An inexpensive method to test for mercury vapor in herbarium cabinets

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Mercuric chloride has been used for control of insect and fungal infestations in herbarium collections for over two centuries. One of the lasting effects of this use is the long-term evolution of elemental mercury vapor from treated specimens. The vapor can contaminate untreated specimens sharing the same closed environment and can pose a human health hazard. By modifying the technique for use of a commercially available mercury indicating powder (Mallinckrodt Baker, Inc., J. T. Baker Mercury Indicator) it is possible to create an inexpensive and fairly rapid test for mercury vapor in herbarium cabinets. The indicator is mixed with deionized water and applied to glass microscope slides. One or more slides are placed inside a cabinet and any color change in the indicator is compared to unexposed controls. In the authors' experiments, the indicator results were compared against readings taken using a Jerome 431-X Mercury Vapor Analyzer and a Lumex RA-915+ Multifunctional Mercury Analyzer and were found to be broadly related to the concentration of mercury vapor present in each cabinet. The method can be used to check for mercury contamination in incoming shipments of specimens and to identify cabinets that currently contain or formerly contained contaminated specimens. Practical safety guidelines have been developed for accessing cabinets that give a positive test for the vapor and for handling contaminated specimens.

KEYWORDS: corrosive sublimate, herbaria, mercuric chloride, mercury contamination, mercury detection, mercury indicator, mercury vapor.

INTRODUCTION

Mercuric chloride ($HgCl_2$) solutions have been used as pesticide and/or fungicide treatments for botanical specimens at least since the 18th century (Forster, 1771), were still in use in at least one major herbarium in 1982 (Clark, 1986), and based on the authors' experience, continue to be used in some herbaria. Mercuric chloride is fairly soluble in water (7%) and very soluble in ethyl alcohol (34%) at 30° C (Singer & Nowak, 1981). Alcohol or water solutions were used to immerse or spray specimens, pressing papers, and mounting sheets (Babbington, 1843; Bailey, 1881, 1899; Robinson, 1903; Clute, 1904; Burtt-Davey & Chalk, 1927; Fosberg & Sachet, 1965; Franks, 1965; Spencer, 1963; Wagstaffe & Fidler, 1968; Croat, 1978; Womersley, 1981; Briggs & al., 1983; Hawks & Von Endt, 1990).

Use of mercuric chloride is associated with the gray-black stains seen on many herbarium sheets (Hawks & Bell, 1999). Stains on sheets in the United States National Herbarium, Department of Systematic Biology-Botany Section, National Museum of Natural History, Smithsonian Institution (U.S. National Herbarium), have been found to contain mercuric sulfide (metacinnabar, probably from reaction with sulfur in the mounting paper) and a mercury sulfide/mercury oxide ($2HgO \cdot HgS$, possibly from reactions with paper and air) as well as unreacted mercuric chloride (Sirois, 1998; Sirois & Helwig, 1996). The metallic sheen of some stains suggests that *elemental* mercury, which is difficult to identify by instrumental methods, may also be present. Stains may be a reliable indication of the presence of mercury in some form (fungal stains may be distinguished from mercury stains by microscopy), but absence of stain is not a reliable indication that mercury compounds are absent. Unreacted mercuric chloride is colorless.

The old name for mercuric chloride is "corrosive sublimate". The name derives from the reaction by which

it was first synthesized in the 10th century—mercury, vitriol (*one of several possible metallic sulfates*), and common salt (*sodium chloride*) were heated, generating hydrochloric acid that reacted with the mercury to form a corrosive sublimate, mercuric chloride (Goldwater, 1972). Mercuric chloride volatilizes slightly at room temperature, appreciably at 100° C, and sublimes without decomposition at 300° C (Singer & Nowak, 1981; Windholz & al., 1983). However, mercuric chloride, mercuric sulfide, and metallic mercury all constantly emit *elemental mercury vapor* into the surrounding air and the transition from solid compound to gaseous element is enhanced by light (Gustin, 2000).

Elemental mercury vapor and inorganic mercury compounds pose a threat to human health by targeting the central nervous system, kidneys, respiratory system, eyes and skin [American Conference of Governmental and Industrial Hygienists (ACGIH), 2003], and constant generation or sublimation means that untreated specimens may become contaminated when housed in cabinets with treated specimens. Using a mercury vapor monitor, Briggs & al. (1983) measured elemental mercury vapor in the herbarium at the University of Cambridge. They found airborne concentrations of up to 700 μg/M^3 in cabinets filled with herbarium specimens, well above the occupational Threshold Limit Value (TLV-TWA) of 25 μg/M^3, as established by the ACGIH (2003) as a maximum average exposure for an 8-hour work day. Mercury vapor is also readily adsorbed by wood used in some specimen cabinets, and could be released from these surfaces even after the removal of contaminated specimens (Waller & al., 2000).

Research by Purewal (1999, 2001) in the National Museum of Wales, using digestion of herbarium sheet papers in the type collection, and analysis by atomic absorption spectrophotometry and flow injection mercury system atomic emission spectrometry found levels as high as 1021 μg of mercury (the form of mercury present could not be deduced by this method) per gram of paper. Although skin absorption and possible inadvertent ingestion of mercury or mercury compounds can have health effects similar to those from inhalation of elemental mercury vapor, the particulate hazard is easily controlled through appropriate use of gloves, good personal hygiene, and careful handling of the sheets.

Neither collectors' notes nor herbaria records detail all chemical treatments that may have been applied to collections. When stains are present on sheets, it is an obvious indication that gloves or other personal protection are necessary, but when stains have not formed there is no indication that mercuric chloride or mercury vapor may be present, thus there is no reliable visual clue for presence of a hazard.

Chemical spot tests for mercury compounds are too laborious for use in large collections and based on our own experience and that of others, often give erroneous results (Found & Helwig, 1995). Validated analytical methods for airborne mercury vapor measurement require specialized sorbent tubes, calibrated sampling apparatus, and external laboratory analysis (i.e., they are not real-time measurements) [United States National Institute for Occupational Safety and Health (NIOSH), 1994]. Real-time monitoring instrumentation for mercury vapor detection or for detection of mercury compounds is expensive to rent or to purchase and maintain, and probably is not a practical or readily available resource for many herbaria.

A commercially available mercury indicator has been produced for many years by J. T. Baker Chemical Co., a division of Mallinckrodt Baker, Inc. The chemical is designed to be used as either a powder (for horizontal surfaces) or paste (for vertical surfaces) to locate micro-droplets of mercury during mercury spill cleanup. The powder or paste undergoes a color change in the presence of metallic mercury or mercury vapor (Mallinckrodt Baker, 2003; J. T. Baker Chemical Co., 1979). It seemed worthwhile to investigate the possibility of using this chemical to detect mercury vapor in herbaria.

MATERIALS AND METHODS

Research into the utility of the indicator powder was conducted in the US National Herbarium. There was no record that mercury salts were used by the herbarium staff; however, it is known that some collections had been treated prior to coming to the museum. The extent of the collections (in excess of 4.7 million specimens housed in 5100 cabinets), the age of the collections (collecting dates span the past two centuries), and the number of collectors from around the world who have contributed to the holdings suggested that the findings here might be of value in any herbarium.

Preliminary ambient monitoring for mercury vapor. — A Jerome 431-X Mercury Vapor Analyzer was used to understand the extent of mercury contamination in the collections. The Jerome operates by measuring the change in electrical resistance of a gold film produced when amalgamated with mercury. It pulls air samples through a filter to remove organic acids, Cl_2, NO_2, H_2S, mercaptans (organic sulfurs), HCl and H_2SO_4 (the instrument does not respond to hydrocarbons, CO, CO_2, SO_2, or water vapor) that would interfere with the analysis, then passes the air across a gold film sensor that is connected to a reference gold film by a Wheatstone Bridge Circuit (Arizona Instrument, 1995). The instrument then uses the change in electrical resistance caused by the mercury on the sensor to calculate the concentra-

tion of elemental mercury in the air sample. The instrument becomes saturated after repeated exposures. It is cleaned through an internal heating cycle that burns mercury off the sensor and passes the gas through a filter to prevent contamination of ambient air. After cleaning, the gold reference and sensor films can be readjusted to the same baseline resistance. The Jerome can detect mercury vapor concentrations from 3 to 999 µg/M^3, with an accuracy of ± 5% at 100 µg/M^3. The response time in discreet sample mode is 13 seconds.

The Jerome was used to measure mercury vapor concentrations in over 300 cabinets selected randomly in the US National Herbarium's facilities at the Smithsonian Institution's National Museum of Natural History (NMNH) building in Washington, D.C., and Museum Support Center (MSC) in Suitland, Maryland. The results indicated that while some cabinets appeared to be free of vapor in the range measured by the Jerome, there were a substantial number of cabinets with mercury vapor and that the concentrations spanned the full range of the instrument's detection capability. Examination of the contents of these cabinets revealed that it was not possible to predict the potential amount or even the presence of mercury vapor through the appearance of the sheets, or through collecting dates.

Trial test of indicator effectiveness. — For use in herbarium cabinets, it was decided that mixing the indicator in water and applying it to microscope slides would be a simple way to hold the powder in place and create a test strip that would fit along the edge of a shelf or along the bottom edge of the frame inside a herbarium cabinet. In addition, preliminary tests suggested that the indicator was especially sensitive to mercury vapor after the powder had been wetted, apparently more so than in a completely dry state.

In the first round of testing, new microscope slides were carefully cleaned with deionized water, then with 99% undenatured ethanol (absolute alcohol), and thoroughly wiped dry with lint-free paper laboratory wipes. A reference number was scribed on one end of each slide for tracking purposes. Because the surface of the powder rapidly turns color in response to mercury vapor, it was assumed that a moderately thin dispersion on a slide would be less equivocal to read than a thick dispersion, not all of which would change color at the same rate. Consequently, rather than a thick paste, we prepared a slurry composed of 10 ml of the indicator powder in 25 ml of deionized water and painted this on the slide, as evenly as possible, using a clean, nylon-bristle artist's brush.

To conduct the trial, seven cabinets were selected to represent a range of anticipated mercury vapor concentrations, based on previous ambient measurements. Twelve slides were used inside each of the seven cabi-

nets. Six slides were placed at the front of the top shelf and six along the front of the bottom edge of the frame, all as close as possible to the latch side of the door for each cabinet. In addition, control slides were placed on top of selected test cabinets and/or on countertops near the cabinets. Four of the cabinets were modern, powder-coated, single-door, full-height, steel herbarium cabinets; three were old, empty, wooden half-unit cabinets that had not been used to house specimens for many years.

Each case was opened and one slide from the top and one from the bottom were removed and compared with readings taken with the Jerome, and with the relevant controls at the following intervals: 1 day (1 day = a 24-hour period), 3 days, 7 days, 14 days, 28 days, and 65 days. While the controls remained unchanged (yellow), the indicator slides inside the cabinets changed color, and by day seven, even the weakest sources, the wooden cabinets without specimens, showed an easily detectable color change. Once slides were removed from cabinets, the colors, which ranged from pale orange to a dark gray (often with red flecks), did not undergo any further alteration and have remained stable for the past three years. The color changes after seven days roughly correlated to readings taken with the Jerome, indicating that as the color progressed from pale orange to dark gray, the concentration recorded for the case increased as well. The concentrations found for the empty wooden cabinets were at or below the minimum level of detection for the instrument. The slides at the bottom of all test cabinets showed the best correlation to the Jerome readings, which might be expected given that mercury vapor is heavier than air and over time would tend to accumulate in the bottom of a closed cabinet. The slides left in cabinets for 14, 28, and 65 days did not continue to darken appreciably nor did interior concentrations increase significantly.

An interesting result of the trial was the apparent color change of the indicator in a few cabinets in which no mercury vapor was detected by the Jerome Analyzer (detection limit of 3 µg/M^3). This suggested either an anomaly in the indicator powder, or that the powder was more sensitive to mercury than the analytical instrument. If the latter proved to be true, it would make the indicator an even more valuable tool for collections management.

Large-scale testing. — To test the correlation between indicator color change (defined by objective colorimetry analysis) and ambient mercury vapor concentrations within cabinets, the Jerome Analyzer was used in conjunction with a Lumex RA-915+ Multifunctional Mercury Analyzer which could be programmed to measure in the range of 0.02 to 400 µg/M^3). The Lumex is a portable instrument designed for quantitative detection of mercury in ambient air, water, soils,

sediments, biological media, and foodstuffs. It operates using Zeeman atomic absorption spectrometry coupled with high-frequency modulation of polarized light from a mercury lamp, and like the Jerome, filters air samples to remove potential interferences. The instrument uses a magnetic field to split the beam from the mercury lamp into three polarized components, then compares the differences in intensity between two of the components, a reference (Zeeman σ +) and an exposed part of one of the components (σ -), caused by the presence of mercury (OhioLumex, 2001).

For these tests, single slides, prepared and labeled as described above, were placed at the bottom of randomly selected herbarium cabinets. The test involved 109 herbarium cabinets in storage Pod 2 at the MSC, and 57 cabinets in the herbarium on the 4th and 5th floors of the NMNH. Jerome and Lumex readings were taken at the time the slides were inserted, then the cabinets were closed for seven days. Controls were placed atop test cabinets or on nearby countertops, and additional controls were kept under cover in a well-cleaned Petri dish, in an area separate from the herbarium. At the end of the test period, the cabinets were opened just enough to permit measurements with the two instruments, then the slides were removed and the color was described subjectively by two members of the team in the following terms: yellow (unreacted), light orange, orange/gray, or dark gray.

Quantitative assessment via colorimeter. — The color of the indicator on each slide was measured using a Minolta Chroma Meter CR-221 tristimulus colorimeter, calibrated according to the manufacturer's instructions to a daylight white tile standard. The instrument provides a fiber optic daylight source for the readings. Each slide was placed on a white background and three readings were taken from the densest areas of the indicator on the slide. The instrument was re-calibrated after each 10 slides. Each meter reading provides data for three color factors (L= range from black to white, a = range from greens to reds, b = range from yellows to blues) and also calculates the mean for each factor for the readings taken from each slide, along with the standard deviation for the readings for each factor. In order to apply correlation statistics to the body of data, the tristimulus color coordinates were converted into a single value (ΔE) representing total color difference according to the formula (American Society for Testing and Materials, 2002):

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Each value was first corrected by subtracting the control value. Color difference increases in value in direct proportion to degree of color change from the control slides.

The color difference measurement for each slide was compared to the measured ambient concentration in the interior of the case upon slide removal, after the one-week undisturbed residence time. The measurement upon removal was considered to be more representative of the concentration impacting the indicator slide than readings taken when the slide was first installed in the cabinet.

RESULTS

Fig. 1 presents a plot of log transformed color difference (ΔE) vs. the mercury concentration data. The underlying distribution of mercury concentrations in the cases was log normal so that the data appear linear on the log scale. The datasets were analyzed to determine the degree of correlation. The r-squared correlation coefficient was +0.67, indicating a positive association between corresponding values in each set of data.

Fig. 2 presents the results of the subjective (visual) interpretation of the color change on the indicator slides. Each slide was judged to be one of four colors after exposure. In this plot each slide within a color category is plotted vs. the mercury concentration as measured by the analytical instrument.

Within broad ranges, the color changes visible in the indicator over a seven-day period appeared to correspond to the following measured concentration of vapor in a closed cabinet:

Yellow = <1 µg/M³
Light orange = 1 to 30 µg/M³
Orange with gray = 30 to 300 µg/M³
Dark gray = >300 µg/M³

While color change is a reliable indicator of the presence of mercury, the association is not strong enough to accurately calculate µg/m³ mercury concentration levels from ΔE readings. However, the data do strongly sug-
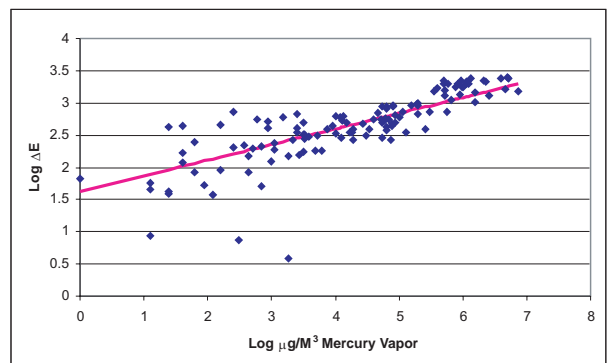

Fig. 1. Line fit plot of log transformed indicator slide color differences and measured mercury vapor concentrations. The fitted line has a y intercept of 1.63 and a slope of 0.242.
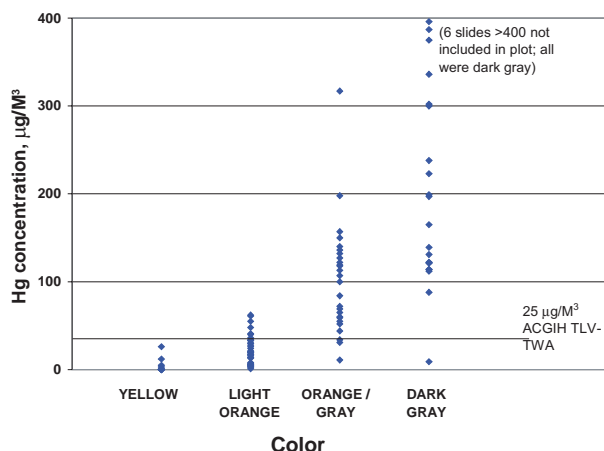
Fig. 2. Color of indicator slide versus Hg concentration.

gest that a yellow indicator slide would represent no detected, or extremely low, mercury vapor concentrations. The presence of any gray color indicates that mercury levels of concern are likely to exist (i.e., with potential to exceed the ACGIH (2003) occupational TLV-TWA of 25 µg /M³; Fig. 3 shows an indicator slide typical of each of the four color ranges.

The mercury indicator is composed of a mixture of cuprous iodide, sulfur, amorphous silica, and starch in proprietary proportions (Mallinckrodt Baker, 2003). The dry, yellow powder becomes reddish-brown on exposure to mercury vapor, and undergoes the changes noted above when wetted and then exposed to the vapor. The authors assume that because the indicator is intended to react with elemental mercury, the color changes are caused by one or more of the following reactions (Levason & McAuliffe, 1977; Prinz & al., 1978; Windholz & al., 1983; Weast, 1988):

- cuprous iodide (CuI, red-brown crystals) to cuprous mercuric iodide (Cu_2HgI_4, the α-form is a deep red crystalline powder, the β-form is chocolate-colored crystals);
- sulfur to mercuric sulfide (HgS, black amorphous powder or black crystals);
- cuprous iodide (CuI, red-brown crystals) to mercuric iodide (HgI, the α-form is red crystals);
- cuprous iodide (CuI, red-brown crystals) to mercurous iodide (Hg_2I_2, bright yellow amorphous powder; darkens on exposure to light with formation of red mercuric iodide, HgI, and elemental mercury);
- sulfur to mercuric sulfide (HgS, the mineral cinnabar, bright red microcrystals or disseminated grains that blacken on exposure to light);
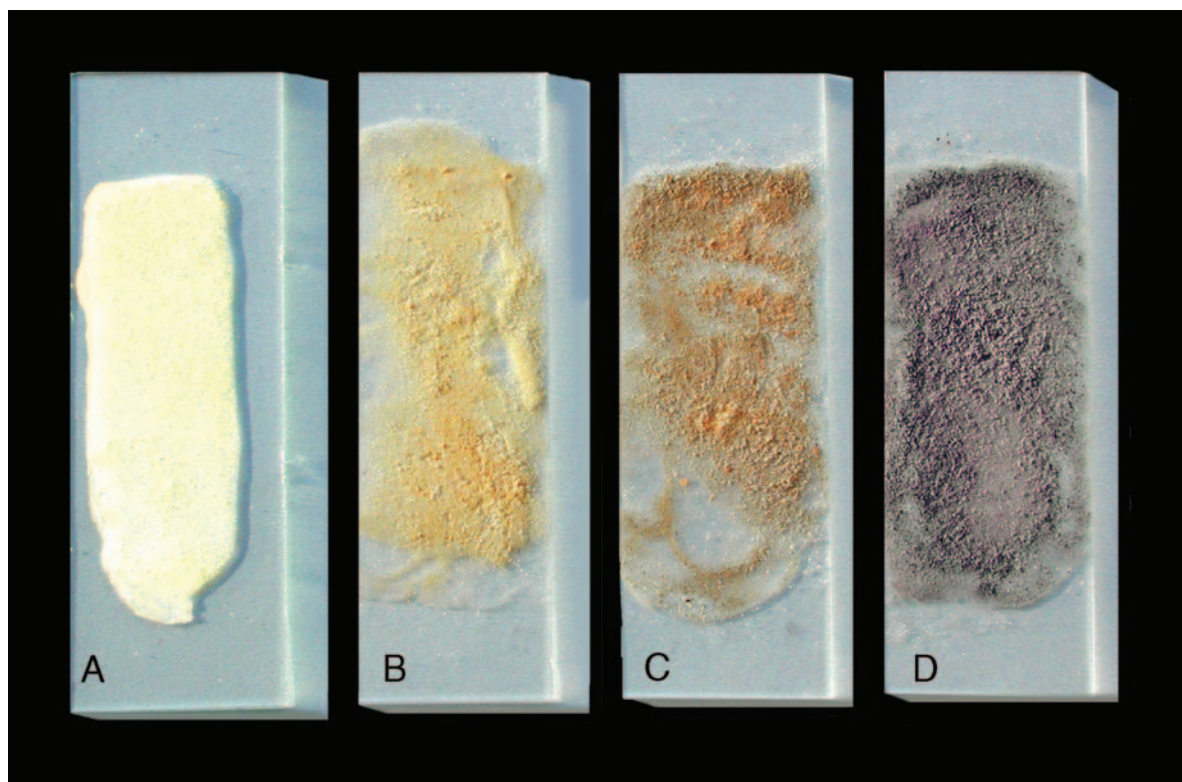

Fig. 3. Mercury indicator powder mixed with deionized water and applied to glass microscope slides. Color is broadly related to the concentration of mercury vapor present after seven days. A, yellow = <1 µg/ M³; B, light orange = 1 to 30 µg/M³; C, orange with gray = 30 to 300 µg/M³; D, dark gray = > 300 µg/M³.

- sulfur to mercurous sulfate (Hg_2SO_4, yellow powder, with light-induced alteration to gray by formation of elemental mercury and mercuric sulfate, $HgSO_4$, a white crystalline powder).

According to Levason & McAuliffe (1977), there is no evidence for the existence of mercurous sulfide (Hg_2S, a black compound), despite suggestions in early literature on inorganic chemistry. The fact that the reaction products observed in our experiments do not change color after exposure to light suggests that either the first three reactions noted above are the ones primarily responsible for the color change, or that any light-induced changes occur rapidly.

■ DISCUSSION

Because the technique described here is sensitive to very low levels of mercury contamination, it can be used to screen empty cabinets to determine if they have become contaminated by mercury vapor over time and to test incoming shipments of specimens to determine whether they are likely to cause cross-contamination if housed with specimens that were never treated with mercury salts. The sensitivity of the indicator was shown to be greater than that of the Jerome 431-X Mercury Vapor Analyzer and comparable to that of the Lumex RA-915+ Analyzer, suggesting that the indicator is an especially useful tool when screening for very low concentrations of mercury vapor in closed environments. Future research might be undertaken to develop a color calibration curve in light of the positive correlation between color differences, visually-perceived color changes, and measured concentrations. A description of the technique for the preparation and use of indicator slides is presented in Appendix 1.

Once the presence of mercury vapor is detected within a particular cabinet, it must be presumed (absent an active scavenging system) that vapor will re-accumulate inside the cabinet after it has been closed again. Safe work practices must be implemented that will minimize the initial, most acute exposure to mercury vapor upon opening a cabinet. Studies conducted by the Smithsonian Institution's Office of Safety and Environmental Management (OSEM) (Makos & Burroughs, 2002) on 184 randomly selected cases within the US National Herbarium indicated that timed aeration of the cabinets (i.e., opening the cabinet door and walking away) as a work practice can be effective in significantly decreasing ambient concentrations to within established standards. The specific time interval relates directly to the level of general air flow and circulation patterns within a herbarium and the method of cabinet packing, and therefore needs to be evaluated per setting.

Other studies, conducted between OSEM and NIOSH involved the analysis of 25 full-shift breathing zone (airborne) personal samples, collected by NIOSH Method 6009 (US National Institute for Occupational Safety and Health, 1994) during varied tasks by US National Herbarium staff. These tasks represented all levels of exposure potential, from case insect inspections (intensive exposure from opening multiple cabinets) to cataloguing and specimen curation on open, well-ventilated workspaces. The data were found to be log normally distributed. The range of mercury vapor exposure was from 0.12 to 2.73 $\mu g/M^3$, indicating 95% confidence that fewer than 5% of exposure would exceed 3.0 $\mu g/M^3$ (Makos & Burroughs, 2002). These data suggest that the following recommended procedures will assure that occupational exposure to mercury vapor will not exceed the ACGIH TLV-TWA of 25 $\mu g/M^3$.

Based on these studies, it is recommended that any herbarium where mercury levels **in cases are above 25 $\mu g/M^3$** be located in well-ventilated spaces, with sufficient space between rows to allow for adequate supply and return air circulation patterns. Safety protocols adopted at the US National Herbarium include:
- cases are allowed to air, with doors fully open, before accessing the contents;
- specimens are removed to a separate, well-ventilated worktable in order to minimize time spent in front of an open case;
- disposable barrier gloves are worn when handling specimens and mounting papers to avoid dermal contact with inorganic mercury or its salts;
- gloves are discarded after a single use; and
- good personal hygiene (i.e., washing hands before and after use of gloves) is recommended practice.

The use of indicator slides provides a valuable clue as to presence of mercury on treated specimens, and an indication of the level of contaminant vapor accumulation within a closed cabinet. However, there is no substitute for exposure monitoring. If screening the collections with the indicator has shown that mercury vapor is present, then herbaria staff should make every effort to secure the services of an occupational hygienist to monitor the ambient environment and conduct personal biological exposure assessments.

■ ACKNOWLEDGEMENTS

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Appendix. Preparation and use of indicator slides.

The indicator is inexpensive (one 250 g container will suffice for hundreds of tests), readily available from laboratory safety suppliers, and when used in compliance with the information on the Materials Safety Data Sheet, need not pose a hazard during use. Disposal of the slides and their residues after use should conform to all applicable hazardous waste disposal regulations.

The technique for use of the indicator is summarized below:

1. Clean glassware and glass microscope slides with deionized or distilled water and then with undenatured, 95–99% ethanol and allow to air dry or dry with lint-free laboratory wipes, such as Kimwipes.
2. Scribe a cabinet number or other tracking number on the end of each microscope slide. Numbers may also be written using a carbon-based, pigmented ink (e.g., using a black-ink, Pigma pen).
3. Mix 10 ml of J.T. Baker Mercury Indicator Powder (Product Code 4509)[1] with 25 ml of distilled or deionized water, using a clean glass stirring rod.
4. Paint the indicator dispersion evenly on slides using a nylon-bristle artist's brush.
5. Allow slides to begin to dry sufficiently for them to be moved without loss of the dispersion.
6. Protect slides from contamination prior to placing them in cabinets (walking past open cabinets that may have mercury vapor contamination, or exhaling across slides if you have mercury amalgam dental fillings can cause color changes in the indicator).
7. Place 1–3 slides inside each cabinet, along the front of the bottom edge of the frame.
8. Place control slides atop test cabinets, at intervals throughout the herbarium, and in a protected environment outside the herbarium.
9. Keep cabinets closed for seven days after placing the slides.
10. Remove slides from cabinets and with two or more people as viewers, compare test slides with the unexposed controls and record any color change. Within broad ranges, the color changes visible in the indicator over a seven-day period appear to correspond to the following measured concentration of vapor in a closed cabinet:

   Yellow = <1 μg/M^3

   Light Orange = 1 to 30 μg/M^3

   Orange with gray = 30 to 300 μg/M^3

   Dark gray = >300 μg/M^3

While color change is a reliable indicator of the presence of mercury, the association is not strong enough to accurately calculate μg/M^3 mercury concentration levels from ΔE readings. However, the data do strongly suggest that a yellow indicator slide would represent no detected, or extremely low, mercury vapor concentrations. The presence of any gray color indicates that mercury levels of concern are likely to exist (i.e., with potential to exceed the ACGIH occupational TLV-TWA of 25 μg /M^3, (ACGIH, 2003).

[1]*Use only the product noted here. Informal testing by conservator Barbara Hamann at the Carnegie Museum of Natural History suggests that mercury indicator powders from other companies are formulated differently and may not respond readily to mercury vapor, or may not respond in the same way.*