The Royal College of Pathologists
Pathology: the science behind the cure

FRCPath Examination

Toxicology Speciality

General Toxicology

Part I, Paper I

Curriculum

Contents

1. Biology and cellular physiology

Specific Topics

Cellular compartments

- Cytosol: Glycolysis and gluconeogenesis, pentose phosphate shunt and fatty acid biosynthesis
- Mitochondria: Kreb's citric acid cycle, electron transport system and oxidative phosphorylation, fatty acid oxidation, amino acid catabolism and interconversion of carbon skeletons
- Endoplasmic reticulum: Lipid synthesis, steroid synthesis; phase one detoxification reactions, biosynthesis and modification of membrane and export proteins
- Ribosomes: protein synthesis (associated with ER for membrane and export proteins)
- Golgi complex: Further modification of membrane and export proteins
- Lysosomes: Hydrolytic (digestive) enzyme localization
- Peroxisomes: Amino acid oxidases, catalase-oxidative degradation reactions
- Plasma membrane: Active and passive transport systems, receptors and signal processing systems (synthesis of various second messengers etc.)
- Nucleus: DNA replication, synthesis and processing of messenger RNA
- Nucleolus: Localized region of the nucleus in which ribosomal RNA is synthesized and processed

Cell Division/death

- Apoptosis
  - Intrinsic and extrinsic pathways
  - Molecular events regulating apoptosis including receptor-mediated, ionic changes, enzyme involvement and genes (e.g. Bcl, Bax, Bad, TRAIL, FLIP)
  - DNA events and controlling factors
- Cell replication
  - Molecular events associated with the cell cycle and mitosis
  - Drugs effecting changes in the cell cycle (e.g. anti-cancer drugs such as taxanes, cis-platin)
  - Cell cycle arrest and its consequences

2. Pharmacology and toxicology

Specific Topics

Central role of pharmacology in the interpretation of drug-induced toxicity

- Pharmacology of drug: receptor-ligand interactions, intracellular signalling cytoplasmic and nuclear receptors and hormones
- Pharmacodynamics and use of PD markers in toxicology studies

3. Toxicological pathology

Specific Topics

Basic physiology, anatomy and histology of major organ systems, their integration and homeostatic control

Organ systems:

- Integument (skin)
- Neurological system (central and peripheral)

- Special senses (eye, ear and other sense organs)
- Endocrine organs (adrenal, pituitary, thyroid and parathyroid glands)
- Cardiovascular system
- Gastrointestinal tract and associated glands (liver, pancreas, salivary glands)
- Respiratory system
- Urinary tract
- Male and female reproduction systems
- Lymphoid/haematopoietic systems (spleen, thymus, lymph nodes & bone marrow)
- Musculoskeletal system

Tissue reactions to toxicity: pathological processes include

- Degenerative changes
- Cell death (apoptosis and necrosis)
- Inflammation (chronic and acute): cytokines and other protein mediators
- Adaptive changes of atrophy, hypertrophy and hyperplasia
- Healing and repair

Drug-induced pathology and mechanisms of toxicity

- Include classis examples of mechanisms of toxicity

Comparative pathology

- Species and strain differences

General histology and other special investigative techniques

- Immunohistochemistry (IHC) and immunocytochemistry (ICC)
- In situ hybridisation (ISH)
- Electron microscopy (EM)

Laboratory animal husbandry

- Infectious diseases
- Specific pathogen free (SPF)
- Quarantine

4. Clinical pathology
Specific Topics
Clinical pathology (clinical chemistry, haematology and urine analysis) • Dynamics and function of the blood • Haematology: including total/differential blood cell counts, blood cell and bone marrow morphology and coagulation tests • Clinical chemistry: including markers for organ function • Urine analysis • Major diseases associated with changes in clinical pathology data • Interpretation of clinical pathology data in context of target organ pathology and exposure to xenobiotics • Role and significance of clinical pathology in studies with xenobiotics for human safety and pharmacovigilance
Practical considerations

- Preanalytical variation: study design, site of blood sampling, inter/intra animal variation, reference ranges and species differences
- Analytical methodologies: biochemical assays, immunoassays, flow cytometry, HPLC, NMR, assay development, qualitative and quantitative microscopic assessment of normal blood films, bone marrow smears, reticulocyte films and urine cytology
- Instrument and method validation: theory, limitations and application of QC
- Application of new molecular methods
- Critical and scientific interpretation of data

5. Principals of preclinical drug development
Specific Topics
• Drug discovery process (from discovery through to market) • Principals of drug structure, design and candidate selection process
Safety assessment process of pharmaceutical agents • Preclinical safety studies (including screening, in vitro metabolism) • Design of studies (number of animals, use of control and recovery animals) • Selection of species and animal models of disease • Formulation and impurities • Dose selection for FTIH
Endpoints in standard toxicology studies (routine and non-routine) • Clinical observations, body weight and body temperature • Clinical Pathology (haematology, clinical chemistry and urine analysis) • Safety Pharmacology • PKPD • Necropsy, organ weights and histopathology
Practical considerations • Methods for collection, handling and storage of biological fluids (e.g. blood, urine, CSF) • Packaging and storage of samples
Additional considerations • Requirements for different drug types (small molecules, mAbs, cell and gene therapy, vaccines) • Combination therapies • Medical devices

6. Safety pharmacology
Specific Topics
Cardiovascular assessment: • in vivo telemetry (BP, HR, ECG) • in vitro hERG, purkinje fibre, perfused heart Respiratory assessment: • in vivo plethysmography (respiratory rate, tidal volume, $HbSatO_2$) CNS assessment

- In vivo Irwin, FOBs (behaviour, locomotion, coordination, sensory reflex, body temp)
- Pain, convulsion threshold, sleep and higher cognitive functions (passive avoidance, maze), EEG

Others: Renal, GI tract, autonomic system

7. Immunotoxicology

Specific Topics

Basic concepts

- The basic features of the innate and adaptive immune function
- Organisation of the immune system (primary and secondary lymphoid organs)
- Basic cellular organisation/histology of lymphoid tissues

Induction and regulation of the immune response

- The nature of antigenic stimulation and roles of APCs (molecular basis for antigen presentation and epitope recognition)
- Lymphocyte function and induction of humoral and cell-mediated immune responses
- The effect and function of lymphocytes and antibodies
- The role of complement, cytokines, macrophages and other cells and molecules in immune and inflammatory reactions
- Regulation of immune function (subpopulations of T cells)
- Genetic influences on immune function

Disease of the immune system

- Autoimmunity and autoimmune disease
- Hypersensitivity (allergy)
- Organ and bone marrow transplantation
- Tumour immunology
- Infectious disease
- Reproduction abnormalities
- Immune-neuroendocrine interactions
- Graft vs host disease

Immunopharmacology/immunotoxicology

- Modulation of the immune system by drugs and chemicals
- The consequences of immunosuppression (primary and secondary immunodeficiency and immunotoxicity)
- Assessment and interpretation of drug-induced gamma globulin changes, acute phase proteins, lymphocyte subsets, cytokines

Testing for immune dysfunction (practical application)

- Laboratory methods for the assessment of immunological function
- Testing strategies and the timing of studies in relation to product development
- Safety testing of biopharmaceuticals (vaccines, mAb, DNA)

8. Reproductive and developmental toxicology

Specific Topics

In vivo reproductive toxicology

Fertility and early embryonic development (EED)

- General fertility and reproductive function in males and females

Embryofetal development (EFD)

- Teratology: causes, mechanisms, manifestation of developmental abnormalities and prevention

Pre and postnatal development (PND) including maternal function

Paediatric and juvenile toxicology

Species: Rat, rabbit, mouse and NHP

9. Neurotoxicology
Specific Topics
Neurotoxicology studies • Organophosphorus-induced delayed neuropathy (OPIDN)

10. Dermal Toxicology
Specific Topics
• Photo-safety • Sensitisation • Skin irritation and local tolerance • Implantation

11. Carcinogenicity and genotoxicity
Specific Topics
Mechanisms involved in experimental and human carcinogenesis including multistage process (Initiation, promotion and progression)
Distinction between genotoxic and non-genotoxic carcinogens and mechanisms
Dose-response relationships
Role of oncogenes and tumour suppressor genes in cell cycle and in apoptosis
How genetics can predispose to drug-induced disease in human and animal populations (e.g. Isoniazid, debrisoquine)
Methods for determining the carcinogenic potential of chemicals Overview of practical procedures for: *In vitro* assays: • Bacterial tests (e.g. Ames): phenotype/genotype of test strains, methodology, criteria for positive response and differing strain sensitivities • Mammalian cell tests (e.g. MLA, CHO): nature of test cells • Mammalian cell tests for aneugens and chromosome damage (cytogenetics): rodent, human cells, karyotype, FISH, comparative genomic hybridisation (CGH) and other new methods

In vivo assays: • Chromosome damage in bone marrow cells (Micronucleus assay, metaphase analysis) • DNA repair tests: unscheduled DNA synthesis • Dominant lethal assay for germ cell effects • DNA damage: Comet assay • Transgenic systems for mutation (Mutamouse, Big Blue) • Two-year rodent bioassays • Truncated (6-12m) transgenic tumorigenicity studies
Biological and toxicological significance of the results of carcinogenicity studies • Common background tumours in rodents • Background pathology in aged animals
Interpretation of experimental data for human safety evaluation • Experimental design, application and limitations of test systems (method sensitivity) • Structure Activity Relationships • Differentiation of human and rodent specific carcinogens • Relative importance of in vitro vs in vivo results • Integration of genotoxicity data in overall assessment • Early use of low-cost in vitro tests for new product screening • Mutagens/carcinogens in the diet, workplace and environment, industrial chemicals, pharmaceuticals, impact and prevention of exposure through education

12. Biochemical and molecular toxicology
Specific Topics
General ADME • Absorption, Distribution, Metabolism and Excretion (ADME) • Species variations in response • Kinetics of response
Routes of exposure • IV, SC, oral, IVT, inhalation, dermal, IP, IM etc
Biotransformation and transport systems and tissue specific metabolism
General PK principles (C_{max}, T_{max}, AUC)
PK: Clearance, volume of distribution, half-life
Single and repeat dose IV data: linear PK behaviour and accumulation
Single and repeat dose oral/dermal data: non-linear PK behaviour and accumulation, effects of the route of administration (first pass)
Kinetic behaviours of small molecules and mAbs
Biopharmaceuticals: relationship of PK, PD and ADA immunogenicity
Species differences and scaling/kinetic modelling to man

13. Mechanistic toxicology
Specific Topics
Understanding mechanisms of toxicity

- Mechanism of action
- Known class effects
- In vitro and in vivo assays to characterise MOA
- Tools and reagents to understand mechanisms of disease
- Relevance for man

Broad knowledge of molecular, in vitro and in vivo test methods

14. Legislative and regulatory toxicology

Specific Topics

Legislative and regulatory guidelines

Differences and main points of various guidance/standards

- ICH, FDA, EMA, EPA, MDR, WHO, OECD

Principals of quality control

- Good Laboratory Practice (GLP), GMP, GCP
- Clinical Pathology accreditation (CPA)
- National Measurement Accreditation Service (NAMAS)
- QA, QC, audit, archive

Nomenclature

- SI and associated units
- Chemical and pharmaceutical nomenclature

Statistical analyses

- Statistical analysis and specific procedures to control variation
- Experimental populations and their differences
- Parametric and non-parametric analytical techniques
- Associations, regression and correlation

Health and Safety, staff training

- COSHH, radioactive handling and genetic manipulation (GMO)
- Ethics of professional practice
- Staff training and development

Animal welfare

- Home office licenses
- Ethics review committee
- Alternative methodologies to the use of animals in toxicology and use of the 3Rs
- *In vitro* assays: validation and interpretation

Pharmacovigilance

- Adverse drug reactions
- Clinical monitoring
- Drug labelling

15. Risk assessment and management

Specific Topics

Principals of risk assessment pertaining to humans, animals and the environment; definitions of hazard, risk v's benefit
Identification of pivotal studies and critical endpoints
Nature of dose response and identification of point of departure
Choice of animal species and strain. The crucial role of kinetics and metabolism in animal extrapolation to humans
Integration and presentation of data from a range of studies, risk and hazard assessment. Exposure data for the human population
Dose response relationships: threshold and non-linear models. The concept of No Adverse Effect Levels (NOAELs), Lowest Observed Effect Level (LOEL), Therapeutic Indices (TI), TDI, MRL, HSTD and derivation of health-based guidance values/reference doses using safety factors
Understanding of Acceptable Daily Intakes (ADIs), Tolerable Daily Intake (TDI), Maximum Residue levels (MRL), occupational exposure limits (OELs), short-term exposure limits (STELs), etc and principals involved in setting these values
Methods for assessing exposure (including MRLs)
Consideration of susceptible sub-populations

16. Biopharmaceuticals
Specific Topics
Range of biotherapeutic modalities • mAb, polyclonal Ab, Fab, DNA, gene therapy products, GMO, recombinant proteins,
Safety considerations: • Exaggerated pharmacology • PK/PD and modelling • Immunogenicity and ADA • Species specificity and preclinical study design issues • Dose calculations for FTIM studies: MABEL • Biodistribution studies • Integration of DNA

17. Environmental and occupational toxicology
Specific Topics
Ecotoxicology Environmental toxicology: the science that studies the effects of drugs, environmental contaminants, and naturally occurring substances found in food, water, air and soil and the use of that information to predict safe exposure levels. • basic terminology and concepts • basic principles of toxicology, including hazard (known or unknown), route of exposure, dose duration and response, susceptibility, metabolism, target organs, excretion and differential diagnosis with reference to environmental toxicants • biological sampling methods, biomarkers and their uses and limitations • removal from exposure, decontamination and the principles of evacuation and shelter

Environmental Science: the study of physical chemical and biological conditions of the environment and their effects on organisms.

- Basics of environmental pathways and source pathway receptor: land, water, food
- Key issues in relation to health impacts of air, water and land pollution
- Principles of environmental pollutants and impacts on health
- Awareness of the common applications of ionizing radiation and exposures to non-ionizing radiation
- Environmental sampling their uses and limitations for air, land and water
- Environmental impact assessment and links to health impact assessment
- Understanding the process of determining environmental standards (what standards are available, how to access them and how to utilize them?)
- Awareness of the main environmental legislation
- Understanding ionizing, radiation physics and biology
- Awareness of ionizing exposure pathways and health effects

Environmental epidemiology: the epidemiology of common environmental exposures, such as for example water contaminants, particulates in air, nitrogen dioxide, ozone, environmental tobacco smoke, diet, radon in homes, toxic waste sites, electromagnetic fields, and lead

- Methods of investigating environmental hazards
- Estimation of exposure and problems of measurement
- Analysis of health and exposure data including using GIS
- Basics of occupational epidemiology
- Disease cluster management, investigation and analysis
- Fundamentals of surveillance
- Critical appraisal of evidence methods

Risk assessment and management: the identification and quantification of the risk resulting from a specific use or occurrence of a chemical, taking into account the possible harmful effects on individual people or society of using the chemical in the amount and manner proposed and all the possible routes of exposure and the control of that risk.

- Acute and chronic response to the main types of environmental incidents and being able to recognize when to ask for help.
- Risk management and standard setting.
- The dimensions of hazards identification and risk characterization, risk management, risk communication.
- The principles of qualitative and quantitative risk assessment and ongoing monitoring
- The main modes of prevention and control of exposure to environmental hazards
- Cost effectiveness and cost benefit analysis and decision making prior to implementation of interventions
- Risks and regulations covering public and occupational ionizing exposure
- Risks associated with exposure to non-ionizing radiation

Environmental public health: the science and art of preventing disease, prolonging life and promoting health where environmental hazards are the key factor, through organized efforts of society

- The relevance of agencies and organizations and their roles
- Incident planning and incident team management.
- Major incident command structure and purpose of JHAC
- Environmental health policy at an international, national, regional and local levels

- National radiation emergency response arrangements
- Main elements of relevant legislation and regulation, e.g. air pollution, IPPC, land contamination and planning
- The principles of sustainability and relevance to public health
- The principles of Health Impact Assessment when and how to use it
- The resource implications of non-communicable incidents, what needs to be paid for and by whom and the ability to prioritize resource use to ensure cost effectiveness
- Managing the process, including media skills, risk assessment, communication and analysis
- Developing and implementing preventive programs and working towards reducing health inequalities and promoting social inclusion
- Communicating and involving the public and other stake holders through the appropriate channels
- Communication and media management
- Ensuring evidence-based activities

Examples of environmental toxicants:

- Endocrine disruptors

Occupational toxicology

National and international guidelines, new product registration and notification, safety requirements for transportation

Health and safety (COSHH)

18. Pesticides, chemicals, food and cosmetic safety
Specific Topics
Pesticides and plant biotechnology (GM food) • Legislation and data required for product registration • Principals of operator/user, environment and consumer safety
Chemicals safety: • national and international guideline • new product registration and notification • safety requirements for transportation
Food safety evaluation: • National and international legislations on food safety • Safety assessment process for food additives, supplements and food contact materials • Residue analysis • Processes and novel goods including genetically modified food and related environmental considerations
Cosmetic and consumer product safety: • National and international legislations on product and ingredient safety, • Test methods and their suitability and limitations for safety assessment • Principals of integrated safety assessment for finished products • Acceptability of in vitro methods

19. Medical toxicology (human clinical toxicology)

Specific Topics
Mechanism of action of classical toxins (e.g. heavy metals, cyanide, CO)
Epidemiology of acute poisoning
General methods for treating overdose; hemoperfusion, haemodialysis, forced diuresis
Specific antidotes, including chelating agents, for common poisons: limitations and the clinical and practical aspects of patient management (symptomatic and supportive care)
Signs and symptoms for common intoxications and accidental poisoning
Adverse drug reactions including methods of detection
Drugs of abuse
Medico-legal aspects of poisoning and drugs of abuse
Poisons Information Services
Analytical toxicology • Analytical methods for measuring chemicals and other biomarkers in biological fluids or tissue samples

20. Analytical and forensic toxicology
Specific Topics
Analytical toxicology
• Sample collection, transport and storage • Sample preparation procedures • Analytical methodology: colour test, UV/Visible spectrophotometry, GC, HPLC, mass spectrometry, immunoassay • Analytical procedures for trace elements • Analytical and Forensic aspects of alcohol and other drugs of abuse • Role of coroner/procurator Fiscal