Reinsch Analysis for Arsenic, Antimony, Bismuth, and Mercury in Foodstuffs and/or Stomach Contents

1 Introduction

While the human intake of sub-milligram amounts of metals such as arsenic, mercury, antimony and bismuth are common to a normal diet, suicidal as well as homicidal fatalities have occurred from the misuse of their toxic salts. The use of arsenic as a chemical murder weapon has historical origins in the odorless and nearly tasteless qualities of its trioxide salt. Human fatality from the ingestion of 'white arsenic' (arsenic trioxide) can occur from as little as 200-300 milligrams while the average lethal dose for an inorganic mercury salt is about one gram.

The following procedure describes the presumptive testing of common foodstuffs, as well as the viscous and liquid stomach contents arising from the ingestion of these foodstuffs, for the presence of these metals. It employs a well-established analytical test, commonly known as the Reinsch test, which is simple and quick to perform.

2 Scope

This procedure allows for the screening of several common heavy metals in foodstuffs and/or stomach contents. Its primary purpose is as a rapid exclusionary test for the presence of these toxic elements and/or their common salts. Positive findings are further analyzed by selective element analytical techniques. This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

The analysis is based on the fact that metallic arsenic, antimony, bismuth and mercury will deposit on a copper foil placed within a sample matrix that is acidified and heated. This deposition is visually recognized as a black or silvery staining of the copper foil.

4 Specimens

Sample matrices can be comprised of common foodstuffs (e.g., cakes, candy, and beverages) and/or liquid and viscous stomach contents. Typically, 5 mL or 5 grams of sample is used.

5 Equipment/Materials/Reagents

Guidance for preparing reagents may be found in the *Preparation of Chemical Reagents* standard operating procedure (Tox 103).

a. Homogenizer and/or mortar and pestle

b. 16 x 125 mm screw-top test tubes

c. 5-mL Serological pipets

d. Copper foil (~0.25 mm thick)

e. 25-mL Graduated cylinder

f. 33% (v/v) Nitric acid:

Prepare fresh by mixing one part nitric acid (Reagent Grade) to two parts water

g. Concentrated Hydrochloric acid (HCl) (Reagent Grade)

h. 1 N Hydrochloric acid (Reagent Grade)

Dilute 8.6 mL of concentrated hydrochloric acid to 100 mL of water.
Store at room temperature in glass or plastic. Stable for at least 6 months.

i. Ethanol (Reagent Grade)

j. Steam bath or equivalent

k. Arsenic trioxide (SPEX Element Kit)

l. Magnetic stirrer

m. Ruler

n. Aluminum foil

o. Antimony trioxide (Reagent Grade, or better)

p. Bismuth nitrate pentahydrate (Reagent Grade, or better)

q. Mercuric chloride (Reagent Grade, or better)

r. Glacial acetic acid (Reagent Grade, or better)

s. Clear plastic tape

6 Standards and Controls

a. Arsenic Standard Solution (0.1 mg/mL):
A 0.1 mg/mL standard solution of arsenic is prepared by adding 13.2 mg of arsenic trioxide to a 100-mL volumetric flask. This is diluted with 1 N HCl to the 100 mL volume mark. Dissolution takes place with magnetic stirring (1-2 hours). Store at room temperature in glass. Stable as a qualitative standard for at least five years.

b. Antimony Standard Solution (0.1 mg/mL):
A 0.1 mg/mL standard solution of antimony is prepared by adding 12.0 mg of antimony trioxide to a 100-mL volumetric flask and dissolving in a few milliliters of concentrated HCl. Following dissolution, the solution is brought to the mark with deionized water. Store at room temperature in glass. Stable as a qualitative standard for at least five years.

c. Bismuth Standard Solution (0.1 mg/mL):
A 0.1 mg/mL standard solution of bismuth is prepared by adding 23.2 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask and dissolving in a few milliliters of glacial acetic acid. Following dissolution, the solution is brought to the mark with deionized water. Store at room temperature in glass. Stable as a qualitative standard for at least five years.

d. Mercury Standard Solution (0.1 mg/mL):
A 0.1 mg/mL standard solution of mercury is prepared by adding 13.6 mg of mercuric chloride to a 100-mL volumetric flask and dissolving in 5-10 milliliters of concentrated HCl. Following dissolution, the solution is brought to the mark with deionized water. Store at room temperature in glass. Stable as a qualitative standard for at least five years.

e. Negative Control:
A deionized water blank or a matrix similar to the submitted specimen (if known and available) is used as the Negative Control. A Negative Control sample is analyzed every time the Reinsch test is performed.

f. Positive Control:
A portion (5 mL) of the arsenic standard solution (or any other heavy metal standard solution listed above) is used as the Positive Control. Alternatively, if the test sample amount permits, the Positive Control can be generated by mixing 5 mL of the arsenic standard solution (or other heavy metal standard solution) with 5 grams of the specimen.
A Positive Control is analyzed every time the Reinsch test is performed.

7 Sampling

Non-homogenous materials should be made more uniform by grinding, dilution or other means.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the authorized individual performing the procedure.

8.1 Preparation of Specimen Material and Copper Foil Strip

a. Thoroughly homogenize the submitted specimen to ensure a representative aliquot is sampled. This may be accomplished by grinding in a mortar and pestle (candy), grinding by hand (cakes, cookies), blending in a homogenizer (bulky stomach contents) or vigorously shaking the specimen (gastric lavages, beverages).

b. Cut a 5 x 10 mm rectangular strip of copper foil. The copper foil strip should not be touched with bare hands.

c. Immerse foil strip in a test tube of 33% nitric acid.

d. Observe the effervescent copper foil strip for about 30 seconds and terminate the chemical reaction by discarding the acid solution and rinsing the foil strip three times with deionized water. Lastly, rinse the foil strip once with ethanol followed by a final deionized water rinse. The clean foil strip should have a shiny appearance.

8.2 Analysis

a. Place a clean copper foil strip into a 16 x 125 mm screw cap test tube. Alternatively, for highly absorbent samples or samples that are difficult to homogenize, the copper foil strip can be placed into a 50-mL Erlenmeyer flask, or similar sized glass vessel.

b. Add 5 grams or 5 mL of uniform specimen to the flask followed by 5 mL of deionized water. A Negative and Positive Control are similarly processed.

Note (1): If a specimen was initially homogenized with an equal volume of deionized water, use 10 grams of homogenate as the sample with no further addition of deionized water.

Note (2): A Positive Control that is generated in the specimen matrix also uses 10 grams of sample (5 grams specimen/5 mLs arsenic or other heavy metal standard solution) with no further addition of deionized water.

c. Add 2 mL of concentrated hydrochloric acid.

d. Cap the tube loosely with a screw cap or cap the flask with aluminum foil and swirl the contents to ensure a uniform distribution of the acid.

e. Partially submerge the test tube or Erlenmeyer flask in a steam bath or equivalent boiling water bath for 45 minutes. Stir the contents by occasional swirling.

f. After the elapsed time, remove the foil and rinse with deionized water. Visually inspect the foil for a deposited black, gray or silvery coating. Photographs will be used to document negative or positive results.

Note: Clear plastic tape may be used to secure the copper foil to a clean sheet of paper before photographing. This is highly recommended when bismuth or mercury are detected as they may be rapidly lost from the foil via sublimation.

9 Instrumental Conditions

Not applicable.

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases, all of the below should be met in order to consider this test a presumptive positive for metals of interest.

a. The test is considered negative if the copper foil is not coated with a black, gray, or silvery coating.

b. A deionized water Negative Control should yield no black, gray, or silvery deposit on the copper foil strip (see Figure 1).

c. Arsenic trioxide used as the Positive Control will stain the copper foil black. Antimony yields a bluish-black stain. Bismuth yields a grey-black stain. Mercury coats the foil with a silvery deposit.

These stains are fixed and should not rinse off with deionized water, but mercury and bismuth may sublime and disappear from the copper foil over a matter of minutes (see Figure 1).

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limits of Detection:
Antimony = 1 µg/mL
Arsenic = 5 µg/mL
Bismuth and Mercury = 10 µg/mL

The primary value of this test is exclusionary and false negatives are unlikely. However, if a false negative is suspected and specimen amount permits, a Positive Control should be generated in the specimen matrix followed by a reassay of the sample to ensure staining of the copper foil.

b. Interferences: Food samples and gastric content specimens that are decomposing may affect detection limits.

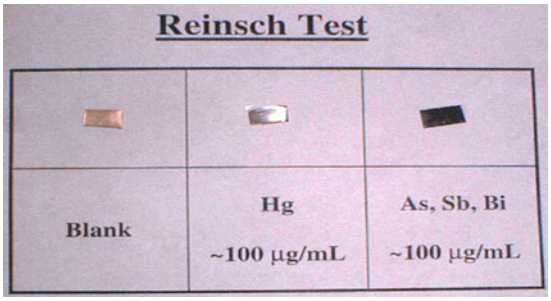

Figure 1: Examples of stains on copper foil

14 Precautionary Statement

The deposit of a fixed (water insoluble) black or silvery stain on the copper foil only indicates and does not identify the presence of arsenic, antimony, bismuth or mercury. Tarnishing of the copper foil may occur from copious amounts of sulfur in decomposed biological material and/or the presence of other metals such as selenium or tellurium. If the Reinsch test is positive, a specific elemental analysis technique such as SEM/EDS (scanning electron microscopy/energy dispersive spectroscopy) of the deposited metal can be used to determine which metal is present. ICP/MS (inductively coupled plasma mass spectrometry) can be used to target the individual metal for qualitative as well as quantitative data.

15 Safety

Take standard precautions for the handling of chemicals and biological materials. Heating of specimens should take place in a hood or vented system due to the volatility of mercury. Metals are cumulative in the body and care should be taken to avoid skin contact with standard solutions. Refer to the *FBI Laboratory Safety Manual* for guidance.

16 References

Alan S. Curry; *Poison Detection in Human Organs*; 4th edition; Charles C. Thomas; Springfield, Illinois; 1988, pp 108.

Clarke's Isolation and Identification of Drugs; Second Edition. The Pharmaceutical Press. London, 1986. Metals; pp 56-62.

Gettler, A.O. and Kaye, S., "A Simple and Rapid Analytical Method for Hg, Bi, Sb and As in Biologic Material", *Journal of Laboratory and Clinical Medicine* vol 35, no 1: 146-151 (1950).

Methodology for Analytical Toxicology. Heavy Metals; CRC Press; Cleveland, Ohio; 1975, pp 395-398.

Sidney Kaye; *Handbook of Emergency Toxicology*. Heavy Metals.; Charles C. Thomas; Springfield, Illinois; 5th edition; 1988, pp 58-64.

Rev #	Issue Date	History
2	09/19/2012	Reduced sample size and updated Sections 4, 6f, and 9.2b and c. Allowed for use of 16 x 125 mm screw cap tubes and updated Sections 5b and 9.2a. Updated copper foil thickness in Section 5d. Updated preparation of Nitric Acid in Section 5f and noted new concentration in Section 9.1c. Added optional alternate positive controls in Sections 5o, p, q and r and 6a, b, c, d and f. Clarified number of rinses of foil required in Section 9.1d. Updated 9.2d to allow for use of test tube cap. Reduced water bath time in Section 9e. Noted in Section 9f that tape may be used to secure copper for photography and prevent sublimation of bismuth and mercury. Clarified colors viewed with various metals in Section 11. Updated LODs in Section 14. Added additional safety recommendation in Section 16. Updated bench sheet to coincide with updated procedure.
3	02/16/2021	2 - Updated scope statement 5, 16 - Removed reference to TOX103, added in preparation for 1N hydrochloric acid (h). (7) - Removed Calibration Section (formerly section 7), renumbered. 7 - Added suggested methods for dealing with heterogeneous samples. 8 - Replaced text with “authorized individual”, updated text 8.2 - Minor typographical/grammar revisions. Analysts will record results with photography. 12 - Updated language to “Measurement Uncertainty” 14 - Clarified abbreviations 16 - Fixed typo References - Removed FBI document references Footer - Removed footer.

Approval Redacted - Signatures on File

Toxicology
Technical Leader: Date: 02/12/2021

Chemistry Unit Chief: Date: 02/12/2021

Appendix 1: Abbreviated version of the Reinsch Procedure for bench use.

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