GENERAL TOXICOLOGY

> What is there that is not a poison? All things are poison and nothing [is] without poison. Solely the dose determines that a thing is not a poison. … While a thing may be a poison, it may not cause poisoning. (Often paraphrased as: ***The dose makes the poison***)
>
> Paracelsus [Philippus Aureolus Theophrastus Bombastus von Hohenheim] (1492-1541)

1: General Principles, Disposition & Mode of Action of Toxins

Origins of toxicology

Toxicology is the study of the adverse effects of xenobiotics. The ancient Greek word *toxikon* meant "poison for arrows" and toxicology, the study of poisons and their effects, originally supported the survival skill of hunting and its extension to military activity in many early societies on both sides of the Atlantic Ocean (Ramoutsaki *et al.* 2000). Some of the best known arrow poisons of the past were aconite from *Aconitum* spp. plants in Europe, curare from the vine *Chondrodendron tomentosum* in South America and strophanthidin and ouabain, cardiac glycosides from *Strophanthus* spp. plants in Africa .

Toxins in antiquity (and more recent eras) were also used for execution of criminals and for murder and knowledge of their effects was gained in part through testing on slaves. A preparation of hemlock plants (*Conium maculatum*) was the medium for execution of Socrates in ancient Athens (399 BC) after his conviction for "corrupting youth" (Stone 1988). The Roman emperor Augustus was reputedly poisoned by his wife Livia with tropane alkaloids from deadly nightshade (*Atropa belladona*) incorporated into his food. The sap of the upas tree (*Antiaris toxicaria*) containing cardiac glycosides was employed to execute people and poison the wells of Dutch colonists in eighteenth century Java. Hallucinogens, stimulants and inebriants from plants were employed in many early societies throughout the world to heighten religious experiences and enhance social interactions. These practices were also bound up with utilisation of the local flora by indigenous peoples for food and medicine. The determination of which plants were safe to use and which doses were efficacious for treating illness must have been the ultimate foundation of toxicology. In Australia, the use by aboriginal people of such high-energy plant food sources as the seeds of cycads (*Cycas* spp., *Macrozamia* spp.), candlenut trees (*Aleurites moluccana*) and black bean trees (*Castanospermum australe*) was based on techniques developed to remove their toxins (Horsfall 1987).

Further reading

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Why study veterinary toxicology?

- **Animal welfare**: The ultimate aim of veterinary toxicology is the welfare of animals under the influence of humans through the prevention of poisoning and the relief of suffering in poisoned animals. The Queensland Animal Care and Protection Act 2001 and Regulations 2002 (Queensland Parliament 2002) place a duty of care on persons in charge of animals to protect their welfare. Theoretically, this extends to the prevention of poisoning.
- **Economics**: prevention or reduction of economic losses through death (McKenzie 1985) or illness in animals or the presence of residues of toxins or their metabolites in animal products

- **Ecosystem health**: toxicoses in domestic animals or wildlife may indicate environmental pollution with industrial products or disturbance of ecological balance through over-exploitation of natural resources (e.g. overgrazing promoting hazardous population size of toxic plants such as *Pimelea* spp.)
- **Implications for human health**: toxicoses in domestic animals or wildlife may indicate exposure of humans to the same agents, e.g. methyl mercury in cats, or act as models for human intoxications.

References:

McKenzie RA (1985) The cost of cattle deaths from toxic plants in Queensland, 1972-1981. In Seawright AA, Hegarty MP, James LF, Keeler RF (eds) *Plant Toxicology*. Queensland Poisonous Plants Committee, Brisbane. pp.14-23.

Queensland Parliament (2002) - Access legislation through the Office of the Queensland Parliamentary Counsel website at http://www.legislation.qld.gov.au/

Concepts & Definitions

See Wexler *et al*. (2000) Appendix 1 for a glossary of terms used in toxicology (IUPAC recommendations).

Some definitions

Veterinary Toxicology: The study of the adverse effects of chemicals of natural (biological, geological) or industrial origin on domestic animals and wildlife.

Poison or *Toxicant*: A substance that, in relatively small amounts, may cause structural or functional disturbance in the body.

Toxin: Usually, a poison/toxicant of biological origin; *zootoxin* (animal origin), *mycotoxin* (fungal origin), *bacterial toxin*, *phytotoxin* (plant origin), *phycotoxin* (algal origin)

Xenobiotic: any chemical compound not normally derived endogenously.

Toxicosis: Disease state resulting from exposure to a poison/toxicant.

Toxicity: The potential for a chemical to produce injury in biological systems; usually expressed as mg of toxicant per kg of body weight required to produce a stated biological effect, such as acute death in 50% of the group dosed (= median lethal dose or LD_{50})

Poisonous organism: an organism that contains a toxic substance within its constituent parts or on its surface

Venomous organism: an organism that contains a toxic substance or toxic substances and a specialised organ for delivery to other organisms for defense or predation or both.

Biotransformation: Chemical alteration of a toxicant in animal tissue or microbes, usually mediated by enzymes, producing either detoxication or potentiation (see below)

Bioaccumulation: In an individual, accumulation of a chemical or its metabolite(s) in tissue faster than it can be excreted; in ecosystems, increasing concentrations of a chemical in tissues of species with progression up the trophic levels of the food web (e.g. DDT in raptors, dieldrin in pelicans)

Mutagenicity: Ability of a toxin to produce changes in DNA leading to mutation (damage to germ cells transmitted to next generation; damage to somatic cells may lead to neoplasia)

Tolerance: State of decreased responsiveness to a toxin; may be of genetic origin (e.g. fluoroacetate & native Australian animals) or conditioned (e.g. plant toxins & travelling vs. local stock)

NOEL (No observable effect level): The highest dose administered experimentally that produced no significant observable adverse effect in the test animals.

Some sub-specialities of toxicology

- *Clinical* toxicology = cause, diagnosis and management of established poisoning
- *Forensic* toxicology = establishing the cause of death for legal purposes
- *Product* or *Pharmacological* toxicology = assessing the potential for adverse effects from commercial or therapeutic products
- *Environmental* toxicology = assessing ecological and other effects of pollutants released from sites of human activity (commercial or domestic)

Classification of Poisonings

Classification → framework to aid memory and systematise knowledge. No single system fulfils all requirements.

Examples

- *Clinical syndrome/organ system* (CNS, circulatory, systemic, etc.)
- *Chemical group* (predicts biological effects of similar substances to a large degree)

- *Biological effect* (caustic, mutagen, carcinogen, etc.)
- *Source* (plant, animal, industrial etc.)
- *Use* (pesticide [insecticide, rodenticide], solvent, etc.)
- *Biochemical effect* (sulphydryl inhibition, cholinesterase inhibitor, etc.)

This text can be approached through the framework of a Clinical Syndrome classification which aims to aid diagnosis, or through a combined Source, Chemical Group and Use classification for economy of space and to highlight biological linkages.

Fundamental Principles of Toxicology

1. **A chemical must reach its effector site in a biological system in order to produce a biological effect.** This conjunction is influenced by physical-chemical properties, translocation, absorption, biotransformation, distribution and elimination mechanisms (see below).
2. **Not all chemical-induced biological effects are harmful**, for example the pharmacological effects of therapeutic drugs
3. **The occurrence and intensity of chemical-induced biological effects are dose related**; there is some dose below which no effect can be demonstrated (except carcinogens?), and there is a dose above which the agent is lethal.
4. **Whenever two animal species have similar biotransformation systems and physiological functions, these two species will respond similarly to particular chemicals**.

Reference: Loomis12

Consequences of Toxicity

Irreversible = damage exceeds the physiological repair capacity of the biological system - death, mutagenicity, carcinogenicity, teratogenicity

Reversible = damage does not exceed physiological repair capacity - physical organ damage (e.g. hepatic necrosis) or functional damage (e.g. convulsions) - regenerative capacity of organs varies e.g. liver (high), CNS (none); some have extra functional capacity (kidney, liver)

Reference: Loomis 12

Measurement of Toxicity: Dose-Response Relationships

Substances (chemicals) capable of ingestion are regarded popularly as either safe (food, healthy, beneficial) or harmful (poisonous). This simple distinction is not strictly scientifically valid - no clear line of demarcation exists - harmfulness or safeness is primarily related to the amount of chemical in the body.

The factor determining the amount of harm produced by a compound is the quantity of the compound that comes into contact with the biological system concerned - commonly called the *dose*. See the quotation from Paracelsus at the head of this chapter.

Dose = quantity of chemical introduced into a biological system in a unit period of time; commonly expressed as weight/unit weight of animal(/time interval) e.g. mg/kg (single dose), mg/kg/day (repeated doses) Dose values must state route of administration (PO, IM, IV, IP, topical, inhalation) to be meaningful. NOEL = no observable effect level = maximum dose used in an experiment which produces no observed effect.

The response of the biological system is a *graded* response related to progressive change in dose - no sudden onset of severe effect at a particular dose (= *dose-response relationship* -describes the biological variation of the measured effect of a poison on a population of animals).

Dose-response relationship usually plotted graphically (dose-response curve) relating dose to cumulative %age of animals (or cells or cultured tissues) responding (death or any other quantifiable biological effect e.g. degree of injury to organs (estimated by serum enzyme assay), lesion counts (neoplastic or necrotic foci).)

Dose-response curves

- simple, S-shaped but essentially linear for xenobiotics
- biphasic for essential endogenous compounds that → deficiency at low concentrations & toxicity at high concentrations, e.g. Cu

Acute toxicity is commonly expressed as an ***LD_{50} (median lethal dose)*** = the amount that kills half the animals dosed. Variability results from many factors including species, breed, sex, age, weight, diet, state of biotransformation and transport mechanisms. Exposure by air or in water → LC(lethal concentration)$_{50}$

The larger the LD_{50}, the less potent (toxic) the chemical - toxicity is a relative term

A rough scale of acute toxicity (LD_{50}) is:

- Extremely toxic 1 mg/kg or less
- Highly toxic 1-50 mg/kg
- Moderately toxic 50-500 mg/kg
- Slightly toxic 0.5-5 g/kg
- Practically non-toxic 5-15 g/kg
- Relatively harmless > 15 g/kg

A reasonable estimate of the LD_{50} + 95% confidence limits can be measured with as few as 6 to 9 animals.
Concern for animal welfare & practical difficulties → ↓↓ use of LD_{50} estimates.
References: Loomis17, K18, Os1

Toxin Disposition

Dose-response is *mediated through toxin disposition → toxicity determined by toxin concentration at site(s) of action* - same dose of different toxins may → very different concentrations at site of action

Disposition = absorption, distribution, biotransformation (metabolism) and excretion mechanisms (ADME) interacting simultaneously

Toxicokinetics = quantitation and determination of time course of toxin disposition (Dyke & Whittem 1999). Mathematical models → e.g. half-life

Site(s) of action = *target organ(s) or tissue(s)*

Barriers to toxin entry to systemic circulation & thus distribution throughout body

- primary barriers - skin, lungs, alimentary mucosa
- secondary removal = presystemic or first-pass elimination
  - GIT cell biotransformation
  - portal circulation → liver (± biotransformation) → bile
  - lung biotransformation

Toxins must cross barriers before exerting effects

- exception = caustic/corrosive toxins (site of action = surface of organs/tissues)
- exception = injected venoms (bites), drugs (iatrogenic, accidental, malicious)

Toxic effects

- direct at target organ
- indirect through alteration of regulatory functions (e.g. oxalate → NSH in horses)

Tissue/organ toxin concentration may not correlate with toxicity (e.g. CHCs in adipose vs. nervous tissue)

Toxin *removal from systemic circulation* by

- *biotransformation* - liver & other organs → ↑ water solubility → excretion
- *excretion* - kidney, bile, lungs
- *storage* - plasma proteins, liver, kidney, fat (CHCs), bone (Pb)

References: K91

Dyke TM, Whittem T (1999) Kinetics for clinicians. *Aust. Vet. J.* **77**:724-726.

Absorption

Reference: K94

Passage across cell membranes

Commonly, toxins pass through membranes of cells in gut, then capillary endothelium, then target organ

2 process types

- passive transport (simple diffusion, filtration)
- specialised transport (active transport, facilitated diffusion)

Diffusion

- most toxins
- no cell energy use needed, moves with concentration gradient
- diffusion surface area availability: lipid domain >> aqueous pores
- small hydrophilic molecules (up to 600 molecular weight) → through aqueous pores (↓ molecular size → ↑ rate of diffusion e.g. ethanol)

- hydrophobic molecules → across membrane lipid domain (↑ lipid solubility → ↑ rate of diffusion; ionised weak organic acids & bases have low lipid solubility - pH influences ionisation → influences absorption from GIT)

Active transport
- can move against concentration gradient
- system saturated at high substrate concentrations → transport maximum (T_{max})
- system structurally-selective → potential for competitive inhibition
- needs energy → blocked by metabolic inhibitors
- important to excretion of xenobiotics, various major organs may have 2-4 separate systems

Facilitated diffusion - similar basis to active transport; cannot move against concentration gradient; no energy expended → not blocked by metabolic inhibitors

Phagocytosis, pinocytosis - more important for removal of particulate matter

Reference: K92

Absorption by gastrointestinal tract

Most toxins of veterinary importance gain entry *per os*.

Most absorbed by diffusion, some by active transport

Many factors affect gastro-intestinal absorption, including
- residency time (intestinal motility) interacting with surface area for absorption (intestine >> stomach)
- presence of food residues (e.g. starvation → ↑ dieldrin absorption)
- absorption site pH determining *degree of ionisation* & thus *lipid solubility* (↑ lipid solubility → ↑ absorption by diffusion)
- use of specialised active transport systems for nutrients (e.g. Pb uses Ca transport system; thallium uses Fe transport system; very lipophilic compounds like TCDD, CHCs, PCBs absorbed in lipid micelles)
- ↑ particle size → ↓ absorption (e.g. metallic Hg relatively non-toxic)
- interaction of ions (e.g. Cd decreases absorption of Zn & Cu; Ca decreases Cd; Zn & Mg decrease Cu; Mg decreases F)
- for some toxins ↑ dose volume → more rapid stomach emptying → ↑ toxicity from ↑absorption from larger surface area of small intestine
- resistance to acid hydrolysis (e.g. snake venom less toxic orally than parenterally)
- chelators (e.g. EDTA) → ↑ lipid solubility, thus ↑ absorption of complexed ions; oral chelators contraindicated if toxic metal (e.g. Pb) still present in GIT
- special anatomical features (rumen, gizzard)

Ruminoreticulum
- great bulk of material, very hard to remove completely
- reservoir for toxins (e.g. Pb, lantadenes)
- biotransformation by microbes → either detoxication (e.g. oxalate) or potentiation (e.g. nitrate, mimosine)

Gizzard
- retains particles for long periods (e.g. Pb, Zn)

Reference: K94

Absorption by lungs

Gases (e.g. manure gas poisoning) & vapours of volatile liquids
- important factors = water solubility, tissue reactivity, blood-to-gas phase partition coefficients
- small, water-soluble molecules → rapid absorption

Aerosols (e.g. urban pets-tetraethyl lead) & particles
- important factors = aerosol particle size, water solubility of contained compounds
- particle size → site of deposition → fate
  - 5 μm or larger → nasopharynx → expelled or swallowed
  - 2-5 μm → tracheobronchial regions → muco-ciliary escalator → swallowed
  - 1 μm or smaller → alveoli → slowly dissolved (↑ solubility in alveolar fluids → ↑ removal rate) & absorbed into blood or scavenged by alveolar macrophages → lymphatics or muco-ciliary escalator

References: K97, Os124,126

Absorption through skin

Intact skin not very permeable - normal barrier function, but many chemicals may be absorbed through intact skin in sufficient amounts to cause harm (chloroform, pesticides)

Haired species generally have thinner epidermis than non-haired

Stratum corneum (outer dead cells of the epidermis) is main absorption rate-determining barrier - toxins cross by passive diffusion

↑ lipid solubility, ↓ molecular weight → ↑ rate of diffusion; but very lipophilic compounds (e.g. TCDD) poorly absorbed - poor solubility in phospholipids that dominate skin

Factors increasing absorption

- hydration (soaking) → ↑ permeability to water-soluble compounds (stratum corneum usually 7% H_2O in humans)
- some solvents (e.g. DMSO)

Species variation in skin permeability: rats, rabbits > pigs, guinea pigs, primates > cats

Insect vs. mammal toxicity of insecticides - chitin vs. keratin → differential toxicity

- DDT similar LD_{50s} parenterally, much less toxic to mammals percutaneously
- note ↑↑ surface-area:body-weight ratio in smaller organisms

Reference: K99

Other routes of entry for toxins

Parenteral routes = SC, IM, IP, IV

- iatrogenic, experimental
- venoms SC
- usually → ↑↑ distribution and delivery to target site → more rapid onset of signs
- avoids primary barriers & hepatic biotransformation (not IP)

Distribution

Initial distribution phase dominated by *blood flow*, but eventual distribution determined largely by *affinity of compounds for various tissues* (e.g. Pb initially very high in liver (50% 2 h after dose), then moves to bone (90% 1 month after dose))

Storage sites

- liver, kidney, plasma proteins (mostly albumin), fat, bone
- may be target organ/tissue (e.g. paraquat-lung; CO-haemoglobin; F-bone, Hg-kidney
- often **not** target organ/tissue (e.g. Pb-bone; CHCs-fat)
- toxins in storage sites in equilibrium with free toxin fraction in plasma.
- toxin elimination from plasma (excretion, biotransformation) → release of toxin from storage depot → re-establish equilibrium

Liver & kidney storage

- high capacity for binding many chemicals → prime sites for toxin concentration
- active transport into parenchymal cells + binding to tissue components

Albumin-toxin binding (e.g. warfarin, cardiac glycosides, dieldrin)

- binding reversible → equilibration with unbound toxin in plasma, extracellular space
- binding does not limit active toxin transport mechanisms
- bound toxins not able to cross capillary walls → no movement into extracellular space; no kidney filtration
- displacement of bound toxin by other compounds → ↑ free toxin → ↑ equilibration concentration at target organ → ↑ toxicity risk

Fat storage

- concentrates highly lipophilic toxins (e.g. CHCs, OPs)
- accumulated by dissolving in neutral fats
- reduced nutrient intake → reduction of fat stores → release of stored toxins may → toxic concentrations in target organ (e.g. CHCs fat → brain)

Bone storage

- e.g. F, Pb, Strontium
- incorporated into the crystal matrix of bone (e.g. Fl^- displaces OH^-; Pb, St displace Ca)
- storage reversible - ionic exchange at bone surface; osteoclastic activity

References: K101

Blood-brain barrier

Not well-developed in neonates → more susceptible to neurotoxins
Physiological barrier to many chemicals, not all
- tight capillary endothelial junctions → ↓ access to water-soluble toxins
- astrocyte processes → multiple membranes to cross
- low interstitial protein concentration cf. other tissues → ↓ paracellular transport of lipophilic toxins

↑ lipid solubility, ↓ ionisation → ↑ penetration
References: K104

Placenta

Nutrients cross by active transport, most toxins by diffusion
Placental thickness (species differences in layer numbers/type) apparently not significant
Placenta has some biotransformation capacity → detoxication
Distribution in foetus relates to tissue affinities and proportions
- foetal liver does not concentrate some xenobiotics
- blood-brain barrier undeveloped → higher brain concentrations than in adult
- little fat in foetus

References: K104

Biotransformation

Animals are repeatedly and unavoidably exposed to potentially injurious chemicals in their environment (food, water, air) and *defense systems* have evolved to deal with them, analogous with the immune system for dealing with foreign macromolecules and organisms
Lipophilicity (that enables many xenobiotics to be readily absorbed) is an obstacle to excretion - except for elimination of volatile compounds by exhalation
Xenobiotic biotransformation
- principal mechanism for maintaining homeostasis during exposure to small foreign molecules
- *enzyme systems* evolved to *render xenobiotics easily excreted* (→ ↑water solubility, viz. lipophilic → hydrophilic; polar; → ↓↓ half-life) *and less toxic* before their removal from the body, usually through urine and bile
- composed of a *limited number* of enzymes with *broad & overlapping substrate specificities* - metabolise xenobiotics + many endogenous chemicals (e.g. steroid hormones, vitamins A & D, bilirubin, bile acids, fatty acids) - xenobiotic biotransformation enzymes/systems = drug-metabolising enzymes/systems
- most enzymes synthesised in absence of discernible external stimulus, some synthesis triggered by particular xenobiotics (enzyme induction)
- can be wide *species variation in biotransformation capacity* - possibly related to ecological niche occupied by the species and thus the degree to which they are exposed to xenobiotics e.g. insects that feed on a variety of plants have greater capacity than those feeding on a limited number or one species of plant; herbivores have a greater capacity than carnivores

Enzyme induction
- positive feedback process of stimulus to synthesis of xenobiotic biotransformation enzymes by exposure to xenobiotics
- adaptive & reversible
- a very wide range of chemicals may act as inducers (e.g. halogenated pesticides, steroids, plant extracts)
- different classes of chemicals induce different isozymes/sites (phenobarbitone → cytochrome P450 in periacinar hepatocytes)
- P450 more readily induced than some other systems
- may → ↑ volume of smooth endoplasmic reticulum (SER) in cytoplasm
- induction can alter significantly the half-life or toxicity of xenobiotics (e.g. pre-treatment of rats with phenobarbital → ↓ hexabarbital sleeping time & ↑ CCl_4 toxicity)
- effect of an inducer may be measurable within hours and maximise in days, returning to normal in 5-10 days if no further exposure occurs

Biotransformation reactions *may ↓ or ↑ toxicity (biological activity)* of compounds

Bioactivation or *toxication* = production of *more toxic/active intermediates* from non- or mildly-toxic compounds (e.g. pyrrolizidine alkaloids; CCl_4)

Sites of biotransformation enzyme systems

- principally in *liver* - range and capacity >>>> those of any other tissue - portal delivery of ingested chemicals direct from GIT
- also significant systems in kidney and lungs; GIT flora; GIT mucosa
- virtually every tissue contains enzymes with activity for some xenobiotics e.g. epidermal basal layer and nasal mucosa contain as much activity weight for weight as hepatocytes
- cells within organs vary in their biotransformation capacity (e.g. some hepatotoxins → periacinar vs. periportal lesions because cytochrome P450 enzymes are more concentrated in periacinar hepatocytes)

Rates of biotransformation may be affected by

- concentration of compound at active site; lipid solubility; intra- and extra-cellular protein binding; rate-limiting steps; induction of biotransformation enzymes
- species; strain; gender (differences can be marked); age (immature neonate systems)
- time of day (circadian rhythms)
- pre-existing liver disease
- nutrition
  - mineral (Cu, Zn, Ca, Fe, Mg), vitamin (B, C, E) and protein deficiency all → ↓
  - food deprivation
    - → partially clears GIT & alters rate of absorption
    - may → specific effect on biotransformation systems (e.g. → ↓↓ hepatic glutathione)
    - ruminants - starvation → ↓ rumen flora number & variety → ↓ microbial xenobiotic biotransformation capacity

One or many biochemical steps involved in dealing with a given xenobiotic

Two groups of biotransformation enzyme reactions - Phases I & II

Phase I enzyme reactions

- the dominant biotransformation pathway
- prime function is to *add or expose functional groups* (e.g. -OH, -SH, $-NH_2$, -COOH) which are often sites of phase II reactions
- generally → only a *small* increase in water solubility
- located mainly in *endoplasmic reticulum* (ER) (some in cytosol, mitochondria)
- lipophilic substrates for reactions preferentially partition into the lipid part of ER membranes
- ER membranes also maintain the enzymes in a spatial orientation needed for efficient function

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The procedures used to homogenise liver tissue for biochemical experiments fragment the ER. The fragments spontaneously "ball-up" into closed vesicles, called **microsomes**, which can be separated from larger fragments, nuclei etc., by differential centrifugation (hence microsomal metabolism, microsomal enzymes, etc.). With a suitable supply of energy and substrates, microsomes will go on metabolising *in vitro*, which is useful for many biochemical and toxicological studies.

Phase I enzyme reactions =

Hydrolysis - carboxylesterases, peptidases, epoxide hydrolase

Reduction - azo- and nitro-reduction; carbonyl reduction; disulphide reduction; sulphoxide and N-oxide reduction; quinone reduction; dehalogenation

Oxidation - alcohol, aldehyde, ketone oxidation-reduction systems; monoamine oxidase, diamine oxidase & polyamine oxidase; aromatisation; peroxidase-dependent cooxidation; flavin-containing monooxygenases; cytochrome P450

Cytochrome P-450-containing monooxygenases

- most important enzyme systems involved in phase I reactions
- cytochrome P-450 system = polysubstrate monooxygenase, or mixed-function oxygenase [MFO] system

- actually two enzymes, cytochrome P-450 and P-450 reductase held in a fixed spatial relationship by the phospholipid membrane of ER → structural integrity vital to proper function
- genetically-determined differences in the protein part of cytochrome P-450 → different substrate specificities
- different species and different individuals have different proportions of isozymes → sometimes responsible for the different susceptibilities to certain xenobiotics
- all mammals have the cytochrome P-450 system and their protein moieties are very similar between species, but functional similarities do not always apply - in some species, the same function appears to reside in different P-450 isotypes, in others, certain substrates are not readily metabolised despite isozymes being spectrally similar. This conundrum largely reflects a deficiency in our methods of characterising the enzymes.

Phase II enzyme reactions

= glucuronidation, sulphation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis) and conjugation with amino acids (e.g. glycine, taurine, glutamic acid)

- glucuronidation missing in the cat family → paracetamol toxicity in domestic cats (cytochrome P450 system → toxic metabolite unable to be detoxified by glucuronidation in cats)
- substrates = xenobiotics or phase I metabolites
- usually (except methylation & acetylation) → *large* ↑ in water solubility → promote excretion
- located mainly in the *cytosol* (some in ER, mitochondria)
- reaction rate >> phase I reactions
- cofactors = uridine-5'-diphospho-α-D-glucuronic acid (UDP-GA), 3'-phosphoadenosine-5'-phosphosulphate (PAPS), acetyl coenzyme A, S-adenosylmethionine (SAM), glutathione, glycine, taurine, glutamine
- activated or "high energy" cofactors used in glucuronidation, sulphation, acetylation & methylation to react with functional groups either present on the xenobiotic or introduced/exposed by phase I reactions
- activated xenobiotics involved in conjugation reactions
- rarely, phase II reactions precede phase I reactions

References: K113, 115, 163

Excretion

Toxins and metabolites need to be water-soluble to access the major excretion routes
Main routes: urine (molecular weight < 500) > bile → faeces (MW > 500)
Any body secretory apparatus can excrete chemicals (e.g. sweat, tears, milk, gas via lungs)
Hair and hoof/horn can contain diagnostically-significant amounts of toxins (e.g. As, Se)
References: K105

Urinary excretion

Kidney - excretion by same mechanisms as for end-products of metabolism (glomerular filtration, tubular excretion by passive diffusion, active tubular secretion)

Glomerular filtration

- molecules up to molecular weight 60,000 (proteins smaller than albumin) pass through; albumin-bound toxins not filtered
- lipophilic compounds reabsorbed, polar compounds & ions excreted (bases at low pH, acids at high pH); manipulate urine pH → promote excretion of certain toxins (e.g. ethylene glycol, strychnine)

Passive diffusion through tubules - minor significance cf. glomerular filtration; some organic acids & bases that readily ionise at urine pH (diuretics useful to enhance excretion of these)

Active tubular secretion in proximal tubules

- two processes known, one for organic anions (acid) one for cations (bases)
- may be competition for active transport sites

References: K106

Faecal excretion

Second major route for elimination of xenobiotics
Unabsorbed residues

- uptake seldom complete, proportion absorbed depends on size, solubility, ionisation and concentration gradient (e.g. most of paraquat dose unabsorbed → faeces)

Biliary excretion

- possibly most important source of faecal xenobiotics; metabolites from hepatic biotransformation - low molecular weight compounds poorly excreted; >325 mol wt better
- enterohepatic circulation may establish when conditions favour reabsorption; conjugated compounds polar & poorly reabsorbed, but microbial hydrolysis may release lipophilic moiety for reabsorption; interruption of cycle by adsorbants useful therapy
- bile concentration:plasma concentration ratio → 3 classes of compound, viz. Class A (ratio = *ca.* 1) e.g. Hg, Thallium; B (10-1000) e.g. Pb, As; C (<1) e.g. Zn
- toxin need not be concentrated for bile to be its principal route of excretion (e.g. Hg)
- Class B compounds actively transported across both sides of hepatocytes
- liver has at least 4 transport systems for organic compounds + 1 for metals blood → bile; organic acids (2 systems), bases (1), neutral compounds (1), metals (1)
- marked species variation in biliary excretion rates
- ↑ microsomal activity may increase bile flow and excretion of toxins/metabolites (e.g. some cardiac glycosides), but not all inducers effective
- hepatic systems not functional/not fully developed in neonates, may → ↑ toxicity

Intestinal excretion

- many chemicals excreted across intestinal mucosa; direct, passive transfer from blood
- can manipulate rate to some extent (e.g. mineral oil → ↑ rate of lipophilic compound clearance)
- some biotransformation occurs in intestinal wall

Effect of intestinal flora

- rumen microflora may → toxins e.g. ammonia, nitrite, but can be protective e.g. synthesise thiamine; destroy oxalate
- biotransformation by intestinal flora favours reabsorption over excretion

References: K107

Respiratory excretion

Gases & volatile liquids (e.g. ethanol) mainly excreted by diffusion through lungs
Rate of elimination related to solubility in blood: low solubility → rapid excretion
Rate of elimination of low blood solubility gas is perfusion-limited; of high solubility is ventilation limited
References: K109

Milk

Numerous compounds diffuse from plasma to milk - because milk more acidic than plasma, basic compounds may be concentrated, acidic compounds may be less concentrated
Large lipid content → many lipid-soluble substances excreted in milk (DDT, PCBs and PBBs)
Pb (chemically similar to Ca) excreted
References: K109

CSF, Sweat, Saliva

- all minor routes. Diffusion of non-ionised, lipid-soluble compounds; in sweat may cause dermatitis, in saliva swallowed and reabsorbed.

Cellular Mechanisms of Toxicity

Stages (steps) in the development of toxicity after exposure = Delivery; Interaction with target molecule; Cellular dysfunction/injury; Dysrepair

Pathways leading to toxicity

- A (1 step): Chemical causes toxicity by its *physical presence* at critical sites in the body without interacting with a target molecule. Most important consideration is delivery. e.g. blockage of renal tubules by oxalate crystals
- B (3 steps): Chemical is *delivered* to target site, *interacts* with target molecule and produces *cellular dysfunction* not susceptible to prevention by repair processes. e.g. tetrodotoxin → blockade of Na^+ channels of motoneurones → skeletal muscle paralysis

- C (4 steps): Chemical is *delivered* to target site, *interacts* with target molecule and *triggers perturbations* in cell function and/or structure which *initiate repair mechanisms*. Toxicity occurs when toxicant-induced perturbations *exceed repair capacity* or when *repair mechanisms malfunction*. e.g. tissue necrosis, cancer, fibrosis

Intensity of toxicity depends on concentration & persistence of *ultimate toxicant* at its site of action. Clinical manifestations depend on the degree of damage and how essential the affected cells or organs are to the animal

Ultimate toxicant

= chemical species that reacts with the endogenous target molecule (e.g. receptor, enzyme, DNA, microfilamental protein, lipid), initiating structural and/or functional alterations that result in toxicity, this may be

- *original chemical* (parent compound) e.g. Pb, tetrodotoxin, HCN, CO
- *metabolite* of parent compound e.g. ethylene glycol → oxalic acid
- *reactive oxygen species* generated during biotransformation of the toxicant e.g. hydrogen peroxide → hydroxyl radical ($HO^{\bullet}$)
- *endogenous molecule* (occasionally) e.g. sulphonamides displace bilirubin from albumin in human neonates → bilirubin

References: K35, Os17

Step 1: Delivery

- concentration of ultimate toxicant at target molecule depends on relative effectiveness of processes increasing or decreasing that concentration, viz.
- *Facilitating processes*: absorption, distribution to the site of action, reabsorption (renal, enterohepatic), bioactivation (toxication)
- *Opposing processes*: presystemic elimination, distribution away from the site of action, excretion, detoxication

Toxication (bioactivation)

- → indiscriminate reactivity towards endogenous compounds with susceptible functional groups through conversion into
  - *Electrophiles* = molecules containing an electron-deficient atom with a partial or full positive charge; react by sharing electron pairs with electron-rich atoms in neucleophiles e.g. aflatoxin B_1 → aflatoxin B_1 8,9-epoxide
  - *Free radicals* = molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital; redox cycling by xenobiotic → superoxide anion radicals ($O_2^{-\bullet}$) → the more reactive hydroxyl radicals ($HO^{\bullet}$)
  - *Nucleophiles* e.g. amygdalin → HCN
  - *Redox-active reactants* e.g. nitrate → nitrite
- much less common are
  - → physicochemical properties adverse to microenvironment e.g. ethylene glycol → oxalic acid → acidosis, hypocalcaemia, renal tubule blockage by Ca oxalate crystals
  - → structural features & reactivity allowing more affinity with receptors or enzymes e.g. OP parathion → paraoxon (active cholinesterase inhibitor); fluoroacetate → fluorocitrate (blocks Krebs cycle)

References: K36

Step 2: Ultimate toxicant-target molecule reaction

- Types of reaction
  - *noncovalent binding* - low bonding energy → bond usually reversible; steric arrangement of toxicant atoms allows close fit with with complementary sites on target molecule e.g. strychnine to glycine receptors in spinal cord motor neurons, warfarin to vitamin K 2,3-epoxide reductase
  - *covalent binding* - practically irreversible → permanent alteration of target molecule; commonly electrophilic toxicants → covalent adducts with nucleophilic atoms abundant in macromolecules (e.g. proteins, nucleic acids) e.g. Pb → bonds with critical thiol groups on δ-aminolevulinic acid dehydratase → blocks haem synthesis; rarely nucleophilic toxicants → covalent adducts with electrophilic atoms rare among biomolecules e.g. CO, HCN → bonds with Fe in various haemproteins

- *hydrogen abstraction* - neutral free radicals readily abstract H atoms from endogenous compounds, converting them into radicals; $HO^{\bullet}$ reacts with amino acids (proteins) → cross-links with other proteins or DNA; H abstraction from DNA → strand breaks; H abstraction from fatty acids initiates lipid peroxidation
- *electron transfer* - oxidation of Fe (II) in Hb to Fe(III) → metHb e.g. nitrite
- *enzymatic reactions* - a few toxicants act enzymically on proteins e.g. ricin → hydrolytic fragmentation of ribosomes → blocks protein synthesis

- Target molecules
  - frequent targets: macromolecules (protein, nucleic acids), membrane lipids
  - rare targets: high energy compounds (e.g. ATP) and cofactors (e.g. coenzyme A, pyridoxal)
- Target molecule attributes
  - *reactivity &/or steric configuration* to allow bonding
  - *accessibility* to ultimate toxicant in significant concentration, thus endogenous molecules near sites of formation of reactive metabolites e.g. enzyme responsible for their production - CCl_4 destroys cytochrome P450 that activates it + neighbouring microsomal membranes
  - *critical function*
- Effects of toxicants on target molecules
  - *Dysfunction*
    - inhibit target function e.g. atropine, curare, strychnine → block neurotransmitter receptors (attach to ligand binding sites; interfere with ion channel function); colchicine, taxol, vinblastine, phalloidin → impair cytoskeletal protein function (bind to tubulin or actin [phalloidin])
    - activate target proteins e.g. morphine → opiate receptors; Pb → protein kinase C
    - alter critical protein structures (for catalytic activity, assembly into macromolecules) e.g. thiol-reactive chemicals
    - interfere with DNA template function (toxicant covalent binding → nucleotide mis-pairing during replication) e.g. aflatoxin 8,9-oxide to *N*-7 of guanine → pairing with adenine rather than cytosine → incorrect codon → incorrect amino acid in protein (involved in aflatoxin-induced mutation of *ras* proto-oncogene & *p53* tumour suppressor gene); $HO^{\bullet}$ → 8-hydroxyguanine & 8-hydroxyadenine → mis-pairings
  - *Destruction*
    - cross-linking cytoskeletal proteins, DNA, DNA with proteins
    - hydrolytic degradation of proteins → fragmentation
    - spontaneous degradation after chemical attack e.g. free radicals → initiate lipid peroxidation by hydrogen abstraction from fatty acids → generate endogenous toxicants (free radicals & electrophiles) → damage adjacent molecules (membrane proteins) or diffuse to more distant molecules (DNA)
  - *Neoantigen formation*: covalent binding to proteins may render them antigenic in some individuals e.g. hepatitis-like syndrome in halothane-sensitive humans (cytochrome P450 biotransforms halothane → electrophile that binds as a hapten to various microsomal & cell surface proteins in liver → antibody production); ?*Vicia*-induced eosinophilc granulomas in certain cattle breeds

References: K43

Step 3: Cellular dysfunctions and resultant toxicities

- Type of cellular dysfunction caused by toxicant depends on the role of target molecule
- If target molecule involved in
  - *cellular regulation* → dysregulation of gene expression or ongoing cell activity
  - *internal cell maintenance* → cell injury or death
  - *external maintenance functions* → impaired function of integrated systems
- Toxicant-induced cellular dysregulation
- cells are regulated by signalling molecules → activate specific cellular receptors linked to signal transducing networks that transmit signals → regulatory regions of genes &/or functional proteins
  - **Dysregulation of gene expression** through
  - *transcription of DNA to mRNA* controlled by interplay of transcription factors (TFs: ligand-activated & signal activated) and promoter region of gene; toxicants may interfere in all facets, but mostly with TFs; steroid hormones & vitamins influence gene expression by

binding to & activating TFs; toxicants binding to appropriate receptors → dysfunction e.g. zearalenone binds to oestrogen receptor → pseudo-oestrus in pigs

- *signal transduction* from extracellular signalling molecules (e.g. cytokines, hormones, growth factors) activates TFs often through phosphorylation controlled by protein kinases & phosphatases; perturbations of this system → altered gene expression regulated by TFs e.g. phorbol esters & Pb ions activate protein kinase C more effectively than the normal physiological regulators diacylglycerol & Ca^{2+} → mitogenic, carcinogenic effects; various toxicants → apoptosis
- *signal production*: perturbation of pituitary hormone production (under negative feedback control from hormones of peripheral endocrine glands) effects peripheral gland function e.g. amitrole herbicide inhibits thryoid hormone production → ↑ pituitary TSH production → goitre; oestrogens in males → ↓ pituitary FSH → testicular apoptosis/atrophy

- **Dysregulation of ongoing cellular activity** (functional proteins)
- control of specialised cells through signalling molecules → membrane receptors → Ca^{2+} cytoplasm entry regulation or enzymatic formation of intracellular 2nd messengers → alter phosphorylation of functional proteins → almost instant activity change; toxicants can disrupt any step in this signal coupling
- *electrically excitable cells* (neurons, muscles) affected through alteration in
  - *neurotransmitter concentrations*: synaptic concentrations of neurotransmitters altered by interfering with synthesis, storage, release or removal from the receptor vicinity e.g. botulinum toxin → inhibits acetylcholine (ACh) release from motor neurons; OP or carbamate insecticides → inhibit ACh esterase → prevent hydrolysis of ACh → massive stimulation of cholinergic receptors
  - *receptor function*: direct toxicant interaction with receptors → block or mimic physiological responses e.g. barbiturates & benzodiazepines activate CNS inhibitory $GABA_A$ receptors → sedation, anaesthesia, medullary respiratory centre blockade (depending on dose); Pb ions → inhibit neuronal nicotinic ACh receptors (muscular nicotinic subtype not as sensitive)
  - *intracellular signal transduction*: voltage-gated Na^+ channels, that transduce & amplify excitatory signals from ligand-gated cation channels, activated by, e.g. grayanotoxin, aconite, ciguatoxin, DDT, → overexcitation; blockers, e.g. tetrodotoxin, saxitoxin, → paralysis
  - *signal-terminating processes*: signals generated by cation influx are terminated by cation removal through channels or by transporters; inhibition of cation export → prolonged excitation e.g. cardiac glycosides inhibit Na^+,K^+-ATPase → ↑ intracellular Na^+ → ↓ Ca^{2+} export by Ca^{2+}/ Na^+ exchange → ↑ contractility & excitability of cardiac muscle; 70% of ATP in neurons is used to drive the Na^+,K^+ pump - HCN → blocks ATP synthesis → depolarisation → release of neurotransmitters such as glutamate → hypoxic seizures
- *other cells*: e.g. many exocrine secretory cells controlled by muscarinic ACh receptors; OP → stimulus → salivation, lacrimation, bronchial hypersecretion; atropine → blockade → hyperthermia

- Toxic alteration of cellular maintenance
  - Impairment of internal cellular maintenance: **mechanisms of toxic cell death**
    - *impared mitochondrial ATP synthesis*: ATP major energy source derived from oxidative phosphorylation; many toxicants impede various parts of this process e.g. HCN, atractyloside, fluoroacetate
    - *sustained rise of intracellular Ca^{2+}* : results in depleted energy reserves, dysfunction of microfilaments (→ plasma membrane blebbing & rupture) & activation of hydrolytic enzymes (→ degrade proteins, phospholipids, nucleic acids);10,000-fold difference between extracellular & cytosol Ca^{2+} (Ca^{2+} pumped out of cells through Ca-impermeable plasma membrane; sequestered in ER & mitochondria); toxicants → open ligand- or voltage-gated Ca^{2+} channels, damage plasma membrane, inhibit Ca^{2+} transporters, induce leakage from mitochondria; e.g. $HO^{\bullet}$, methyl Hg,

- *other mechanisms*: direct damage to plasma membrane, damage to lysosomal membrane, destruction of cytoskeleton (phalloidin, colchicine), disrupt protein synthesis (ricin, α-amanitin, ethanol)

- Impairment of external cellular maintenance
  - interference with the protein synthesis for export and excretory functions of hepatocytes e.g. warfarin, coumarin inhibition of hepatic coagulation factor synthesis

References: K46

Step 4: Toxicity resulting from dysrepair

Repair fails when

- damage *overwhelms* repair mechanisms e.g. protein thiols oxidised faster than they can be reduced
- repair *capacity* becomes *exhausted* when necessary enzymes or cofactors are consumed e.g. lipid peroxidation can deplete α-tocopherol
- toxicant-induced injury *affects repair process itself*
- effective repair is *impossible* e.g. toxicant covalently bound to protein

Cell injury progresses to cell necrosis when molecular repair mechanisms are inefficient or the molecular damage is irreversible

Most severe toxic injuries of this type = tissue necrosis, fibrosis, carcinogenesis

Tissue necrosis

- progression of cell injury to tissue necrosis can be intercepted by 2 repair mechanisms working in concert: apoptosis & cell proliferation
- injured cells can initiate apoptosis → prevents necrosis and the consequent inflammatory response; inflammation may → injury through cytotoxic mediators (including phagocytes)
- injured cells → surge of mitosis in adjacent cells → prevents spread of necrosis
- efficiency of repair is an important factor in the dose-response relationship of toxicants that cause tissue necrosis; tissue necrosis occurs when a sufficient concentration of the ultimate toxicant reaches the target site **and** that dose causes damage sufficient to compromise repair e.g hepatotoxicants → apoptosis & cell proliferation with latent tissue injury caused by low doses, but are inhibited by high (necrogenic) doses

Fibrosis

- associated with toxicants generating free radicals
- overproduction of extracellular matrix controlled by cytokines (mainly transforming growth factor-beta: TGF-beta) from non-parenchymal cells; positive feedback loop may become established (TGF-beta induces transcription of its own gene in target cells)
- failure to halt TGF-beta production may result from a defect in its regulation or continuous injury

Carcinogenesis

Results from multiple failures and malfunctions of various repair mechanisms

Genotoxic carcinogens → adduct formation, oxidative alteration, strand breakage e.g. aflatoxin, ptaquiloside

- *failure of DNA repair* → mutation (the initiating event); mutation (→ activation) of proto-oncogenes & mutation (→ inactivation) of tumour suppressor genes → neoplastic cell transformation
- *failure of apoptosis* → promotion of mutation & clonal growth; apoptosis eliminates cells with DNA damage (& thus mutations); some tumour promoters inhibit apoptosis
- *failure to terminate proliferation* → promotion of mutation, proto-oncogene expression & clonal growth

Non-genotoxic (epigenetic) carcinogens or *tumour promoters* → promotion of mitosis & inhibition of apoptosis; do not alter DNA or induce mutation; carcinogenic after prolonged exposure; cause neoplasia by promoting carcinogenesis initiated by genotoxic agents or the spontaneous DNA damage commonly occurring in normal cells e.g. phorbol esters, endogenous mitogens such as oestrogen, TGF-alpha

References: K66