNA of specimens and made subsequent PCR steps difficult, but the results seemed to vary depending on specimen type (plant or animal, etc.). Some fumigants are known carcinogens or suspected carcinogens which affect DNA molecules, generating DNA adducts by alkylation (Bolt et al. 1988, 1991, Bolt and Gansewendt 1993, Gansewendt et al. 1991a, 1991b, Starratt and Bond 1988, Thier and Bolt 2000, Walker et al. 1990). In spite of these very suggestive studies, there are not many reports on the impact on DNA molecules from fumigating specimens. Also, the prominent causes of PCR inhibition seen in fumigated samples remain to be elucidated.

Collection Forum 2003; 18(1–2):74–89

The purpose of this paper is to examine and draw out a broad sketch of the effects of various kinds of treatments for pest eradication on DNA molecules of natural history specimens, and to investigate the magnitudes of the harmful effects on subsequent DNA analysis by PCR. Treatments by various fumigants, including commonly used chemicals as well as newly developed ones, thermal methods (low temperature and heating), and carbon dioxide were performed on samples derived from the same individual specimens. DNA was extracted from pieces of the treated samples simultaneously, and the efficiency of PCR using the extracted template DNA was subsequently examined. Direct sequencing of a few kinds of PCR products was also performed to check the possibility of modifications that may prove detrimental to DNA sequencing.

Materials and Methods

Preparation of Samples

An edible mushroom *Lentinus edodes* and muscle of chicken *Gallus gallus* were freeze-dried at −40°C at approximately 7.5×10^{-4} Pa for four days. Each fruiting-body of the mushroom was cut radially into 12 pieces (approximately 60 to 80 mg each). Freeze-dried chicken muscle was also cut into small pieces (approximately 500 mg each). These pieces were kept in a glass jar with desiccant silica gel at 4°C until treatments.

Treatments

Treatment conditions are shown in Table 1. Fumigants were used at the highest concentration seen in general treatments. Fumigants that are also used for killing fungi (methyl bromide/ethylene oxide mixed gas, ethylene oxide, propylene oxide and methyl iodide) were used at concentrations needed for sound fungicidal effects, which are usually much higher than those for killing insects. Propylene oxide is a fumigant that is thought to have a similar but less fungicidal effect, compared to ethylene oxide (Yamazaki et al. 1993). Methyl iodide is analogous to methyl bromide in its ability to act as an insecticide (Becker et al. 1998, Ohr et al. 1996, Sims et al. 1995). Sulfuryl fluoride was used at the concentration for killing only insects.

Pieces of freeze-dried samples were put into 1.5 ml polypropylene tubes (Eppendorf). Fumigation with each fumigant was performed in glass chambers of approximately 8.0 liters in volume at 23°C, with lids of the polypropylene tubes left open. After fumigation, the treated materials were kept in fresh air at 23°C, 55 percent RH for five days, with the tubes left open.

Low temperature treatment at −30°C for one week in a freezer was performed on samples in 1.5 ml polypropylene tubes, followed by returning to room temperature, with the lids of the tubes tightly closed. Heating was performed in the same manner, at 60°C for 24 hours in an air incubator followed by returning to room temperature, with the lids of the tubes tightly closed. Carbon dioxide treatment was performed with 60 percent volume of carbon dioxide and approximately eight percent volume of oxygen at 23°C for two weeks, with the lids of the tubes left open.

Treated samples were kept in a glass jar with a desiccant (silica gel) at room temperature, until DNA extraction.

Table 1. Treatment conditions.

Sample	Treatment	Trade name	Concentration	Treatment period	Purpose
A	No treatment	—	—	—	—
B	Methyl bromide 86% wt./ethylene oxide 14% wt.	EKIBON	100g/m^3	48 hours	killing insects and fungi
C	Methyl bromide	—	86g/m^3	48 hours	*
D	Ethylene oxide	—	14g/m^3	48 hours	*
E	Ethylene oxide 15% wt./HFC R-134a 85% wt.	EKIHUME	200g/m^3	48 hours	killing insects and fungi
F	Propylene oxide	—	48g/m^3	48 hours	killing insects and fungi
G	Methyl iodide	IOGUARD	120g/m^3	72 hours	killing insects and fungi
H	Sulfuryl fluoride	Vikane^R	50g/m^3	48 hours	killing insects
I	Low temperature at −30°C	—	—	1 week	killing insects
J	Heating at 60°C	—	—	24 hours	killing insects
K	Carbon dioxide	—	60% volume	2 weeks	killing insects
L	No treatment	—	—	—	—

Fumigation (B to H) was performed at 23 ± 1°C. Both A and L are non-treated samples. The reason for preparing two control samples is to see the magnitude of experimental error between different pieces of samples.
* Individual components of treatment B.

DNA Extraction

For each species, twelve treatments (designated A to L in Table 1) were performed on samples derived from the same individual specimens. Two pieces weighing approximately 20 mg were cut from each of the treated samples, and DNA extraction was performed simultaneously. The reason for using a duplicate for each treatment option was to determine the magnitude of experimental error for the same sample. DNA extraction from both freeze-dried mushroom and freeze-dried muscle of chicken was performed with DNeasy Plant Mini Kit (QIAGEN), and finally 100 μl of extracted DNA solution was obtained for each preparation. Ten microlitres of each of the extracted DNA solutions were checked by an electrophoresis on a one percent agarose gel (Agarose L03, TAKARA BIO INC.).

PCR Analysis

PCR primers used are shown in Table 2. Three sets of primers were designed for each of the mushroom or chicken muscle. The criteria for designing the PCR primers are as follows:

(1) Primer sets to amplify a relatively small fragment (approximately 300 base pairs (bp)) in a gene which exists in cells in multiple copies, from a few hundred to a few thousand copies (for example, ribosomal RNA (rRNA) genes or mitochondrial DNA genes);

(2) Primer sets to amplify a larger fragment (approximately 1,000 bp) in a gene which exists in cells in multiple copies, as in (1); and

(3) Primer sets to amplify a larger fragment (approximately 1,000 bp) from a single copy nuclear gene.

The purpose of using these three types of primer sets is to see fragment-size dependency and number-of-templates dependency on PCR efficiency. This information provides evidence for possible mechanisms that influence PCR efficiency.

NS5/NS6 and ML3/ML4 are primers commonly used for the amplification of fungal ribosomal RNA genes (nuclear rRNA gene and mitochondrial rRNA gene, respectively) in phylogenetic studies (White et al. 1990). Other primers were designed by the authors with reference to sequence data described in the literature (Irwin et al. 1991, Kaneko et al. 1998, Shen 2001, Zehner et al. 1987).

PCR was performed using a TAKARA Ex Taq™ kit. The reaction mixture was as follows: template DNA solution 1 μl; 10 × ExTaq buffer 5 μl; 2.5 mM dNTPs mixture 4 μl; Primer 1 (10 μM) 0.5 μl; Primer 2 (10 μM) 0.5 μl; ExTaq polymerase 0.5 μl; and distilled water 38.5 μl for a total reaction volume of 50 μl.

The PCR conditions were as follows: Preheating at 94°C for two minutes, and 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for one minute, followed by 72°C for three minutes using a TAKARA PCR Thermal Cycler SP (TAKARA BIO INC.).

PCR products were checked by electrophoresis on a one percent agarose gel (Agarose L03, TAKARA BIO INC.).

DNA Sequencing

Different PCR products (a 354 bp fragment in *G. gallus* cytochrome *b* gene and an approximately 1,300 bp fragment in *L. edodes* mitochondrial large rRNA gene) were directly sequenced for both DNA strands, using the primer sets LG14841/HG15149 and ML3/ML4. PCR products were purified by Centri-sep

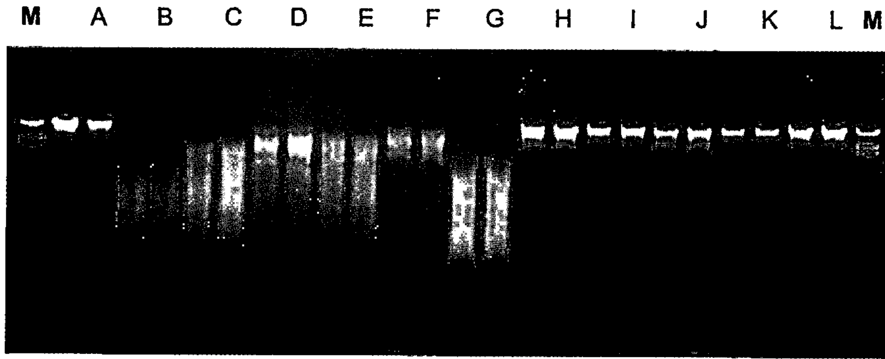
Table 2. Primer sets for PCR analysis.

Primer set*	Sequence	Expected size of PCR product (bp)	Targeted gene	Related literature
NS5	5′-AACTTAAAggAATTgACggAAg-3′	310**	Nuclear small rRNA gene	White et al. 1990
NS6	5′-gCATCACAgACCTgTTATTgCCTC-3′		(fungal)	
ML3	5′-gCTggTTTTCTACgAAACATATTTAAg-3′	934**	Mitochondrial large rRNA	White et al. 1990
ML4	5′-gAggATAATTTgCCgAgTTCC-3′		gene (fungal)	
Luck 24	5′-ATgCCTACTATCATCgACAAATTg-3′	987	*L. edodes*	Kaneko et al. 1998
Huck 966	5′-gggATTCATggTCCATggATAA-3′		nuclear *uckl* gene	
LG 14841	5′-CCATCCAACATCTCTgCTTgATgAAA-3′	354	*G. gallus* mitochondrial	Irwin et al. 1991
HG 15149	5′-TCAgAATgATATTTggCCCCA-3′		cytochrome *b* gene	Shen 2001
LG 14841	5′-CCATCCAACATCTCTgCTTgATgAAA-3′	1,028	*G. gallus* mitochondrial	Irwin et al. 1991
HG 15833	5′-gATTgTgggCAAgAggATAAgT-3′		cytochrome *b* gene	Shen 2001
Lvim 182	5′-TgCAgATCTCTCCgAAgCTgCT-3′	1,095	*G. gallus* nuclear	Zehner et al. 1987
Hvim 1233	5′-CAggTTCAAAgAAgCAAAggTTg-3′		vimentin gene	

* Primer sets are indicated in the order of 5′ primer and 3′ primer.

** Product sizes are approximately based on the rRNA genes of *Saccharomyces cereviceae*. Product size may vary according to species especially for mitochondrial rRNA gene. In case of *L. edodes*, product size of ML3/ML4 primer set seems to be approximately 1,300 bp.

A.



B.

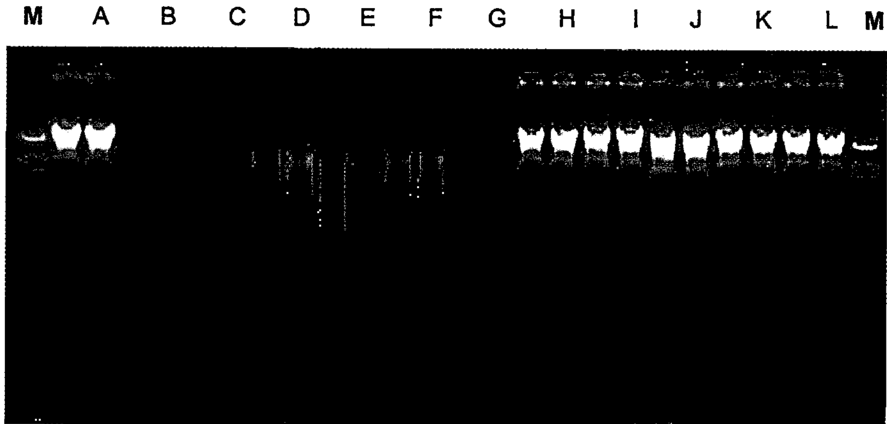


Figure 1. DNA extracted from freeze-dried mushroom *Lentinus edodes* (A) and freeze-dried muscle of chicken *Gallus gallus* (B). One tenth of the extracted DNA was examined by electrophoresis on a one percent L03 agarose gel. A–L correspond to treatments in Table 1. M: λ/Eco T Marker DNA (100 ng total). Fragment size (base pairs): from the top to the bottom: 19,329; 7,743; 6,223; 4,254; 3,472; 2,690; 1,882; 1,489; and 925.

spin column (Applied Biosystems). Sequencing was performed using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems), followed by analysis using an ABI PRISM 377 DNA Sequencer (ABI).

Results

Effects of Various Pest Control Treatments on DNA Extracted from Specimens

The electrophoretic patterns of the extracted DNA from treated samples are shown in Figure 1. These patterns clearly show degradation (smeared pattern) of the specimens in lanes B to G. Lanes of untreated samples (A and L) show the pattern of intact DNA, which contains both genomic and mitochondrial DNA, in

a broad band. The degradation pattern is obvious in lanes of samples fumigated with methyl bromide/ethylene oxide mixed gas (B), methyl bromide (C), ethylene oxide (D and E), propylene oxide (F) and methyl iodide (G). Sizes of degraded fragments of DNA are continuous from high molecular weight to low molecular weight (less than 1,000 bp).

In the lanes containing samples treated with sulfuryl fluoride (H), low temperature (−30°C for one week) (I), heating (60°C for 24 hours) (J), and 60 percent volume of carbon dioxide treatment (K), there appears to be no significant indication of DNA degradation, compared with lanes containing untreated samples (A and L).

Degradation was more severe in the samples of freeze-dried muscle, (Fig. 1B) than those of freeze-dried mushroom (Fig. 1A). This might be a reflection of the difference in the cell structure (difference in the existence of cell walls, etc.), which will be mentioned later in the discussion.

Effects of Various Pest Control Treatments on DNA Amplification by PCR

PCR was performed using equal volumes of each of the extracted DNA solutions shown in Figure 1 as template DNA. Results of PCR with three kinds of primer sets with DNA extracted from freeze-dried mushroom are shown in Figure 2.

The result of amplifying a fragment (ca. 310 bp) of the ribosomal RNA gene (nuclear small rRNA gene) interestingly shows no significant difference in the efficiencies of the PCR in all lanes (Fig. 2A). This result suggests that, when the targeted fragment size is small (ca. 310 bp), and if the template DNA molecules exist in sufficient number for PCR, PCR can be successfully performed even with the samples that show DNA degradation. This result also suggests that the reaction of PCR itself can be successfully performed in all the treated samples, without inhibition by residual chemicals.

However, when the size of the targeted fragment is larger (ca. 1,300 bp), there appears to be a clear difference in efficiencies of PCR (Fig. 2B). This gene region is part of a mitochondrial rRNA gene, which exists in a high number of copies in cells, but the difference in the efficiencies of PCR among treatments are obvious. The samples which amplified poorly show a good correlation with those samples whose template DNA was shown to be degraded (smeared) in Figure 1.

In the same manner, we can see that, when the targeted fragment is from a single copy nuclear gene, a greater number of samples fail to amplify (Fig. 2C).

On the other hand, all sets of PCR were successfully performed with the samples treated with sulfuryl fluoride (H), low temperature (I), heating (J), and 60 percent by volume of carbon dioxide (K). The yield of PCR products seems to be almost equal to that of untreated samples (A and L).

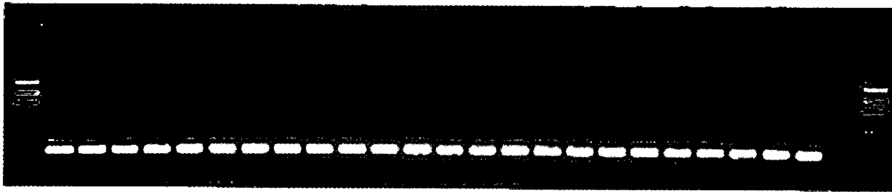
The results of PCR with extracted DNA of freeze-dried chicken muscle (Fig. 3) can be explained in the same manner as in the case of freeze-dried mushroom, but in this sample, the decrease in the amount of PCR products was more severe, a fact which is thought to reflect the more severe patterns of degradation of the template DNA.

Direct Sequencing of PCR Products

To examine the DNA sequences of the PCR products, direct sequencing of the amplified products was performed for a 354 bp fragment of the mitochondrial

A. Nuclear small rRNA gene, 310bp

M A B C D E F G H I J K L N



B. Mitochondrial large rRNA gene, ca. 1,300bp

M A B C D E F G H I J K L N

C. Nuclear *uck1* gene, 987bp

M A B C D E F G H I J K L N

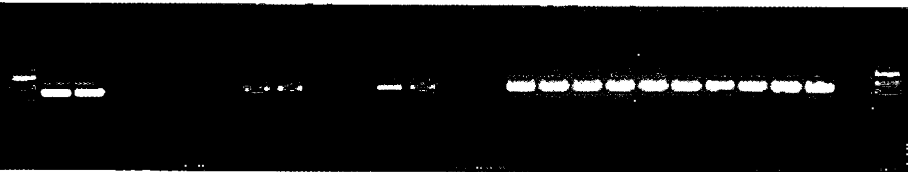
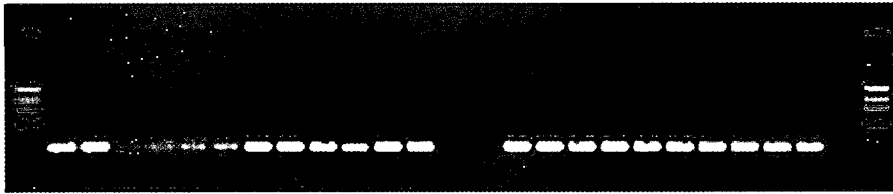


Figure 2. Results of PCR with DNA extracts of freeze-dried mushroom *Lentinus edodes*. PCR products were examined by electrophoresis on a one percent L03 agarose gel. A–L correspond to treatments in Table 1. M: pHY Marker DNA (200 ng total). Fragment size (base pairs): from the top to the bottom: 4,870; 2,016; 1,360; 1,107; 926; 658; 489; and 267. N: no template control. For B, the expected size based on the rRNA gene of *S. cerevisiae* is 934 bp, but the actual size varies from species to species. In case of *Lentinus edodes*, the PCR product is approximately 1,300 bp (B).

cytochrome *b* gene of *G. gallus* and an approximately 1,300 bp fragment of the mitochondrial rRNA gene of *L. edodes* for both DNA strands, using primer sets LG14841/HG15149 and ML3/ML4. This time, we examined the DNA sequences of the PCR products amplified from samples given treatments A, B, C, E, F, G, H and J (Table 1). Direct sequencing of the 354 bp PCR products of the *G. gallus* mitochondrial cytochrome *b* gene was successfully performed for all the samples examined (A, B, C, E, F, G, H and J). There were a few ambiguous sites; however when sequences from both strands were compared, complete sequence data could be obtained. When the yield of PCR product was low, as is shown in lanes G in

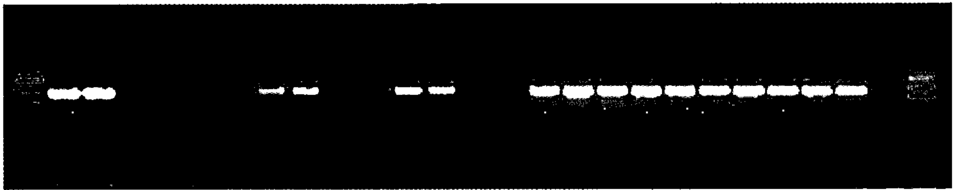
A. Mitochondrial cytochrome *b* gene, 354bp

M A B C D E F G H I J K L N



B. Mitochondrial cytochrome *b* gene, 1,028bp

M A B C D E F G H I J K L N



C. Nuclear vimentin gene, 1,095bp

M A B C D E F G H I J K L N

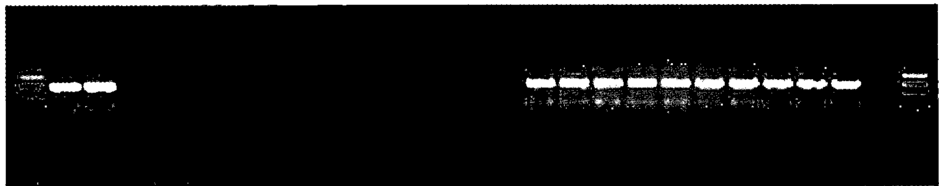


Figure 3. Results of PCR with DNA extracts of freeze-dried muscle of chicken *Gallus gallus*. PCR products were examined by electrophoresis on a one percent L03 agarose gel. A–L correspond to treatments in Table 1. M: pHY Marker DNA (200 ng total). Fragment size (base pairs): from the top to the bottom: 4,870; 2,016; 1,360; 1,107; 926; 658; 489; and 267. N: no template control.

Figure 3A, a four-fold greater concentration of the PCR product was used to obtain a readable sequence. As a consequence, there was no difference in DNA sequence data of the PCR products among these treatment options. The DNA sequence is shown in Figure 4A.

When we tried direct sequencing of the approximately 1,300 bp fragment of the mitochondrial rRNA gene of *L. edodes* (Fig. 2B), complete sequence data was obtained except for treatments B and G. For treatment B, we could not obtain sequence data and for treatment G, only a partial sequence was obtained, even when we used all the PCR product. There were no differences between the se-

A.

CCATCCAACATCTCTGCTTGATGAAATTTCGGCTCCCTATTAGCAGTCTG
CCTCATGACCCAAATCCTCACCGGCCTACTACTAGCCATGCACTACACAG
CAGACACATCCCTAGCCTTCTCCTCCGTAGCCCACACTTGCCGGAACGTA
CAATACGGCTGACTCATCCGGAATCTCCACGCAAACGGCGCCTCATTCTT
CTTCATCTGTATCTTCCTTCACATCGGACGAGGCCTATACTACGGCTCCT
ACCTCTACAAGGAAACCTGAAACACAGGAGTAATCCTCCTCCTCACACTC
ATAGCCACCGCCTTTGTGGGCTATGTTCTCCCATGGGGCCAAATATCATT
CTGA

B.

TGACATCTACATTTATAGATTTATGGAGGTACAGAAAGGCAACTCCCAAC
AAGAAATAGTTTTATGTTGTGCCCTATTTCTTAAAAAATAGACAGCATCC
TTAGTCGAGCATCGAGCATCCTTATCTCCAACCTTTCGTCACTTCACCTT
TCGTATATAAGTACGTAGTACAGAGTACGGAGCCCTTAAAAGGGAGTGGT
GGTTGTTGTATGTAGAGGGAGGAGGAAATGGAAGCAAACATGCGTAGCCG
TAGGCAGGCGGGAAAATTTTTAATTTAATTTTAATCGATTAAATAAGTTT
TTACATAATACTATTTGGAGTTCAGGTTGCGGGGACGTAGAAATCTTACC
TTTGGAGGCGAATCTCGGAATTACATAAATTGATAGTAGATATTCAGACT
ATTCATGCTAAGTTGAATAGTCAAGACGGAAACAGCCGAGAACACAGATC
AAGGTCCCAAATGATTGTTAAGTGAAATTAAGGATGTAGCATAATCGTAT
ACAGCTGTTAAGTGAGCCTGGCAGTCGCTACTTATAATGATCGTATGTAA
CAACGCATCAGCAGTAAGACTGTAGCACCGAAAATTTAACGGGTCTTAAA
CAATCAACCGATATCGTGTTGGTATTAAATAAAATATTGTACTCTCCCGA
TACCTGCCATACGACAATGACACCGTGTTATTCTATTCTATTGATTAGGT
TAACTCTATGTAGTTGAGTATGGATTACGGAGTACGGATTCTTGAGTACA
GCACCATTCTACGGTTATGTTACCCCTTCGGAGCGTAGACGTAGCCATGA
TGCTTATTTTTATTTTATTTTTTATCTAGGTAGTAGAACATTCAGTCAAT
CTATTGAGAAAATAGTGTTTCATTATTTTTTAGGGAACTGAAGAGAGAAT
GCTGACATGAGTAACGTAAAAGAAGTTAATAATACTTCTCGCCGAAAACG
AAAGGGTTGCACAACTAGATTAAATCTTTGCCACTGTGTTAATGCGGCCT
CTAAGGACCCTAACCAAAGTTATGGCTGATGAGTAAATAGTAGAAAGTCC
TAAAATAGTTTTAATAAAATAAAATTAATGGACCAGGAAATTAATATTAT
TATCTTAACTTTTTAATAGAATATATTGTATTTTTATTAATAGAAGAGAT
TATTACTACCGTACCCTAAACCGACACAGGTTCGTAGGTAGGGAATACTA
AGGCGCAGAGCTAAAAGTTG

Figure 4. DNA sequences of PCR products by direct sequencing. A. DNA sequence in *G. gallus* cytochrome *b* gene (345 bp), which contains both primer regions. DNA sequence was the same for all the samples. B. DNA sequence in *L. edodes* mitochondrial rRNA gene (1,220 bp), which does not contain primer regions. DNA sequence was the same for the samples for which we could obtain sequence data. For sample B, we could not obtain sequence data. For sample G, only partial sequence was obtained and it was the same as those of other samples.

quences obtained following treatments A, C, E, F, H and J. The sequence is shown in Figure 4B.

Discussion

Suggested Mechanisms of DNA Damage by Fumigants

In this study, degradation of extracted DNA was observed in the samples treated with methyl bromide, methyl bromide/ethylene oxide mixed gas, ethylene oxide, propylene oxide and methyl iodide. Price (1985) and Florian (1988) describe the reactivity of methyl bromide and ethylene oxide with their characteristics of alkylating agents. Alkylating agents are generally known to take part in the alkylation of macromolecules such as proteins and DNA. Methyl bromide and methyl iodide (both are methyl halide) are involved in methylation, and ethylene oxide and propylene oxide are involved in hydroxyl-alkylation. Recent studies have shown that these alkylating agents induce DNA adducts by alkylation (Bolt et al. 1988, 1991, Bolt and Gansewendt 1993, Gansewendt et al. 1991a, 1991b, Starratt and Bond 1988, Thier and Bolt 2000, Walker et al. 1990). DNA adducts are covalent complexes formed between genotoxic carcinogens and DNA bases. These DNA adducts are thought to be involved in causing mutations in DNA *in vivo.* The best known DNA adducts caused by alkylating agents are on guanine bases, 7-alkyl-guanine (Lawley and Brooks 1963). The N-glucosylic bond joining guanine to deoxyribose in DNA is destabilized as a consequence of this N-alkylation (Lawley and Brooks 1963). This destabilization causes an elevated number of spontaneous base losses that results in an increase in apurinic sites.

Major DNA adducts caused by methyl bromide and methyl iodide are reported to be 7-methyl-guanine, O6-methyl-guanine and 3-methyl-adenine (Bolt and Gansewendt 1993, Gansewendt et al. 1991a, 1991b, Starratt and Bond 1988). The major DNA adduct in cells exposed to ethylene oxide is reported to be 7-(2-hydroxyethyl) guanine (Bolt et al. 1988, Thier and Bolt 2000, Walker et al. 1990). Similarly, propylene oxide is reported to cause the formation of 7-(hydroxypropyl) guanine (Bolt et al. 1991). In living cells, single-strand breaks are generated in DNA as a consequence of DNA repair processes (Prakash and Gibson 1992). The DNA single-strand breaks or alkali labile sites are known to be induced by a majority of genotoxic substances which are carcinogenic in animals (Sina et al. 1983). However, these reports on the formation of single-strand breaks in DNA were based on the detection of DNA damage in metabolically active, living cells, where DNA repair processes are active, forming single-strand breaks in DNA to eliminate unusual bases. There are not many studies which describe the effects of these alkylating agents on DNA molecules in dead or inactive cells, such as those found in many museum specimens. Therefore, the mechanism of degradation of extracted DNA we have observed here is not yet well understood.

It is possible that the DNA molecules become labile as a result of treatments with these alkylating agents and that, even if significant degradation of DNA does not occur directly because of alkylating agents, degradation of DNA might occur as a consequence of subsequent DNA extraction processes with the labile DNA molecules. This phenomenon is not usually observed with healthy DNA molecules of untreated samples. Living cells have mechanisms of DNA repair, but in

museum specimens lesions on DNA accumulate, thus resulting in severe fragmentation of DNA molecules, as is seen in Figure 1.

Sulfuryl fluoride did not seem to affect the DNA of treated specimens, and subsequent PCR was successful in our study. This result is consistent with the results of Whitten et al. (1999). They fumigated herbarium specimens with sulfuryl fluoride (Vikane[R]), and the DNA extracted from both fumigated and control samples yielded good quality DNA and PCR products (Whitten et al. 1999). Therefore, sulfuryl fluoride (Vikane[R]) does not appear to degrade DNA in specimens, as long as it is used at a concentration sufficient to kill insects. However, in this study, each chemical was applied at a limited concentration; investigation in various concentrations would be necessary to sufficiently know the magnitude of damage by fumigants.

Causes of Decrease in DNA Amplification by PCR

Several potential causes may be considered for reduced yields in PCR products from DNA template solutions, including a decrease in the available number of target DNA molecules or the presence of some residual inhibitory agents in the template DNA solutions. For example, in analysis of ancient DNA, inhibition of PCR by residual contaminants has been commonly stated (Handt et al. 1994, Tuross 1994).

In our case, DNA degradation seemed to be the first and major cause of the observed reduction in PCR efficiency, as the amount of PCR products was shown to be dependent on fragment size. This correlates well with the degradation patterns of template DNA shown in Figure 1. In analysis of ancient DNA, where template DNA is usually severely fragmented, fragment-size dependency of PCR efficiencies are also well known. In other words, an inverse relationship between amplification efficiency and length of the amplification products is observed, and only small fragments of about 100 to 200 bp can be commonly amplified (Handt et al. 1994).

Another possible cause for the decrease in PCR efficiency is the inhibition of the enzyme (Taq polymerase) for PCR by residual chemicals and modification of the template DNA. If the main cause is inhibition of Taq polymerase, the amplification of both small and larger fragments would be likely to be equally inhibited and such clear fragment-size dependency of PCR efficiency would not be observed. Considering the results in our case, residual chemicals do not appear to be the major cause of the PCR inhibition. As to the possibility of the modification of template DNA, alkylation of template DNA molecules was very likely to have occurred to some extent, but its involvement in PCR efficiency was not clear in this research.

Effects on DNA Sequencing Data from PCR Products

If the amount of PCR products is not sufficient for sequence analysis, we cannot analyze the DNA sequence of the region. Akiyama (2001) showed a case in which DNA sequencing was deficient for the PCR product of approximately 1,000 bp fragment in mitochondrial DNA amplified from dried muscle that had been fumigated with methyl bromide/ethylene oxide mixed gas. The causes of failures in DNA sequencing could be due mainly to the amount of PCR products, but it is possible that alkylation of DNA by chemicals might occasionally influence the DNA sequence data of PCR products. For example, it is reported that some al-

kylating agents such as N-methyl–N-nitrosourea and N-methyl–N′-nitro-nitrosoguanidine can cause alkylation of DNA at sequence-specific sites (Mironov et al. 1993, Shoukry et al. 1993). If this kind of highly sequence-specific alkylation occurs, there is a possibility that we can detect the effects of alkylation at sequence-specific sites in DNA sequence analysis. But when the alkylation occurs in a non-sequence-specific random manner, the bulk sequence data obtained by direct sequencing of PCR products would not reflect each change.

In our examination of the DNA sequence of the PCR products, we did not observe sequence-specific change of DNA bases. Therefore, it might be said that the chemicals we used here did not appear to cause changes in the DNA sequence as long as we did direct sequencing of the bulk PCR products.

Difference in the Effects on the Two Specimen Types

The degradation pattern of DNA was more severe in samples of freeze-dried muscle (Fig. 1B) than those of freeze-dried mushroom (Fig. 1A). This might be a reflection of the presence or absence of a cell wall. Cells of fungi commonly have cell walls but cells of animals do not. Furthermore, Akiyama (2001) showed that DNA in plant leaves which are rich in external cuticle was more tolerant to fumigation than that in mushroom or animal muscle. This is an interesting difference which might be explained by the accessibility of the fumigants to DNA molecules in cells. Other specimen types, such as hairs or feathers, might differ in the magnitude of damaged DNA, compared with muscle specimens. However, parallel results in the DNA degradation patterns obtained from the two types of specimens in our study might suggest the general tendency for damage caused by the chemicals when they have access to the DNA molecules.

Possible Link to Knowledge on Seed Viability

Strang (1998) reviewed seed viability of plant specimens in the context of thermal treatments to control insect pests, equating lowered viability to macromolecular damage. He concluded that, if the seeds are in well-dried conditions (specimens preconditioned below 50 percent RH before heating at 55–60°C, and specimens preconditioned below 14 percent EMC dry basis for low temperature treatments down to −30°C; both of these conditions are normally met in controlled museum storage environments), thermal methods would not affect seed viability (Strang 1998). Consistent results in heating were reported on the preservation of both DNA and proteins by Chalfoun and Tuross (1999). They examined modern barley seeds heated in a regime from gentle to charring conditions for the preservation of both DNA and proteins, and reported that extended heating at 100ºC had little effect, although higher temperature caused the chemical modification and degradation of both protein and DNA as well as the failure of immunological detection by Western blotting and PCR (Chalfoun and Tuross 1999).

Since seed viability is a complex process in which many molecules such as DNA and proteins are involved, in the situations where seed viability is well preserved, DNA molecules are also expected to be well preserved.

In our experiments we used freeze-dried, dry samples, and no significant problem was observed on extracted DNA by low temperature treatment or heating. While this is consistent with observations on seed viability, it is possible that

repeated freezing and thawing can affect DNA, if the samples are damp or wet, probably because of mechanical stress by ice formation.

High doses of fumigants such as methyl bromide, ethylene oxide and methyl bromide/ethylene oxide mixed gas, are reported to affect percentages of germination of seeds of malts (Matsunobu and Mori 1968, Moriyama et al. 1974). This seems to correlate with the damage on DNA molecules we have observed.

Effects of Repeated Treatments

In our study, we did not examine the effects of repeated treatments on DNA molecules of specimens. Further investigation would be necessary to confirm the impact of repeated treatments.

Conclusions

Significant degradation of extracted DNA of the specimens, such as genomic DNA and mitochondrial DNA, was shown in the samples treated by some fumigants; methyl bromide, methyl bromide/ethylene oxide mixed gas, ethylene oxide, propylene oxide and methyl iodide, even with a single fumigation. Subsequent amplification of certain DNA fragments by PCR was difficult in these samples. There were more problems in amplifying larger DNA fragments than smaller fragments. The main cause for the decreased efficiency of PCR was thought to be the degradation of template DNA. On the other hand, thermal methods (heating and low temperature), carbon dioxide treatment, and fumigation by sulfuryl fluoride seemed scarcely to affect the DNA molecules of the specimens, making subsequent PCR successful.

This study describes only the results of a single treatment under limited conditions. More investigation will be necessary to determine the effects of treatments; repeated treatments; treatments in different conditions, such as with specimens of different moisture contents; or with different concentrations of chemicals. But at least it is possible to consider how damaging to collections it would be to perform repeated fumigation by chemicals which cause obvious degradation of extracted DNA molecules even after a single treatment.

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EXAMINATION OF MACROSCOPIC PARTICLES FROM DUST ACCUMULATIONS IN COLLECTION STORAGE AREAS

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Abstract.—The dirt from the floor of a collection storage area was examined to determine its usefulness in understanding collection conditions and practices. Accumulated floor debris was separated into different groups based on particle size. The larger-sized particle groups were subjected to macroscopic examination for material identification. All particle-size groups were subjected to analyses to determine moisture absorption potential, as well as the proportion of ferrous material, soluble proteins, and organic material present in the sample. This study demonstrates the usefulness of analyzing dust and debris in collection storage areas for evaluating practices. Also, the study provides fresh perspectives about dust and debris in terms of pest management, the nature of particulate matter in collections, and human health concerns.

Bisbing (1989) points out that dust "is found as small particles in a pulverized state created by the disintegration of other materials" and that "its compositional and morphological features [are] derive[d] from its origin." As a result of this realization some professions, such as criminology and archaeology, go to great lengths to analyze dust to obtain evidence of the past. It is speculated that similar analyses can provide evidence about a collection as well.

Cleaning processes are usually predicated on some awareness of undesirable materials being present and in direct association with surfaces and materials of special concern. Although the number and diversity of cleaning processes are perhaps endless, it is common to be more focused on the process itself than the nature of the undesirable materials that are being removed. This is certainly the case when a floor is swept, and the accumulated dust and debris are collected and ultimately discarded. While any serious thought about the nature of discarded dust and debris might be somewhat revolting to some, the article entitled "Clues in the Dust" (Bisbing 1989) gives cause for reconsidering the significance of these undesirable materials.

It has been documented that accumulated dust and debris below storage cases can be a major contributor to pest infestations in collections (Williams and McLaren 1990). In such situations, questions arise about the materials in the dust that would support a thriving population of pests. This in turn leads to more questions about the nature of other particulate matter that might contribute to preservation problems, such as abrasion, soilage, chemical reactions, and research-based contamination. Also, the relationship of these materials to human health and safety concerns should not be overlooked.

There may be multiple benefits of analyzing the dust and debris from collection storage areas. Evidence of collection conditions and practices may be revealed; or, by understanding the nature and source of the materials involved, it may be possible to avoid or block those materials that pose the greatest risks to collections

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and individuals. With these thoughts in mind, some simple tests were conducted to better understand the stuff in collection corners commonly known as "dirt."

The study described herein represents a continuation of a study previously described by Williams and McLaren (1990). In that particular study, a serious infestation of the odd dermestid (*Thylodrias contractus*) was documented in the collection. The source of the infestation was the floor debris that accumulated below storage equipment. A direct correlation was made between levels of human activity in the collection and quantity of accumulated debris. The problem was resolved by removing debris accumulations and positioning the storage units so that they were well above the floor level. The contribution described in the following text reports on the floor debris analyses that immediately followed the original study.

Methods and Materials

Floor debris was collected as a part of a previously described project (Williams and McLaren 1990). As groups of storage cases were removed from their initial position, the underlying accumulated dust and debris were swept together, collected in a dustpan, and totally enclosed in polyethylene bags. The accumulated material was weighed to the nearest 0.1 gm with a triple-beam balance. The current study goes beyond documenting trends of debris accumulation in the collection area by examining the composition of the actual material collected from the original study.

The debris from each set of cases was partitioned according to particle size using Standard Sieve Series (A.S.T.M. Designation E11) with sieve openings of 2.00 mm (No. 10), 1.00 mm (No. 18), 0.500 mm (No. 35), and 0.250 mm (No. 60). Each resulting particle-size group was weighed and placed in a separate polyethylene bag. For the sake of discussion, particle sizes were categorized as follows: Group A, ≥2.0 mm; Group B, 1.0 to 2.0 mm; Group C, 0.5 to 1.0 mm; Group D, 0.25 to 0.5 mm; Group E, ≤0.25 mm.

Basic identification of debris material.—The project included the examination of the sample having the greatest quantities of materials from the study described by Williams and McLaren (1990:30). The components of the three groups representing particle sizes larger than 0.5 mm (Groups A, B, and C), were manually separated into identifiable materials. Groups D and E were not included in this part of the study because the procedure, involving greater numbers of near-microscopic particles, became increasing difficult and did not justify what little information might be gained. Instead, relevant information was obtained by other methods, as described in the following text.

Percent of ferrous materials.—Because the purpose of the project was to analyze the composition of floor debris, it was appropriate initially to separate known components, if possible. Also, the possibility of analyses being influenced by the weight of the iron particles had to be considered. For this reason, the ferrous materials were removed from the group samples using a magnet. The proportion of the weight attributable to ferrous material was determined by the resulting sample weight after the ferrous materials were removed.

Analyses of heavy metal preservatives, such as arsenic, were not conducted even though it is acknowledged that they may exist with some specimens in the collection. It is unlikely that there would be detectable amounts of such preser-

vatives because the sources (specimens) are enclosed in storage units and less likely to become part of the floor debris. The miniscule amounts that might become part of the floor debris would be significantly diluted with the much greater volumes of debris originating from external sources that are totally disassociated from such preservatives. For other analyses, ten separate one-milliliter units were removed from each particle-size group for determination of moisture-absorption potential, protein content, and total organic content.

Analysis of moisture absorption potential.—Because hygroscopic materials can serve as a source of moisture for insect pests and mold growth (Florian 1997, Scott 1994), an analysis of the moisture absorption potential of the floor debris was conducted. The group samples were weighed at ambient conditions (65 percent RH), desiccated in an oven for 48 hours at 45°C, and reweighed to determine moisture absorption potential. Next, the samples were acclimated to 100 percent RH for 24 hours and reweighed, to determine maximum moisture-absorption potential; this was assumed to be relevant because of water getting under cases when floors are mopped.

Percent of soluble proteins.—Although some pest species can survive on cellulosic materials, species of primary concern in the situation described by Williams and McLaren (1990) were those species that typically feed on proteinaceous materials. For this reason, group samples were analyzed for protein content. The presence of proteins in dust accumulations, as well as differences in protein levels between sizes of particulates, was determined using a Micro Protein Determination Kit (Sigma Chemical Company; P.O. Box 14508, St. Louis, Missouri 63178). This procedure is based on the phenol reagent method described by Ohnishi and Barr (1978) and Sigma Chemical Company (1985). Spectrophotometric absorbency readings were taken at 580 nm. The procedure measures amounts of soluble proteins.

Percentage of organic material.—The amount of organic material in each of the group samples was determined by methods described by Dean (1974). Each sample previously used for moisture absorption analyses was desiccated, weighed, heated in crucibles at 550°C for two hours, and reweighed to determine the amount of organic material burned off by the procedure.

Results

Most of the volume and mass of each sample consisted of materials that would not pass through a 2.0 mm mesh screen. Although there were identifiable materials in these samples, most of the bulk consisted of matted fibers of organic (mostly plant) origin. As particle sizes were separated with screens into sub-samples less than 2.0 mm, there was a progressive trend of smaller particles being represented by greater numbers and a greater proportion of the total sample weight, due to open spaces being filled with particles.

Basic identification of debris material.—Table 1 provides an overview of the materials identified in the floor debris from the collection storage area. Following the large amounts of lint material previously mentioned, the next most conspicuous materials included pieces of wood, mop strings, papers/paper products, cotton wool, various plastics, and several kinds of unidentified materials. The paper material included corrugated cardboard, colored paper, specimen label material,

Table 1. List of materials represented by particles greater than 0.5 mm, removed from the dust and debris of a collection storage area. Items are listed in general order of diminishing quantities, in terms of volume, of the particles exceeding 2.0 mm in size (Group A).

Material	Group C (0.5–1 mm)	Group B (1–2 mm)	Group A (≥2 mm)
Lint	X	X	X
Wood	X	X	X
Mop strings			X
Paper/paper products			X
Plastics	X	X	X
Cotton wool			X
Unidentified materials	X	X	X
Paint	X	X	X
Metal	X	X	X
Hair		X	X
Gravel	X	X	X
Label strings			X
Leaf parts			X
Insect parts		X	X
Bone			X
Glass	X	X	
Wax	X	X	

and an assortment of broken or cut paper fragments. The plastics included polystyrene, polyethylene, an unknown plastic from an ornament, and a rubber band.

Several other materials also were identified, but their representation in terms of volume was noticeably lower than the previously mentioned materials. These materials included paint chips, various metals, hair, gravel, label strings, leaf parts, insect parts, and bone. The metals included ferrous forms identified with a magnet, as well as monel wire. The hair samples appeared to have both human and specimen origins.

Only two materials (glass and wax) were not found with the particles larger than 2.0 mm, yet were present among the groups of smaller particle size. While the groups with smaller particles had many of the same materials previously discussed (Table 1), noted discrepancies were attributed to a function of the screens (for example, mop strings) or fragments too small for reliable identification (for example, bone).

A cursory examination of materials from other samples from the collection area revealed a variety of other materials not listed in Table 1, such as electrical wires, tape, feathers, and wood products. While the identification of other materials may have some interest, the current study focused on the general qualities of the remaining floor debris. Analyses of the groups are summarized in Table 2.

Percent of ferrous materials.—Various amounts of ferrous particulates were initially removed from the samples using a magnet. The percentage of the mass of such particulates was determined from the total mass of the group sample in question. The samples from Group A (≥2.0 mm) were not included because of the obvious bulk of fiber and wood, as well as the presence of metal objects, such as staples and paper clips. Group B (1.0 to 2.0 mm) had the greatest average

Table 2. Summary of analyses of debris collected under collection storage units.

Group	Particle size (mm)	% Total weight	Sample size	% Ferrous particulates Mean (Min–Max) ± SD	Non-ferrous materials % Moisture weight at 65% RH Mean(Min–Max) ± SD
A	>2	*54.8	10	*	4.9 (4.1–5.7) ± 0.46
B	1.0–2.0	7.2	10	9.6 (2.9–25.5) ± 6.98	1.8 (1.2–2.7) ± 0.48
C	0.5–1.0	9.4	10	6.6 (3.6–11.7) ± 2.84	1.7 (1.3–2.2) ± 0.30
D	0.25–0.5	10.5	10	7.0 (2.5–13.6) ± 3.50	2.3 (1.7–3.3) ± 0.49
E	<0.25	18.1	10	8.3 (4.4–18.3) ± 4.25	2.3 (1.7–2.6) ± 0.29

* Value influenced by the presence of specific objects, such as paper clips and larger pieces of wood. Non-ferrous analyses were performed on only the fibrous portion of the sample.

percentage of ferrous material (9.6 percent). Group E (≤0.25 mm) represented the next largest average percentage (8.3 percent; Table 2).

Analysis of moisture absorption potential.—The group samples representing non-ferrous materials were subjected to moisture absorption analyses. Group A (≥2 mm) had two to three times the amount of moisture absorption capacity compared to other samples. Groups B (1.0 to 2.0 mm) and C (0.5 to 1.0 mm) had very similar moisture levels, both of which represented the lowest levels of all group samples examined. Groups D and E (≤0.5 mm) retained about 60 percent more moisture than Groups B and C. The relationships of moisture absorption potential between all group samples remained the same for comparisons at 65 percent RH and 100 percent RH (Table 2).

Percent of soluble proteins.—The group samples representing non-ferrous materials were subjected to analyses of soluble proteins. All groups examined had detectable amounts of protein present. The greatest average percentage of proteins (0.16 percent) was found with Group A. With Group C and Group D, the average percentages dropped to 0.10 and 0.11, respectively. Groups B and E revealed another drop of protein content with respective average percentages of 0.06 and 0.07.

Percentage of organic material.—The quantification of organic material present showed Group A to have the highest average percentage (90.1 percent). In dealing with the other groups having actual particles, there was an inverse relationship noted between the amount of organic material present and the particle size. The smallest particles, represented by Group E, had an average percentage of organic materials equal to 53.1 percent, whereas the much larger particles, represented by Group B, had a lower average percentage (32.9 percent).

Discussion

One of the recognized agents of deterioration is contamination (Michalski 1994, Waller 1994). Although contamination in the form of particulates is readily recognized throughout the museum community as a problem, it is somewhat surprising that comparatively speaking very little has been published on particulate matter in collection areas.

Perhaps the most useful information about particulate matter is found in literature about the atmosphere and environment, such as Brimblecombe (1986), Fennelly (1976), and Hinds (1982). However, the primary focus of such literature

Table 2. Extended.

	Non-ferrous materials		
Group	% Moisture weight at 100% RH Mean (Min–Max) ± SD	% Soluble proteins Mean (Min–Max) ± SD	% Organic material Mean (Min–Max) ± SD
A	10.7 (8.6–13.5) ± 1.38	0.16 (0.15–0.23) ± 0.01	90.1 (85.4–92.8) ± 2.33
B	3.2 (2.2–4.7) ± 0.85	0.06 (0.05–0.15) ± 0.01	32.9 (27.0–39.0) ± 3.70
C	3.1 (2.6–3.9) ± 0.42	0.10 (0.06–0.14) ± 0.04	43.3 (33.2–52.2) ± 5.73
D	4.6 (3.5–6.8) ± 0.06	0.11 (0.08–0.18) ± 0.01	48.0 (38.7–57.7) ± 6.35
E	4.5 (3.5–5.1) ± 0.53	0.07 (0.05–0.07) ± 0.01	53.1 (47.0–61.9) ± 4.42

generally involves microscopic particles less than a micron in size. Particles of such size are of considerable interest because of their contribution to general pollution as well as preservation problems, such as abrasion, soilage, and chemical reactivity (Kadokura 1980, Moncrieff and Weaver 1987). For this reason, the primary interest within the museum community has been in ventilation (Scott 1994), filtration systems (Thomson 1986), barriers (Rose and Torres 1992), and less invasive cleaning techniques (Moncrieff and Weaver 1987). In reviewing the available literature, it becomes obvious that very little attention has been given to the macroscopic dirt material in collections that is typically removed by wiping, sweeping, and vacuuming. While it may be generally understood that such material must have adverse effects on collections, very little is reported about the actual materials present and what risks they bring to the collection. Also, in reference to the work of Bisbing (1989), very little is reported about how collection practices might contribute to the problem of dust and debris in collections.

This study documented that much of the dust and debris in collection areas is organic in nature. Although 90 percent of the larger sized particles (Group A) are organic, it is important to realize that all particle groups contained large quantities of organic material, with the amounts increasing as particle size decreased. Based on the examination of materials, most of the organic material seemed to have plant origins, as opposed to the lesser amounts that had origins from animal sources or synthetics. The prevailing cellulosic composition of the dust and debris would provide nourishment, as well as possible moisture and shelter for pests that feed on plant materials. Furthermore, enclosed spaces below storage units may develop higher than normal relative humidity levels, thus raising risks of mold growth, which in turn may attract other forms of insect pests and create possible health problems.

With respect to pest species that feed primarily on animal-based materials, such as proteins and lipids, it is logical to question whether there is sufficient nourishment in the dust and debris to support great numbers of pests, as was the case with the infestation described by Williams and McLaren (1990). The current study determined that the hair, feathers, and parts of insects found with the larger particles, are definite sources of protein. It is likely that sloughed human epithelial cells, spider webs, and wool (from clothing) could represent other sources. Regardless of the source, it is important to realize that detectable amounts of protein were found in all group samples, regardless of particle size. The significance of this point is reiterated with the understanding that the detection methods used in

this study focused on less stable proteins, thus substantially greater amounts of protein would be expected.

While the significance of the smaller particulate matter should not be ignored, Table 1 and Table 2 indicate that most of the problems encountered and the bulk of the dust and debris, are associated with the large-sized particulates (Group A). This group combined with supplemental information from groups B and C, provide useful information about practices and the sources of dust and debris in collections. Based on the nature of the materials identified, both internal and external sources contribute to the problem.

Those materials of internal origin can be divided into advertent and inadvertent practices. The former involves storage designs and various day-to-day activities conducted in the collection.

Storage design proved to be a major influence on the nature of floor debris in the collection. Most of the wood material undoubtedly originated from boards on the floor that were used for leveling storage units. However, the paint chips and ferrous materials are likely the result of abrasion of storage units as they are pushed across concrete floors. The bulk of the inorganic material from the dust and debris might represent similar abrasion of cement surfaces. While the issue of dust and debris is significant by itself, the presumed damage to the storage units is perhaps a greater concern. The loss of paint surfaces raises the risk of corrosion of exposed metal, and the abrasion of the metal weakens the storage unit itself. When one considers the cost of such equipment as well as the ongoing reliance of the equipment to provide long-term management and care of the collection, there is a need to re-evaluate practices and designs involving collection storage equipment.

The day-to-day activities also are evident in the dust and debris of the collection. For instance, cotton wool, polystyrene, and polyethylene provide evidence of the packing or unpacking of parcels, such as those associated with loans. Wax may have been used on drawer runners to facilitate smoother drawer movement. Straight pieces of monel wire suggest activities such as wire stretching and cutting (for processing specimens in the field) or specimen preparation. Because a separate and isolated preparation facility exists for the collection, the second explanation is unlikely. Finally, the mop strings serve as a clue to the cleaning methods used in the collection.

The floor debris also provided evidence of inadvertent collection activities or situations. For instance, the presence of glass and bones would suggest occasional accidents where a vial containing a specimen has been dropped. Specimen hair may simply have been lost from specimens through normal handling. Another inadvertent situation might exist with inorganic materials originating from the deterioration and wear of exposed cement surfaces (Thomson 1986).

Based on this study, it is reasonable to believe that almost all of the dust and debris is the result of internal sources. The only evidence of materials brought in from external sources occurred with human hair, gravel, and feathers. Apparently, these materials have been introduced by the body and clothing of the individuals associated with the collection.

The problems exposed by the study justify a reconsideration of some practices, even if it simply involves a more regular removal of the dust and debris from the collection storage area. Although it is doubtful that the findings of this study

would be duplicated exactly in another setting, the results clearly show how simple analyses of collection "dirt" might be used to improve the management and care of collections.

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PHENOXYETHANOL AS A RELAXANT BEFORE FIXATION IN THE SEA CUCUMBER *CUCUMARIA MINIATA* (ECHINODERMATA)

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Abstract.—Phenoxypropanol has been used as a relaxant before fixation with varying success on many taxonomic groups. Experimental evidence in this study shows that a similar chemical, phenoxyethanol, effectively relaxes specimens of the sea cucumber *Cucumaria miniata* (Brandt 1835) before fixation. The desired state of relaxation was with the tentacles exposed. The use of phenoxyethanol before fixation in 70 percent ethanol reduces the amount of shrinkage as measured after three months. A review of the literature shows that phenoxyethanol has not been tested on many groups as a prefixation relaxing agent.

INTRODUCTION

Over time, the collections of specimens within natural history museums increasingly represent the world's biodiversity. Their value to society is affected by how closely the specimens resemble their living state (Araujo et al. 1995). This is also important in the proper identification of species from many taxonomic groups. If fluid-preserved specimens look significantly different from their living counterparts, it is much more difficult to make accurate identifications.

Traditionally, unsorted collections of marine invertebrates were fixed in a solution of five to ten percent formalin in fresh or seawater, and, after a sufficient period, preserved in a solution of ethanol or isopropanol. Such practice may contort the specimen and obscure taxonomic characters important for identification. Specialists that develop their own collections of specific taxa routinely use a relaxant, anesthetic or narcotic (hereinafter referred to as 'relaxant') prior to fixation to keep important features visible and resilient to handling after fixation. From personal experience, if a relaxant is not used, the shock of the fixative often causes the animals to contract or withdraw appendages, bristles, or mouthparts, making them hard to see, or parts may even become detached. This often renders the specimens more difficult to identify to species level and therefore less reliable to taxonomists. One notable example is a polychaete worm of the genus *Capitella* that is found in most temperate waters of the world. It can assume a different posture at death if it has been handled substantially before fixation. This has been known to cause confusion with at least one other genus from the same family (Mendez and Cardell 1994).

Current methods of relaxing marine and freshwater invertebrates, for most taxa, are numerous and usually specific to the taxon. For the general collection of marine invertebrates, sorting alone can stress animals to the point of defeating the purpose. Some comparatively simple prefixation treatments, however, have been developed and may work satisfactorily on many groups of marine and freshwater invertebrates. If two or three simple methods could be found and tested for their effectiveness as valuable treatments in achieving a desired post mortem state after fixation, the reliability of identifications of invertebrates would increase. Mea-

Table 1. Historical applications of phenoxyethanol (POE) and phenoxypropanol (POP).

Author	Date	Chemical	Application	Taxon/group
Gohar	1937		?	"marine animals"
Gregg	1944		?	terrestrial slugs
Owen	1955	POP	relaxant	lamellibranchs (bivalves)
Owen and Steedman	1958	POP	relaxant/ preservative	terrestrial slugs
Russell	1963	POP	relaxant	clams, gastropods, tunicates
Runham et al.	1965	POP	relaxant	gastropods
Hill	1966	POP	preservative	holothurians (sea cucumbers)
Nakanishi et al.	1969	POE	preservative	general
McKay and Hartzband	1970	POP	relaxant	benthic invertebrates
Hulings and Gray	1971	POP	anesthetic	meiofaunal copepoda
Banse and Hobson	1974	POP	relaxant	polychaetes
Hill and Reinschmidt	1976	POP	anesthetic	holothurians (sea cucumbers)
Townsend	1983	POP	anesthetic	nematodes
Coney	1993	POE	anesthetic	freshwater bivalves
Araujo et al.	1995	POE	relaxant	freshwater mollusks
Norton	1996	both	relaxant	oysters
Lellis and Plerhoples	1997	POE	anesthetic	freshwater mussel
Mills et al.	1997	POP	anesthetic	oysters
Aquilina and Roberts	2000	POP	relaxant	abalone

surements of body parts would be closer to those found in life, and the specimens themselves would be more flexible and resilient to handling.

Phenoxyethanol (hereinafter POE) and phenoxypropanol (hereinafter POP) have been used in several applications with various animals over the last 70 years with varying success (Table 1). More than 50 years ago POP was used as an active agent against gram-negative bacteria and to restore blood pigments after formalin fixation (Owen 1955). Due to the mixture of trade or brand names with those relating to chemical structure for both chemicals, deducing what compound was used is sometimes unclear. A list of synonyms from previous studies for POE and POP is given in Table 2.

While POP has been used on many groups, POE has not (Table 1). POP is a

Table 2. Synonyms for phenoxyethanol and phenoxypropanol compiled from references in Literature Cited.

Phenoxyethanol	Phenoxypropanol
2-phenoxyethanol	propylene phenoxetol
Phenoxetol	propylene phenoxytol
ethyleneglycol-mono-phenylether	1-phenoxy-2-propanol
glycol-mono-phenylether	1-phenoxyisopropanol
Monophenylglycol	1-phenoxyisopropyl alcohol
Phenylethyleneglycol	phenyl-beta-hydroxypropylether
Phenylglycol	propylene phenoxotol
beta-Hydroxyethoxybenzene, POE	POP

colorless, nonflammable, stable aromatic solid. POE is a colorless, non-volatile, nonflammable, stable and nearly odorless oily liquid. The latter has been known to work well in maintaining the integrity of native proteins of sample specimens while used for weeks as a preservative (Nakanishi et al. 1969). Other uses for POE and POP have varied over time. Some of the more common uses today are as biocides in some embalming fluids and cosmetics, carriers for fragrances or flavours, coatings, in coolants, optical brighteners in dyestuffs and hardeners in glues and adhesives. Information from the Material Safety Data Sheets (MSDS) for POE and POP suggests that POE is less toxic to rabbit skin than POP. POE is more toxic than POP when ingested by rats. Material Safety Data Sheets were located on the internet at the web site for Fisher Scientific at http://www.fishersci.ca/msds.nsf.

In this study POE was used on a common species of sea cucumber to check its effectiveness as a relaxant before fixation. *Cucumaria miniata* (Brandt 1835) [Echinodermata] is a burrowing sea cucumber found commonly in the waters off British Columbia. Its distribution ranges from the Aleutian Islands in Alaska south to San Benito Island, California. It can be found in large groups at depths between zero and 25 meters, where a current exists along the bottom (Lambert 1997).

Methods

POE was applied to 20 individuals of *Cucumaria miniata.* Specimens were collected in the spring and summer of 2001 from Wain Rock and Telegraph Cove, both near Victoria, British Columbia, Canada. They were collected by hand using SCUBA and transferred into buckets of seawater from the collection site. The specimens were maintained undisturbed in a 200-liter seawater aquarium held at 12°C for 14 days before being treated with POE. Each sea cucumber was placed in a separate glass stacking-dish containing a solution of 0.15 percent POE in seawater held at 12°C. Each individual was checked frequently to ensure that regular cloacal pumping continued for the duration of its treatment in POE. The specimens remained in the relaxant for about 1.75 hours before being transferred to 70 percent ethanol. Ideally, there should be an equal number of specimens used as controls, but only two were used.

Total length of each sea cucumber was measured with string to the nearest 0.5 cm from the cloaca to the base of the tentacles immediately prior to being immersed in the relaxant solution. After being placed in POE, specimens were measured at 25 minutes and 1.75 hours, then transferred to 70 percent ethanol. All measurements were done using string to avoid disturbing them initially and for the remainder of the exercise to be consistent.

Results and Discussion

The results presented here are not as conclusive as they could be if given the lack of control specimens. That should be considered with any comparisons made between the controls and the treated specimens.

After 25 minutes in POE there was a 16 percent average reduction in body length (Fig. 1). This is most likely due to the shock of the solution on the specimens. Less than half of the treated specimens had relaxed to the point where the tentacles were exposed. Conversely, the two control specimens had shown a relative increase in body length of five percent. At the time all specimens, treated

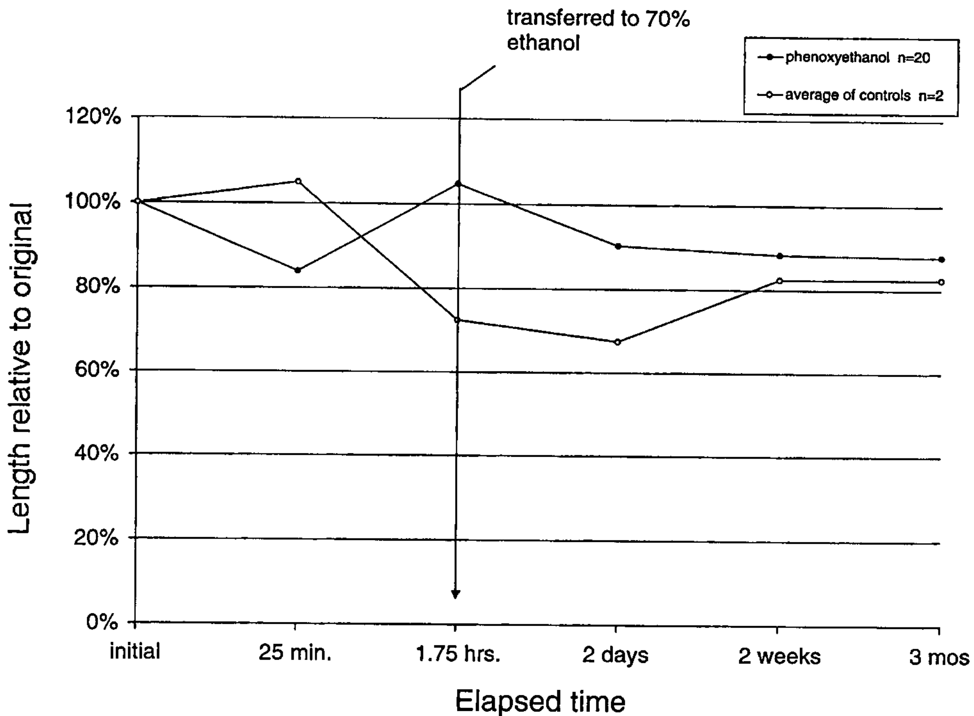

Figure 1. Change in body length of sea cucumbers after immersion in a solution of 0.15 percent phenoxyethanol (POE) and subsequent transfer to 70 percent ethanol.

and untreated, were transferred to 70 percent ethanol (about 1.75 hours) all but one of the POE treated specimens had relaxed to the point of exposing the tentacles completely. On average the treated specimens showed an increase in body length of approximately four percent. The control specimens had decreased in length by an average of 27 percent. The reason for the large decrease in the control specimens may have been due to the stress of the small water volume in the stacking dish. The difference between the control and treated specimens was 31 percent. The difference shows a pronounced effect on the treated specimens and what appears to be a stress related response in the control specimens.

Two days after fixation all specimens had shown some decrease in size. The treated specimens had decreased by an average of 13 percent and the controls by five percent. The difference between the treated and control specimens was 22.5 percent. After two weeks in ethanol the treated specimens had decreased in length slightly (two percent). The controls, however, had increased in length significantly (15 percent). By the time the control specimens were fixed, both were somewhat contracted. The subsequent increase in body length is presumably caused by a post mortem relaxation of the muscle tissues. This was not seen in the treated specimens as substantial muscle relaxation had already occurred due to the POE. The difference between the treated and control specimens at that point was approximately six percent. After three months very little change had occurred. Overall the two control specimens had decreased in size by 17.5 percent and the treated specimens by 12.2 percent. Although the difference is slight, and not statistically

sound due to the small number of control specimens, the treated specimens had assumed a much more relaxed and less rigid posture with the tentacles exposed.

Although the difference in proportional body length between the treated and control specimens is small, the fact that the tentacles were left exposed after fixation in all of the treated specimens may be important. The arrangement, size, colour and number of tentacles in many sea cucumbers is critical. Having them exposed after fixation allows an examiner to make determinations without dissection. This is perhaps the main benefit of relaxing specimens in POE.

Many other taxonomic groups have been tested with varying success. Smaldon and Lee (1979) list Turbellaria, Kinorhyncha, Nematoda, Sipuncula, Echiura, Bivalvia, Oligochaeta, Polychaeta, Copepoda, and Holothuroidea as known groups that respond well to POP. One study recommends POP as a general narcotic (relaxant) for meiofaunal organisms (dimensions between 0.5 mm and 0.1 mm) (Hulings and Gray 1971). McKay and Hartzband (1970) highly recommend POP for use on unsorted benthic invertebrates in general and report that relaxation is complete in ten to fifteen minutes. For Sipuncula the recommendation is a one percent solution of POP in seawater for two to 12 hours, but other studies have reported much shorter duration periods, with a solution of about 0.15 percent (McKay and Hartzband 1970, Townsend 1983).

Most historical applications of POP have been as a relaxant/narcotizing agent before fixation (Table 1). POP works as a narcotic by inhibiting acetylcholine synthesis (Mueller 1972). The method of action for POE is probably similar. POP was reputed to be carcinogenic at some point, although there is no such indication on the MSDS. This may explain the switch to POE in the early 1990s. Recently there have been more studies using POE as an anesthetic on aquaculture species where the objective is to have the organism recover unchanged. The majority of these studies used commercial species such as pearl oysters (Norton et al. 1996) or abalone (Aquilina and Roberts 2000).

The results of this study have shown that POE may have potential as a useful prefixation treatment for sea cucumbers. Additional studies need to be done to investigate how effective POE may be with other taxonomic groups.

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DMDM-HYDANTOIN: THE PROMISING RESULT OF A SEARCH FOR AN ALTERNATIVE IN FLUID PRESERVATION OF BIOLOGICAL SPECIMENS

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Abstract.—Since occupational health and safety authorities throughout the world have more strictly regulated the use of formalin and storage of ethanol, and the natural history community is more aware of the occupational risks involved, the interest for non-hazardous alternatives grows. For this reason, I initiated a study of the properties of "modern" biocides, currently used for preservation in food, cosmetic, and pharmaceutical products. In order to determine their suitability for preservation of biological specimens, a set of parameters was defined that could be weighed against the properties of the biocides.

Of the 22 biocides that were reviewed in this way, only DMDM-hydantoin was considered to be suitable as an alternative for fluid preservation of biological specimens. This biocide is a so-called formaldehyde-releasing agent that is primarily used as a preservative in cosmetic and personal care products. Apart from a short-term experiment that I conducted, DMDM-hydantoin has never been used for the preservation of biological specimens. Therefore, additional research is necessary to reveal the long-term effects of DMDM-hydantoin on the structural integrity of biological specimens.

INTRODUCTION

Since its introduction, more than 300 years ago, fluid preservation has given scientists a powerful technique to preserve biological specimens for study resulting in an accelerated build-up of knowledge in biology and medicine. Starting as an important aid to study and unravel the anatomy of the human body, fluid preservation has been essential to most research related to biology. In natural history collections all over the world, fluid preserved specimens store vital data concerning the change in earth's biodiversity.

Over the past 100 years fluid preservation practices have hardly evolved. Every year patents for new antiseptic mixtures are registered in the pharmaceutical and cosmetic industry, yet fluid preservation of biological specimens is still based on ethanol and formalin. The antiseptic properties of ethanol and formalin were discovered more than a century ago. These classic preservatives are now known to be hazardous materials especially in the specific concentrations used in fluid preservation. Ethanol is highly flammable whereas formaldehyde is recognized to be a highly toxic substance. For these reasons, a survey was undertaken of the properties of "modern" antiseptics to determine if less hazardous alternatives are available for fluid preservation of biological specimens.

BIOCIDES IN PRESERVATION

One of the main functions of a preservative fluid is to protect the specimen from decay and deterioration (Stoddart 1989). Secondary functions of fluid preservation can include preserving color (color preservatives such as Kaiserling III solution), maintaining DNA integrity (ethanol 95 percent), preventing decalcification (borax buffered ethanol or phosphate buffered formalin), studying stained internal structures (glycerin or methylbenzoate), or promoting decalcification for

Table 1. Summary of mechanisms of antibacterial action of biocides (from McDonnell and Russell 1999).

Biocide group	Biocide	Mechanism of action
Alcohols	Ethanol Isopropanol	Membrane damage, denaturation of proteins (exact mechanism still not known)
Aldehydes	Formaldehyde	Cross-linking of proteins, RNA, and DNA
	Glutaraldehyde	Cross-linking of proteins
Biguanides	Chlorhexidine	Loss of membrane integrity (low concentrations), congealing of cytoplasma (high concentrations)
Oxidizing agents	Halogens	Oxidation of thiol groups in enzymes and proteins, inhibition of DNA synthesis
	Hydrogen peroxide	Oxidation of thiol groups in enzymes and proteins, DNA strand breakage
	Peracetic acid	Disruption of thiol groups in enzymes and proteins
Heavy metal derivates	Silver compounds	Interaction with thiol groups of membrane bound enzymes, DNA strand breakage (silver ions)
Phenols	Phenol, Phenoxetol	Cytoplasmic membrane leakage
Quaternary Ammonium Compounds (QAC's)	Cetrimide, Benzalkonium chloride	Membrane damage involving phospholipid bilayers

histological purposes (Bouin solution). It is clear that the choice of fluid preservative not only depends on what preservative gives the specimen greater longevity but also depends on the related research or presentation goal. The one purpose that all fluid preservatives have in common is to prevent microbial attack, and consequently, decay of the specimen. This is usually achieved by the use of biocides. "Biocide" is a general term describing a chemical agent, which inactivates microorganisms. Because biocides vary in antimicrobial activity, other terms may be more specific, such as bacteriostatic, fungistatic, and sporistatic referring to agents which inhibit growth, and bactericidal, sporicidal, and virucidal, referring to agents which kill the target organism (Block 1991).

Considerable progress has been made in understanding the mechanisms of antimicrobial action of biocides, although most studies are restricted to the modes of action against bacteria (McDonnell and Russell 1999). Table 1 summarizes the mechanisms of action of several types of biocides.

Search Method

Today, many different preservatives can be found in food, cosmetic, and pharmaceutical products. In order to refine the search for suitable biocides in fluid preservation, a set of six parameters was defined to assess the antiseptic properties of the biocides. Parameters one through four are directly related to the primary purpose of a fluid preservative, prevention of microbial attack. In more specific terms, the parameters relate to those properties of the biocides that might influence the antiseptic ability of the biocide when used for fluid preservation of biological specimens.

1. Antiseptic range—Minimally, the biocide should be bacteriostatic and fungistatic.

2. *Water/fat solubility coefficient*—To prevent excessive migration of biocide into fat tissue, which can result in a dramatic loss of antiseptic strength in the preservative fluid, the biocide should have a water/fat solubility coefficient greater than one.

3. *pH range*—Due to the fact that the pH in preservative solutions can vary from about four to eight (Simmons and Waller 1994), the antimicrobial activity of the biocide should not be pH-dependant.

4. *Stability/Reactivity*—There should be no severe degradation of the biocide initiated by light or air (oxidation), and there should be low reactivity to non-organic substances such as buffer salts and container materials.

The other two parameters used to assess fluid preservatives are related to health and safety aspects of the biocides:

5. *Toxicity*—The biocide should be of low toxicity or non-toxic. The standards of the National Fire Protection Agency (NFPA) express this as a Hazard rating H (Health) ≤ 1 (NFPA 2001).

6. *Flammability*—The biocide should be of low flammability or non-flammable, with a corresponding NFPA Hazard Rating F (Flammability) ≤ 1 (NFPA 2001).

When the properties are out of range with one of the six parameters, the biocide is considered not to be a suitable alternative for formaldehyde or ethanol in fluid preservation of biological specimens. With such a protocol, each parameter can be used as a "knockout instrument" which results in a very efficient method to disqualify inappropriate preservatives.

The order of ranking of the parameter set one to four relating to antiseptic ability and the parameter set five and six relating to safe use does not imply priority of the parameter and does not influence the final outcome. On the other hand, the order of ranking of the two above mentioned sets is deliberately chosen, because if the order between the sets had been reversed, "less safe to use" biocides would be knocked out because of their unacceptable level of toxicity or flammability, although they may still be interesting alternatives in fluid preservation. In such a scenario their possible positive features such as higher stability, lower evaporation rate, neutral pH etc. compared to the classic preservatives will not be revealed.

Results

Table 2 summarizes the results of the biocides weighed against the six parameters, which serve as a knockout instrument. The list is far from complete but the major biocide groups are represented. Despite their use in fluid preservation, glycols were not listed because they are not considered to be true biocides. Corrosive biocides, such as halogens, halogen-releasing agents, anilides, and peroxygens that are not used for preservation but for disinfection, sterilization, and cleaning were not considered. Also, internationally banned biocides, such as DDT, or biocides that are only allowed for very restricted purposes, such as mercury compounds, are not listed. The final selection was determined by the function of the biocide as a representative of its biocide group, the availability of data that could be compared with the parameters, and/or the popularity of the biocide as a preservative in food, cosmetic, or pharmaceutical products.

Alcohols.—Alcohols exhibit rapid, broad-spectrum activity against vegetative

Table 2. Biocides weighed against the six parameters. Each parameter functions as a knockout instrument. The highest ranked parameter is the first to be implemented in this procedure.

		Parameter						
Biocide group	Biocide	1. Antiseptic range	2. Solubility coefficient	3. pH range	4. Stability/ Reactivity	5. Toxicity	6. Flamma-bility	
Alcohols	Ethanol	+	+	+	+	+	−	ko
	Isopropanol	+	+	+	−	ko		
Aldehydes	Formaldehyde	+	+	+	±	−	ko	
	Glutaraldehyde	+	+	+	−	ko		
Benzoates	Sodium benzoate	±	+	−	ko			
	Methylparaben	±	−	ko				
	Ethylparaben	±	−	ko				
	Propylparaben	±	−	ko				
	Butylparaben	±	−	ko				
Biguanides	Chlorhexidine	−	ko					
Bis-phenols	Triclosan	−	ko					
	Triclocarban	−	ko					
	Hexachlorophene	−	ko					
Formaldehyde-releasing agents	DMDM-hydantoin	+	+	+	+	±*	+	
Halophenols	Chloroxylenol	−	ko					
Heavy metal derivates	Silver nitrate	+	+	+	−	ko		
Oxidizing agents	Bronopol	+	+	+	−	ko		
Phenols	Phenol	+	−	ko				
	Phenoxetol	±	−	ko				
Propionates	Sodium propionate	−	ko					
Quaternary ammonium compounds	Benzalkonium chloride	+	+	+	±	−	ko	
Sorbates	Potassium sorbate	±	+	−	ko			

+ in acceptable range.
− out of acceptable range.
± borderline.
ko knocked out by previous parameter.
* In pure solid form toxic (NFPA Hazard rating H = 4); in concentration of aqueous solutions up to one percent considered to be safe (in cosmetic products).

bacteria, viruses, and fungi, but are not sporicidal (McDonnell and Russell 1999). Ethanol and isopropanol are miscible with water but not with fats, although they have the ability to dissolve fats. Isopropanol has greater fat-solvent effects than ethyl alcohol (AMA, Department of Drugs 1977). There are no indications that antimicrobial activity of both alcohols is pH-dependant. Isopropanol reacts with air (oxygen) to form dangerously unstable peroxides (Sax and Lewis 1989). Its long-term stability as a preservative is questionable (Simmons 1995). Ethanol is stable under normal storage conditions. Both alcohols are only slightly hazardous to health, but are highly flammable.

Aldehydes.—Formaldehyde is bactericidal, sporicidal, and virucidal, but it works more slowly than glutaraldehyde (Power 1995, Scott and Gorman 1991). Both aldehydes are soluble in water and are not miscible with fats. The antimicrobial activity of formaldehyde seems not to be pH-dependant. Formaldehyde in an aqueous solution (formalin) may become cloudy upon standing, especially at cool temperatures. It also slowly oxidizes in air. Formalin is sensitive to exposure to light. It is polymerized if unstabilized by low temperatures (Hartley and Kidd 1987, Simmons 1995). Glutaraldehyde polymerizes in the presence of water (Budavari 1996). Lack of stability is an important drawback to its use (AMA, Department of Drugs 1977). Formaldehyde is hazardous to health and considered to be a potential carcinogen. Recommended exposure limits are 0.1 ppm (15 minute ceiling value) and 0.016 ppm (ten hour time weighted average). Both limits are below the odor threshold of 1.00 ppm (Fazzalari 1978, Chan 1997).

Benzoates.—Sodium benzoate is generally considered to be most active against yeast, bacteria, and less active against molds (Furia 1972). The salt is very soluble in water. To be effective, it must be used in acid media of pH not above four (Osol et al. 1975). Parabens are most active against molds and yeasts. They are less effective against bacteria, especially gram-negative bacteria (Furia 1972). All parabens are soluble in oils and fats. Their solubility in water is maximally 0.25 percent (w/w) at 20°C (Budavari 1996). Parabens are extensively used as a preservative in lipid- and oil-based cosmetic products.

Biguanides, bis-phenols, and halophenols.—The antimicrobial activity of chlorhexidine is pH-dependant and greatly reduced in the presence of organic matter (Russell and Day 1993). In general, bis-phenols exhibit broad-spectrum efficacy but have little activity against molds (McDonnell and Russell 1999). Chloroxylenol is bactericidal, but many molds are highly resistant (Bruch 1996, Russell and Furr 1977).

Formaldehyde-releasing agents.—DMDM-hydantoin has, similar to the other aldehydes, a broad-spectrum activity against bacteria, fungi and yeasts. It is effective in acid, neutral and mild alkaline solutions. It is miscible with water and not miscible in oils and fats (Hörchner 1987). As a solid, it is stable under normal temperatures and pressure (OHS Material Safety Data Sheet Reference Database 1984–1999). A 55 percent stock solution contains a maximum of two percent released free formaldehyde and 17 to 18.5 percent chemically bound formaldehyde (Hörchner 1987). Only the free formaldehyde is volatile. It is regarded to be safe in concentrations of maximally one percent in cosmetic and personal care products such as shampoos, liquid soaps and skin lotions.

Heavy metal derivates.—Silver nitrate exhibits antiseptic, germicidal, astringent, and caustic activity (Gilman et al. 1985). It is soluble in water and does not

dissolve in fats or oils. There are no indications that its antimicrobial activity is pH-dependant. On exposure to air or light in the presence of organic matter, silver nitrate becomes gray or grayish-black (Reynolds and Prasad 1982).

Oxidizing agents.—Bronopol is bactericidal. It also exhibits action against fungi and yeasts (Stretton and Manson 1972). It is very soluble in water and only slightly soluble in oils. When exposed to light, especially under alkaline conditions, it may become yellow or brown. It is unstable in anhydrous solutions of glycerol (Reynolds and Prasad 1982).

Phenols.—Phenol is bacteriostatic in concentrations of approximately 0.2 percent, bactericidal above one percent, and fungicidal above 1.3 percent (Gilman et al. 1985). Phenoxetol has a limited spectrum of antibacterial activity. It has been used as a preservative at a concentration of one percent. A wider spectrum of antimicrobial activity is obtained with preservative mixtures of phenoxetol and parabens (Reynolds and Prasad 1982). Both compounds are very soluble in volatile and fixed oils, and slightly soluble in water (Budavari 1996).

Propionates.—Propionates are more active against molds than sodium benzoate, but have essentially no activity against yeast. They have little action against bacteria. The pH for optimum activity of propionates ranges up to 5.0 (Furia 1972).

Quaternary ammonium compounds (QACs).—These salts are often called cationic surfactants or cationic detergents because their aqueous solutions have low surface tensions. At concentrations between 0.01 and 1.0 percent, they are used as antiseptics, bactericides and fungicides (Gosselin et al. 1984). Benzalkonium chloride is very soluble in water, alcohol, and acetone; it is almost insoluble in ether (Budavari 1996). An increase in temperature and pH of solution will increase the effectiveness of quaternary ammonium compounds (Jones et al. 1977). Of practical importance is its compatibility with phosphate buffers (Osol et al. 1975). Aqueous solution is slightly alkaline and foams strongly when shaken. It is incompatible with anionic detergents such as soaps, with nitrates (Budavari 1996), and with aluminum, rubber, cork, and cotton. (OHS Material Safety Data Sheet Reference Database 1984–1999). Concentrations of aqueous solutions (ten percent and sometimes less) are primary skin irritants and concentrations as low as 0.1 to 0.5 percent are often irritating to conjunctivae and mucous membranes (Gosselin et al. 1984).

Sorbates.—Sorbic acid and its salts have broad-spectrum activity against yeasts and molds, but are less active against bacteria. As with other weak acid microbial inhibitors, the activity of sorbate increases as the pH of the medium declines. The range of optimum effectiveness extends up to pH 6.5 (Furia 1972). Potassium sorbate is primarily used in food products, such as cheese and beverages.

Discussion

Antiseptic range.—Table 2 shows that only the alcohols, aldehydes (including formaldehyde-releasing agents), heavy metal derivates, oxidizing agents, phenol, and QAC's have broad-spectrum activity against bacteria and fungi. The other biocides have either a limited spectrum of activity against bacteria, which means that they are not effective against all types of bacteria (gram-positive bacteria, gram-negative bacteria, and mycobacteria), or they are only effective against bacteria and not effective against molds or vice versa. In preservation, these biocides

are often combined in order to have full spectrum antimicrobial activity. An advantage of these mixtures is that the individual concentrations can be kept significantly lower than with one-compound preservation. As an example, when combining methylparaben and DMDM-hydantoin, the minimal concentration to inhibit bacterial and fungal growth is three times lower (0.05 percent methylparaben plus 0.05 percent DMDM-hydantoin) than when used separately (0.15 percent Methylparaben or 0.15 percent DMDM-hydantoin) (Hörchner 1987). Possible disadvantages include synergistic effects. As well, differences in properties such as solubility and stability will limit successful use of biocide-mixtures in fluid preservation of biological specimens.

Water/fat solubility coefficient.—Crimmen (1989) described the serious deterioration of a collection of formalin-fixed fishes, which were preserved for a period of ten years in one percent phenoxetol. It was particularly noticed that the pectoral 'wings' of some rays (Rajidae) had completely disintegrated. In his paper no possible explanation for this decay was given. Although poor fixation and layering of preservative could be possible causes for this decay, another reasonable explanation could be the difference in solubility of phenoxetol in fat/oil and in water. Biocides that are more soluble in fats or oils than in water will partly migrate to the fat tissue in the specimens and to the leached-out fats and oils in the aqueous preservative fluid. This will result in a decrease of the antiseptic strength in the aqueous solution. Parts of the specimen that do not contain much fat, such as "wings" of fish, can become very vulnerable for microbial attack. For these reasons, biocides that have a water/fat solubility coefficient lower than one are not considered suitable for preservation of biological specimens in aqueous solutions.

pH-range.—The assessment of a fluid preserved collection (Simmons and Waller 1994) show an average pH of 6.37 with a range of 4.2 to 7.75 for formalin preserved specimens and an average pH of 6.67 with a range of 5.41 to 8.23 for alcohol solutions. Therefore, the antimicrobial activity of the biocide should not be pH-dependant. A lot of "non-toxic" biocides used in food products, such as potassium sorbate, sodium propionate, and sodium benzoate, only exhibit antimicrobial activity in acid solutions.

Stability/Reactivity.—The ideal biocide for fluid preservation should only attack "alien" living cells and should leave the cells of the specimen physically and chemically unchanged. This kind of biocide will probably never be found. Biocides affect the cell membranes of the specimen and most of them also affect DNA (Table 1), which makes them effective antiseptics.

Biocides themselves can also degrade. A lot of biocides are not very stable in aqueous solutions; sometimes a stabilizer can reduce severe degradation. Some can react with certain types of buffers or with container materials. Other biocides, such as formaldehyde, are photosensitive and/or react with air (oxygen) (Hartley and Kidd 1987). In such a case, the speed of degradation also depends on the storage conditions (light, temperature) and the oxygen permeability of the container materials (van Dam 2000).

Toxicity and flammability.—Ethanol is one of the rare biocides that even at high concentrations is considered to be of low toxicity. Between concentrations of 50 to 80 percent it has very effective antimicrobial activity (Morton 1977). Unfortunately, at these concentrations ethanol is also highly flammable and com-

bustible. In most countries, the storage of large quantities of ethanol is strictly regulated and only permitted when local and/or national fire safety regulations for storage requirements are met. In practice, this can mean that the storage facility has to be equipped with specified systems for ventilation, ethanol vapor detection, flame suppression, and electrical service, as well as grounding of storage cabinets and doors (to prevent electrostatic discharge), and an explosion outlet wall bordered outside by a safety zone. It is clear that the storage requirements of flammable liquids are an expensive proposition.

Formaldehyde gas is toxic for humans at concentrations below the odor threshold of 1.00 ppm. Most medical and biology students well remember the smell of formaldehyde in the dissection room; this implies that they were exposed to levels well beyond the ceiling limit of 0.1 ppm. Formaldehyde is used in a relatively high concentration, about four percent, in fluid preservation of biological specimens. The only way of working with formalin that is permitted in a building is in a fume-hood. In areas with formalin preserved collections, a loose lid of one specimen jar may well lead to an air concentration above the ceiling limit. Even by permeation of formaldehyde through the container materials, this limit can be exceeded in collection areas.

Table 2 shows that ethanol is the only suitable formaldehyde-free biocide that could replace formalin. However, not all types of biological specimens can be preserved in ethanol. Marine specimens may be adversely affected when preserved in ethanol. It is commonly known that jellyfish disintegrate in ethanol. In such a case, only water-based preservatives can be applied. It seems that the only other alternatives are formaldehyde-releasing agents, which still contain formaldehyde but with the important difference that the major part of the formaldehyde is chemically bound to a non-volatile compound.

DMDM-hydantoin, the final search result.—Table 2 shows that DMDM-hydantoin is the only biocide that falls within all the defined parameter ranges of acceptability. Since DMDM-hydantoin is a formaldehyde-releasing agent, its properties can be qualitatively best compared with formalin.

With respect to preservation quality, DMDM-hydantoin seems to have some great advantages over formalin. DMDM-hydantoin solution is neutral, whereas formalin is acidic. This implies that DMDM-hydantoin might be suitable for the preservation of calcium containing specimens (vertebrates) although the long-term pH stability with or without the use of buffers is not known. Additional research is necessary in order to address this issue adequately. Unlike formalin, DMDM-hydantoin does not polymerize at low temperatures and therefore does not need a stabilizer (Hörchner 1987). A major disadvantage of both biocides can be found in their mechanism of antimicrobial action based on the presence of formaldehyde, which affects DNA and RNA (Table 1). When maintaining the DNA integrity of the complete specimen is an important issue, it is not recommendable to consider the use of these biocides as a preservative or fixative.

With respect to safety, there is a very important argument to be made for the use of DMDM-hydantoin as a substitute for formalin in fluid preservation. In case of solutions of equal strength, the concentration of free volatile formaldehyde is at least 55 times lower than in formalin. Rosen and McFarland (1984) showed that in normal and protein-based shampoos the concentration of DMDM-hydantoin is linearly related to the concentration of free formaldehyde, with the differ-

ence that in protein-based shampoos the concentration of free formaldehyde is about two times lower than in normal shampoos. In such products, which are applied on the human skin, DMDM-hydantoin is considered to be safe in concentrations of one percent and less.

Supplied as a saturated solution of 55 percent, DMDM-hydantoin is considered to be a skin and eye irritant. Therefore, contact with skin and eyes should be avoided by wearing protective clothes, gloves, and eye protection. With respect to respiratory system protection, even in this highly concentrated form no special requirements (fume-hood) are needed when handling DMDM-hydantoin. In case of ingestion, the acute toxicity (rat) is 50 times lower than that of formalin 37 percent. In contact with skin, the acute toxicity (rabbit) is at least 70 times lower than that of formalin 37 percent. With respect to the transport of DMDM-hydantoin, it is considered not to be a dangerous material in concentrations up to 55 percent. It may be transported without any special precautions over land, sea, and through air, provided that the fluid is stored in suitable containers made of polyethylene, polypropylene or glass (Jan Dekker International 2000).

As a solid in the form of powder, pure DMDM-hydantoin is very toxic and may be potentially fatal if inhaled and is also, like formaldehyde, a carcinogen (OHS Material Safety Data Sheet Reference Database 1984–1999). When spilled, the solution dries to the air leaving the solid powder as a residue; this severely increases the risk of poisoning by inhalation. This risk can be can be minimized by the addition of glycerol to solutions. When DMDM-hydantion is mixed with glycerol it is not likely to ever dry to a powder. The added glycerol will also retard the evaporation rate of water and prevents the complete drying out of the specimen. Glycerol solutions such as Kaiserling III (Kaiserling 1896) are widely used as durable color preservatives in anatomy collections.

A recommended concentration of DMDM-hydantoin for the purpose of preserving biological specimens is based on the acquired data on DMDM-hydantoin. Considering that formalin supplied as a 37 percent solution generally represents ten percent of the preservative solution used in fluid preservation of biological specimens, it seems logical to prepare the same concentration of approximately four percent aldehyde when using DMDM-hydantoin. However, since the chemically bound formaldehyde is more stable than free formaldehyde, it can be expected that a two times lower aldehyde concentration is more than sufficient for long-term preservation. Together with the safety considerations mentioned above, the following basic solution is proposed: ten percent DMDM-hydantoin 55 percent, five percent glycerol, de-mineralized water.

As a pilot-experiment, three one cm thick slices of a lower human arm were fixed in ten percent formalin 35 percent buffered with four percent sodium acetate. After 24 hours they were put in running water for one hour. Two specimens were transferred to the proposed basic solution. One of these mixtures was buffered with four percent sodium acetate. The third specimen was transferred to ten percent formalin 35 percent. After one year all specimens did not visually show any form of decay. It was noted that the progressed hardening of the tissue of the specimen in formalin was not present in the other specimens. Substantial loss of colour was seen in both unbuffered solutions, while the buffered DMDM-hydantoin only showed some color loss. This pilot-experiment cannot lead to any de-

cisive conclusions, but it suggests that DMDM-hydantoin has potential to become a serious alternative for formalin in fluid preservation of biological specimens.

Apart from this short-term experiment, DMDM-hydantoin has never been used for the purpose of preserving biological specimens. At the moment, it is impossible to determine its long-term effects on biological tissues. Additional research, such as accelerated aging studies concerning the stability of the structural proteins of different types of biological tissue preserved in DMDM-hydantoin, similar to the work of von Endt (2000), will be necessary to determine the long-term effects of DMDM-hydantoin on the structural integrity of the specimens.

Conclusion

Most biocides used in food, cosmetic, and pharmaceutical products are not considered to be suitable for the preservation of biological specimens due to one of the following properties:

1. The biocide has a limited antimicrobial spectrum.
2. The biocide is better soluble in fats and oils than in water, which may cause migration of the biocide to the tissue fats resulting in a decrease in antiseptic strength in the aqueous solution.
3. The biocide has antimicrobial activity only in acid solutions.
4. The biocide is not stable in aqueous solutions.

When the aspect of health and safety is also considered, even the traditional preservative fluids such as formalin and ethanol are not ideal media for storing natural history collections, because, both are considered hazardous materials for one or more reasons.

DMDM-hydantoin is the only biocide that was not ruled out by all the six parameters considered in this study. This so-called formaldehyde-releasing agent, primarily used as a preservative in cosmetic and personal care products, has enough potential to be a serious, less-toxic alternative for formalin in fluid preservation of biological specimens. Because DMDM-hydantoin contains formaldehyde, like formalin, it seems to be less suitable for preserving DNA than ethanol. The flammability of ethanol, and resulting storage requirements deemed necessary by fire regulatory groups suggest the need to pursue new fluid preservation alternatives.

Because DMDM-hydantoin has never been used for the purpose of preserving biological specimens, its long-term effects on biological tissue are not known. Additional research will be necessary to determine the long-term effects of DMDM-hydantoin on the structural integrity of the specimens.

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WEIGHT CHANGES ON OXIDATION OF DRYING AND SEMI-DRYING OILS

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Abstract.—Objects in ethnographic and ancillary natural history collections contain many types of vegetable oils as components of varnish coatings, paints and lubricants. Tests were performed on thin oil films dried in a laboratory environment. These oils, composed of the glycerol esters of unsaturated fatty acids, will oxidize through a free radical process and crosslink to form polymers of variable properties. Oxygen uptake produces an initial weight gain but further oxidation yields compounds such as carbon dioxide, and short chain acids, aldehydes, ketones and alcohols that can volatilize and contribute to a loss of weight. Monitoring weight changes can provide information on the duration and magnitude of these reactions. Weight changes are greatest in the first year and are still detectable up to two years later. Acid or base processing of the oils affects the incubation period for oxygen uptake but not the long term weight change behavior. Weight loss can be quite dramatic depending on the oil composition. Oils with low linoleic acid content eventually lose more weight than linseed oil and even a non-drying oil with high oleic acid content, olive oil, is surprisingly active after two years. In addition to the chemical effects, changes in weight also indicate alteration of the physical and mechanical properties of the oil films.

INTRODUCTION

Objects in ethnographic and ancillary natural history collections may contain vegetable oils as components of varnish coatings, paints and lubricants whether originally applied or the result of conservation or restoration. These oils are composed primarily of the glycerol esters of unsaturated fatty acids. They react with oxygen through a free radical process to form hydroperoxides, which in turn decompose and then crosslink to form polymeric gels. This process is called autoxidation and has been covered thoroughly in reviews (Bateman 1954, Russell 1959, Wexler 1964, Hutchinson 1973, Porter et al. 1995). Weight changes are a measurable and an inevitable consequence of the autoxidation processes. Oxygen uptake increases the weight of the oil as hydroperoxides are formed while bond cleavage produces volatile oxidation byproducts such as carbon dioxide, and short chain acids, aldehydes, ketones and alcohols. These diffuse out, volatilize and decrease the weight (Hancock and Leeves 1989). The measured weight changes represent the net change in weight of the oil film due to oxygen uptake during the free radical and polymerization process and the diffusion and loss of volatiles created by oxidation and molecular rearrangements. This behavior was first observed in the 19th century and reviewed early in the 20th century (Weger 1899, Sabin 1911, Eibner 1920). However, only certain oils, notably linseed but occasionally poppy and walnut, were tested and the weight changes were not monitored continuously for periods beyond one or two months. The changes in weight over periods of time other than that required for gelling or becoming dry to touch were not followed in any detailed fashion. Most of such testing was conducted in an industrial or commercial context, and the primary emphasis was on short term changes rather than changes occurring on a museum timescale.

Measurements of the weight changes with time show the continuing nature of

the chemical process and the plots provide information such as the presence and length of the incubation period, the maximum weight gain, and the loss of volatiles after gelling. The length of the incubation period for oxygen uptake is a function of oil processing, presence of antioxidants, or the presence of driers, whether natural or added. The maximum amount of weight gain gives a measure of the amount of crosslinking through oxygen uptake. The removal of chemical compounds as volatiles produces a mass loss and will alter the mechanical properties and diffusion behavior. Weight plots can be altered by the addition of certain metal salts and by metal substrate effects (Morley-Smith 1957). These types of data can be used to investigate oil and paint interactions with substrates.

This paper studies the drying behavior of three types of processed linseed oil commonly encountered in collections and adds new data for five other oils. Safflower, sunflower, walnut and poppy oils were selected because these have been used as substitutes for or additives to linseed oil to reduce the yellowing of white or light colored paints. Olive oil was selected as an example of a common nondrying oil. The effect of copper as a drier on the chemistry of linseed oil was also confirmed using a small amount of copper and an untreated oil. The weight changes over a two year period were monitored to determine activity within the films.

Materials and Methods

Test specimens were made from oils representative of those found in collections. These included cold-pressed linseed oil, acid and alkaline refined linseed oils, olive, cold-pressed safflower, refined poppy, cold-pressed walnut and refined sunflower oils. The oils were obtained from Kremer Pigments, 228 Elizabeth Street, New York, NY 10012 USA. Olive oil was a commercial cooking product. Cold pressed linseed oil with 0.1 percent copper(II) was prepared by adding copper(II) hydroxide to slightly warmed linseed oil and stirring until dissolution was complete. Neat oils were applied to aluminum coupons and held in place by surface tension resulting in gelled films from 0.13 to 0.26 mm (0.005 to 0.010 in) in thickness. Effects of metal substrates were determined in a similar manner using mechanically cleaned copper and lead foils.

Weight changes were monitored using a Mettler model AT201 balance measuring to 0.1 milligrams. The laboratory environment was maintained between 40 and 50 percent relative humidity and 18° to 20°C throughout the study and reflects the environment of most museums. Light was from fluorescent lights, which mimicked natural light, and from indirect natural light. Metal concentrations in oils were determined by inductively coupled plasma mass spectrometry (ICP-MS) using standard techniques (Hill 1999).

Results and Discussion

Weight changes in the oils were not immediate and started only after an incubation period when the retardation of the free radical oxidation process by naturally occurring antioxidants was finally overcome. The chemical processing of oils removes these antioxidants and, on testing, the oxygen uptake, as measured by weight increase, starts earlier even though the rate of oxygen uptake is about the same. This phenomenon is evident in Figure 1, which shows the weight change plots of the three types of processed linseed oil over a 45 day period. A

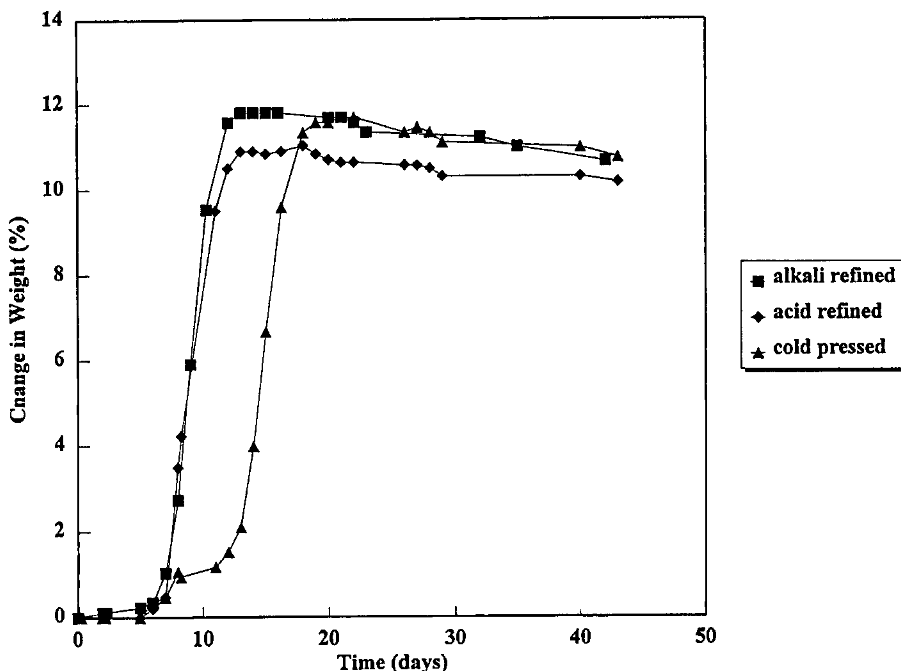

Figure 1. Changes in weight over 45 days of three linseed oil films: unprocessed cold pressed linseed oil, acid and alkali refined oils.

cold-pressed oil, which contained naturally occurring antioxidants, took longer to begin its weight gain (oxygen uptake) but after it started eventually attained the same weight increase as the alkali refined oil. The acid refined oil reached a somewhat lower value as a maximum weight gain. Historically, paints have been made from cold pressed oils, but at present commercial artists' oil paints that use linseed oil are made with alkali refined linseed oil. Much like a pigmented oil film, the pure oil films reach a "dry-to-touch" state at the point of maximum weight uptake.

Figure 2 shows the longer two-year behavior of these same oils. The trend in the data shows the rapid initial weight increase from oxygen uptake and then a weight loss due to loss of volatiles. After about one year the weight loss starts to level off and by two years there are only minor differences among them although the weight loss continues. The differences in behavior for these three oils are apparent only in the early initial stages and are not significant at later times.

Figure 3 shows the short term weight change for cold-pressed linseed oil in comparison to five other oils—olive, safflower, sunflower, poppy and walnut. The oils show varying incubation periods because of the differences in natural antioxidants and in processing. Figure 4 shows the same oils over a longer two-year period. With the exception of olive oil, the oils are drying oils, i.e., they form gels on oxidation. Their weights decline steadily for about a year and the weight of the oxygenated gel eventually becomes less than the original weight of the oil even after an initial oxygen uptake of ten percent by weight or more. This indicates that the decrease in weight due to the loss of volatiles is greater than weight

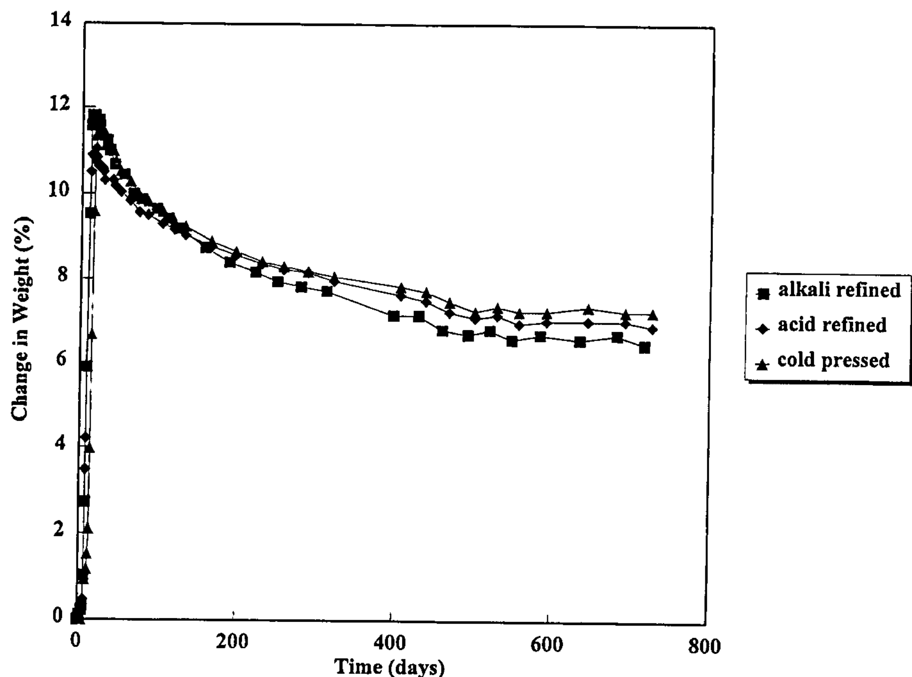

Figure 2. Changes in weight over 700 days of three linseed oil films: unprocessed cold pressed linseed oil, acid and alkali refined oils.

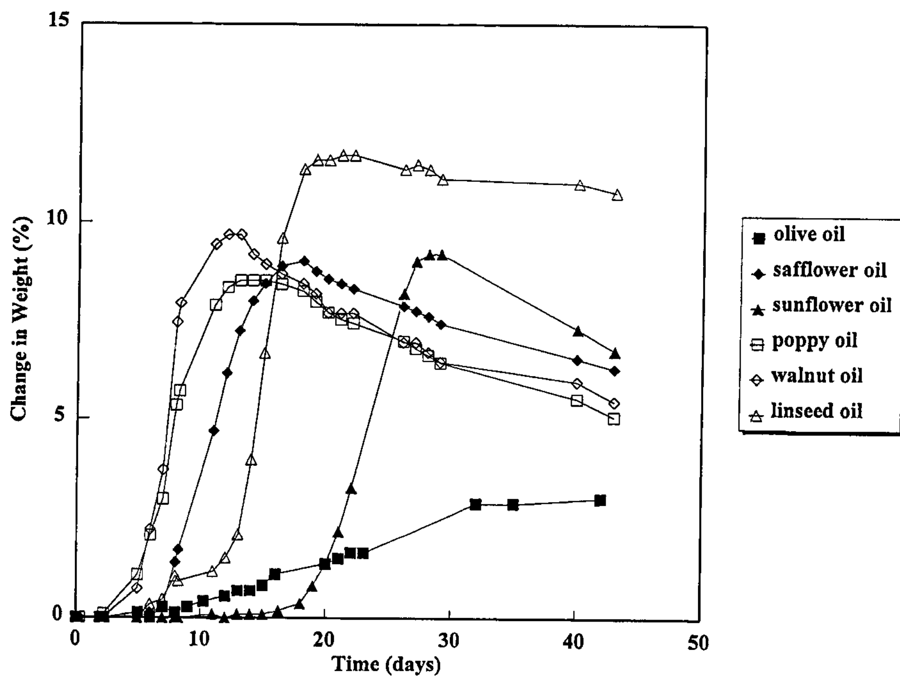

Figure 3. Changes in weight over 45 days of five oil films compared to cold pressed linseed oil. See text for oil preparation.

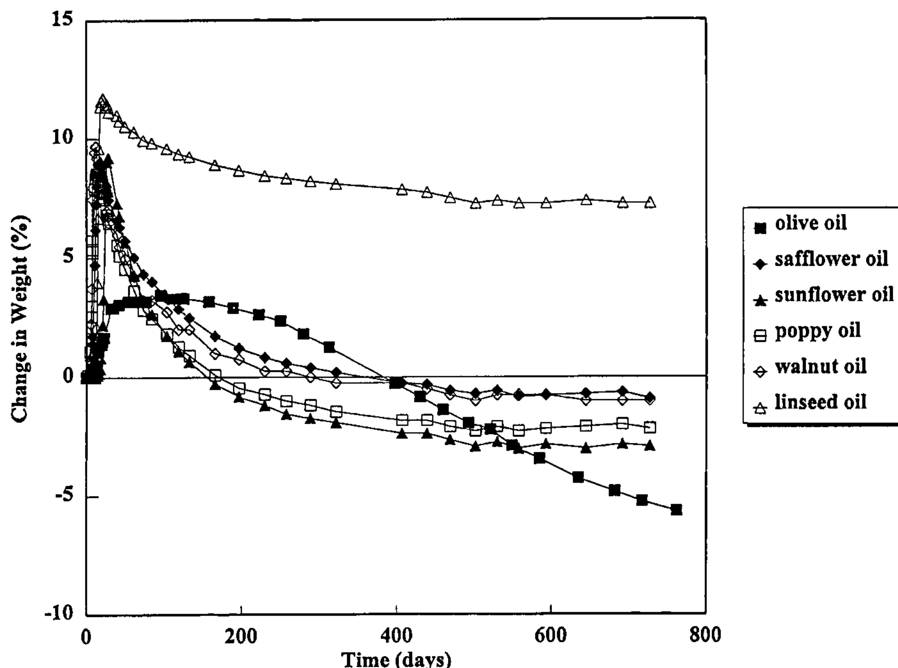

Figure 4. Changes in weight over 700 days of five oil films compared to cold pressed linseed oil. See text for oil preparation.

increases due to oxygen uptake. The degree of weight loss is dramatic for the oils substituting for linseed oil when compared to the linseed oil itself.

Seed oils contain the glycerol esters of fatty acids: principally, two saturated acids—palmitic and stearic; and three unsaturated acids—oleic with one carbon-carbon double bond, linoleic with two double bonds and linolenic with three double bonds. Oxidation may occur directly at the double bonds or through peroxyl formation at the allylic position. In any case, all three unsaturated acids form azelaic acid, a 9 carbon dicarboxylic acid, on oxidation at the double bond nearest the carboxyl group while other more volatile compounds may be formed from other unsaturated sites. Olive oil contains mostly oleic acid with small amounts of linoleic acid and does not "dry". Oxidative scission of its single carbon-carbon double bond yields the diacid, azelaic acid, and a 9 carbon saturated acid. By this same mechanism linoleic and linolenic acids similarly form azelaic acid but also smaller more volatile compounds from the other double bond sites if crosslinking does not prevent or inhibit the oxidation at these sites. Oleic acid can also be oxidized biologically and under certain conditions gives hydroxy or oxo saturated acids and can even produce the saturated analog stearic acid by a hydrogenation mechanism (Yan et al. 2001). This chemical route may not be important in the usual chemistry of coatings but may be quite important in archaeological materials, i.e., those buried in moist soils or immersed in water. In addition, the relatively slow action of the hydrolysis process with ambient moisture becomes more rapid and aggressive with high relative humidity or liquid water leading to loss of glycerol-fatty acid linkages. However, in the present experiments hydrolysis is

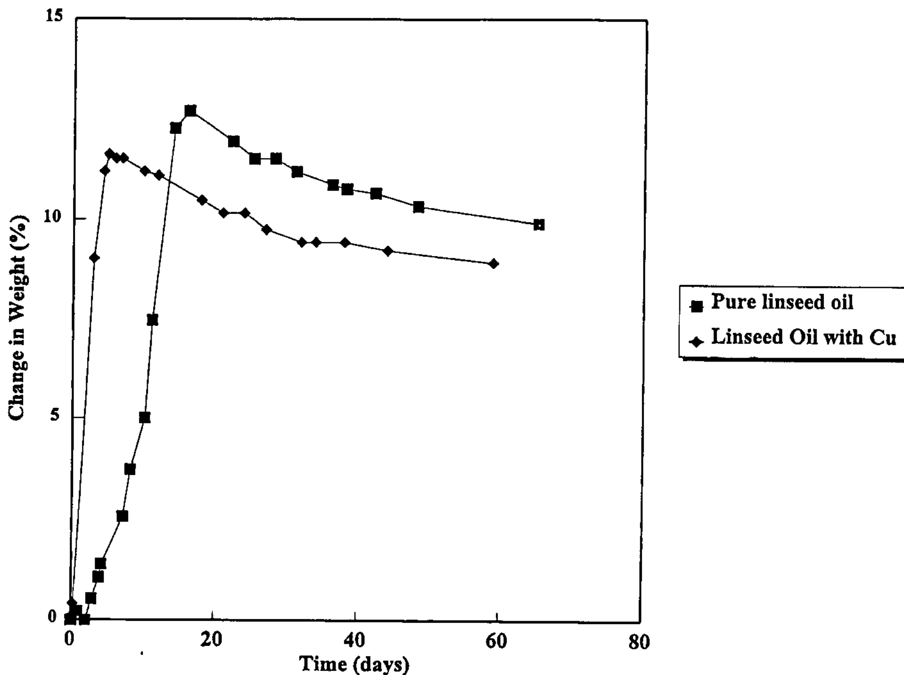

Figure 5. Changes in weight over 62 days for cold pressed linseed oil and the same oil with 0.1 percent Cu(II) added to accelerate oxygen uptake.

less important because of the average 50 percent RH encountered by the specimens.

The weight change plot of olive oil also shows that while oxidation at first results in a moderate weight gain, the final result is a significant weight loss. The olive oil loses almost eight percent of its maximum weight after an initial three percent weight increase on oxygen uptake without polymerizing or forming a gel. The other "drying" oils pick up a minimum of 8.5 percent by weight early and steadily lose this weight after gel formation similar to the later behavior of the oleic acid containing olive oil. Because of their multiple carbon-carbon double bonds, linoleic and linolenic acids readily form polymers through crosslinks that limit the amount of small chain volatile compounds formed. The poppy, walnut, safflower and sunflower oils contain primarily linoleic acid and oleic acid with little linolenic acid while olive oil is primarily oleic acid with small amounts of linoleic (Mills and White 1987, Miyashita and Takagi 1988). Linseed oil contains considerable linolenic acid and thus crosslinks or polymerizes with less evident volatile formation. This is reflected in the smaller weight loss after initial oxidation than the other oils.

While antioxidants can inhibit the autoxidation of oils and delay oxygen uptake, certain compounds can accelerate the process. The most common accelerants are heavy metal salts, commonly called driers. Traditionally, artists' oil paints and housepaints had lead salts added to speed up drying but now most paints eliminate lead and contain cobalt and/or manganese salts for a similar effect. Copper and iron also belong to this class of compounds. Figure 5 shows a weight gain plot

for cold pressed linseed oil and the same oil with 0.1 percent by weight copper(II) added. Copper(II) hydroxide was used to introduce copper into the oil because this could be done at near ambient temperatures without altering the oil by excessive heat. Vegetable oils can dissolve some metals. When metal surfaces are coated with oils, metals may be introduced into the oil as the films oxidize, crosslink and dry. In fact, lead kettles have been used in the past to heat linseed oil in preparing the oil for making artists' paints. The commercial processing of edible oils can introduce enough metal from storage containers to accelerate spoilage or rancidity. Experiments using ICP-MS to measure metal concentrations showed that cold pressed linseed oil when applied to a lead foil reached a concentration of 2.8 percent by weight lead in the resulting gel. Similarly, a cold pressed linseed oil film dried on a clean copper strip contained 0.13 percent copper in the gelled oil at the copper-gel interface and the same oil in bulk with a copper strip immersed reached a level of 1.3 percent dissolved copper after seven days. The dissolved copper shortened the time required for the oil to gel.

The chemistry of oxidation and drying of neat oils is different from that of pigmented oils. Pigments can react with the acids and other compounds produced on oxidation and can inhibit diffusion of oxygen into the film as well as inhibit the diffusion of volatiles out of the film. This will change the amount of weight loss and alter the rate of weight change. Unpigmented oils are, however, a good indicator of the types of processes that do occur but that may be modified either chemically or physically by the presence of pigment particles.

Conclusions

The data clearly show that there are chemical and physical processes occurring in the oil films for a considerable time after they have gelled. The time of onset of the process of drying (oxidation and gel formation) depends upon the presence of antioxidants that may be removed by either alkali or acid processing but does not affect long term (two-year) behavior. Weight loss depends primarily upon the presence of linolenic acid. The higher the linolenic acid content, the less weight is lost on oxidation. The addition of driers alters the time required for gelling. In the past these driers have been lead, cobalt and manganese compounds intentionally added into pigmented films but lead and copper compounds may be introduced by dissolution from substrate surfaces by the action of the oil itself.

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REVIEWERS

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BOOK REVIEWS

MATERIAL CHARACTERIZATION TESTS FOR OBJECTS OF ART AND ARCHAEOLOGY, 2000, N. Odegaard, S. Carroll, and W. S. Zimmt. (Archetype Publications, London, England, 230 pp., spiral bound). This book is intended for the non-chemist professional working with historic and prehistoric objects of material culture found in museum collections or archaeological excavation. Its purpose is to provide easy, safe, and economical testing techniques for the professional who needs to know the materials of which objects are made, the compounds that are associated with them, or the characteristics of the materials used to package or store them. The book is directed toward conservators, archaeologists, and museum specialists. Its readership is expected to be international.

The book opens with a Foreword for United Kingdom readers, covering substances hazardous to health, and general requirements of the Control of Substances Hazardous to Health Regulations 1994 (CoSHH). Pertinent citations are listed at the end of the Foreword.

1. *Introduction.* The authors give a brief history of material characterization in conservation, noting the development of modern instrumental analytical techniques, the decline of reliance on chemical characterization techniques, and the limited publication of systematic spot test systems in the conservation or archaeological literature.

2. *Chemical Safety.* Understanding and controlling chemical hazards are stressed. Safe use, storage, and disposal of chemicals are emphasized. Proper labeling, correct terminology, personal protective equipment, and material safety data sheets are described.

3. *Scientific Method and Techniques of Spot Testing.* Control of the tests should include experimental trials, comparative observations, accurate record keeping, good laboratory hygiene, and efficient laboratory technique. Each of these factors is elaborated and expanded in the text. Repeatability of test results is stressed. If a new problem arises during investigation, it should be included in the test results.

The authors present detailed methodology for carrying out each test, evaluation of the effectiveness of each test, and advice in the interpretation of test results. The text provides guidance in a variety of test techniques.

Standard and auxiliary laboratory equipment is discussed. Standard equipment includes a variety of glass, steel, porcelain, and plastic lab tools and lab ware. A 10×-hand lens is thought to be adequate magnification to observe results, if a microscope is unavailable. Auxiliary equipment described includes balances, a specific gravity measurement device, sources of heat or flame, electrolysis sampling set-up, a centrifuge, and a melting point device. Two testing techniques using pyrolysis are presented.

4. *Spot Tests for Metals.* Tests for aluminum, antimony, arsenic chromium, copper, gold, iron, lead, mercury, nickel, silver, tin, and zinc are presented.

5. *Spot Tests for Inorganic and Ionic Materials.* Calcium, carbonate, chloride ions, halogens, chlorine, nitrate, phosphate, and sulfate tests are provided.

6. *Spot Tests for Organic Materials.* Tests for starch, simple and complex carbohydrates, triglycerides, unsaturated oils, blood, protein, sulfur and organic sulfur, phenols in vegetable-tanned leather, determination of vegetable-tanned leather,

indigo, lignin, rosin, cellulose, cellulose nitrate, poly (vinyl alcohol), polyester groups, polyamides, polycarbonates, silicone-based rubber are described.

7. *Other Tests.* pH, volatile acid, and acid vapor tests using pH pencils or pens, pH papers, and reagents are discussed. Additionally, there is a test for hardness with a pencil sequence, a test to determine specific gravity using an electronic analytical balance, and a test for radioactivity using photographic film.

Appendixes. The information in the appendixes gives a chemistry refresher to those who may not have worked with chemicals recently. Included are a dilution table and chemical concentration calculations, product suppliers, a table of reagents with safety information, a material characterization trial form, glossary, and bibliography.

The authors make clear the time-consuming tasks of preparation, set-up, testing, observation, and record keeping. They emphasize the importance of safe chemical storage, a fume hood or extraction system, an appropriate workspace, and personal protective equipment. The book is a useful guide to material characterization using spot testing. It should be noted that spot test results might be inconclusive, resulting in the need for additional testing or instrumental analytical techniques.

This publication is available for 29.95GBP, 40.00USD through Archetype Publications. Publication details are on www.archetype.co.uk.—*Diana Hobart Dicus, Objects Conservator in Private Practice, 2712 Lancaster Drive, Boise, Idaho 83702.*

HEALTH AND SAFETY FOR ARCHAEOLOGISTS AND CRM PROFESSIONALS, 1999, P. J. Gorton, F. Schieppati, and M. Cinquino. (Panamerican Environmental, Inc., Buffalo, New York, 108 pp. Produced through a grant from the New York State Occupational Safety and Health Training and Education Program, Contract No. C008414). Field research and collection expeditions require resourcefulness in almost every aspect, but particularly in adapting health and safety (H&S) regulations to the practicalities of remote, outdoor operations. There is a paucity of published material on this subject, with the exception of a 1990 report for the American Anthropological Association. Therefore, the reviewed text provides a welcome H&S guide for anyone doing field work, not just the archaeologists and CRM (Cultural Resource Management) professionals to whom the book is primarily addressed. The authors were thorough in their identification of the possible hazards, some unique, of field/outdoor work. As the Preface points out, noncompliance is more the result of ignorance than negligence, and field methods courses do not adequately address safety issues. The book presents (as an appendix) a simple H&S Plan to help a group organize its needs.

The section on Physical Hazards is particularly strong, with discussions on heat/cold stress management; trenching, excavation, and general construction safety; confined space entry hazards; and drilling and subsurface investigations. Other topics covered (motor vehicle safety; underwater/dive safety; historic building safety; remote site safety, and unexploded ordinances) did not have the same level of details about hazard controls. Environmental monitoring methods and a description of instrumentation available for field use were a critical omission in the confined space section.

The Biological Hazards section was excellent in its identification of sources,

symptoms, and practical remedies. More photographs of sources and effects would have been useful (e.g., photos distinguishing the deer tick, or of characteristic bite and skin reactions). Exception is taken, though, to their description of who is at risk from blood borne pathogens (BBP). On a remote field site, everyone who has to assist an injured and bleeding colleague is at risk from exposure.

Personal Protective Equipment was extremely thorough, but the reader should not be overwhelmed by the layout of the discussion. The authors chose to frame the chapter in terms of risks on a hazardous waste cleanup site, which placed requirements for more typical field work closer to the end of the chapter. The chapter details the regulatory and programmatic requirements, but falls short of noting resources to consult for selection of actual materials and types of equipment to match the hazard.

The major shortcoming is that the book does not go far enough. The book and its intended audience would both profit from inclusion of case studies, practical solutions to specific fieldwork problems, Internet and literature references for further information, and lists of government agencies and professional organizations that can help steer one toward appropriate vendors, consultants, equipment and materials. For instance, the section on hearing conservation would have been improved by a chart on average noise levels produced by typical work operations or equipment, so that the reader could begin to judge the level of protection required. The ergonomics section would have benefitted from a description of the types of tools on the market that would reduce injuries in "typical" field tasks.. A note was made about the importance of labeling hazardous materials for shipment, but an entire section could have been devoted to this subject. The pesticides/herbicides section gives no actual guidance on prevention or protection. Non-U.S. readers should note the book's reliance on U.S. regulations in its recommendations, and thus, will need to double-check their own country's health and safety regulations for specific programs.

Even with these shortcomings, the book fills an important void in the application of structured, rigorous, and consistent H&S programs in field archaeology and Cultural Resource Management field work. It would also, with some modification, serve as a resource for allied specimen collection tasks whose occupational risks are not directly addressed, such as the use of fixatives and preservatives in less than controlled environments or silica/dust exposure in paleontological work. It serves as a necessary hazard awareness guide to those in the high risk occupation of fieldwork.

This spiral-bound publication (available for $12.50, which is the shipping or handling figure) is apparently available only through Panamerican Environmental, Inc., and Panamerican Consultants, Inc., 2390 Clinton Street, Buffalo, New York 14227; 716-821-1650, panamny@mindspring.com—*Kathryn A. Makos, Smithsonian Institution, Office of Safety and Environmental Management, 750 Ninth Street, NW, Suite 9100, Washington, DC 20560.*

PROCEEDINGS OF THE 3RD NORDIC SYMPOSIUM ON INSECT PEST CONTROL IN MUSEUMS, 1998, M. Åkerlund, J. Bergh, A. Stenmark, and I. Wallenborg, eds. (Swedish Museum of Natural History, Stockholm, Sweden, 179 pp.). Pest Control and Integrated Pest Management in museums are dynamic

and rapidly evolving fields in museum conservation of natural history, art and cultural collections. Different techniques for the control of insect damage, mold damage and the deterioration of museum specimens are being developed independently throughout the worldwide museum community. This book is meant to provide insight into the research in protecting museum specimens from biodeterioration, focusing on what is being accomplished in Europe. The title of this compilation is misleading, however, as the book deals with forms of biodeterioration other than merely insect problems.

The Proceedings starts with a reiteration of the opening address for the 3rd Nordic Symposium on Insect Pest Control in Museums. The ten separate units summarizing each talk as presented are discussed in turn below.

The first unit, Integrated Pest Management (IPM), has only one presentation. This article describes how building design for IPM can also accommodate other design requirements such as fire safety, storage, physical safety, etc. When IPM design requirements have to stand alone they must be balanced with available resources and they must show measurable benefit. Examples and technical arguments for supporting such design requirements are given.

Heating is the subject of the second unit and three presentations are described. Techniques for using heat treatments to control insect pests are discussed in the first presentation. The other two focus on the common furniture beetle, *Anobium*, and discuss the effects of heat on that species and the effects of heat on infested wood.

Four papers on freezing are presented in the third unit. Three discuss the effects of freezing on different organic materials, including paper and several different textile types. The fourth paper begins by discussing the effects of freezing on moths but ends with an analysis of the effects of freezing on wool fibers. These types of investigations are rare in the literature of museum conservation and provide technical information not available elsewhere.

The fourth unit, low oxygen, also contains four papers. This unit is more a discussion of current projects than a discussion of results. When these reported research experiments are completed, there should be some exciting results to review. At present, however, little relevant data are provided.

Unit five has one article on pheromones. This article demonstrates the use of pheromone trapping in IPM protocols and also includes a very thorough discussion of insect monitors and their usage in general.

There are three articles on pesticides in unit six discussing fumigants, pesticide efficacy when used on storage materials (not on the specimens themselves), and pesticide resistance.

A single paper on health hazards comprises unit seven. This article briefly discusses the health risks of exposure to the most common pesticides, toxins and dusts found in museums.

The next unit is on wood infestation. There are three papers included: one deals with insect biodeterioration, the other two with deterioration of wood through physical and environmental factors. The first paper in this section discusses anobiid life histories through examples. The other two papers are concerned with structure, and deterioration of wood through general physical and environmental factors such as high humidity, and fungal growth.

The ninth unit is on rodents. The sole article here describes the problems caused by rodents in a museum and includes an IPM strategy to control it.

The final unit includes two articles on education. The first article describes programs and surveys executed by the Conservation Department of the National Heritage Board in Sweden. The second paper is a book review of a new guide for health hazards and IPM in museums.

Individuals who are immersed in the field of Museum Conservation and Museum IPM will find this compilation worthwhile. Some of the articles provide useful data and describe procedures that can be used or modified in other museums, but other articles are mere mentions of a general topic discussed at the symposium and contain no real substance. The editing and literal translations are sometimes awkward, and English vocabulary and grammar are at times very creative but wading through these rough spots can be rewarding.

Information on obtaining a copy of the Proceedings, which costs approximately $20 US, can be found at http://www.nrm.se/premal/pmsymp.html.en.—*Jeremy F. Jacobs, Department of Vertebrate Zoology, Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0108.*

HERPETOLOGICAL COLLECTING AND COLLECTIONS MANAGEMENT. Revised edition, 2002, John E. Simmons (Society for the Study of Amphibians and Reptiles Circular No. 31: vi + 153 pp.). This publication is a revision of John Simmons' 1987 work, enlarged from 70 pages (1987) to 153 pages (2002). The book's sections have been somewhat rearranged from the earlier version, but the basic organization is similar. Although it is aimed at herpetological collecting and collections, the book has relevance to any discipline which uses fluid-preserved collections.

The book is both broad and deep. It examines the philosophy behind maintaining and managing collections and associated activities, through policies for collecting and maintaining collections, down to nuts-and-bolts procedural details. The section on philosophy and history makes good reading, and should be compulsory study for institutional administrators. The bulk of the book provides accepted, tried-and-true policies and procedures which can be easily followed by less-experienced collection workers; they also serve as a checklist to be periodically reviewed by all of us, no matter how experienced. Protocols are also laid out for loans, tracking the status of specimens, monitoring the year-to-year use of a collection and adjusting one's procedures accordingly. Adhering to all the procedures in the book would make one a model collection manager. I read with increasing chagrin, as I came across yet another procedure that I should be following but wasn't.

The author emphasizes the long-term nature of collection management, and that a collection's lifespan greatly exceeds that of its managers. We are reminded that our actions will affect future generations of managers, who will rely upon (or attempt to mitigate) anything we do now. The point is also made that there are still a number of practices whose long-term (>100 yrs) ramifications are not yet known, such as the effects of fixation in formalin.

Long-term maintenance of collections is treated extensively. Although most emphasis is on alcohol-preserved specimens, the book also deals with ancillary

collections such as photographs, sound recordings and paper documents. The author has done an exhaustive survey of the pertinent literature, and the text includes many references to procedures, both recommended and not recommended. There is a compendium of collecting techniques, with emphasis on the actual specimen fixation and preservation, and equipment. Most of the commonly used procedures are laid out in an unequivocal step-by-step manner. Less-frequently-used or specialized techniques are not covered in detail; readers are instead referred to other published resources. Tissue collections and electronic databases are treated only briefly; although the significance of both is increasing, more detailed treatment would have resulted in a much longer book.

Examples of forms and other procedures are provided. The examples are all taken from the author's home institution, the University of Kansas, but can be adapted to suit any institution. Likewise, most examples dealing with permits or legislation are relevant only to American institutions, but these can be used as guidelines for anyone, anywhere.

The Literature Cited section alone is a valuable resource, running to 26 pages of references. The book ends with three appendices. The first is a list of required American permits. The second contains lists of recommended equipment and supplies for field work. In some cases specific brands of supplies, including medications, are recommended; these may not be available everywhere. The third is a list of suppliers and sources of equipment, all of which are U.S.-based.

My quibbles with the book are minor. I had some difficulty with one recommended field procedure—that of tying field tags on specimens after the specimens have hardened in formalin. I would hesitate to harden a batch of unnumbered animals in the same tray. My preferred procedure is to tag specimens right after they are photographed and killed. Tissue is then removed for molecular work, and the field tag number is written immediately on the tissue tube and into the photography record, so that specimen, tissue and photograph carry the same number at an early stage of processing. This minimizes the chance of mixups between tissue, photograph and specimen. I also prefer sewn or spiral-bound books, rather than loose-leaf, for field notes, because of the risk of losing pages from a ring binder; the author, however, makes the valid point that pages may tear out of books with sewn binding. In several places throughout the text, other sections in the book are referred to (e.g., "... *see Appendix III* ..."). It would have been useful to include a page number in these situations to help a reader find the recommended section quickly. Typos are few, although my curiosity was piqued by the inclusion of "vice grips" in the list of essential field equipment.

The book is written clearly, with instructions and recommendations laid out systematically. The author injects wry, humourous comments where appropriate. All in all, this is a fine, useful book. Some readers may wish for more detail on certain techniques or types of collections, but these needs can be covered by the comprehensive citations. As the Introduction states, "... development of techniques ... made care and management of collections far more sophisticated than can be covered in just one volume." The book strikes a good balance between extensive coverage and succinctness, but who knows—perhaps we'll see a 300-page third edition 15 years from now. (US$16.00; available from the SSAR Publications Secretary, ssar@herplit.com).—*Ross D. MacCulloch, Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario M5S 2C6, rossm@rom.on.ca*

Museum Collection Resources Display Available for Loan

The Resources Subcommittee of the Conservation Committee (SPNHC) maintains two displays of supplies and materials used by many museums for the storage and preservation of natural history collections. Examples of items included in the displays are: materials used in the construction of storage containers and specimen supports; equipment for monitoring storage environments (e.g., humidity, temperature, air quality, insects); and a variety of containers for the storage of collections and documentation. Some of the products are discipline-specific (e.g., pH-neutral glassine for interleaving between herbarium sheets) but most can be used in multidisciplinary collections (e.g., Ethafoam™ for lining shelves and drawers; Tyvek™ tape for box and tray construction). The displays are available for loan to interested parties for meetings, conferences, and other museum-related activities. Shipping costs to and from the requested venues are the responsibility of the borrower. There is no loan fee but SPNHC invites borrowers to make a voluntary contribution to cover the costs of routine maintenance. For additional information, or to borrow a display, contact:

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On the first page indicate only the name, e-mail address, and mailing address for the author to whom correspondence and proofs should be addressed. The second page then includes only the title of the article, names of the authors, affiliations and addresses of authors, and the abstract. Begin the text on the third page.

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Jones, E.M. and R.D. Owen. 1987. Fluid preservation of specimens. Pp. 51–64 in *Mammal Collection Management* (H.H. Genoways, C. Jones, and O.L. Rossolimo, eds.). Texas Tech University Press, Lubbock, Texas. 219 pp.

Thomson, G. 1986. *The Museum Environment*, 2nd ed. Butterworth's, London, England. 293 pp.

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