Veterinary Forensic Toxicology: field implications

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Terminologies in Veterinary forensic toxicology.

- **Toxicology** : It is the study of nature and effects of poisonous or toxic substances.
- **Toxicity** : It is inherent capacity of substance to produce harmful effects.
- **Poison** : It is any substance(liquid/solid/gas) that causes deleterious effects in a living organism.
- **Venoms** : These are substances produced by reptiles for their defence mechanism. e.g. Snake Venom.
- **Toxins** : These are the poisonous substances produced in animal tissues by the action of bacteria or waste products **Antidote** : These are the drugs or chemical agents that neutralize the effects of poison.



- The disciplines of toxicology and pharmacology are both centered on the concept of dose response, that is, that for a given dose, a predictable effect will be seen.
- The paraphrased statement of Paracelsus, "The dose makes the poison," is an essential concept for those dealing with toxicology cases
- Even the most toxic compound known will not cause an adverse effect if exposure to a toxic dose does not occur.

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- Animals die or perform poorly after accidentally ingesting these poisons.
- Gradual poisoning may also occur in areas with heavy industrial pollution.
- Symptoms vary, but certain common syndromes may suggest particular classes of poisons.
- Diagnosis is primarily clinical, but for some poisonings, blood and urine tests can help.
- Treatment is supportive for most poisonings; specific antidotes are necessary for a few.

Various type of poisoning in the field





Sources of poisoning

- There are two major sources of poisoning in animals 1) Natural Sources
- Plants : Ipomea carnea, Datura stramonium, Abrus precatorius, Strychnus nuxvomica, Young shoots of sorghum, Nitrate rich plants..
- Animals : Poisonous snake bite (Cobra, Krait, Russel viper and Rattle snake), Scorpion bite, Toad toxin, Tick toxins and Spider venom.
- Minerals or metals : Arsenic, Lead, Mercury, Selenium, Fluoride.



2) Human oriented sources Accidental causes

- a) Fertilizers : Urea, phosphate or Nitrate fertilizers.
- b) Insecticides : Organophosphates, Organochlorines, Carbamates, Pyrethroids.
- c) Rodenticides : Zinc phosphide.
- d) Industrial effluents : Lead, Fluorine, Cyanide, Mercury, Nitrate.
- e) Radiation hazards
- Malicious poisoning

Unlawful discriminable killing of animals by administering poisons

e.g. Zinc phosphide, Strychnine, etc.

Classification of Poisoning

The classification of poison on the basis of its effect on body is as follows:

- 1)Poisons causing respiratory insufficiency (anoxia) by
- Hindering oxygen uptake from pulmonary alveoli e.g. Petroleum products, Nitrous oxide, Sulfur oxide.
- Hindering oxygen transport to tissues e.g. Nitrites/Nitrates – Formation of Methaemoglobin.
- Inhibiting oxygen utilization by tissue cells

 e.g. HCN/Cyanide Inhibiting enzyme cytochrome
 oxidase.

2) Poisons causing nervous stimulation or depression by

- Directly damaging the brain or spinal cord e.g. Salt, Organomercurials.
- Acting on known receptor sites

 e.g. Organophosphates and Carbamate pesticides :
 inhibits enzyme acetyl cholinesterase.
- Causing nervous stimulation or depression by unknown mechanism

e.g. Chlorinated hydrocarbons, Lead, Acute Fluoride poisoning.

3) Poisons causing severe liver damage e.g. Aflatoxins and other mould toxins. 4) Poisons causing severe kidney damage e.g. Mould toxins, Carbamates. 5) Poisons causing severe colic Direct corrosives/irritants e.g. Chemicals. Metabolic poisons e.g. Arsenic, Urea. **6)** Poisons causing bone, tooth, hoof and hair abnormalities e.g. Fluorosis, Selenium toxicosis 7) Poisons causing lesions on skin e.g. Corrosives (acid/alkalis), Mould toxins, Photosensitizing plants like Lantana camara, Tribulus species.

Types of toxicity

1. Acute toxicity :

- It results from exposure of animals to high dose of a compound usually a single exposure or exposure over a short period. The animals exhibit immediate onset of severe toxic symptoms and usually die suddenly.
- 2. Subacute toxicity :
- Slow development of toxicity on repeated exposure to subtoxic doses over a period upto 90 days.
- **3. Chronic toxicity :**
- It results from exposure to still less doses than in subacute toxicity over a period of 6 months or more.

Diagnosis of Poisoning

1. **Vomiting, Diarrhoea, Abdominal pains** - Zinc, Copper, Arsenic, Iron salts, Acids, Alkalies, Oxalphenols, Turpentine.

2. **Convulsions** - Ammonia salts, Cyanides, Nitrates and Nitrites, Phenol, Strychnine.

3. Coma -Bromides, Carbon monoxide, Nicotine, Alcohol.

4. **Muscular incoordination -** Ammonium salts, Boric Acid, Cyanide, Nicotine, Nitrates.

- 5. Dilatation of Pupil -Nicotine, Water hemlock, Hyoscine.
- 6. Contraction of Pupil -Opium derivatives.
- 7. Slow respiration Atropine, Hypnotics
- 8. Rapid Respiration Ammonium salts, Nicotine, Urea.
- 9. Dyspnoea Carbon monoxide, Cyanides, Sulphur dioxide
- 10. Lameness Fluorine, Ergot, Insecticides.





Severe icterus in a cow due to **arsenic poisoning** whole body, eye

Arsenic : Sloughing of rumen



depraved appetite (pica) leading to lead toxicity first sign of lead poisoning : dead stock – often near a fence or obstacle



battery **lead** particles in the reticulum of poisoned weaner.



Mercury toxicity : Non-ambulatory tetraplegia , deep pain sensation and cranial nerve reaction. Radiographic examination revealed spotty metal opaque materials at the <u>metaphyses</u> of long bones.



Cadmium-Induced Abnormalities in chicks

Nitrate poisoning : excessive amount of nitrate or nitrite from grazing crops, silage, fertilizer, etc. difficult and painful breathing
cyanotic membranes
rapid breathing



Pullets exposed to high levels of aerosolized **ammonia** in wet litter developed blindness due to corneal ulcers.



Ascites and pulmonary edema, **sodium poisoning**,



osteo-dental fluorosis











Salt poisoning : poultry: increased thirst, dyspnea, fluid discharge from the beak, weakness, diarrhea, and

leg paralysis.

Subcutaneous edema in a six days old broiler chick

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Enlarged heart with pericard filled with clear transudate in a 6 days old broiler chick

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Aflatoxins (coumarinic group) In liver form toxic substances damaging liver cells. anorexia, lethargy, jaundice ; subacute or acute cases-death.

Ochratoxins (lactinic group)

nephrotoxic mycotoxins ; corn, wheat, oats

alter the physicochemical and nutritional properties of feed and forages fed to ruminants.



anorexia, polydipsia, polyuria, tenesmus, bloody diarrhea, dehydration, and prostration.

Trichothecenes (sesquiterpene group)

Trichothecenes : genus *Fusarium*, corn, wheat, barley, and oats, throughout the world. These interfere with DNA synthesis, gastrointestinal bleeding, ataxia, and immunosuppression.

Zearalenone (lactonic group)

Zearalenone : genus *Fusarium*, found in corn and causes hyperestrogenism; reduced corpora lutea in females and arrest of spermatogenesis in males

Fumonisins (amino alcohol group)

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cell damage, apoptosis, necrosis. heart damage. Acute:liver and kidneys;chronic :immune system



Tetanus in cow, horse, calves

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Hepatotoxicity, photosensitization in *L. camara* poisoning **Lantana camara**









1) Receptor-blocking agents. Atropine blocks receptors : reduce bronchoconstriction and raise the heart rate.

2) Cholinesterase reactivators.
Pralidoxime chloride (2-PAM) is used as a cholinesterase reactivator that reactivates the enzyme to reduce accumulation of acetylcholine.
3) Emetics, cathartics, and adsorbents
: decrease further absorption.

PLEASE STOP POISONING OUR FOOD.

Rodenticides kill more than just rodents.

Poison is not species-specific. Though you may target mice and rats when using rodenticides, a chain reaction is created.

Non-target wildlife species like muskrats, chipmunks, and squirrels can be attracted to rodenticide.





Eastern Chipmunk



Eastern Gray Squirrel

Anticoagulant rodenticides















DDT and PCBs built up in the fish the ospreys ate and made their egg shells so thin that adults crushed their progeny when they nested on them.



Since DDT and PCBs were banned, the Osprey chicks have rebounded





Lawn Chemicals and Herbicides Affected animals



Herbicides specifically 2,4dichlorophenoxyacetic acid (2,4-D), 4-chloro-2methylphenoxypropionic acid (MCPP), and dicamba remained detectable on grass for at least 48 hours after application

PARAQUAT

A POTENT WEED KILLER

IS KILLING PEOPLE

GENERAL PRINCIPLES OF TREATMENT OF POISONING



- After tentative, presumptive or confirmative diagnosis treatment can be: 1) General Procedures
- Removal of source of poison
- Shifting animal to fresh feed and water.
- Removal of unabsorbed toxicant from GI tract and skin
- a) If poison is **ingested**
- Gastric lavage with neutralizing agent or water.
- Emetics : 30-60 gm of common salt in a glassful warm water.
- Purgatives: Saline purgatives :non-irritant poisons (Magnesium Sulphate @ 250-500 gm orally in large animals). Oily purgatives : irritant poisons.

b) If poisoning is **through eye or skin** Wash the skin thoroughly with lot of water / normal saline. 2) Removal of absorbed toxin : Intensive fluid therapy (Inj. Dextrose 5%).

3) Common antidotal therapy : neutralizing or detoxifying agents

- Universal antidote (2 parts activated charcoal + 1 part Tannic acid + 1 part Magnesium oxide) @ 250 gm : large animals, 15-30 gm : small animals.
- Administration of **egg white** (from 6-8 eggs) and milk (500 ml) orally to neutralize heavy metals.
- Use of **15-20 drops of Tr. Iodine** in 100-125 ml of drinking water to precipitate alkaloids, strychnine, mercury and lead.
- Use of 5% acetic acid or vinegar or lemon juice to dilute alkali poisons.
- Use of lime water, powdered chalk to dilute acid poisons.

4) Specific antidotal therapy

It is the use of exact antidote against certain poisoning on its confirmation..

Sl. No.	Poisoning	Specific Antidote
1	HCN poisoning	Sodium nitrite and sodium thiosulphate
2	Nitrate poisoning	Methylene blue
3	Organophosphate poisoning	Atropine sulphate
4	Lead poisoning	Ca disodium EDTA
5	Arsenic	BAL (British antilewisite)

5) Symptomatic Treatment : based on symptoms

- Maintenance of clear air way.
- Use of respiratory stimulants. e.g. inj Nikethamide @ 7-11 mg/kg IM,IV.
- Control of convulsions by using sedatives. e.g. inj Diazepam @0.5-1 mg/kg IM, IV.
- Giving CNS stimulants in case of severe depression. e.g. Caffeine 0.5 g IV (Dog 0.1-1.0 g SC)
- In case of shock, protection of the animal from cold and use of corticosteroids / adrenaline and fluid therapy.

- Intensive fluid therapy to counteract dehydration owing to diarrhea or vomition.
- Oral administration of demulcents (mixture of egg, sugar and milk or rice gruel) to alleviate irritation caused by poisons.
- 6) **Supportive Treatment :** during and after recovery from poisoning.
- Liver tonics to promote appetite and liver function.
- Fluids and electrolytes to compensate water and electrolyte losses.
- Dextrose to compensate lost energy.
- Antibiotics to check secondary bacterial infections

Handling the poisoning cases of pets & farm animals



In Veterinary Medicine, suspected poisoning often cannot be detected and certified by a Toxicology Laboratory due to many procedural errors and unclear requests for analysis.

A definitive diagnosis on poisoning in domestic animals need the multi-step approach involving

- Components and procedures for a good clinical anamnesis.
- Utility and modality to perform a necropsy
- Method to collect and dispatch biological materials to Veterinary Toxicology Laboratory
- Analytical techniques for detection of major toxic substances, responsible for frequent poisoning in domestic animals

Forensic toxicology is the application of toxicology for the purposes of the law. These intentional poisoning of animals are **animal cruelty** under most jurisdictions and may be prosecuted as such.

Intentional poisoning of food-producing animals that threatens public health through contamination or adulteration of the human food supply may be criminally prosecuted.

Most animal poisonings are adjudicated through the civil court system, with party seeking damages as veterinary fees or loss of animal use or life from a second party responsible for the poisoning.







Forensic veterinary toxicology include cases involving

- animal cruelty (malicious poisoning),
- regulatory issues (contamination of the food supply),
- insurance litigation, or
- poisoning of wildlife.

An understanding of these types of cases, including proper sample collection, handling, and transport is essential to obtain proper samples for toxicological analysis.



Consultation with veterinary toxicologists at the diagnostic laboratory who will be processing the samples before, during, and after the forensic necropsy ensures that the appropriate analytical tests .

- Contrast to human forensic toxicology, veterinary forensic toxicology is less well-defined, with most veterinarians various duties that may or may not include the occasional forensic case.
- With the exception of regulatory and wildlife toxicology cases, veterinary forensic toxicology investigations are not commonly performed, and their relative infrequency when compared to human forensic toxicology may be due to factors unique to veterinary medicine.



- The ideal situation in a veterinary forensic toxicology case
- (1) the clinical syndrome including historical information, clinical signs, and response to antidotal or appropriate therapy consistent with the suspected toxicant
- (2) pathological and/or clinical pathological findings consistent with the suspected toxicant
- (3) results of analytical testing confirm the presence of the suspected toxicant in the tissues of the patient at a level consistent with poisoning. Obtaining this perfect trifecta of facts requires coordination
 between animal caretakers, veterinarians, veterinary pathologists and veterinary toxicologists.

For **wildlife field investigations**, close work with wildlife officials is important due to the legalities entailed with handling wildlife; especially for protected species investigations.

Field investigations should include

- interviews with on-site personnel,
- evaluation of both affected and unaffected animals,
- walkthrough of animal housing areas, pastures, feed preparation areas, and water sources
- to identify potential sites of exposure & to rule out other potential causes of toxicosis.



Forensic Toxicology Specimen Collection, Handling and Storage

Often when the toxicant is not known or toxicosis may not be suspected at the time of the forensic necropsy, a systematic approach to collection of samples must be undertaken by the veterinary pathologist to ensure adequate samples are available for toxicological analysis.

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In forensic toxicology cases, the **proper collection**, **storage**, **handling**, **and analysis of samples** are essential to providing admissible evidence needed to successfully adjudicate the case.

Sample collection

- Specimens for toxicology and histopathology can be collected at the same time
- Each sample container should be marked with the patient's name, case number, date and time of collection, type of sample (eg: tissue, fluid type, and collection site (eg, heart blood)
- Each tissue sample should have its own container and the collector should work with one organ or tissue at a time to avoid cross-contamination of samples
- knives and other sectioning equipment should be cleaned between tissues.

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Collection of duplicate samples is frequently recommended.

Sample Collection For Toxicology

SI. No.	Sample	Amount	Storage	Analysis
1	Liver	300 g	Chilled, frozen	Heavy metals, pesticides, pharmaceuticals
2	Kidney	300 g	Chilled, frozen	Heavy metals, ethylene glycol (Ca: P ratio), pharmaceuticals, plant toxins
3	Brain	Half brain	Chilled, frozen (unfrozen for acetylcholinesterase activity)	Sodium, acetylcholinesterase activity, pesticides
4	Fat	300 g	Chilled, frozen	Organochlorines, PCBs, bromethalin
5	Ocular fluid	Entire eye	Chilled	Potassium, nitrates, magnesium, ammonia
6	Retina	Entire eye	Chilled, not frozen	Acetylcholinesterase activity
7	Lung/spleen	100 g	Chilled, frozen	Paraquat, barbiturates
8	Lung	Entire lobe	Chilled, airtight container	Volatile agents
9	Injection site	100 g	Chilled	Pharmaceuticals
10	Whole blood	5–10 mL	Chilled	Heavy metals, acetylcholinesterase activity, insecticides

SI. No.	Sample	Amount	Storage	Analysis	
11	11 Serum 5–10 mL Chille		Chilled	Some metals, pharmaceuticals, alkaloids, electrolytes	
12 Urine 5–100 mL Chilled		Chilled	Pharmaceuticals, heavy metals, alkaloids		
13	Milk	30 mL Chilled Organochlorines, P		Organochlorines, PCBs	
14	14 Ingesta/ 500 g Chilled M faeces		Chilled	Metals, plants, mycotoxins, other organic toxicants	
15	Hair	3–5 g	Dry, store in paper	Pesticides, some heavy metals	
16 Feed 1 kg composite		Dry: paper Wet: freeze	Ionophores, salt, pesticides, heavy metals, mycotoxins, nutrients, botulism		
17	Plant	Entire plant	nt Dry: newspaper Alkaloids, glycosides, nitrates		
18	Water 1–2 L Glass containers Pesticides, heavy met blue-green		Pesticides, heavy metals, salt, nitrates, blue-green algae		
19	Soil	500 g	Glass containers	Pesticides, heavy metals	
20	Insects, insect casings	3–5 g (*100 maggots)	Live: glass vials Dead: casing, glass vials	Live: forensic entomology Dead: testing for pharmaceuticals, heavy metals, organic toxicants	

Sample Collection

- During the forensic necropsy in a suspected poisoning case, all visible abnormalities should be documented verbally (audio recording or written record) and photographed, videoed, and/ or sketched as appropriate.
- Standard forensic necropsy procedure should be followed and samples collected for histopathology, microbiology or other testing as deemed fit.
- The external condition of the patient should be recorded in regards to rigor mortis, livor mortis, insect activity, body temperature, and degree of decomposition.



- All wet biologic specimens should be placed in containers with little headspace at the top to minimize oxidative losses from trapped air.
- Dry biologic material like hair should be placed in a sealed, breathable container
 - (eg, paper envelope, paper bag).
- Samples should be shipped to the diagnostic laboratory via bonded courier and the laboratory should be alerted to the impending arrival of a veterolegal case.

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Any retained samples should be kept chilled (4C)

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The veterinary pathologist should be alert to any abnormal odors.

- rotten fish or rotten garlic odors : presence of phosphine gas (from ingestion of zinc or aluminum phosphide rodenticide)
- almond-like odors : cyanide gas

External examination of the body

• foreign material on hair or coat (shave 3×3 inch square of hair and save) material around the mouth or anus

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- injection sites (collect skin and underlying subcutis and muscle)
- between the paw pads (collect)

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- foreign material in the mouth or adherent to the teeth
- Any loose plant material or insects and their casings

- eyes : for lesions (petechiae, etc)
 - 1 eye for vitreous and/or retinal evaluation (for acetylcholinesterase)
- brain should be removed with

1 hemisphere packaged for toxicological analysis and rest for histopathology and/or testing for infectious agents

- **Body cavities**: any excessive fluids should be collected.
- Gastrointestinal contents : minimize the risk of contamination of other viscera.
- For monogastric animals, the **stomach** should be ligated proximal to the esophageal sphincter and at the pylorus, then removed.
- **Gastric contents** : collected to a clean container via an incision along the greater curvature.
- Any **solid or foreign objects** should be removed, photographed, and packaged separately, while **liquid contents** should be mixed thoroughly for testing.

For ruminants

- representative samples of the rumen and abomasum contents should be collected along with any foreign material
- Several centimeters of small and large intestine and their contents should be collected

feces.

Suspected exposure to volatile agents

A sample of lung or lung lobe may be obtained by tightly ligating the trachea or bronchi, immediately placed in an airtight container and sealed to minimize loss of any volatile compounds.

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Liver and kidney :

Multiple sections of should be collected (being metabolic and excretory organs).

- Subcutaneous or other adipose tissue :
- for detection of highly lipophilic compounds such as bromethalin and organochlorines.
- **Blood** :

from cardiac or peripheral sites, if insufficient blood is available, samples of spleen may be taken.

- Urine : from the bladder
- Milk : lactating animals for toxicants preferentially eliminated via milk (white snakeroot).

Toxicology Testing and Interpretation



Types of Toxicology Analytical Methods

Physical inspection : visual and olfactory assessments of sample characters

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HPLC separates chemicals in Liquids **TLC** separates compounds in a solid matrix **GC** separates compounds in a gas matrix

Bioassays : exposing normal animals to the suspect compound and documenting the toxidrome

Chemical reactions are sometimes used as qualitative tests to indicate the presence of certain compounds.

Immunoassays utilize antibodies to identify and measure quantities of chemicals with a color change indicating its presence or absence Spectroscopy and spectrophotometry use light or ultraviolet light emission or absorption characteristic Mass spectrometry superheats or ionizes a sample then separates the ions according to their mass-tocharge ratios showing mass and relative amounts of the different ions, chemical formula of the compound

Rodenticides Associated With Intentional Animal Poisonings

SI. No.	Toxicant	Mechanism of Toxicity	Lesions	Samples for Analysis
1	Anticoagulant, rodenticide	Interference with vitamin K recycling, decreased formation of vitamin K dependent coagulation factors	Hemorrhage into various body compartments	Liver (anticoagulant screen)
2	Bromethalin rodenticide	Uncouples oxidative phosphorylation, loss of intramembrane pumps, intramyelenic edema	No specific gross lesions; spongiosis of white matter; minimal inflammatory response	Brain, fat, liver, kidney
3	Cholecalciferol rodenticide	Induces hypercalcemic metastatic mineralization of soft tissues, renal failure	Gastric hyperemia, ulceration, hemorrhage; + mottled kidney; mineralization in kidney, heart, stomach, lungs, arteries	Kidney (renal Ca:P ratio)
4	Strychnine	Inhibits glycine (an inhibitory neurotransmitter) within spinal cord, muscle spasms, convulsions	No specific gross or histologic lesions	Stomach contents, (best), liver, bile, kidney (strychnine)
5	Zinc/aluminum phosphide	Converts to corrosive phosphine gas on contact with water; free radical injury to multiple organs	Gross hyperemia of viscera, pulmonary edema, hepatocellular necrosis, necrosis, adrenal hemorrhage	Serum, liver, kidney, pancreas (zinc levels)

Pesticides Associated With Intentional Animal Poisonings

SI. No•	Toxicant	Mechanism of Toxicity	Lesion Samples for Analysis	Samples for analysis
1	Organophosphate carbamate insecticides	Inhibition of AChE acetylcholine-induced muscarinic and nicotinic stimulation. "Ageing" on acetylcholinesterase	No lesions if peracute death. Thick, ropy saliva, vomiting, diarrhea; petroleum- like odor of coat or stomach contents; petechiae; tissue congestion +pulmonary edema; +pancreatitis (dogs)	Brain, retina (cholinesterase activity)
2	Organochlorine insecticides	Interfere with neuronal sodium channels \rightarrow convulsions	No specific gross or histologic lesions Adipose tissue (organochlorines)	
3	Metaldehyde	Mechanism not known; may act on gamma aminobutyric acid mediated pathways	Formaldehyde odor of stomach contents; congested viscera; subepicardial, subendocardial hemorrhage; no specific microscopic lesions	Stomach contents (best), serum, liver, urine (metaldehyde)
4	Paraquat	Concentrates in lung; free radical production in presence of oxygen →damage to cell macromolecules	Erosive stomatitis, gastritis; subacute interstitial pneumonia with fulminating fibrosis and type II cell proliferation	Urine (acute); lung (paraquat)

Interpretation of Toxicological Results

- Collecting the proper samples for toxicological analysis and performing the appropriate test(s)
- Analytical equipment and methodology : allow detection of many compounds at the parts per trillion (ppt) level

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Veterinary toxicology diagnostic laboratories have established reference ranges (such as heavy metals and minerals, some rodenticides, some plant toxins, and some mycotoxins), and interpretation can be problematic for those toxicants for which reference values have not been developed.

Toxicants Used in Intentional Animal Poisonings

- Malicious poisonings in animals involve universal toxicants like rodenticides, insecticides that are generally readily accessible on a farm
- Intentional poisonings of companion animals involve easily acquirable household products like rodenticides, insecticides, slug/snail killers, ethylene glycol, caffeine, nicotine, and human medications such as acetaminophen.
- Patterns of malicious animal poisonings in these regions reflect the availability of these compounds in that particular area



Conclusion

- Intentional or accidental poisonings of animals of high financial value or of protected wildlife species have the greatest potential to trigger criminal and/or civil prosecutions of the suspected poisoners.
- Veterinarians can aid in the forensic investigation of animal poisonings through identification and collection of appropriate samples for toxicological analysis.
- Open and frequent consultation between animal caretakers, referring veterinarians, veterinary pathologists, and veterinary toxicologists can expedite the investigative process and maximize the likelihood of a diagnosis and, ultimately, successful prosecution of these cases.



Any questions?

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