

### Components

- A. Protocol E instructions for total cyanide analysis in leaves.
- B. Plastic balance with a 100 mg weight in one spoon, for weighing 100 mg of leaf.
- C. Thirty (30) flat-bottomed plastic bottles with screw capped lids.
- D. Two (2) graduated 1 ml plastic pipettes.
- E. One hundred (100) **buffer** papers.
- F. One hundred (100) yellow **indicator** papers glued to strips of clear plastic. STORE IN FREEZER. Stable for one month only at room temperature.
- G. Colour chart with ten (10) shades of colour which correspond to 0-800 ppm total cyanide.
- H. Ten (10) pink **standard** papers with linamarin (ppm cyanide on label). STORE IN REFRIGERATOR.
- I. Ten (10) **buffer/enzyme** papers, identified by a small black spot.

### Method (Complete steps 2 to 6 quickly as the enzyme acts rapidly to release HCN)

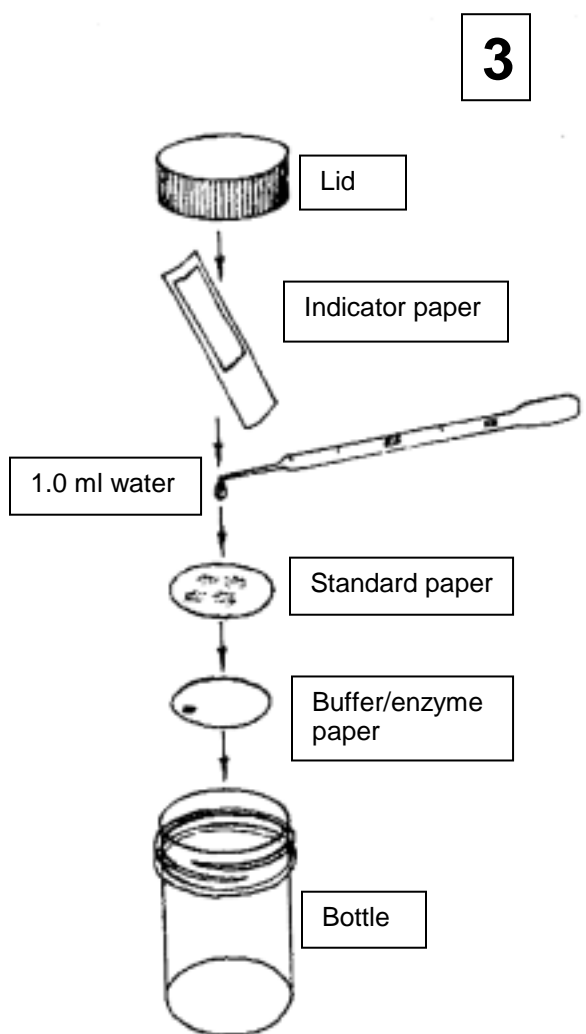
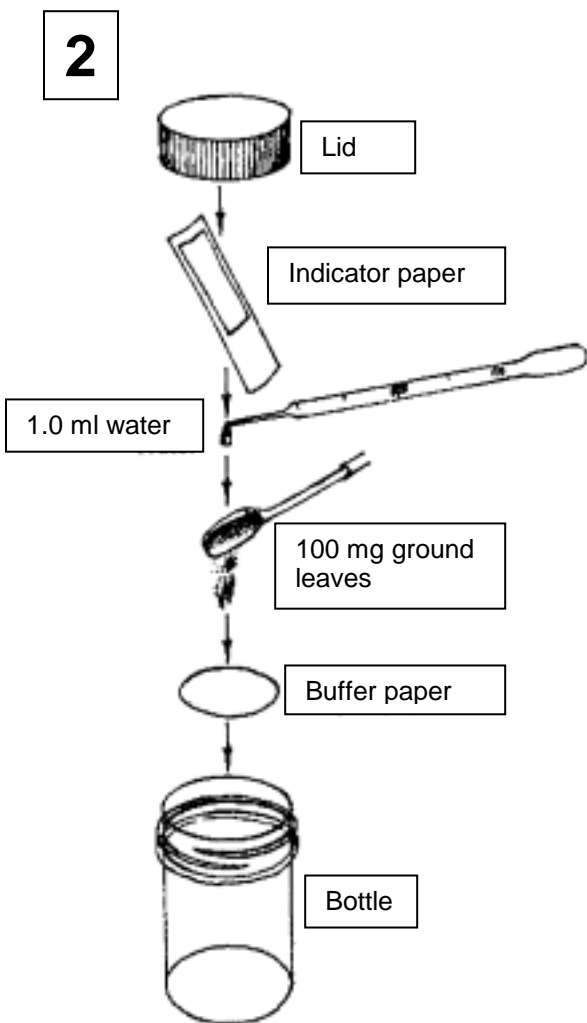
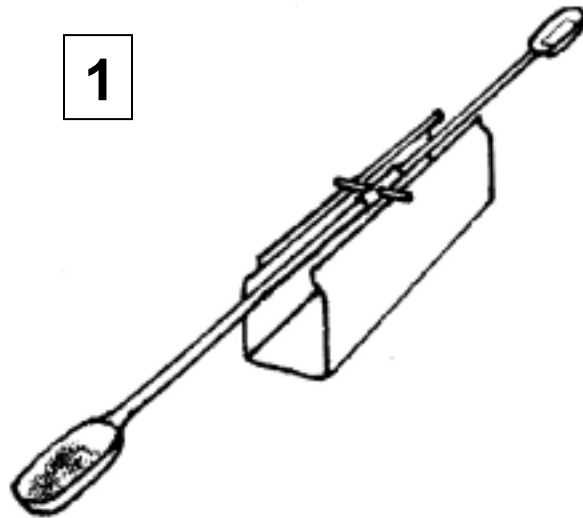
1. Follow sketch 1. Place the small portable balance on its U-shaped plastic mount so that it swings freely. It has a 100 mg weight glued inside one spoon.
2. Young leaves of many plants contain a cyanide compound (cyanogenic glucoside) and an enzyme which catalyses the hydrolysis of the cyanogenic glucosides to liberate HCN. *The enzyme acts rapidly to produce HCN so complete steps 2 to 6 quickly.* The leaves must be chopped up with scissors and the small pieces IMMEDIATELY ground up using a pestle and mortar. Add the ground leaves evenly to the empty spoon until balance is achieved.
3. Follow sketch 2. Place a **buffer** paper disc in a flat-bottomed plastic bottle and place a 100 mg ground leaves on top of it.
4. Add 1.0 ml of clean water using the plastic pipette and mix gently.
5. IMMEDIATELY add a yellow **indicator** paper attached to a plastic strip so that the paper does not touch the liquid in the bottle. When not in use STORE INDICATOR PAPERS IN FREEZER.
6. IMMEDIATELY close the bottle with a screw capped lid.
7. A positive and negative control should be run for each set of experiments.
  - a. For a negative control, prepare another sample as shown in sketch 2 but with no ground leaves present.
  - b. For a positive control, follow sketch 3. Place a **buffer/enzyme** paper disc in the bottle. Add a pink **standard** paper disc and then 1.0 ml water from a pipette and the yellow **indicator** paper. IMMEDIATELY close the bottle with a screw capped lid.
8. Allow the bottles to stand for 16-24 hour at room temperature.
9. Open the bottles and match the colour of the **indicator** papers against the shades of colour on the colour chart supplied.
10. Read off from the colour chart the total cyanide content in ppm in the leaves. Check that the negative control is zero and the positive control gives a colour equivalent to ppm on label.

### THIS SECTION TO BE FOLLOWED IF YOU HAVE A SPECTROPHOTOMETER

11. For each sample, carefully remove the plastic backing sheet from the **indicator** paper.
12. Place the paper in a test tube and add 5.0 ml of water measured accurately.
13. Leave the test tube at room temperature for about 30 min with occasional gentle stirring.
14. Measure the absorbance at 510 nm of the solution, subtract the value of the negative control.
15. The total cyanide content in ppm is calculated by the equation<sup>1</sup>  
total cyanide content (ppm) = 396 x absorbance
16. The total cyanide content obtained for the same sample of leaves, from both measurements 10 and 15 should be about the same. Also check the **standard** value agrees using both methods.

### Troubleshooting

- The total cyanide content of the pink **standard** paper should be  $\pm 10$  ppm of that shown on label. If it is not then it is likely something is wrong with the **standard** paper.
- If the **indicator** paper is left at room temperature it gradually becomes darker and after one month its colour will be around 10 ppm on the colour chart.
- If the **indicator** paper has been left in bright sunlight it becomes bleached on one side and is no good.
- If you use a bottle which is not gas tight (e.g. the screw cap is cracked) then gas could escape and this would give a low result.



**Reference**

<sup>1</sup>Bradbury, M. G., Egan, S. V. and Bradbury, J. H. (1999) Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *J. Sci. Food Agric.*, 79, 593-601.

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