Chapter-4

COLLECTION AND PRESERVATION OF MATERIAL FOR TOXICOLOGICAL EXAMINATION

Some points to remember

1. A successful toxicological examination requires appropriate specimens and a thorough history, including clinical signs, treatment, necropsy findings and circumstances involved. If a known poison is suspected, a specific analysis should always be requested.
2. In all vetero-legal cases, an accurate record of all the persons keeping the custody of the material from the time of collection of sample till the final analysis in the laboratory should be maintained.
3. If feed or water is suspected as the source of poisoning, samples of these and any descriptive feed tag should accompany the tissue specimen. A representative feed sample should be submitted from the lot involved in the poisoning.
4. Specimens should be packed individually. Containers must be labelled with all information necessary to identify the specimen, and if mailed, must confirm to postal regulations.
5. Specimen should be packed in glass or plastic to prevent contamination by lead in soldered joints of cans. Metal tops on jars should also be separated from the tissue by a layer of plastic or other impervious materials.
6. No preservative should be added except in the case of nitrate poisoning. If a preservative is necessary because of distance from the laboratory, packing in dry ice or ethyl alcohol (l ml/g of tissue) is advisable. But in the latter case, a specimen of the alcohol should also be sent. Ingesta and tissue should be kept separate, as diffusion is likely to occur between the two.
7. The preservation of materials is done in 50% of ethanol (l ml/g/tissue). Tissues and fluids for analysis should be as fresh as possible, kept in refrigerator or preserved chemically. Packing with ice is preferred. Adequate refrigeration is of special importance when submitting body fluids and materials for nitrate analysis, as these salts are rapidly metabolised by micro-organisms and only low or insignificant levels may be found on analysis. Refrigeration prevents microbial growth and

helps to ensure that the salts are preserved.

8. In some case, if an adequate amount of involved feed is available, some of it may be fed to experimental animals in an effort to produce the signs and lesion observed in the field cases.
9. Samples for toxicological examination should not be washed during collection as washing may lead to the dilution of the incriminating toxic material.

Materials for detection of poisons

- Materials to be collected in suspected cases of poisoning are as follows:

(a) 1000-1500 g of stomach contents and stomach walls

(b) 1000-1500 g of intestinal contents

(c) 1000-1500 g of liver (d)1000g of spleen

(e) Urine-1 litre in a separate bottle and put thymol as a preservative

(f) Kidney-one

- In survival cases, the following materials may be sent for analysis: stomach wash, stomach contents, vomitus, blood, urine, faeces, water and feed.
- Where the poison is suspected to be consumed by inhalation, parts of small intestine with its contents, liver, one kidney, lung, heart and brain tissues must also be sent.
- Uterus and foetus may also be useful in suspected cases of abortion.
- Burnt bones ashes should be preserved for analysis, if dead body has been cremated. The skeleton or the remnant bones are important materials for analysis in cases of exhumed bodies where no visceral tissues are available for toxicological examination.

(Note: Please also refer to Appendix-II for further details).

Containers for preservation of materials

Wide-mouthed glass bottles of about 2-litre capacity having airtight stoppers should be used for visceral tissues. These bottles should be numbered and labelled properly which should mention about the details of the case, nature of the contents preserved, place and date of preservation etc., and should bear the signature of the veterinarian.

Preservation of tissues

The tissues are taken into the container and sufficient alcohol is added so that whole tissues are dipped into the solution.

Tissues are also preserved in saturated sodium chloride solution (some excess quantity of undissolved salt should remain at the bottom). Solid common salt may also be used for preservation of the post-mortem tissues. The tissues are taken into the container and sufficient quantity of common salt is added to it. The tissues are immersed well with the salt. Some salt should remain at the bottom of the container and over the tissues.

A sample of alcohol or saturated solution of common salt used for preservation must also be sent in separate glass bottles for analysis to exclude the presence of any poison in it.

Preservation of blood sample

The following preservation may be used for preservation of blood sample:

1. Sodium fluoride 20 mg/ml of blood
2. Solution containing 10 g of sodium citrate and 200 mg of mercuric chloride dissolved in 100 ml of distilled water. One drop of this solution is sufficient for each ml of blood. The appropriate amount of fluid is taken into the clean dry glass bottle; the fluid is dried so that the salt remains sticking in the bottle. Then bottles are cooled at room temperature and the sample of blood to be preserved is taken into these bottles.

Appendix-I

CHECK LIST FOR FIXED TISSUES*

All tissues listed below should be preserved in 10% buffered formalin (one part by volume of tissues to 10 parts by volume of fixative). Tissue-samples should not be thicker than 0.5 cm. A sample of all lesions seen and of all listed tissues should be included.

Tissues

Salivary glands

Oral and pharyngeal mucosae

Tonsil

Tongue (cut across tip)

Trachea

Lungs (specimens from several lobes)

Thyroid and parathyroid glands

All major lymph nodes (sectioned to one-cm cube)

Thymus

Heart (sections from auricles, ventricles and valves)

Liver (3 representative specimens and duct)

Spleen (representative cross section with capsule)

Oesophagus (representative section - 3 cm)

Stomach (several specimens from all areas)

Intestines (3 cm representative specimen from each region)

Omentum (specimen of 3 cm)

Adrenal glands (if possible whole incised or cut in half)

Kidney (one- cm cube cortex and medulla of each kidney]

Urinary bladder (ureters, urethra, cross section of bladder including mucosa; 3-cm sections of ureters and urethra)

Uterus and Ovaries (if possible complete with ovaries, incise horns to allow entry of fixatives) being too big representative samples of cervix. uterine wall and ovaries cut transversely

Testes (0.5 cm cube section of each with capsule)

Epididymis (representative sample)

Prostrate gland (representative one- cm cube)

Eyes (whole eye, incise sclera to allow entry of fixative)

Brain (cut in half and part preserved in formalin and the other preserved for virology and toxicology)

Spinal cord (sections from cervical, thoracic and lumbar)

Diaphragm and skeletal muscles (representative samples)

Bones (sections sawn)

Skin (sections of abdomen, lip and ear pinna)

Neonates (umbilical stump and surrounding tissue)

Appendix-II

GUIDELINE FOR SUBMITTING SPECIMENS FOR TOXICOLOGICAL EXAMINATION *

Suspected Xenobiotic for Analysis	Specimen Required	Amount Required	Remarks
Ammonia/Urea	Whole Blood or Serum Urine Rumen contents	5 ml. 5 ml. 100 g.	Frozen or may add 1-2 drops of saturated mercuric chloride*
Arsenic	Liver, Kidney Whole Blood Urine Ingesta Feed	100 g. 15 ml. 50 ml. 100 g. 1-2 kg.	
Chlorinated Hydrocarbons	Cerebrum, Ingesta Fat Liver, Kidney	100 g.	Use only glass containers. Avoid aluminium foil for wrapping specimen.
Copper	Kidney, Liver Serum Whole Blood Feed Faeces	100 g. 2-5 ml. 10 ml. 1-2 kg. 100g.	
Cyanide	Forage Whole Blood Liver	1-2 kg; 10 ml. 100 g.	Rush sample to laboratory. Frozen in airtight container.
Fluoride	Bone Water Forage Urine	20 g. 100 ml. 100 g. 50 ml.	Ideal sample will be the lesion seen in teeth and done.
Herbicides	Treated weeds Urine Ingesta Liver, Kidney	1-2 kg. 50 ml. 500 g. 100 g.	
Lead, Mercury	Kidney Whole Blood Liver Urine	100g. 10 ml. 100 g. 15 ml.	Heparinized, do not use EDTA

Mycotoxins	Brain, Forages, Liver, Kidney	100 g.	Airtight container, plastic bag. For dry
Nitrate	Forage Water Body fluids	1-2 kg. 100 ml. 10-20 ml.	
Organophosphates Organocarbonates	Feed Ingesta Liver Urine	100g. 100 g. 100 g. 50 ml.	
Oxalates	Fresh forage Kidney	100 g. 100 g.	Fixed in formalin
Sodium (NaCI)	Brain Serum CSF Feed	100 g. 2 ml. 1 ml. 1-2 kg.	
Zinc phosphide	Liver, Kidney, Gastric content	100 g.	

Appendix-Ill

ESTIMATING THE AGE OF ELEPHANTS

Molar	Appearance at age	Replacement age
1	4 months	2-2 '/. years
2	6 months	6 years
3	3 years	9 years
4	6 years	25 years
5	20 years	45-50 years
6	40 years	Lasts up to 80 years

NUMBER OF LAMELLAE PER TOOTH*

Sequential number of tooth	Asian Elephant	African Elephant
	Maxillary teeth / Mandibular teeth	
1	4-5 / 5	3-5 / 3-5
II	7-8 / 6-9	5-8 / 5-8
III	11-15 / 11-14	7-10 / 7-10
IV	14-17 / 14-17	6-10 / 6-10
V	17-21 / 16-21	7-12 / 7-12
VI	20-26 / 21-29	8-14 / 8-14

Before the eruption as well as in the embryonic stage, the plates are loose and can be separated.