Overview – Internal review

- Presentation title: Introduction to toxicology Assessment
- Track title: Contamination / toxicology
- Speaker: Andrew Bartholomaeus
- Date / Time: Tuesday
- Time allotted: 11:00 -12:30
- Dot point overview:
  - A basic introduction to the toxicology relevant to establishing a PDE or OEL
  - Introduction to the basic vocabulary and concepts
  - General overview of the types of studies and toxicological endpoints that must be considered
  - Where to get the data to support the toxicology assessment
  - Establishing a point of departure (NOAELS

RISK IDENTIFICATION FOR MANUFACTURE IN SHARED FACILITIES

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Introduction to Toxicology Assessment

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Basic Concepts

$$\text{risk} = f(\text{hazard}*\text{exposure})$$

Key Terms

- **NOAEL** - No Observed Adverse Effect Level
  - The highest dose in a study at which no adverse effects of treatment are observed
- **LOAEL** – Lowest Observed Adverse Effect Level
  - The lowest dose in a study at which adverse effects were observed
- ADI, TDI - Acceptable or Tolerable Daily Intake (oral) – food & food contaminants
  - The amount of a substance that can be taken orally every day for a lifetime without appreciable toxicological effect
- **PDE** / ADE -Permitted (or Acceptable) daily Exposure
  - A dose that is unlikely to cause an adverse effect if an individual is exposed every day for a lifetime
  - Route specific but not limited to oral intake
  - Equivalent to Acceptable Daily Exposure
- **OELs** Occupational Exposure Limits
- TLV, PEL – Threshold limit value, Permissible Exposure limit
- **OEB – Occupational Exposure Band**
- **LD_{50}**: lethal dose for 50% of animals treated
- **Historical Controls**
  - A collation of data from control groups of the same strain, sex, source and age of animal constructed over a period of time
  - Provides normative data for the range of standard experimental parameters

Defining toxicity

- **Everything is toxic** at some level of exposure by some route
  - Oxygen, Nitrogen and water are all toxic
- The Objective of Toxicological Risk Assessment is Identification of the boundary (zone) between safety and toxicity
- How toxic an agent is will depend on:
  - Physical and chemical properties of the toxicant
  - Route of administration
  - Dose, duration and frequency of exposure
  - How system metabolises and eliminates the toxic agent (ADME)
  - Susceptibility of subject to toxic agent
  - Local versus systemic toxicity
- **DOSE METRICS** are defined by
  - How much is given/taken (total dose)
  - Per kg of body mass (dose per unit of body weight)
  - Over what period (the duration of the exposure eg iv infusion over 1 minute or 1 hour)
  - Frequency (constant, hourly, daily, weekly etc)
  - Duration – for a week, month, year, lifetime

Drug Toxicity

- Drugs may exhibit toxicity due to
  - Excessive pharmacological action (primary pharmacology)
    - Clinically predictable/manageable
    - Occurs at the upper dose range for the individual patient
  - Secondary pharmacological effects due to lack of specificity for the primary target (receptor)
    - Generally clinically manageable
    - Generally occurs across a defined dose range
  - Off target toxicity unrelated to the primary pharmacological action
    - May be idiosyncratic and unpredictable
    - Often not readily managed clinically
    - May occur in some patients at low or sub therapeutic doses

Guidelines Applicable to APIs

- Preclinical studies on APIs generally need to be conducted in accordance with specific guidelines on study design, the studies required by specific regulators and on GLP certification
  - **OECD**
    - Industrial chemicals, pesticides, some drug studies
  - **ICH**
    - Human pharmaceuticals
  - **VICH**
    - Veterinary pharmaceuticals
  - EMA (European Medicines Agency
  - FDA (US Food and Drug Administration)
  - TGA (Therapeutic Goods Administration) – mostly adopts from EU and USA

OECD – Guidelines

OECD Guidelines for the Testing of Chemicals

- Test Guidelines
- Test Guidelines that have been deleted or replaced by updated versions
- Terminology in OECD Test Guidelines to designate what is tested
- Material Transfer Agreement (MTA): Test Guidelines with components covered by MTA

Test Guidelines

The OECD Test Guidelines are a collection of the most relevant internationally agreed test methods used by government, industry and independent laboratories to determine the safety of chemicals and chemical preparations, including pesticides and industrial chemicals. They cover tests for the physical-chemical properties of chemicals (section 1), environmental effects (section 2), degradation and accumulation in the environment (section 3), human health effects (section 4), and other areas (section 5 for Test Guidelines which do not fall within the four sections).

Section 1: Physical Chemical Properties
English; French

Section 2: Effects on Biotic Systems
English; French (SOFTWARE FOR TG 223)

Section 3: Degradation and Accumulation
English; French

Section 4: Health Effects
English; French (SOFTWARE FOR TG 455, TG 432 AND TG 425)

Section 5: Other Test Guidelines
English; French

–http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

ICH - Pharmaceuticals

Safety Guidelines / ICH Guidelines / Work Products /

ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.
Zip file with all ICH Safety Guidelines in Word format

- Carcinogenicity Studies S1A - S1C
- Genotoxicity Studies S2
- Toxicokinetics and Pharmacokinetics S3A - S3B
- Toxicity Testing S4
- Reproductive Toxicology S5
- Biotechnological Products S6
- Pharmacology Studies S7A - S7B
- Immunotoxicology Studies S8
- Nonclinical Evaluation for Anticancer Pharmaceuticals S9
- Photosafety Evaluation S10
- Cross-cutting Topics

ICH Guidelines / Work Products /

The ICH topics are divided into four categories and ICH topic codes are assigned according

Quality Guidelines

Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.

Safety Guidelines

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Efficacy Guidelines

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines.

Multidisciplinary Guidelines

Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

Data Quality: Good Laboratory Practice (GLP)

- Codes of GLP ensure that the processes of design, conduct and documentation of nonclinical studies are of a very high standard
  - But do not of themselves make a study good or bad
- GLP-compliance not required for pharmacodynamic or pharmacokinetic studies, but routinely expected for toxicity studies
  - Published studies not conducted by the innovator will not usually be GLP
- In toxicological assessment, more weight is generally given to GLP-compliant than non-compliant studies
  - But common sense and judgement need to be applied here
- Ideally all pivotal safety studies should be under GLP conditions
- GLP Guidelines specify documentation details and processes to provide a high level of QA and transparency of process
- **Provides no reassurance of quality of study conduct**, ie competence, but does facilitate detection of fraud and poor study design or execution.

Critical elements of toxicity studies

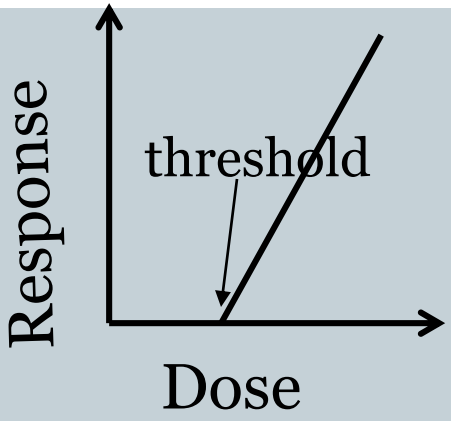
- **Dose**:
  - The **amount** of a substance administered per unit of **weight/animal/surface area** and per unit of **time** at a given **frequency** for a specified **duration** by a specific **route**
  - A dose is meaningless without specifying **all of these parameters, eg**
    - 5 mg/kg bw/day for 5 days orally
    - 1 mg/kg bw eight hourly for 1 month by ip injection
- **Response**:
  - Effects in the test species from the administered substance; Response can be pharmacological, toxicological or physiological/adaptive.
- Dose-response **relationship**
  - establishes causality that a substance has in fact induced the observed effects
  - establishes the lowest dose where an induced effect occurs and the highest dose where no effect occurs- the threshold effect and no-effect dose
    - Beware however that these are partially artefacts of study design
  - Indicates the rate of increase of injury with increased dose - the slope for the dose response.
    - Gives some indication of leeway in exposure – ie does a small increase in dose cause a large or small increase in injury

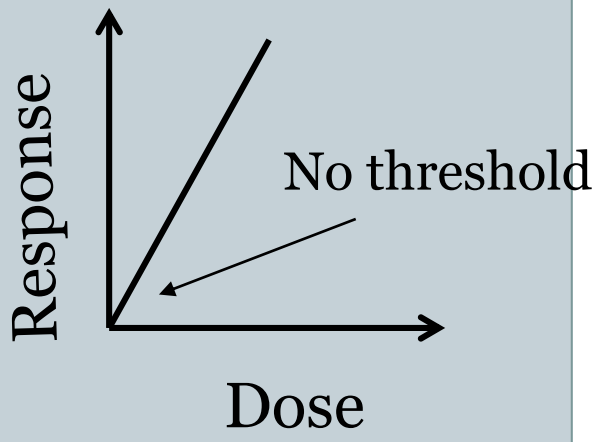
Animal versus human exposure (Dose)

- Not all tissues/effects will be best measured by the same exposure parameter
  - mg/kg bw may be a better comparator for
    - GIT effects likely to be due to purely local concentrations
    - Effects on the liver and kidney - may be related to the total flux of drug rather than the AUC
    - Bone toxicity - osteoporosis drugs generally concentrate in the bone and levels are not directly proportional to blood AUC values
- Exposure ratio = AUC or C_{max} in animals / AUC or C_{max} in humans
- This may be determined at the:
  - NOEL (No Observable Effect Level), and/or
  - NOAEL (No Observable Adverse Effect Level), and/or
  - LOEL (Lowest Observable Effect Level) for a given toxicity, and/or
  - HD (highest dose tested)
- Safety margin = ratio of exposure at no-effect dose in animals *cf* the exposure at the maximal human dose

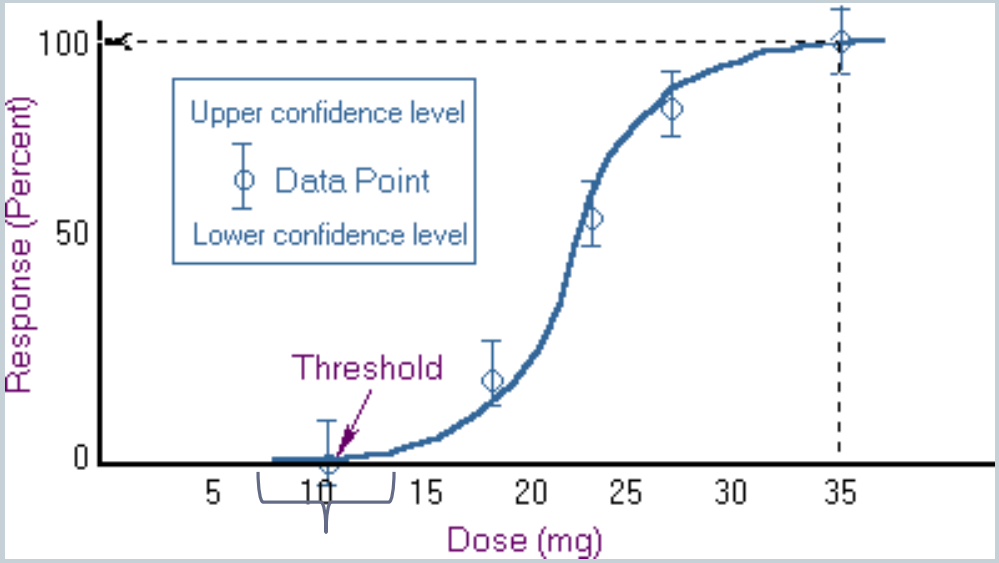
Two models of dose response

- **Threshold**: when the data indicates a threshold below which no adverse effects can be measured.
- **Non-threshold**: when the data cannot identify a threshold for adverse effects. (In this case, the dose-response curve may be assumed to pass through zero).
  - In practice all effects are likely to have a threshold but this may be at exceptionally small doses/exposures for some effects such as genotoxicity
  - The non threshold approach is a regulatory policy determined by government directives in the USA – it is a matter of some contention and disagreement amongst academic toxicologists





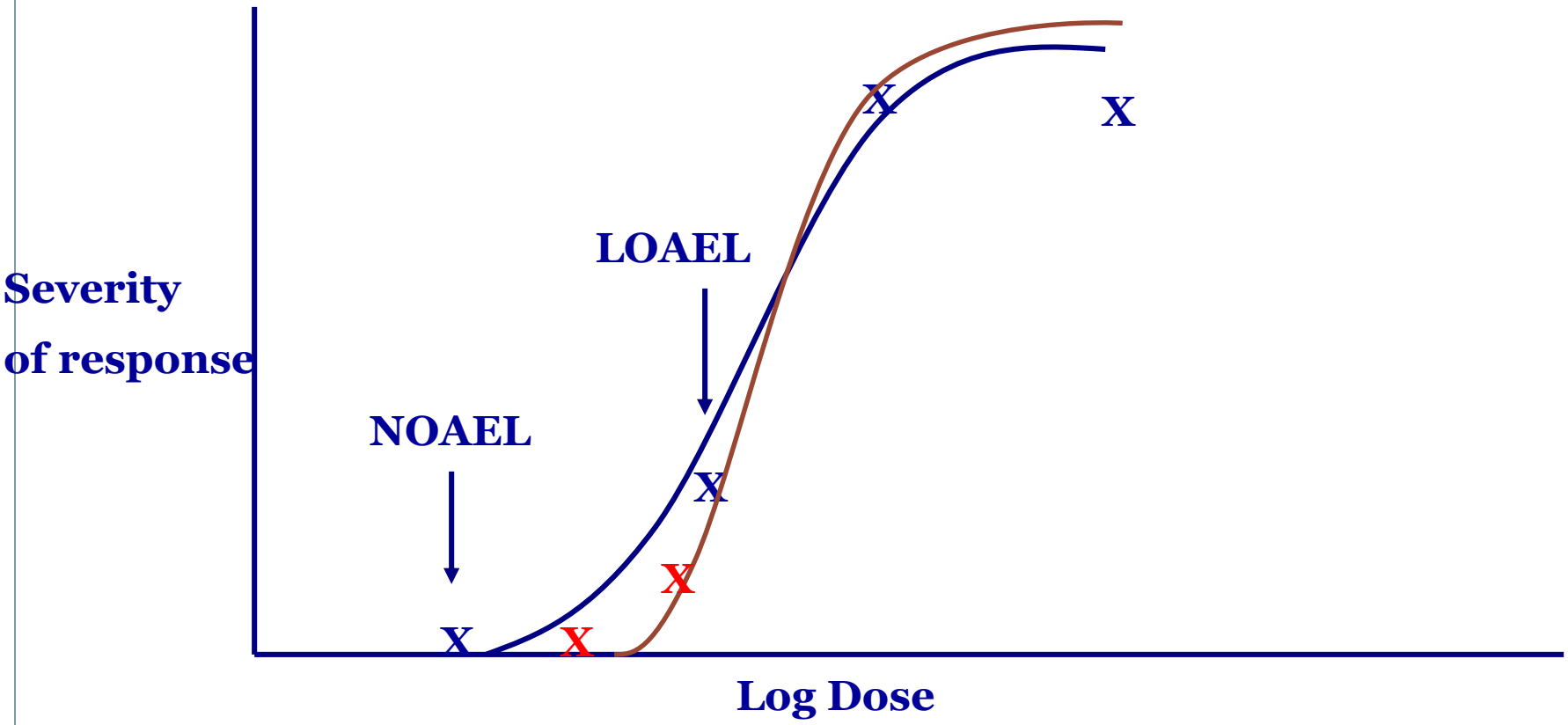
Dose Response Relationship



•Dose-response-relationship between magnitude of dose and incidence or severity of adverse effect

NOAEL and LOAEL

- **No Observed *Adverse* Effect Level (NOAEL)**
  - **The highest dose at which no adverse effects were observed**
- **Lowest Observed *Adverse* Effect Level (LOAEL)**
  - **The lowest dose at which adverse effects were observed**
- They are the ***actual data points*** from experimental animal studies or human clinical trials.
- Partially **artefacts of dose selection** and statistical power of the study
- NOAEL and LOAEL are pivotal in the conduct of risk assessments
  - Used as Points of Departure **PoD for PDE estimation**

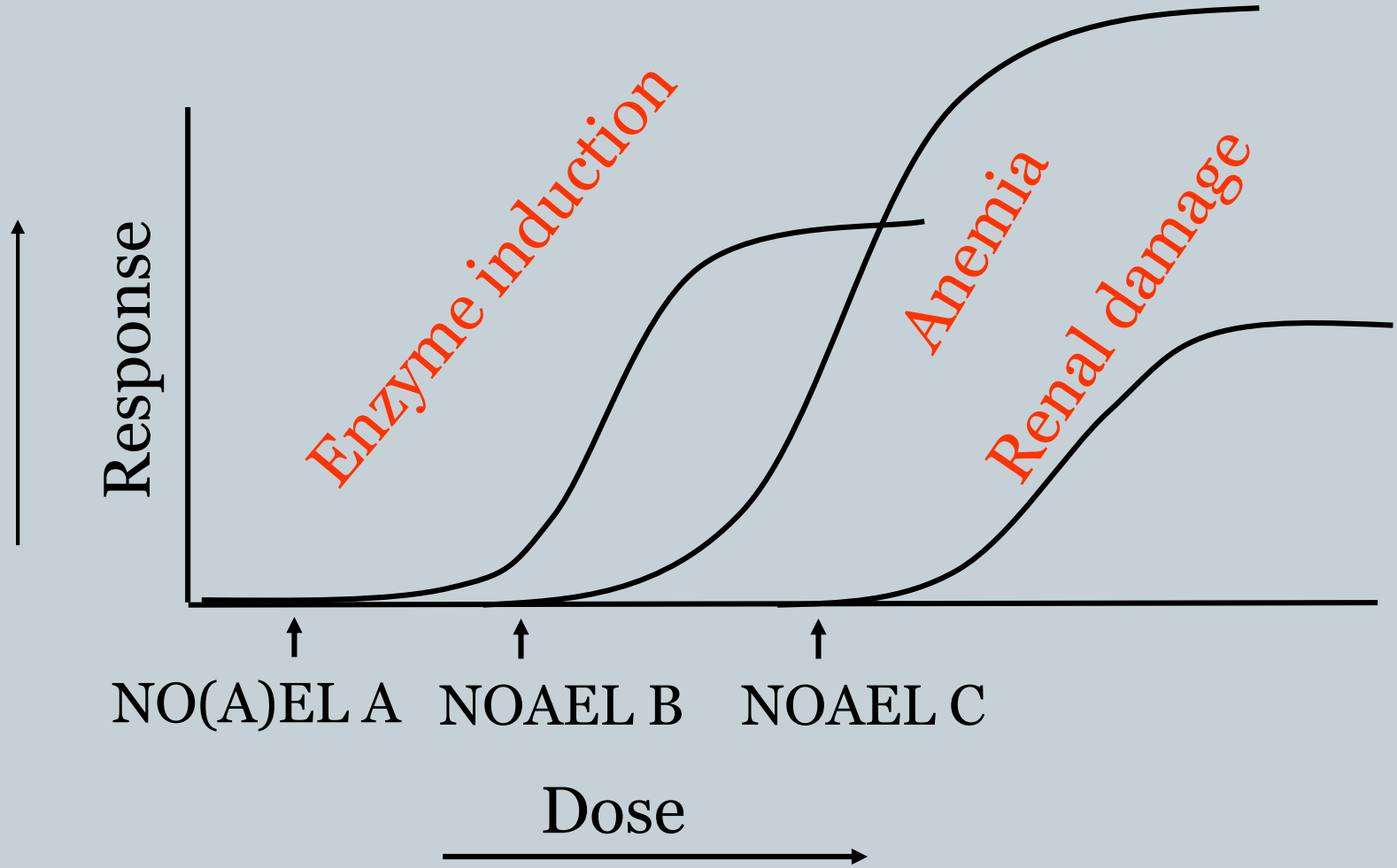


Threshold - lowest dose which causes an effect, below this dose, no toxicity is observed

NOAEL – highest dose employed in a study at which no adverse effect was observed (derived experimentally)

LOAEL - lowest dose at which there was an observed adverse effect (derived experimentally)

Each toxicological or pharmacological end point (effect) may have its own dose response curve



Threshold Approach

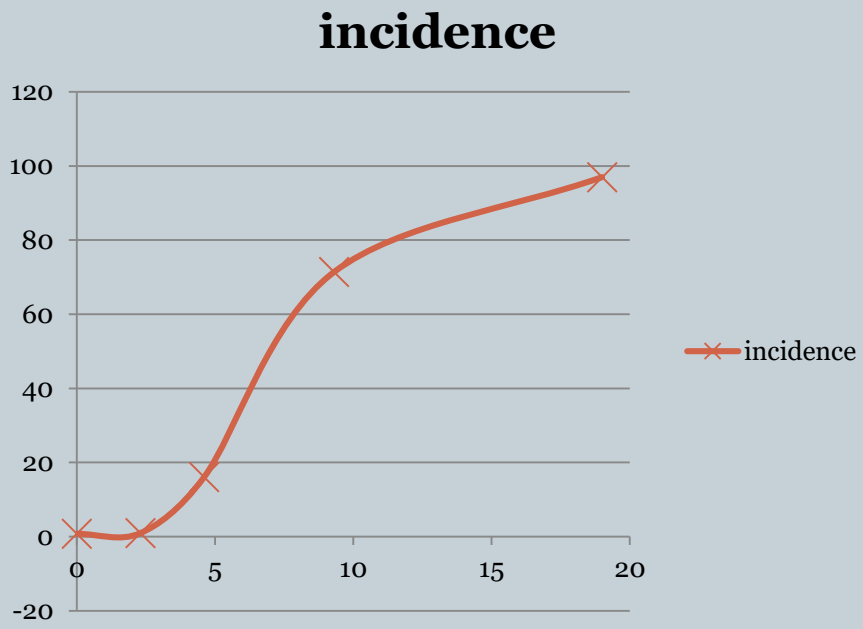
- Used to derive PDE, (ADI, TDI etc) for
  - Non-genotoxic carcinogens
  - Non-cancer effects
    - Where a threshold occurs due to biological mechanisms such as;
      - Excretion, metabolism,
      - repair or adaptation
- A very high likelihood that no effect will occur in humans at or below threshold level (after adjusting for uncertainty)
- The Threshold approach doesn't attempt to calculate a numerical level of risk at low exposure (ie not quantitative)

Threshold Approach

- **Advantages**
  - NOAEL relatively easy to determine
  - Avoids the need to quantify the risk
  - Default uncertainty factor of 100 is generally highly conservative
    - Assumes all uncertain acts to increase risk in humans (unlikely)
  - Long history of use to protect public health
  - Crude, simple, easily explained, works reliably in almost all circumstances
- **Disadvantages**
  - NOAEL depends on dose levels used
  - Depends on biological effects monitored (if you do not look you may not see)
  - Limited by experimental protocol
    - statistical & interpretive Power
    - Relevance of the animal or other model to humans
  - Limited use of dose response slope
  - Choice of uncertainty factor largely arbitrary with poor substantiation
  - Based on a Point estimate

Dose Response

Mg/kg bw/d	0	2.3	4.6	9.3	19
No pregnant animals	20	22	21	23	21
Foetal weight	3.41	3.32	3.33	3.31	3.16
Foetuses	122	126	118	133	131
% Foetuses with cardiac **dysplasia**	0.8	1.0	16.1	71.4	97.0



Dysplasia = abnormal development & probably abnormal function

Selecting LOAELs

LOAELs

Dose (mg/kg/day PO)	Control	15	75	125
Liver (n=15)				
Multinucleated hepatocyte, Minimal	0	0	1	4
Testes (n=15)				
Spermatid degeneration, Minimal	0	0	7	15
Sertoli cell vacuolation,				
Minimal	0	0	3	8
Mild	0	0	1	1
Moderate	0	0	0	1

Interpreting toxicity studies

Interpreting toxicity studies

- Questioning the results
  - Validity of older studies (pre 1970)
  - Species-specific effects
  - Statistical versus biological significance
  - Weight of evidence-judgement
  - adequacy, validity and appropriateness of data base.
- Is the effect a physiological or toxicological one?
  - Physiology
    - Variation within limits of normal function (e.g. variation in liver enzymes following a meal).
  - Toxicology
    - Reversible/irreversible ?; Injurious ? and therefore adverse and harmful

Remember

- Hazards are the potential adverse effects a chemical is capable of causing, risk is the likelihood of that occurring at various exposures
- All substances are hazardous under certain conditions of exposure (route & dose)
- Sometimes need only small amounts for it to be a risk.
- Toxicity is a function of exposure and intrinsic hazard - dose metrics are very important

Safe Level of Exposure (PDE)

Clinical and Preclinical
Toxicity study Data Set

↓

No Observed Adverse Effect Level(s) (NOAEL)
Based on the most sensitive adverse effect(s)* in the most sensitive animal study for that effect (mg/kg bw/day)

↓

Uncertainty factors:

↓

Safe level of intake for humans for a lifetime.
PDE (mg/kg bw)

* Under some circumstances you may need different PDEs for different sub populations

Setting a PDE

- Establishment of a PDE
  - the amount of a substance in a medicine that can be administered daily over a lifetime without appreciable health risk
  - Highly conservative (safe) value
    - Assumes the substance is administered every single day of a persons life from infancy until death (**VERY** unlikely for an API as contaminant)
    - Assumes all uncertainty acts to increase the sensitivity of humans compared to the test animals (also very unlikely)
- Other aspects of the Risk assessment such as exposure assessment add additional layers of conservatism

Species Variation

- **Dose vs. plasma concentration**
- **Sildenafil [Viagra]:**
  - Mouse: 1.0 mg/kg PO dose - plasma AUC 31 ng.h/ml
  - Human: 1.0 mg/kg PO dose - plasma AUC 815 ng.h/mL
  - Cause of the difference: Faster clearance in mice (91 ml/min/kg) than in humans (9.8 ml/min/kg)
  - Consequence: significantly higher oral doses would need to be administered to mice in the evaluation of the potential adverse effects in humans.
  - Preclinical safety testing: Oral doses up to 200 mg/kg bw/day were studied in mice cf. clinical dose of 50 mg (1 mg/kg for a 50 kg patient).

Statistical Versus Biological Significance

- Scientists often use a p value of 0.05 to justify a conclusion that an effect is more likely to be real rather than a chance variation
- What this actually means however is that there is a 5% (or 1 in 20) chance of the finding being due to simple random variation - or put another way if the study was run multiple times the finding would occur on average in 1 in 20 studies by random chance alone
- In a toxicology study there are hundreds or even thousands of parameter combinations being measured so it is inevitable that a number of parameters will vary from control simply by random chance
- This is where the concept of biological significance comes in. A finding might be concluded to be biologically significant if for example:
  - There is a dose relationship
  - The effect is consistent with that on related parameters
  - A similar effect is seen in other studies or in other species
  - The magnitude of the effect is outside that observed in historical controls

Statistical vs biological vs toxicological significance

- A lack of statistical significance ≠ no effect
  - the study may lack statistical power
  - if pre-treatment values are available compare these to the treated values for individual animals (unlikely to be possible for most published studies)
  - look for similar findings across studies/species if possible
  - look for correlated findings eg spleen enlargement + anaemia
- Not all substances which cause cancer in animal studies are "carcinogens"
  - altered hormonal balance, persistent irritation, persistent stimulation of a tissue can cause cancer by non (primary) genotoxic mechanisms
  - the mechanism involved may not be relevant to human exposures
- Statistical significance ≠ Biological significance
  - eg in a full chronic toxicity study many thousands of end points are measured so statistically significant results will occur at random - look for trends over time and doses
- Biological significance ≠ Toxicological significance
  - a small change in a parameter at high doses which remains in the normal range may be treatment related but not adverse & ∴ not toxicologically significant
  - A clear treatment related adverse effect may still not be toxicologically significant
    - eg pregnant rabbit gavage dosed with a bad tasting irritant substance, stops eating for 2 days then resumes normal eating
    - pups have decreased body weights, decreased survival and increased anomalies (eg delayed development)
    - effect is 2^0 to maternal nutritional deficit
    - What if overt terato-genesis is seen? Eg cleft palate
      - May need specific studies on the effect of nutritional deficit at the same time on development

Species Specificity

- In general the animal models in use ARE highly predictive of effects in man but there are some important exceptions
- Most species have some natural susceptibility to diseases which may differ quantitatively or qualitatively to man
  - eg thyroid follicular cell tumours - rats more sensitive
  - liver tumours - mice more sensitive
- We can use historical control data to provide some indication of natural propensities but remember genetic drift.
- Animal characteristics
  - species, strain, source, age, sex, pregnancy
  - background incidences & natural disease susceptibility
  - suitability as a model for humans
  - route and nature of administration
    - diet, drinking water, gavage, parenteral, topical
    - relevance to human exposure route

“Historical Controls”

- aggregated incidences of ‘spontaneous’ pathological values collated from control groups over a period of time
- to be of value they must be
  - same sex, age, species, strain, source, nutritional status, dosing vehicle
  - should be as temporally close to the comparator study as possible (genetic drift)
- Provide a measure of ‘background’ incidences and species variance
- Particularly useful for
  - small group sizes (but first consider comparison against pre treatment values where available)
  - assessing low incidence, dose related, non significant effects
- CAUTION; The more studies in the HC database the greater the range between extremes
  - therefore more likely for a treatment related effect to fall within the ever broadening range
  - comparison against the mean + 2 SD may often be more appropriate
- Dosing vehicle (eg corn oil) may not be without physiological effect
- Genetic drift may quickly render HC data non comparable
- Some parameters change with age so need to age match the HC data

Validity of Findings

- Questions to ask of positive results
  - is the effect real?
  - what is the possible/probable/proposed mechanism?
  - is it likely to occur in humans at clinical exposures?
  - is there a specific population at risk
    - eg poor/extensive metabolisers?
- Questions to ask of negative results (often related to study design)
  - was sample size insufficient?
  - was exposure inadequate (both degree & duration)?
  - were appropriate parameters measured?
  - is the animal model unsuitable?
  - are there differences in animal *cf* human metabolism?
  - is pre-existing pathology absent?
  - does tolerance develop?

Study Types

RANGE OF STUDIES NORMALLY CONDUCTED

PHARMACOKINETICS

ACUTE TOXICITY

REPEAT DOSE TOXICITY

CARCINOGENICITY

GENOTOXICITY

REPRODUCTION

DEVELOPMENTAL

CLINICAL

Studies Providing Toxicology Data

- Preclinical
  - *In Silico* - ie computer modelling
    - Unlikely to be useful for PDE determination
  - *In vitro*
    - most important are genotoxicity assays
  - *In vivo*
    - Animal studies will often yield key data for PDE determination but can be hard to obtain the data
- Clinical
  - Always important but may not cover some of the critical endpoints (reproduction and development)

STANDARD PRECLINCAL STUDIES

- Primary pharmacodynamics
  - Mechanism, comparators, implications
- Secondary pharmacodynamics
  - Mechanism, comparators, implications
  - Cardiovascular and respiratory effects
  - Autonomic and Central Nervous system
  - Gastrointestinal
  - Renal
  - Endocrinological
  - Immunological
- Pharmacokinetics and metabolism
  - Species comparisons
  - Effect of repeat dosing
  - Effect of route of administration, vehicle, dose response

PRECLINCAL STUDIES - 2

- General Toxicity
  - Acute
  - Local
  - Subacute and chronic
- Carcinogenicity
- Genotoxicity
  - Gene mutations
  - Chromosomal damage
  - (DNA damage)
- Reproductive toxicity
  - Pharmacokinetics
  - Fertility
  - Oraganogenesis
  - Peri and postnatal development
- Additional studies

In vitro Studies

- Generally cheaper than animal studies and more consistent (reproducible)
- May require animal tissues but generally reduces animal use
- Often adaptable for high throughput screening
- All have some, generally considerable, limitations in predictivity (also somewhat true for animal studies)
- Irritation
  - Eye
    - Chemistry (strong acids or alkalies irritant)
    - Excised living bovine cornea
    - Cell culture
    - Chicken egg chorioallantroic membrane
    - Enucleated chicken eye
  - Skin
    - Simple chemistry
      - eg strong alkalies are known skin irritants so no need to test further
    - Keratinocytes in culture
    - Skin organ culture
    - Reconstituted human skin models
- With the exception of genotoxicity studies, *in vitro* studies will generally not be important in establishing a PDE

ANIMAL TOXICITY STUDIES

- Majority of toxicity data is obtained from animals, primarily rodents and dogs
- Monkeys and other species generally used sparingly
- Acute Toxicity Studies

es

humans,

or OH&S) and the pharmaceutical route of

& giunea pigs, rabbits , - dogs, & monkeys may also be used less

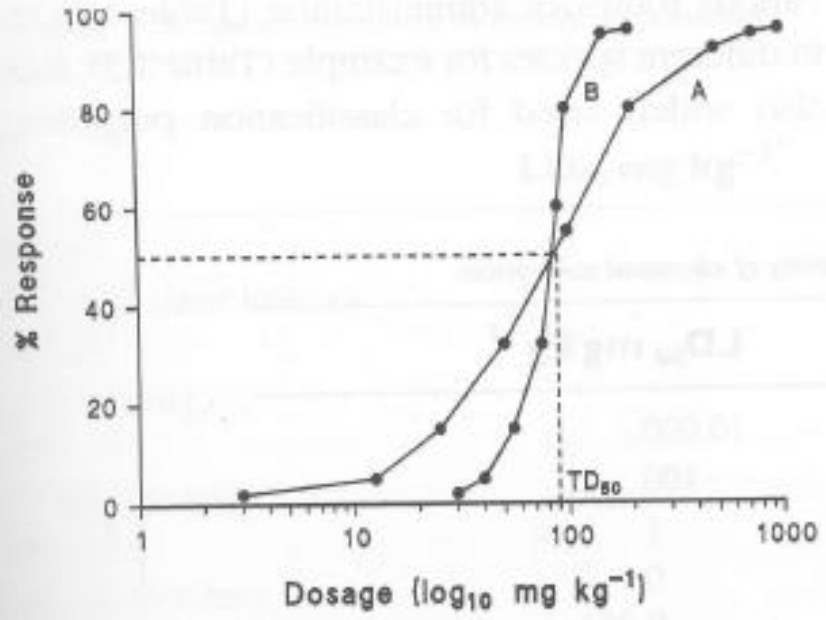


FIGURE 1.6 *Comparison of the toxicity of two compounds A and B. Although they both have the same TD_{50} compound A is more* potent *than compound B.*

proximate LD_{50} values for a variety of chemical substances

ound	LD_{50} mg kg^{-1}
	10,000
	100
e	1
otoxin	0.1
	0.001
us toxin	0.00001

TABLE 1.2 *Effect of route of administration on the toxicity of various compounds*

	Pentobarbital[1]	Isoniazid[1]	Procaine[1]	DFP[2]
Route of administration	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}
Oral	280	142	500	4.0
Subcutaneous	130	160	800	1.0
Intramuscular	124	140	630	0.9
Intraperitoneal	130	132	230	1.0
Intravenous	80	153	45	0.3

[1] Mouse toxicity data.
[2] Di-isopropylfluoro phosphate; Rabbit toxicity data.
Source: T. A. Loomis (1968), *Essentials of Toxicology* (Philadelphia: Lea & Febiger).

If LD_{50} at lower concentration for IV than for oral this implies that metabolism is important for detoxification of this chemical
May also be a Cmax effect or bioavailability effect
If LD_{50} higher (less toxic) for IV than oral what does this imply ?

Study Types

PHARMACOKINETICS

Pharmacokinetics

Ideal key data requirements

- Absorption and bioavailability
  - For test species yielding pivotal NOAELS
  - For humans
  - For all relevant routes of exposure in each species
- Interspecies/strain differences
  - Any relevance for cross species extrapolation
- Gender differences
- Linearity
  - If absorption is saturated is this above or below the NOAEL
- Accumulation
  - Does the drug build up, bioaccumulate, eg bisphosphonates in bone, and this potentially adverse
- Half life
  - If a once daily medication (long half life) contaminates a thrice daily medication (short half life) could this result in build up in the contaminant blood levels

Animal versus human exposure

- Not all tissues/effects will be best measured by the same exposure parameter
  - mg/kg bw may be a better comparator for
    - GIT effects likely to be due to purely local concentrations
    - Effects on the liver and kidney - may be related to the total flux of drug rather than the AUC
    - Bone toxicity - osteoporosis drugs generally concentrate in the bone and levels are not directly proportional to blood AUC values
- Exposure ratio = AUC or C_{max} in animals / AUC or C_{max} in humans
- This may be determined at the:
  - NOEL (No Observable Effect Level), and/or
  - NOAEL (No Observable Adverse Effect Level), and/or
  - LOEL (Lowest Observable Effect Level) for a given toxicity, and/or
  - HD (highest dose tested)
- Safety margin = ratio of exposure at no-effect dose in animals *cf* the exposure at the maximal human dose

REPEAT DOSE TOXICITY STUDIES

Repeat Dose Toxicity

- Identify most sensitive target organ(s) of toxicity
- NOAEL and LOAEL for these effects in the test species and AUC at these doses (to estimate Human Equivalent Dose HED)
- Likely to be the most difficult data to locate for older drugs
  - For newer drugs may be covered in and EPAR or FDA review
- Is there any evidence for this toxicity in human clinical trials or post market review

Group sizes

- The greater the number of animals/subjects per group the greater the statistical power
- For small group sizes, (low statistical power) ideal is to have pre-treatment (baseline) investigations
  - Each animal then serves as its own control
- Number of groups also affects power (trend analysis)
- For some serious but rare pathologies no practicable group size will ever have sufficient power to detect even substantial increases in incidence (eg gliomas in rats)
- Dose selection can compensate for (or increase) statistical power in some circumstances
  - Increased magnitude of effect reduces the number of subjects to reliable detect that effect

REPEAT-DOSE TOXICITY STUDIES

- **Species:**
  - Two mammalian species, one of which must be non-rodent.
  - Rats are usually preferred for oral and inhalation, rabbits and mini pigs for dermal, dogs for oral and intravenous, monkeys for biologicals…
  - Pharmacokinetics, mode of action, species-specific factors *etc*. should be taken into account when choosing an animal model for toxicity testing.
- **Age**
  - Generally young healthy adult animals
  - May use juvenile animals for products intended for children
  - Or where toxicity specific to a life stage is of concern
- **Duration**
  - Short term usually 2- 4 weeks
  - Long term up to a year
  - Chronic, lifetime
- **Dose levels**
  - Usually three Sometimes (4) dose levels plus a control group.
    - The highest dose should cause target organ or non specific toxicity
    - The lowest dose should cause no toxicity (NOAEL), but for a drug is ideally sufficient to produce a pharmacological/therapeutic effect and is associated with an AUC (ie systemic exposure) comparable to the maximum anticipated human value.
    - Doses usually escalate at approx multiples of √10 (3.2X)
- **Group size**
  - 10/sex/dose for rodents, 4/sex/dose for non-rodents in short-term studies.
  - 20/sex/dose for rodents, 4 – 6 /sex/dose for non-rodents in long-term studies (usually including recovery subgroups).
  - Satellite groups are also used for toxicokinetic testing.
  - If the product is intended to be used clinically in only one gender (*e.g*. oral contraceptive), then use of the relevant gender alone may be acceptable.

Duration of repeat-dose toxicity studies to support clinical trials

Duration of repeat-dose toxicity studies to support Phase I & II clinical trials in EU		
Duration of clinical trials	Minimum duration of repeat-dose toxicity studies	
Single dose	2-4 weeks	2 weeks
Up to 2 weeks	2-4 weeks	2 weeks
Up to 1 month	1 month	1 month
Up to 3 months	3 months	3 months
Up to 6 months	6 months	6 months
>6 months	6 months	Chronic

You may be able to get some information about the short term repeat dose toxicity studies from published reports on phase I trials

REPEAT-DOSE TOXICITY STUDIES
Observations

- Pre-treatment (baseline) values (especially important in wild-caught large animals eg primates)
- Mortality and clinical signs of toxicity (appearance & behaviour)
- Body weights, food and water consumption
- Laboratory tests (Clinical Chemistry, Haematology etc) , ECG, ophthalmoscopy and body temperature
- Post-mortem examinations (organ weight, gross and histopathology)
- Reversibility of drug-related lesions (in recovery animals)
- Toxicokinetics (in satellite animals)

Typical Parameters in 1- 3 Month Studies

Investigation	Performed	Parameters
General Observations		
Clinical Observations	Daily	
Food & Water Consumption	Weekly	
Body Weights	Weekly	
Ophthalmoscopic Examinations	Once pretest & termination	
Clinical Pathology		
Urine Chemistry	Pretest and 4 Weekly	
Urinalysis	Pretest and 4 Weekly	Color, appearance, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, microscopic examination of sediment Sodium, potassium, chloride, calcium, phosphorus, creatinine clearance, volume osmolality
Serum Chemistry	Pretest and 4 Weekly	Glucose, urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, triglycerides, sodium, potassium, chloride, calcium (total) Phosphorus, lactate dehydrogenase, testosterone (males only)
Hematology	Pretest and 4 Weekly	RBC, Hb, HCT, MCV, MCH, MCHC, Ret, Plat., total leukocyte count, differential leukocyte count, Coagulation (PT, APTT)
Post Mortem Investigations		
Necropsy		Gross pathology of all organs
Organ Weights & Histopathology		Adrenal Glands, Muscle - Biceps Femoris, Aorta, Thoracic Nerve, Bone, Ovaries, Pancreas, Bone Marrow, Parathyroid , Pituitary Gland, Brain, Prostate Gland, Cecum, Salivary Glands, Colon, Seminal Vesicles, Duodenum, Skin, Epididymides, Spinal Cord Esophagus, Spleen, Eyes with Optic Nerve, Stomach, Harderian Glands, Testes, Heart, Thymus, Ileum, Thyroid Gland, Jejunum, Tongue, Kidneys, Trachea, Liver, Urinary Bladder, Lungs (plus Bronchi), Uterus (plus Cervix), Lymph Nodes ,Vagina, Lymph Nodes, Mammary Gland
Ultrastructural Pathology		Selected pathology

Study Types

CARCINOGENICITY STUDIES

Carcinogenicity

- Is the drug carcinogenic in animal studies
  - Both rats and mice or just one
- Is there any indication of carcinogenicity in humans
  - Clinical trials (very unlikely)
  - Post market surveillance -
- If data is confined to animals, is the tumour type, location & mechanism relevant to humans ?
- Prescriber information will usually provide adequate data for this endpoint and indicate the conclusions of the respective regulators as to the relevance of the findings if any.
  - Just use the regulatory conclusions
  - Usually provides an indication of NOAEL or MOE calculation at specific rat dose

Carcinogenicity - long term study

- Internationally harmonised guidelines
- Species selection
  - Rat, mouse
  - Factors for species selection:
    - Background tumor rates
    - pharmacology
    - repeat dose toxicity
    - metabolism and toxicokinetics
- Duration – lifetime (24 months for rats,18-24 months for mice)
- Dose levels: generally 3, plus a control group
- Group size: 50 (desirable survival at termination 25/group)
- Complete necropsy and histopathology

Carcinogenicity - long term study

- Dose level selection
  - Toxicity MTD (maximum tolerated dose/Minimally Toxic Dose)
  - Pharmacokinetic end points
  - Pharmacodynamic end points
  - Maximum feasible dose (5% in diet, local tolerance, practicality) - for low toxicity substances
  - Limit dose (1500 mg/kg/day for non-genotoxic drugs with human dose of < 500 mg/day)
  - Other (case-by-case basis)
- Minimally Toxic dose (MTD) (More than mild toxicity invalidates the study)
  - produce at least minimum toxic effect over the course of the carcinogenicity study
    - Some toxicity necessary to establish “LOD”
  - Must not cause alterations in physiological function which alter the animal's normal life span or interfere with interpretation of the study
  - not more than 10% decrease in body weight gain relative to controls
  - Must not cause marked target organ toxicity
  - No substantial alterations in clinical pathology parameters

Carcinogenicity studies

- Evaluation of carcinogenicity studies
  - tumour incidence and latency (time to onset)
    - ie how many of each type and when did they appear
  - statistical analysis
  - dose-related trend
  - historical control data
  - pharmacokinetics and exposure
  - mechanism of action: genotoxic or non-genotoxic
  - If non-genotoxic,
    - mechanism and threshold?
    - relevance to humans?

Assessing a non-genotoxic carcinogen

- To discount a non-genotoxic carcinogen finding in animals consider:
  - There should be a plausible non-genotoxic mechanism explaining the animal carcinogenicity, supported by data or published information.
  - A threshold dose exists in animal studies, and the threshold exposure level in animals is higher than the likely human exposure.
  - Tumours only occur in one species or one sex (sex specific metabolism or mechanism?). The mechanism does not operate or is weak in humans.
  - There is extensive human data to indicate lack of carcinogenicity in humans by that mechanisms.

Non-genotoxic carcinogenicity

- Lymphoma
  - Immunosuppression (cyclosporin, azathioprine)
  - Relevance to humans: high incidence of lymphoma was found in organ transplant patients,
  - BUT strong risk/benefit
  - SO real risk but acceptable to the patient group

Non-genotoxic carcinogenicity

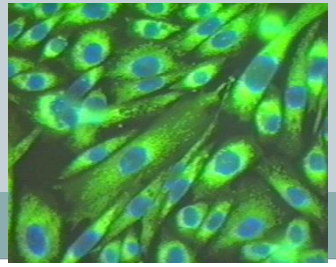
- Kidney tumours - binding to α2μ-globulin (**specifically synthesised by male rats**)
  - male rats only
    - Not applicable to female rats or other rodents
  - mechanism:
    - binding to α2μ-globulin (present in lysosomes)
    - globulin accumulates in the lysosomes of the proximal tubules
    - Causes lysosome dysfunction
    - Results in release of digestive enzymes
    - Causes renal epithelial necrosis
    - Leads to cell proliferation
    - Results in tumours
  - relevance to humans:
    - species specific (male rats only)
    - unlikely to occur in humans so disregard in risk assessment

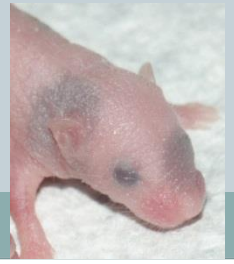
Study Types

GENOTOXICITY

Common Genotoxicity Tests

- Bacterial Reverse Mutation (Ames test)
- Gene mutation in mammalian cells (V79, CHO etc)
- Unscheduled DNA synthesis (DNA damage) – in vitro and/or in vivo
- Sister chromatid exchange assay (SCE)
- *Saccharomyces cerevisiae* (yeast) (gene mutation or mitotic recombination)
- Dominant lethal test (heritable genetic alteration assay)
- Mouse spot test
- Heritable translocation assay
- Drosophila (fruit fly) sex-linked recessive lethal mutation
- *In vivo* micronucleus assay in mice



Genotoxicity

- The Genotoxicity of a compound is determined on a weight of evidence basis from the results of a battery of *in vivo* and *in vitro* tests
  - ie no one test is usually sufficient in isolation to classify a compound as genotoxic or not.
- Generally little or no value in assessing the underlying studies for API PDE purposes
- The Prescriber Information (authorised label) will usually provide adequate data for this endpoint and indicate the conclusions of the respective regulators as to the relevance of the findings if any

Study Types

REPRODUCTIVE AND DEVELOPMENTAL TOXCITY

Reproduction and Development

- Does the drug affect;
  - male or female fertility
  - Foetal survival
  - Foetal development
  - Neonatal development
- The presence or absence of relevant effects will be noted in the authorised prescriber information but there may not be any information on the threshold doses.
- Will often need to look at post market studies to gauge the relevance of animal data in clinical practice
- Clinical trials will generally be of no value (exclude pregnant women)

Reproductive & Developmental toxicity

- ICH and OECD protocols differ
- ICH approach recognises 6 distinct phases and allows greater flexibility in study designs but essentially covers the same ground as OECD
- OECD uses two stages – reproduction and development
- Developmental Toxicity
  - Treatment of pregnant females only, from time of implantation until just before birth
  - Generally Rat and Rabbit, about 20/sex
  - Pups are removed by caesarean section and examined
- Reproductive toxicity
  - Treatment of 2 generations from before mating through to birth, lactation, weaning, maturation of pups, mating ….etc
  - Treatment of males for 2-4 weeks prior to mating, during cohabitation, until termination when the female is pregnant
  - Treatment of females for 2 weeks prior to mating, during cohabitation, through birth, lactation and weaning
  - Then treatment of 4 pups from each litter at each dose right through until they give birth and wean their pups
    - ie 2 whole generations

Developmental toxicity endpoints

- Pre-implantation loss
- Early resorption (post implantation death)
- Late death
- Malformations/variations/anomalies
- Growth retardation, size & weight
- Sex ratio

Study Types

CLINICAL DATA

First Dose in Man - MABEL

- **Minimum Anticipated Biological Effect Dose**
  - Combines a pharmacological with a toxicological estimate of first dose in man and uses the lesser of the two
- **Pharmacology**
  - Estimate human "Minimal Anticipated Biological Effect Level" (MABEL)
    - **justify based on pharmacology**
    - adjust for anticipated **exposure** in man
    - include anticipated duration of effect
    - adjust for **inter-species differences in affinity / potency**
- **Toxicology**
  - Determine "No Observable Adverse Effect Level" (NOAEL)
  - Convert NOAEL to a "Human Equivalent Dose" (HED)
  - adjust for anticipated **exposure** in man
  - adjust for **inter-species differences in affinity / potency**
  - Apply >10-fold safety factor

Phase I Clinical Trials

- Open Label and small numbers 10-100 healthy participants (often men between 18-40)
- Low single dose initially based on the NOAEL from the most sensitive animal species (or MABEL see later)
- Convert NOAEL to Human Equivalent dose (HED) using body weight scaling ($W^{0.67}$) which compensates for surface area and comparative metabolic rate between smaller and larger species
  - Consider
    - Differences in ADME
    - Experience with the chemical class
    - Cross species expression of relevant receptors
- Divide by uncertainty factors to account for interspecies variability, steep dose response curve etc (usually at least 10 to 100)
- Progressively increase if no evidence of toxicity
- Primarily looks at basic pharmacokinetics, safety, tolerability
- Lasts months to 1 year
- May provide an excellent estimate of a no effect dose in humans

Phases II & III & IV

- Larger numbers of patients as subjects over periods of years
- Randomised, controlled, blinded
- Efficacy and safety
- Phase IV is post market surveillance
  - e.g. Vioxx (rofecoxib) was used for RA for 5 years before a post approval clinical trial revealed increased risks of heart attack and stroke
  - Withdrawn from the market

Key Points

- Prescriber information
  - Carcinogenicity and genotoxicity are usually well covered
  - Reproductive effects often not covered in sufficient detail to yield POD
  - Repeat dose toxicological effects generally not well covered
  - Side effects, exaggerated pharmacology effects usually well covered
- Published papers
  - Phase I trial reports may provide information on MABEL or human pharmcokinetic endpoints (EC50, IC50) that are a useful source of potential POD values
  - Phase II & III and other studies may yield data on off target effects, reproduction
- EPAR, US FDA reviews
  - May be required for Pharmacokinetic data and PODs for repeat dose effects in animals

Not What You Know

- Toxicology covers every aspect of biological science so no one can be truly an expert in the traditional sense
- The key is;
  - Asking the right questions
  - Knowing where to get the answers
  - Accurately interpreting the available data
  - Balancing caution with pragmatism

Got all that ?
Time for a break !