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

FRCPath Examination

Toxicology Speciality

Subspecialty
Safety Evaluation Toxicology
Part I, Paper II

Curriculum

Regulatory toxicology
Specific Topics
Regulations • Detailed knowledge of published regulatory guidelines • Experience dealing with Health Authorites

In vitro and *in silico* (computer) prediction of toxicity
Specific Topics
In vitro and in silico (computer) prediction of toxicity • SARs

Genotoxicology
Specific Topics
Mutations and human health • The basic structure of human genome, the karyotype, gene organisation, genetic code, transcription, translation, gene regulation and mitochondrial organisation • DNA structure, DNA repair, replication fidelity, base selection, enzymes and components of replication, mismatch repair correction and replication errors • Cell cycles/division, mitosis and meiosis, male and female germ cell cycles. Targets for chemical interactions, cell division, spindle etc • Cell signalling errors. Types of mutations, base substitutions, insertions, deletions, micro/mini satellite changes, point mutations, frameshift and duplication • Transposons and significance • Structural chromosome/chromatid changes, types of rearrangements and modifications of the karyotype, gaps, breaks, translocations, exchange figures and fusions • Numerical chromosome changes, aneuploidy and polyploidy • Gene mutation changes in human germ and somatic cells (including multifactorial disease), examples of gain and loss of function mutations. Imprinting. Relevant examples of disease, nuclear and mitochondrial changes • Chromosome changes, structural and numerical in human congenital disease with relevant examples • Genetic changes in human cancer, oncogene and tumour suppressor gene changes • Chromosomal structural and numerical changes in human cancers, such as translocation and aneuploidy found in leukaemia. • Link between mutation and cancer, oncogenes (viral, cellular), tumour suppressor genes, cancer susceptibility, multi-step carcinogenesis and initiation-promotion • Chemical structure-activity-relationships (SAR) in carcinogenesis. Genotoxic vs non-genotoxic carcinogens, mechanisms and limitations • Germ cell genetic damage, heritable disease, monogenic polygenic, multi-factorial and X-linked • Role of mutation in other clinical conditions/diseases (atherosclerosis, reproductive effects, ageing and experimental models)

Testing methods in Genetic Toxicology

- Historical basis of mutagenicity testing, diversity of methods ranging from the use of viruses to whole animals. Information sources on the methods available in the literature
- Basic principles of genetic toxicology. Role and place of test methods in compound development. Selection of the key informative test methods.
- The concept of sensitivity and specificity. The international collaborative studies and the range of assays studied. Development and understanding of those methods with appropriate range of qualities and sensitivities for testing and the limitations of many methods. The handling of published data, limitations (e.g. lack of GLP compliance and assay validation).
- The development of Bacterial Gene Mutation Assay. Basic battery, specialised strains (e.g. oxidative damage, cross-linking agents), modifications (fluctuation tests)
- In vitro cytogenetics, human and rodent cultures. Basic metaphase analysis, structural and numerical changes, molecular methods, FISH methods, fluorescent probes
- In vitro micronucleus assay, structural numerical changes, centromere and kinetochore staining, binucleate assay, measurement of non-dysjunction
- In vitro gene mutation assays, HPRT methods, thymidine kinase (TK) mutations. The value of the mouse lymphoma assay, range of endpoints (chromosome and point mutation). Experimental design.
- The transition from in vitro to in vivo assessment, test selection. Factors that may influence the relationship between in vitro and in vivo responses
- In vivo methods, bone marrow cytogenetics, bone marrow and peripheral blood micronucleus assays.
- Comet assays in vitro and in vivo, tissue specificity, the role of the in vivo/in vitro rat-liver UDS assays. Use of DNA adduct measurements
- Structure activity models, application and development
- The transition to germ cell studies. Germ cell methods, dominant lethal, heritable translocation, specific locus assay. Female methodologies. Embryo analysis
- Transgenic animal mutation models. Application in somatic and germ cell studies
- Application of mutagenicity testing to the environment, terrestrial and aquatic models: species selection
- Selection and general application of statistical methods and report preparation

Lesions, repair and mutations

- Lesions produced by UV and ionising radiations (important as models)
- Chemically induced lesions, small adducts (e.g. alkylating agents)
- Bulky adduct formation (e.g. polycyclic aromatic hydrocarbons such as benzo(a)pyrene, natural compounds such as aflatoxin, synthetic molecules
- Endogenous lesions, oxidative damage (e.g. relationship to exogenous events, the spontaneous lesion background)
- DNA reactive anti-cancer drugs (e.g. cis-platin, tamoxifen) examples of non-DNA reactive anti-cancer drugs (vinca alkaloids, taxol)
- Detection methods (modified with development) currently 32P post labelling, Comet assay, immunological methods, radiolabelled compounds, mass spectrometry
- DNA repair, replication fidelity leading to mismatch repair. Identification of repair defects leading to colorectal cancer
- Base excision repair. Nucleotide excision repair, post-replication and double strand repair systems. Repair defective mutations and knock out.
- The human repair syndromes and their implications

- Conversion of DNA lesion into genetic and chromosome changes
- Detection methods such as single stranded conformation polymorphisms (SSCP). Restriction site mutation analysis (RSM), FISH, comparative genomic hybridisation (CGH) assays. Application to analysis of gene and chromosome mutations
- Shuttle vectors, specific mutation detection and analysis (e.g. HPRT). Development of mutation databases. Application to the analysis of mutation spectra and profiles
- Cancer mutation database and application. Relationship with mutation databases
- Gene expression models, application to the analysis of mutant genes

Metabolism, human and experimental animal variation

- Metabolism: Phase I and Phase II: oxidation, P450 families, in vitro activation systems and in vivo tissue specific
- Metabolism, principals of phase I metabolism (oxidation, reduction, hydrolysis and the enzymes involved)
- The cytochromes P450, history of identification and characterisation. Basic biochemistry. Classification cloning and sequencing.
- Genetic factors, polymorphisms and SNPs. Examples of variation
- Environmental factors, enzyme induction, inhibition
- Pharmacokinetics, pharmacodynamics and toxicokinetics
- Activation preparations, roles in genotoxicity testing
- Reactions and enzymology of phase 2 metabolism, basic conjugation reactions
- Influence of metabolism on the effects of genotoxin exposure
- Genetic engineering of cell lines. Development and application
- Enzyme variation, genotyping, pharmaceutical development

Data analysis

- Test systems and endpoints, test designs. International guidelines. Design improvements. Questions to answer in analysis of data. Principals of analysis relevant to specific studies
- Test validity. Integrity of data. Tabulation. Negative and positive controls. Generation and use of historical database. Value of repeat tests.
- How to decide if treatment values exceed the negative control. Statistics, variables, random sampling, variation, distribution, confidence limits, null hypothesis, errors (alpha, beta), power, one-and two-tailed tests, multiple comparisons, parametric and non-parametric, underlying assumptions (and violations), chi-squared approximation, data transformations, common methodologies used in routine tests.
- How to decide if changes are treatment-related? Test for positive trends, relationship of trends test with tests for central tendencies, non-parametric trend tests (e.g. Kendall's coefficient, Spearman's coefficient, Joncheer's test, Cochrane-Armitage etc), parametric trend test (e.g. regression analyses, William's test). Artefacts.
- The determination of whether a treatment-related increase is biologically important? The understanding of hazard versus risk. The understanding of the use of historical control, negative control data (ranges, difference between duplicates etc). the importance of ADME in risk evaluations
- Relative importance of assays and endpoints. Permutations of outcome. Problem areas, invitro positive versus in vivo negative, one in vitro assay proves negative while a second is positive.

Study design, quality and interpretation

- Need for short-term tests, purpose of screening, protocol design, dose-response relationships and data interpretation
- Genotoxic and non-genotoxic activity. Direct and indirect mechanisms, DNA and non-DNA targets

- Criteria for the definition of positive, statistical significance fold increase, relationship to historical databases of control and treated values. Assay reproducibility. False positives and negatives
- Dose response relationships, exponential and threshold models, factors which include a threshold, nucleoside analogous, aneugens, topoisomerase inhibitors, detoxification, repair etc
- Influence of toxicity, acute and chronic toxicity, high and low toxicities
- The development of optimal designs. Quality control, the activity of the International Workshop on Genotoxicity Testing and the criteria for test system validation
- Operation of regulatory bodies
- Strategies for testing
- Test batteries and the problems of conflicting data, weight of evidence considerations
- Potency in in vitro and in vivo tests, potential for human risk estimation
- Artefacts in the in vitro and in vivo assays. False positive and negative
- Biological relevance of responses in vitro and in vivo, influence of animal species-specific metabolism, S9 modifications

Reproductive toxicology

Specific Topics

Background knowledge

- Anatomy, structure and physiology of the male and female reproductive tracts
- Hormonal control (endocrine and paracrine) of the reproductive process (function, gametogenesis, steroidogenesis, extragonadal gamete maturation and delivery. Integrated function and behaviour.

Comparative reproductive physiology of experimental species and humans

Mechanisms of Reproductive and Developmental toxicity

- Congenital malformations at the molecular, genetic and cellular level of embryogenesis
- Functional inter-relationships and the importance of syndromes of malformation, typical malformation syndromes induced by teratogens.

Reproductive toxicity

- Testicular abnormalities: consequences of action on specific cell types in the testes
- Ovarian or uterine abnormalities

Developmental toxicity

- Embryofetal development: the stage of development affected. Consider relationships between malformation in different organ systems and the maturational sensitivity and interdependence of different systems (skeletal, GIT, genitourinary)
- Post-natal development of offspring

Use examples of classical reproductive toxicants (drugs, chemicals) for illustration

Practical testing for reproductive toxicity

- Importance of animal husbandry, animal identification, animal housing design, photoperiods, nutrition and environment
- Mating techniques for different species, determination of copulation, vaginal smears
- Caesarean section examination and PM techniques. Corpora lutea evaluation. External examination of the foetus and placenta., Methods for internal examination of foetuses for visceral and skeletal defects.
- Importance of fixation methods for foetus, tests and accessory sex organs. Methods for semen evaluation
- Histological examination of the male and female reproductive tract including principals of staging spermatogenic cycle in the male and oestrus cycle in the female ovary, uterus and vagina
- Cross fostering studies
- Requirements for different laboratory animal species for testing
- Importance of organ weights in reproductive toxicity
- Methods to investigate mechanisms of reproductive and developmental toxicity (utero-placental function, hormonal disturbance, vascular and amniotic factors, germ cell interactions
- Utility of in vitro systems

The regulatory requirements for assessing potential reproductive toxicants

- The role of the laboratory studies in reproductive hazard evaluation.

Interpretation of data and the risk assessment process for reproductive toxicants • Knowledge of the different parametric and non-parametric statistical methods • Relationships in the analyses of litter and foetal data • Incidence of spontaneous congenital abnormalities in different species and strains and importance of concurrent and historical control data. • Importance of maternal toxicity and embryotoxicity when interpreting dose-response relationships
Environmental occupational • Occupational hazards from reproductive toxicants • Role of environmental oestrogens and androgens