Industrial and Regulatory Toxicology:

Focus on Toxicity Testing Methods and Support of Clinical Studies and Consumer Use (route of exposure and subject demographic)

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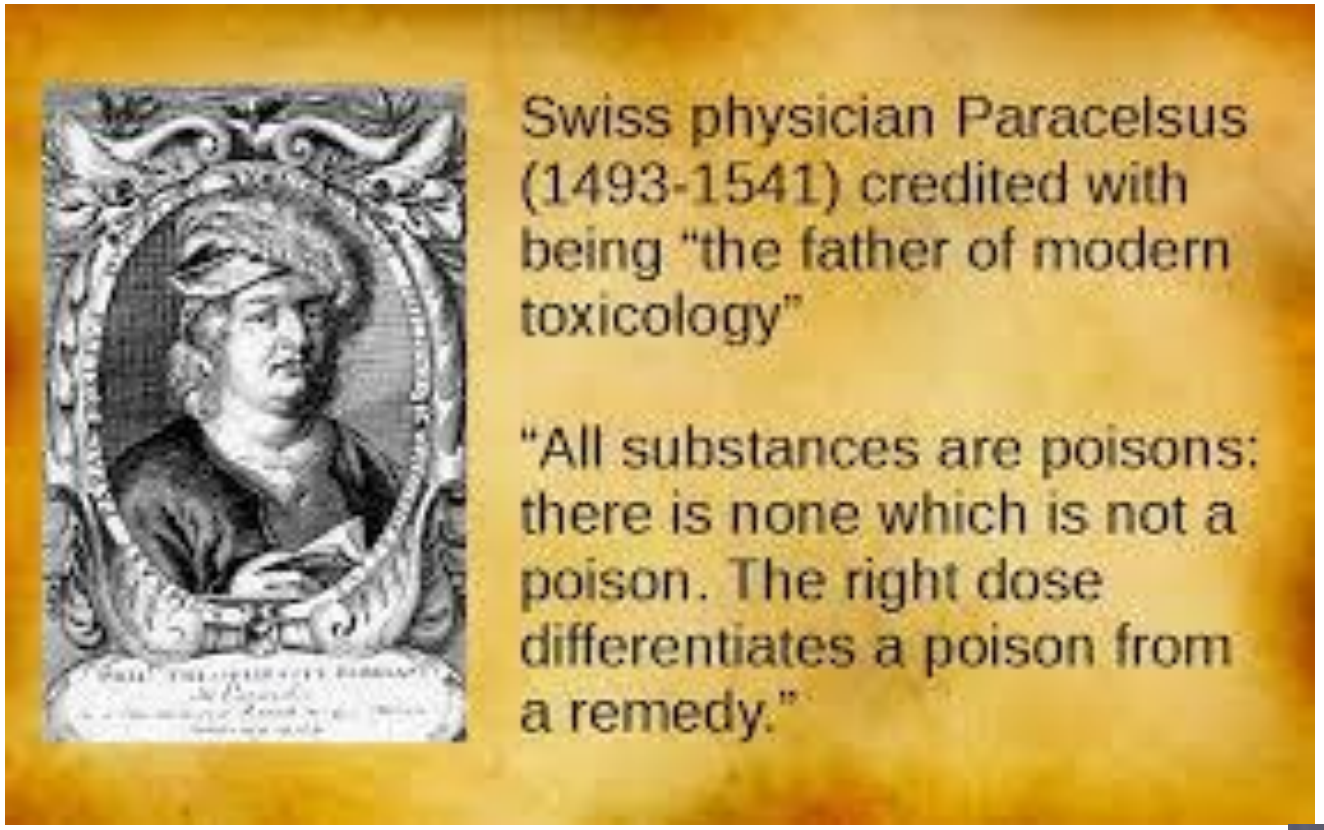
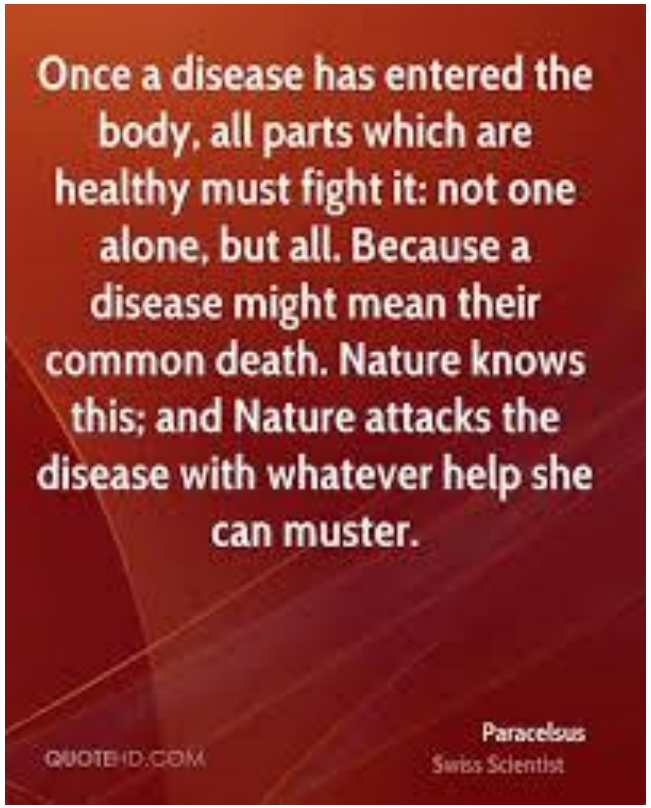
Lecture adapted from 2015 American Toxicology Continuing Education Course, Toxicology for Industrial and Regulatory Scientists: General Toxicology; Buckley, PhD, DABT, ATS Sr Research Fellow Toxicology and Drug Disposition Eli Lilly and Company, Inc
28 April 2015

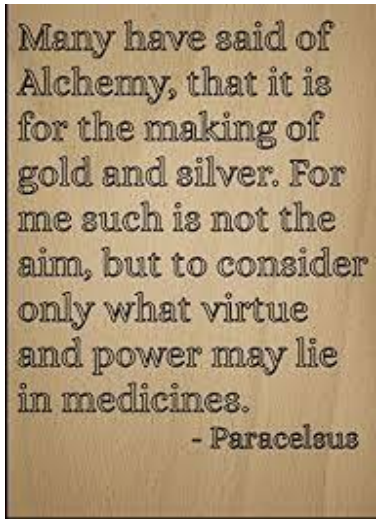
Who are you?.... and who is this guy?

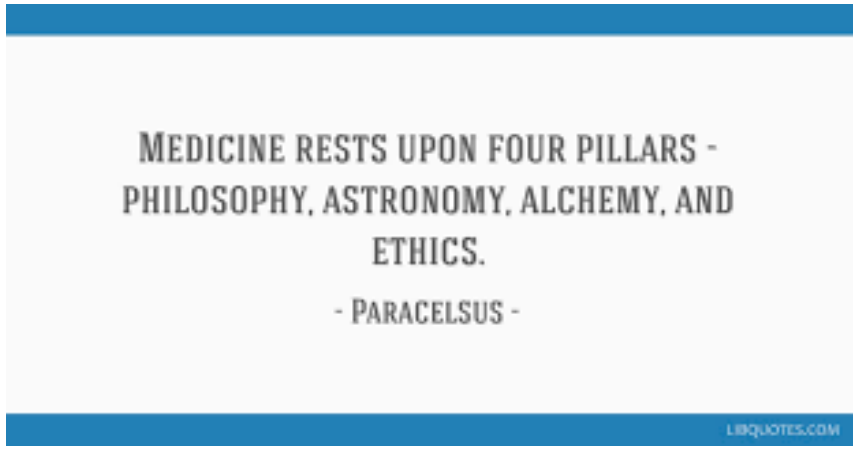
- Toxicologist for over 20 years
- PhD Pharmacology and Toxicology Texas A&M (1998); Two postdocs biochemical and mechanistic toxicology (MD Anderson, UNM College of Pharmacy); Alcon Research Limited/ Pharma; Lovelace Biomedical (Hybrid, local, academic-contract-IP development, 18 years)
- 100s of safety and risk mitigation programs developed for chemicals and pharmaceuticals.
- Currently “consulting” for pharma, chemical and other “clients” pharmacology-translational biology and toxicology
- Adjunct faculty UNMHSC-CP

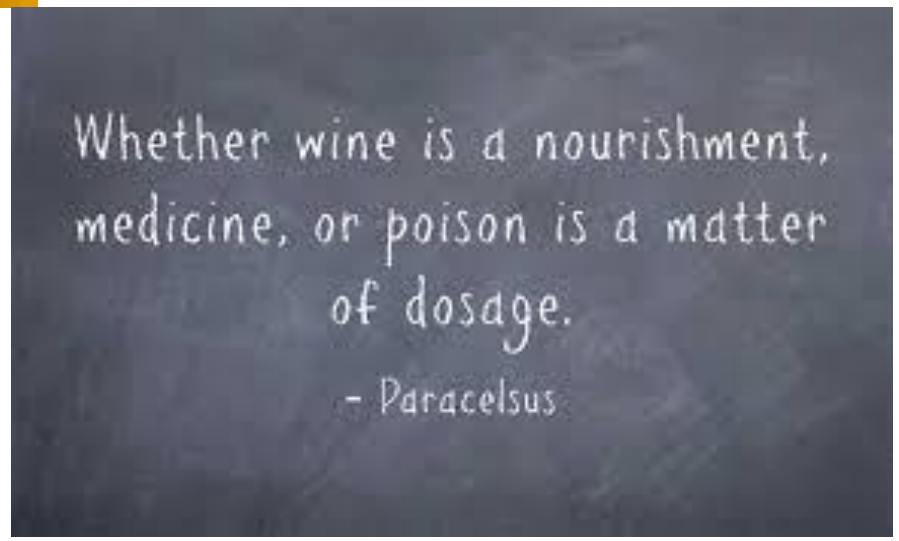
COELUS


Swiss physician Paracelsus (1493-1541) credited with being "the father of modern toxicology"
"All substances are poisons: there is none which is not a poison. The right dose differentiates a poison from a remedy."


Once a disease has entered the body, all parts which are healthy must fight it: not one alone, but all. Because a disease might mean their common death. Nature knows this; and Nature attacks the disease with whatever help she can muster.
QUOTEHD.COM
Paracelsus
Swiss Scientist


Many have said of Alchemy, that it is for the making of gold and silver. For me such is not the aim, but to consider only what virtue and power may lie in medicines.
- Paracelsus


MEDICINE RESTS UPON FOUR PILLARS - PHILOSOPHY, ASTRONOMY, ALCHEMY, AND ETHICS.
- PARACELSUS -
LIBQUOTES.COM


Whether wine is a nourishment, medicine, or poison is a matter of dosage.
- Paracelsus

Lecture Objective

1. Overview of risk assessment principles
2. Describe the principles of general (descriptive) toxicology testing
3. Describe how these principles are applied in nonclinical product development
4. Describe how these results support regulatory objectives

Focus will be on human therapeutics

- But as noted most principles also apply to other regulated industries (homeopathy, food/color additives, pesticides, industrial/specialty chemicals, etc.)
- Emphasis on US Food and Drug Administration (FDA)

General Overview

- Practicing Risk Assessment
- Basic Tenets of Toxicology Testing
- Program Design Considerations
  - General Regulations and Guidelines
  - Species Selection
  - Specific chemical/product class/use
- Study Design Considerations
  - Specific Design Parameters
- Regulatory application of nonclinical data
  - Nonclinical safety assessment in drug development
  - Assessment of FSD for homeopathy

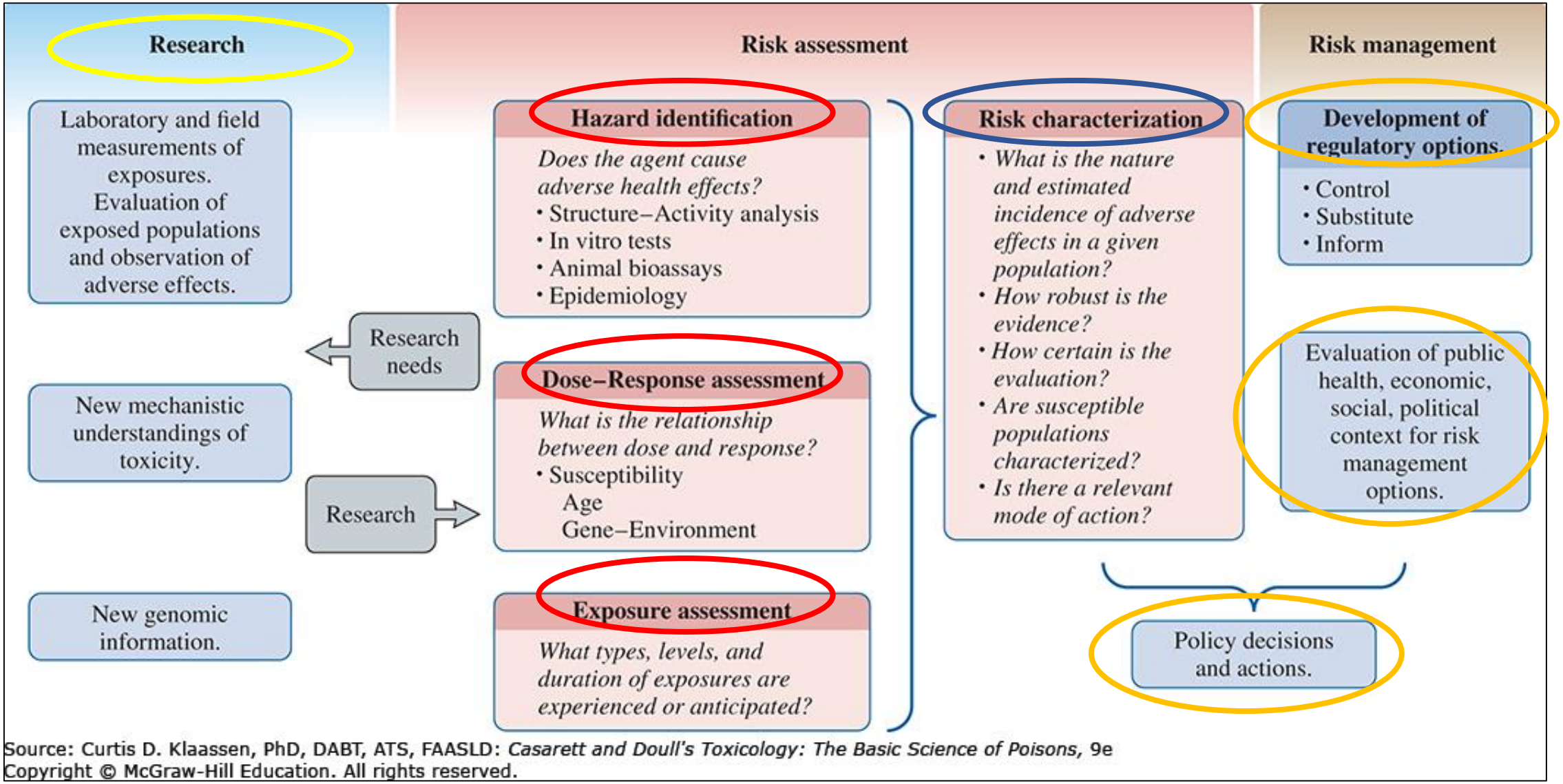
Real Toxicology?

This was my *late* husband's favorite recipe.

Of course, you'll want to ignore my margin note about "*a pinch of arsenic*."

Courtesy Eric Foxman

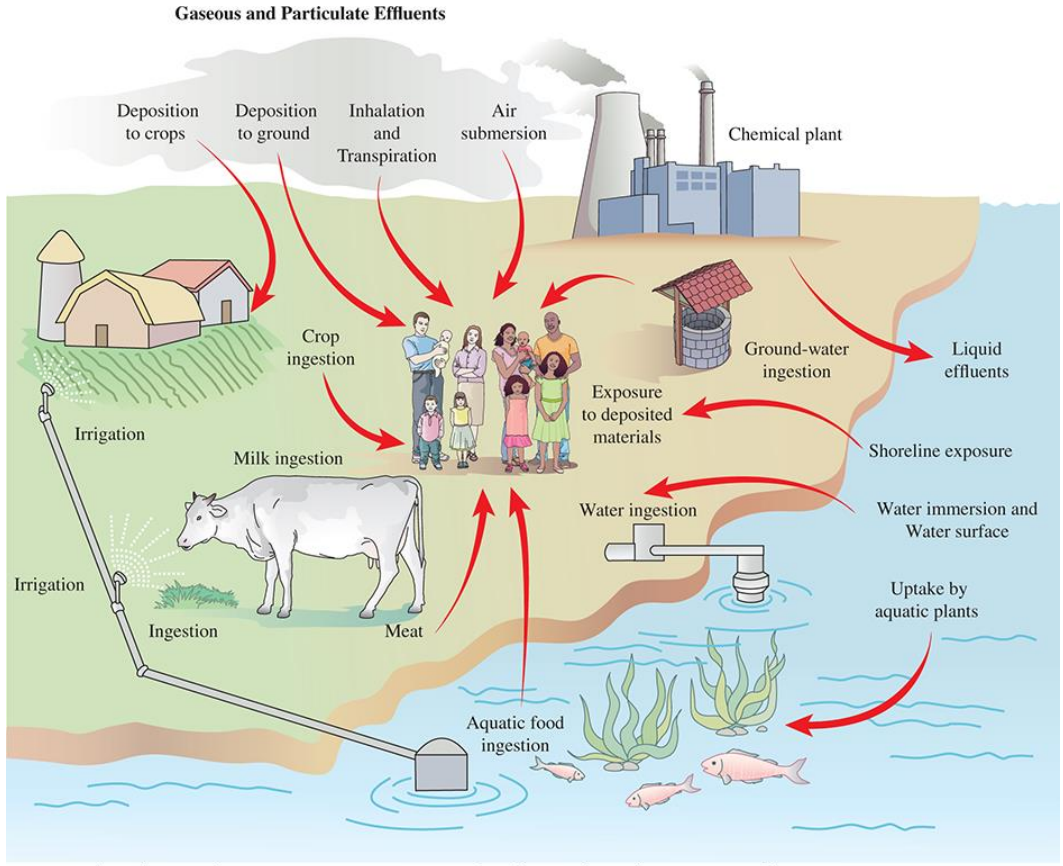
Basic Biology, Risk Assessment, and Regulatory Sciences Intertwined



Source: Curtis D. Klaassen, PhD, DABT, ATS, FAASLD: *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 9e

Exposure Assessment

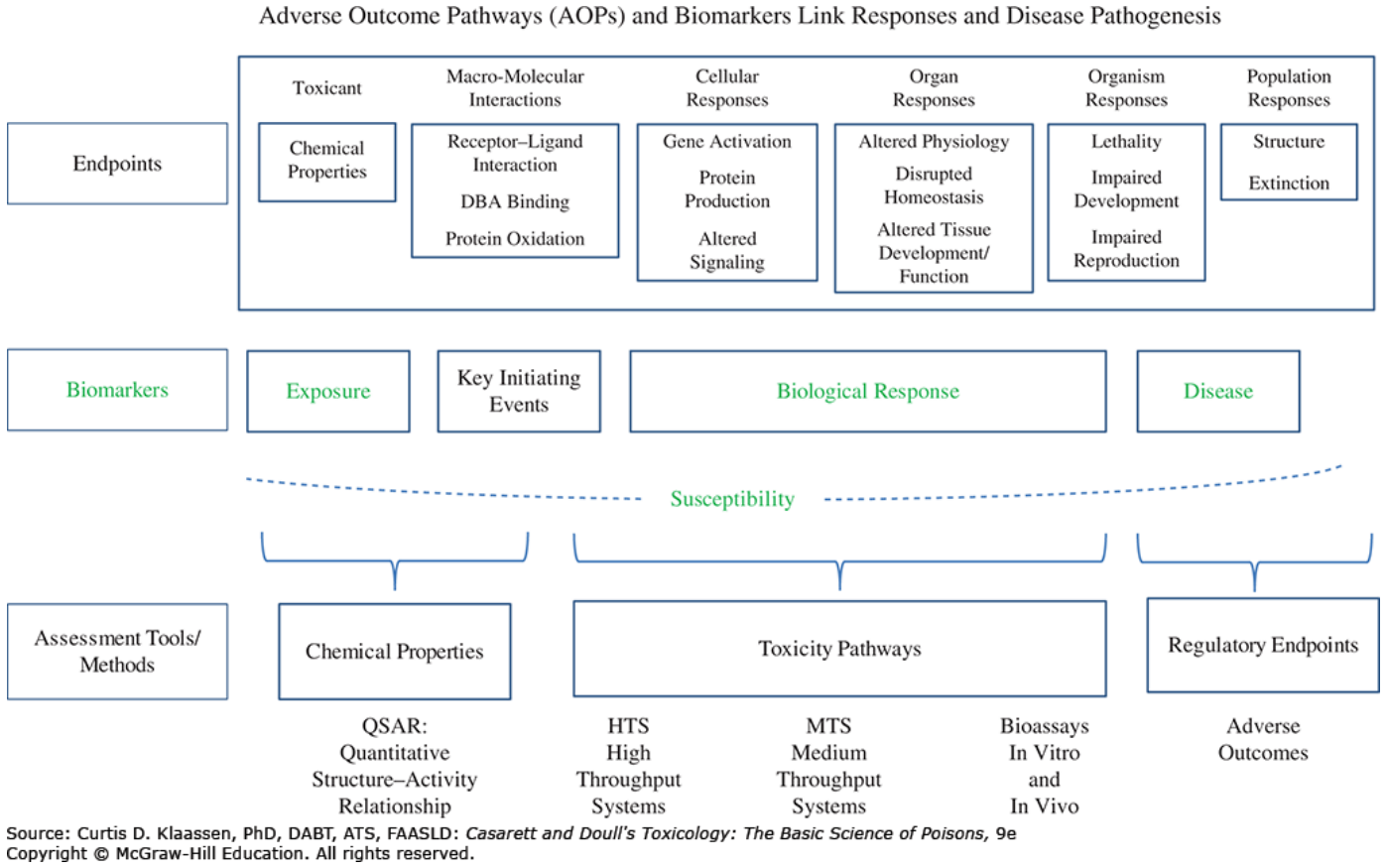
- Source, type, magnitude, and duration of contact with the chemical(s) of interest.
- ***Risk does not occur in the absence of exposure.*** But exposure is a key source of uncertainty
- Who may be exposed? Population, individuals or groups
- How large a dose may be reaching target tissues?
- Exposure calculations can include an estimation of total exposures for a specified population as well as calculation of exposure for highly exposed individuals.



Example:

LADD (Lifetime Average Daily Dose) = Concentration of the toxicant in matrix × $\frac{\text{Contact rate} \times \text{Exposure duration}}{\text{Bodyweight} \times \text{Lifetime}}$

Basic Biology, Risk Assessment, and Regulatory Sciences Intertwined (AOP)

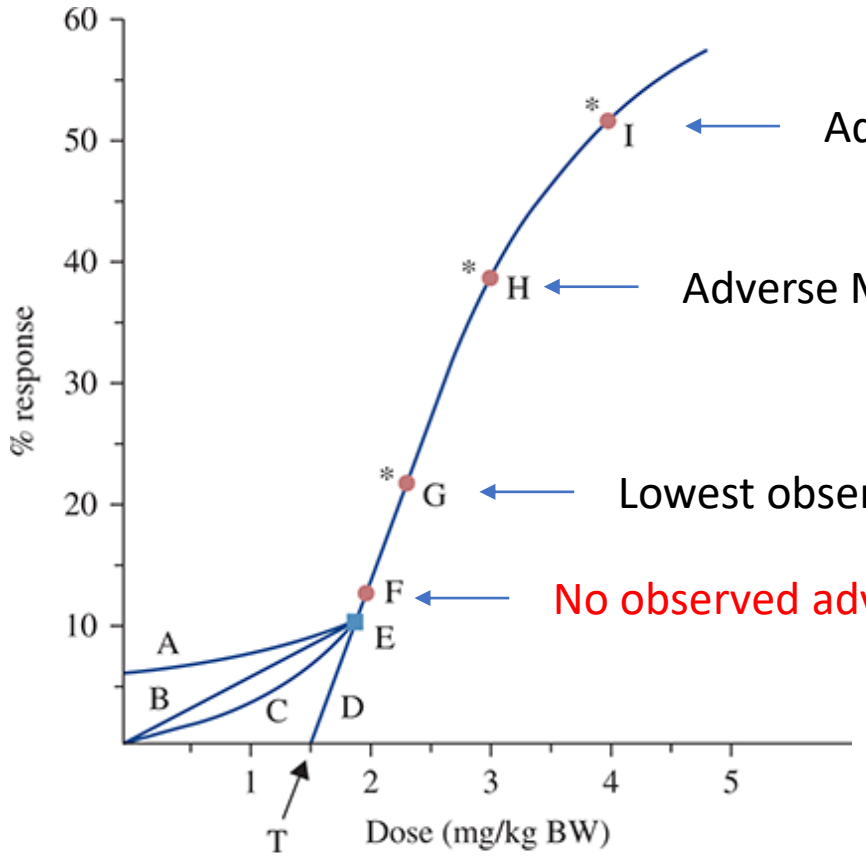


Adverse outcome pathways (AOPs) and biomarkers link responses and describe disease pathogenesis. Relationships between AOPs and biomarkers of exposure, effect, susceptibility, and disease are illustrated across disease response.

Methods used to collect information along the disease continuum include

- quantitative SAR (QSAR)
- high and medium throughput in vitro systems
- bioassays (other in vivo and in vitro assessments)

Basic Biology, Risk Assessment, and Regulatory Sciences Intertwined: Dose Response-Uncertainty



Adverse High Dose

Adverse Mid-High Dose

Lowest observed effect level (LOAEL)

No observed adverse effect level (NOAEL)

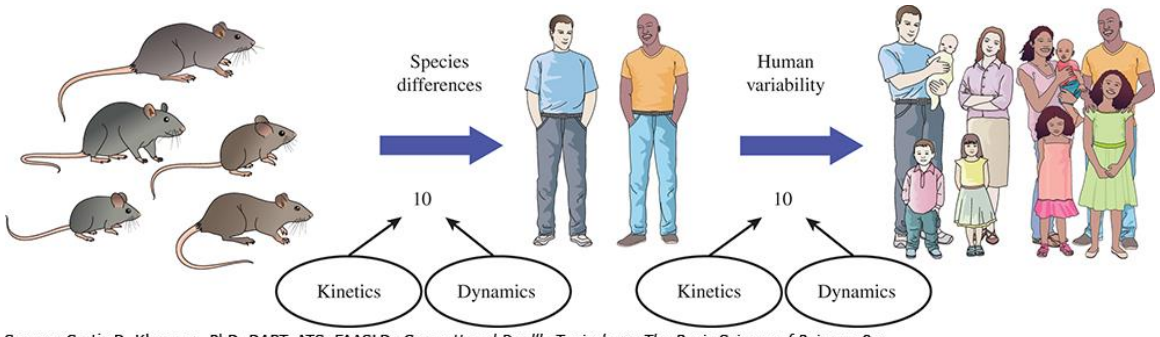
T = Threshold Response

Dose–response curve. This figure is designed to illustrate a typical dose–response curve with points F to I indicating the biologically determined responses. Statistical significance of these responses is indicated with the symbol "*." Point E (■) represents a dose near the lower end of the observed dose–response range, below which extrapolation to lower doses can occur for cancer risk estimates (U.S. EPA, 2005a). Point F is the highest nonstatistically significant response point; hence, it is the "no observed adverse effect level" (NOAEL) for this example. Point G is the "lowest observed adverse response level" (LOAEL). Point T represents a threshold for response curve D. Curves A to D show some options for extrapolating the dose–response relationship below the range of biologically observed data points and POD.

Reference Dose
(RfD) = $\frac{\text{NOAEL}}{\text{UF} \times \text{MF}}$,

Acceptable Daily Intake
(ADI) = $\frac{\text{NOAEL}}{\text{UF} \times \text{MF}}$

- UF = Uncertainty factor (usually 10)
- MF =modifying factor (usually 10 again)
- Move toward use of toxicokinetic and toxicodynamic to refine safety factor assessments
- Don't forget the literature!!



Integrating Quantitative Aspects of Risk Assessment

- **Quantitative Considerations in risk assessment**
  - exposure assessment
  - dose–response assessment
  - characterization of uncertainty/ variation in susceptibility
- **Human Exposure Data**
  - Generally limited (in terms of predicting a response)
- **Animal bioassay data have primarily served as the basis for most quantitative risk assessments and have required extrapolation for human health risk prediction.**
  - It is usual practice to choose ***the most robust data sets with adverse effects occurring at the lowest levels of exposure from studies using the most relevant exposure routes. The "critical" adverse effect is defined as the significant adverse biological effect that occurs at the lowest exposure level.***
- **Dose Response?**
  - Analysis must start with the determination of the ***critical effects*** to be quantitatively evaluated
  - Methods for extrapolating from ***high dose to low dose***
  - Method for extrapolating from ***animal hazard to human risk***
  - ***Threshold*** approaches have been applied for assessment of ***noncancer endpoints***
  - ***Nonthreshold*** approaches have been used for ***cancer endpoints***
- **Uncertainty Assessments**
  - Dependent on the dataset, population for intended use, regulatory intent, and regulatory body

Who does what in toxicology? We all do risk assessment!

Ninth Triennial Toxicology Salary Survey

Shayne Cox Gad[1] and Dexter Wayne Sullivan Jr[1]

International Journal of Toxicology
1-9

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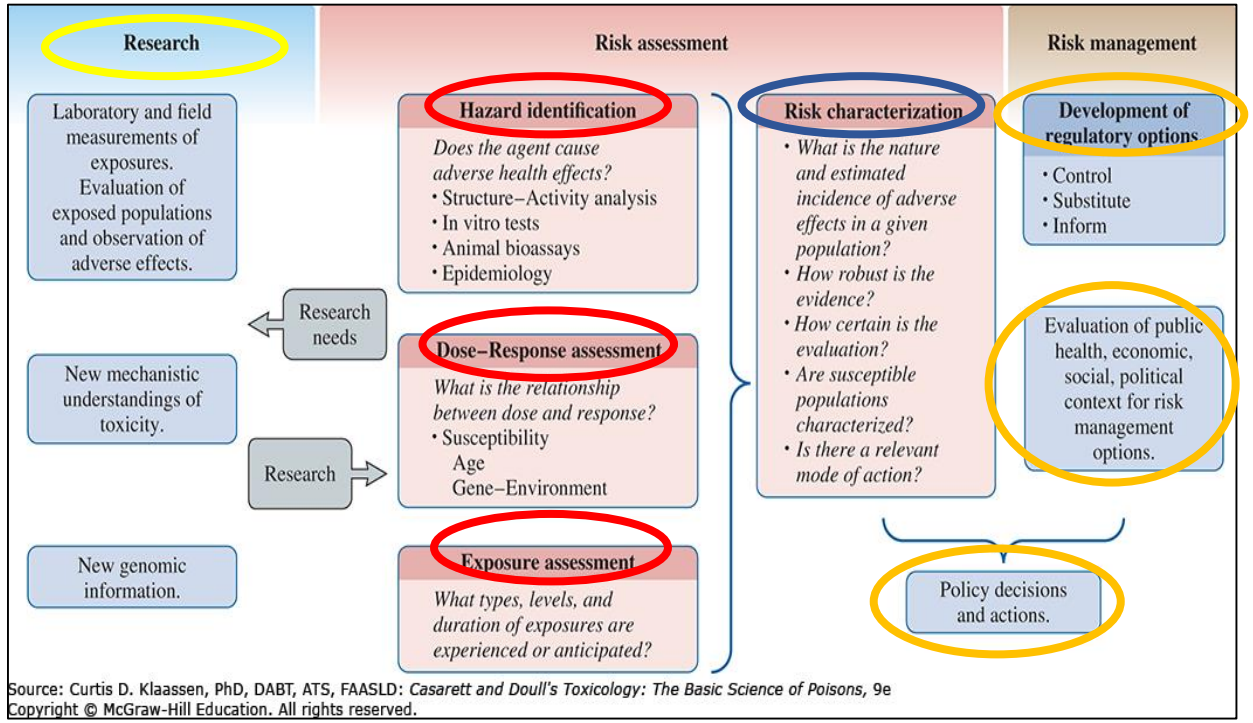
Table 22. Description of Employment.

Employer	Frequency	Percent
Academic	163	13.63
Business development and/or sales of equipment or services	3	0.25
Consulting—as an employee of a consulting firm	79	6.61
Consulting—independent	82	6.86
Contract laboratory	138	11.54
Government—federal (including military)	123	10.28
Government—state or local	32	2.68
Industry	546	45.65
Nonprofit research institution	20	1.67
Other	10	0.84



Source: Curtis D. Klaassen, PhD, DABT, ATS, FAASLD: *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 9e

Table 23. Industry Employment Breakdown.

Employer	Frequency	Percent
Pharmaceutical	361	66.24
Chemical	52	9.54
Consumer product	45	8.26
Food/food ingredients	20	3.67
Petroleum	12	2.20
Medical devices	21	3.85
Other	34	6.24

Table 24. Academic Employment Breakdown.

Employer	Frequency	Percent
Private doctoral granting institution/medical center	26	15.95
Private master's granting university	2	1.23
Private primarily undergraduate institution	6	3.68
Public doctoral granting institution/medical center	121	74.23
Public master's granting university	4	2.45
Public primarily undergraduate institution	3	1.84
Other	1	0.61

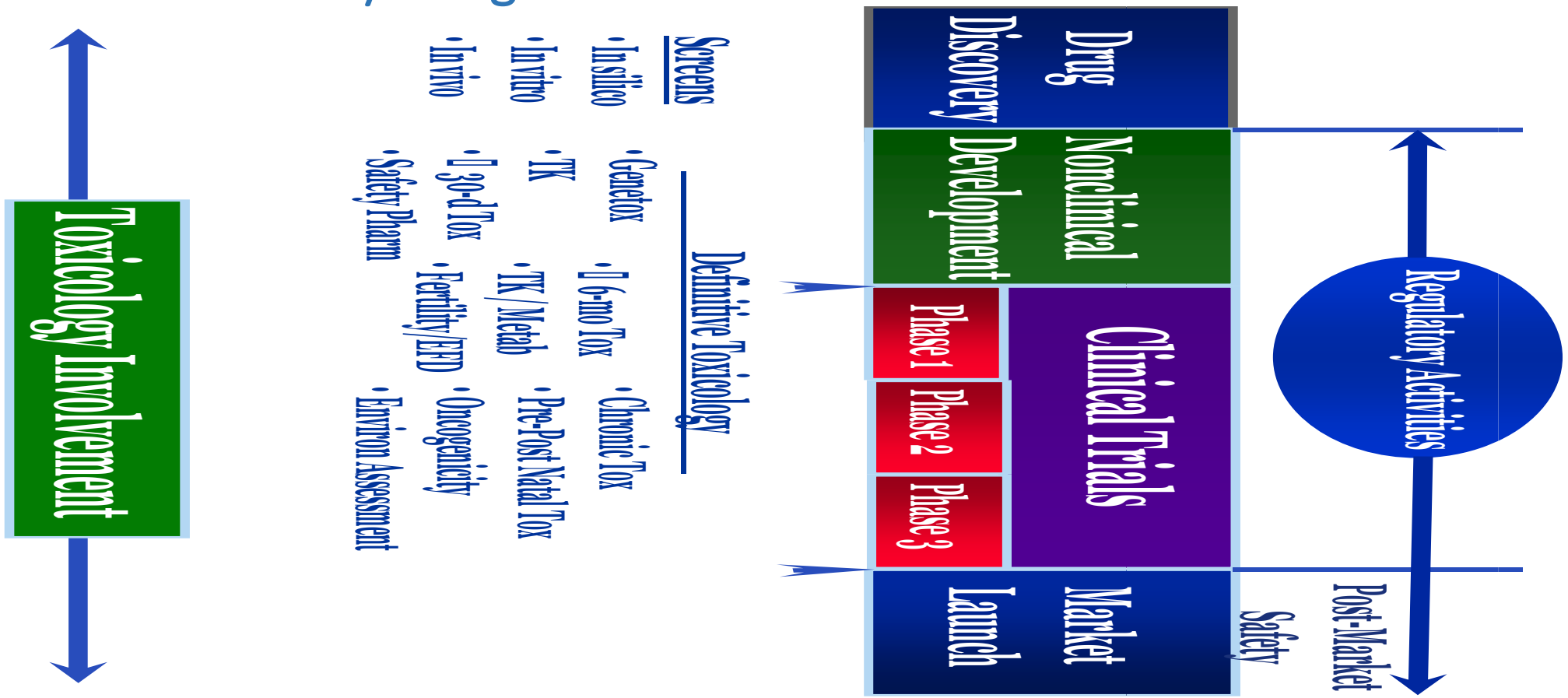
Table 5. Field of Highest Degree.

Field	Frequency	Percent
Biochemistry	56	4.71
Biomedical systems	14	1.18
Chemistry	19	1.60
Genetics	16	1.35
Human health medical	12	1.01
Molecular engineering	4	0.34
Pathology	132	11.10
Pharmacology	117	9.84
Physiology/biology/zoology	109	9.17
Public health	27	2.27
Toxicology	504	42.39
Veterinary medicine	57	4.79
Other	122	10.26

Toxicology/ Risk Assessment in Pharmaceutical R&D?

We do our job to assure safety of clinical subjects and patients?

Risk assessment by design.



Courtesy of Michael Dorato

Phased Progression of Toxicology/ Risk Assessment Support through Approval

- Our studies support dosing in clinical subjects (i.e. real people)
- EVERYTHING about a clinical plan (consumer use) is important!
  - Indication
  - Drug type
  - Target
  - Duration of treatment
  - Route of exposure
  - Patient Population, etc.
  - Regulatory phase (first in humans?/ Phase I; Proof of Principle Efficacy?/Phase II; Definitive Efficacy/ Phase III

- These components and many others drive our selection of species and the types of toxicology studies we perform to support safety in our subjects
- Studies meet requirements and expectations of regulatory authorities

Value of Nonclinical Studies (basic and applied)

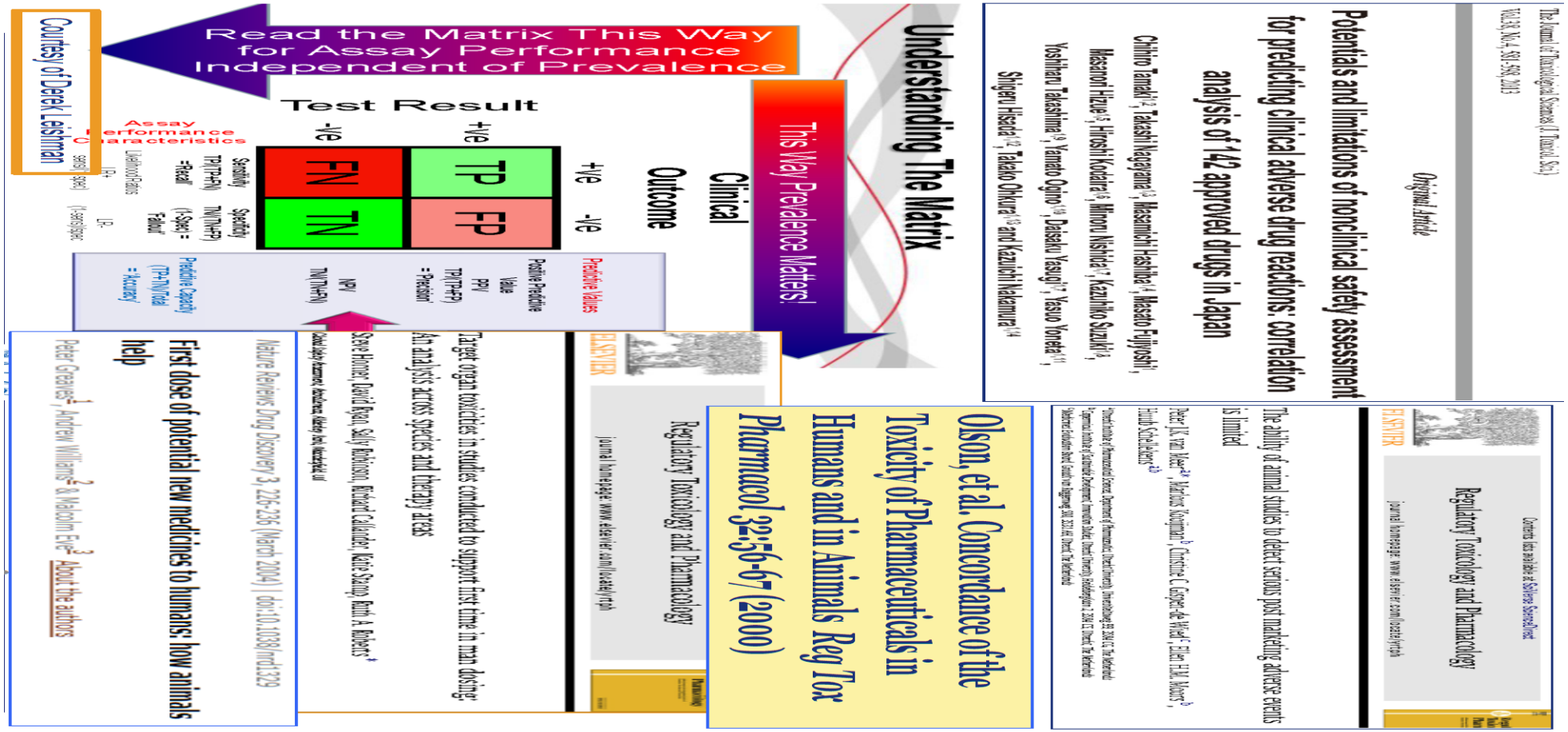
- Surrogate to characterize potential effects in humans (efficacy, safety)
  - Identify ***target organs/systems***
  - Study dose-response relationships across wide range of doses/exposures **(NOEL, NOAEL, LOAEL, MTD)**
  - Characterize ***reversibility and monitorability of toxicology***
  - Identify ***biomarkers*** for diagnosis, monitoring, & understanding MoA
- Well-defined methods of investigation based on precedence & experience
- Can be conducted under controlled laboratory conditions
- Study effects not possible/ethical in humans (e.g., systematic ***histopathologic evaluation, embryofetal development***, carcinogenic potential)
- And meet requirements and expectations of regulatory authorities

NOEL= No Observed Effect Level; NO(A)EL = No Observed (Adverse) Effect Level; LO(A)EL= Lowest Observed (Adverse) Effect Level; MTD = Maximum Tolerated Dose; MoA = Mechanism of Action

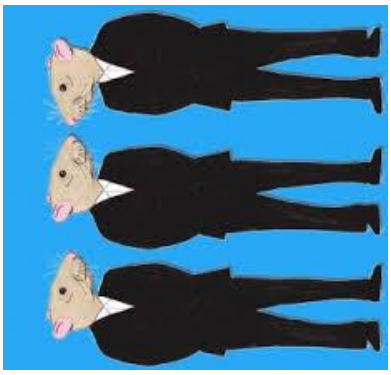
Basic Tenets of Toxicology Testing

General assumptions

- Animal models will predict human efficacy and *safety/toxicity*
- High doses will maximize model *(animal)* sensitivity to detect effects



Differences Between Animals & Humans (clinical subjects/ patients) ?????

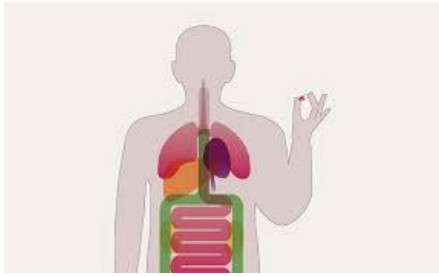


	Animals	Humans
Subjects		
Number	Large Groups	Individual
Age	Young Adult	All ages
State of health	Healthy	Usually sick
Genetic background	Homogeneous	Heterogeneous
Doses		
Magnitude	Therapeutic to toxic	Therapeutic
Schedule	Usually once daily	Therapeutic optimum
Circumstances		
Housing, Nutrition	Uniform, optimal	Variable
Concomitant therapy	Never	Frequent
Diagnostic procedures		
Verbal contact	None	Intensive
Physical exam	Limited	Extensive
Clinical lab	Limited, standardized	Individualized
Timing	Predetermined	Individualized
Autopsy/Histopathology	Always / Extensive	Exceptional

Common (Human) Reactions/ Adverse Events (AEs) Upon Drug Administration

Predictable Reactions from Animal Studies

- Drowsiness, Sedation, Dry mouth, Nervousness, Vomiting, Weakness
- Nasal stuffiness, Hypertension, Anorexia, Insomnia, Constipation
- Weight gain, Hypotension, Diarrhea, Skin rash, Dryness of nasopharynx
- Depression, ↑Appetite, Tremor, Perspiration, Dermatitis, ↑ Energy, Palpitation, Blurred vision, Lethargy



NOT Predictable

- Nausea, Fatigue, Dizziness, Tinnitus, Heartburn, Vertigo, Headache

Funny how most of these are monitorable in a clinical situation?

Adapted from: M.A. Dorato, M.J. Vodicnik (2008). The toxicological assessment of pharmaceutical and biotechnology products. In: Principles and Methods of Toxicology, 5th ed. (A.W. Hayes, ed.)

Concordance of Animal & Human Toxicities: How do we do?

- International Life Sciences Institute (ILSI) workshop (pharmaceutical survey)
- 150 compounds (most small molecules), 221 human toxicities; 12 companies
- Caveats: Retrospective survey; excluded compounds which did not advance to clinic (e.g., “killed” in preclinical development)

Species	% Concordance
All Species	71
Rodent (rat)	43
Non-rodent (dog)	63
Other species	29

- 71% of the time, target organ toxicity seen in humans occurred in one or more animal models
- Dogs tend to be slightly more inline with predicting human toxicity than rodents

- Up to 43% of toxicities related to pharmacological action (largely anticipatable)
- 94% of toxicities were detected in the 1-month studies
- Highest concordance: Hematological, GI, Cardiovascular (Least=Cutaneous)

Olson, et al. Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Reg Tox Pharmacol* 32:56-67 (2000)

Basic Tenets of Toxicology Testing

Question: "Can animals ***predict*** the (human) effect?"

Clear model limitations: Animals ≠ Humans

Question: "Is it safe?"

- As with other studies investigating a dose response, nonclinical safety assessment studies ***define the "space" within which a compound can be administered without unacceptable toxicity***
- ***Animals studies inform clinical study*** design and monitoring
- Generally, nonclinical studies do a good job. I.e. a good safety record for clinical trials in human subjects based on nonclinical data
- But bad things can happen no matter how careful we are……
  - ***i.e., when performing nonclincal development always ask yourself whether you would be comfortable putting this investigational product into your nearest, dearest someone……***

Use of High Doses in Toxicology Studies (i.e. hazard assessment and dose response)

- Nonclinical toxicology studies aim to define the bounds of what (bad) could possibly occur
- High doses to account for uncertainties in interspecies extrapolations and intra-populations susceptibilities (i.e. “punch through” uncertainty)
- Maximum Tolerated Dose (MTD) designed to provide a level of toxicity indicative of sufficient chemical challenge to allow expression of toxicity within the system
- Issues with excessively high doses
  - Extrapolation from high (animal) to low (human) doses is not usually linear
  - Distortion of normal physiologic pathways (e.g., saturation of detoxification processes)

(More discussion in subsequent “Dose Selection” section)

Developing Nonclinical Safety Evaluations to Support a Clinical Development Plan

- Compound-specific considerations that should shape the plan
  - What do we already know?
    - Product type and similarity to existing agents with known safety profiles (New Molecular Entity vs Follow-on/Generic Drug)
    - Drug class effects
    - Pharmacology (and “exaggerated pharmacology”)
      - What is the molecular target or pathway?
      - Conserved across species? Tissue distribution?
      - Toxicity associated with target manipulation?
  - Chemical / Metabolic stability
  - Metabolized similarly across species? (in vitro)

Developing a Nonclinical Safety Evaluation Plan

- ***For a specific therapeutic, consider use patterns:***
  - ***Who*** is the target human population?
    - adults, infants, pregnant women, elderly, etc.
  - ***What*** is the proposed duration and route(s) of administration?
    - Daily vs intermittent dosing; short-term vs chronic
    - Parenteral vs oral or dermal or inhalation dosing
  - ***What*** are use pattern considerations?
    - Concomitant medications, adjuvant therapy
- Dose range finding data and pilot studies
- ***Interaction with regulatory authorities*** will provide additional perspective / information

Developing a Nonclinical Safety Evaluation Plan

- Regulatory Guidance (not necessarily a checkbox by a stretch!!!!)
- ICH safety & multidisciplinary guidance documents (www.ich.org)
- FDA Pharmacology/Toxicology (www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guid ances/ucm065014.htm)
- European Medicines Agency (EMA, www.ema.europa.eu/ema)
- Regulatory Precedence
- Reviewers' comments regarding approved drugs
- FDA: Summary Basis of Approvals (SBA, www.accessdata.fda.gov/scripts/cder/drugsatfda)
- EU CHMP: EPARS
- Japan PMDA: submissions are published

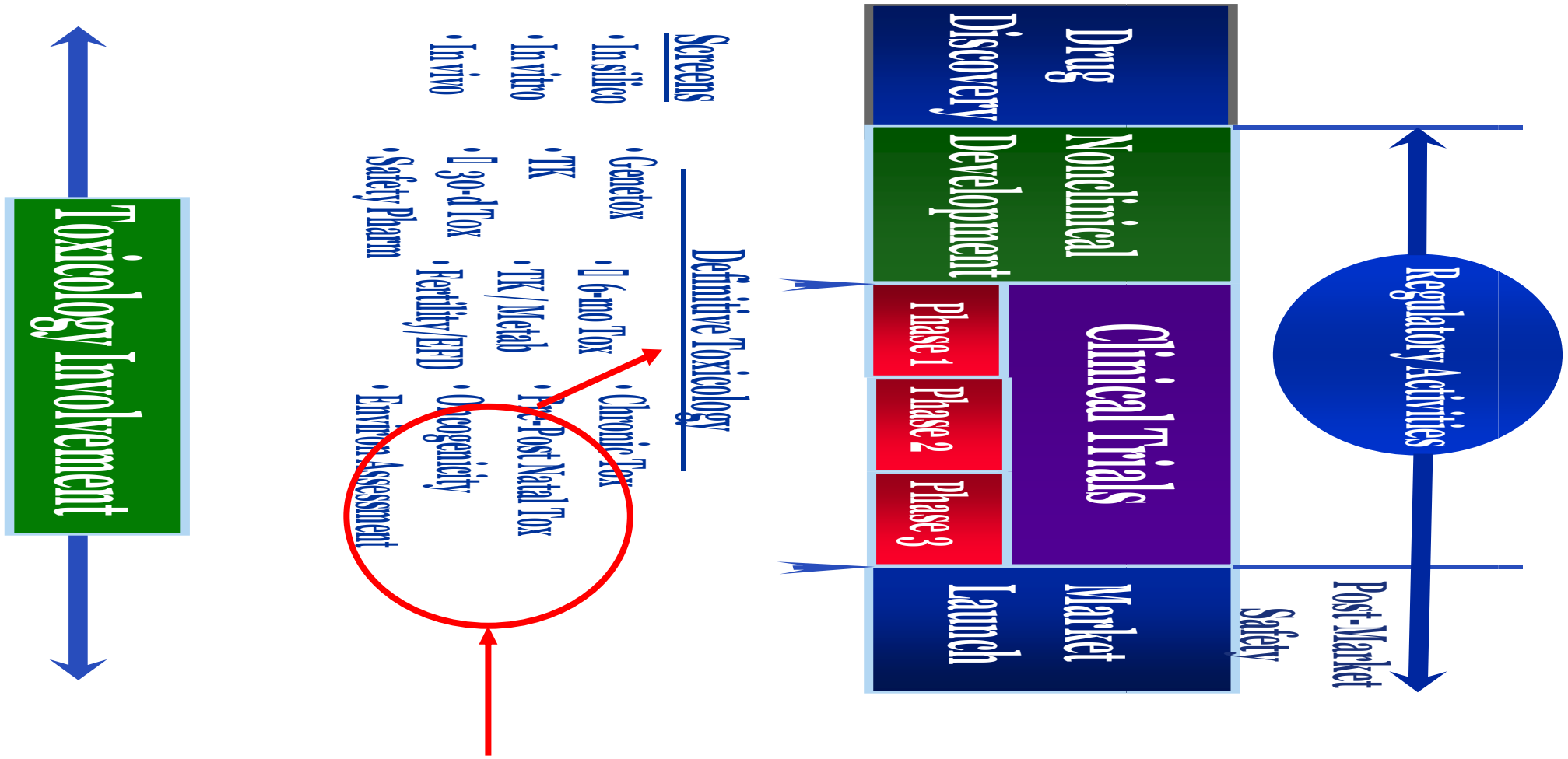
International Conference on Harmonization

- To promote uniformity in technical requirements for registration of pharmaceuticals for human use
  - Discuss and establish common guidelines (EU, Japan, USA) for technical requirements
  - Ensure that safe, effective, and high quality medicines are developed and registered in the most efficient and cost-effective manner
    - Prevent unnecessary duplication
    - Minimize the use of animal testing
- Promote public health

ICH Guidance Related to Nonclinical Safety

S1A,B,C(R2)	Carcinogenicity: When Studies are Needed, Testing Methodology, Dose Selection
S2(R1)	Genotoxicity Studies
S3A, B	Toxicokinetics & Tissue Distribution Studies
S4	Duration of Chronic Toxicity in Rodents & Nonrodents
S5(R2)	Reproductive Toxicology
S6(R1)	Preclinical Safety: Biotechnology Products
S7A, B	Safety Pharmacology Studies; QT Prolongation
S8	Immunotoxicology Studies
S9	Nonclinical Evaluation of Anticancer Pharmaceuticals (+Q&A)
S10	Photosafety Evaluation
S11	Nonclinical Testing for Pediatrics
Q3A-D	Guidance on Impurities, Residual Solvents, Elemental Impurities
M3(R2)+Q&A	Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals
M7	Genotoxic Impurities

Let's get to IND! And support first in human (FIH) safety and proof of principle efficacy in humans

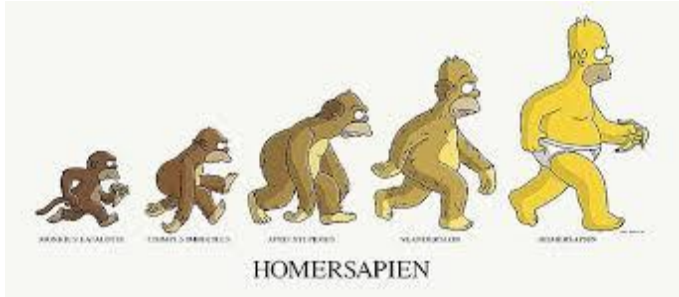


US Investigational New Drug (IND);
Generally supports Phase II

Courtesy of Michael Dorato

Design of Toxicology Studies: Selection of Species, IND and Beyond

- Species selection criteria should be intentional and explicitly justified
- Largely based on expected similarity to humans
- Comparative pharmacology?
  - Is the (similar) target present in the animal model?
  - Does the target have a similar function in the animal model?
  - Is anatomy/physiology of potential target organs comparable?
- Comparative metabolic profile
  - In vitro screening: hepatic microsomes and/or cytosolic fractions
  - Metabolite identification and PK profile (post Phase 1-II/ IND)
- Default species small molecule = rat (rodent) and dog (nonrodent)
- Default species biologic = nonhuman primate and mouse (maybe)
- Other factors include: Precedence with drug class (e.g., known toxicities, mechanisms); Regulatory agency experience; Availability of historical control data; Logistical issues ...



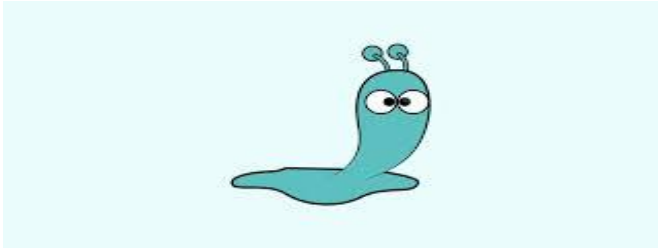
Design of Toxicology Studies: Selection of Species

Animal Models of Safety Assessment (examples of supporting role for clinic)

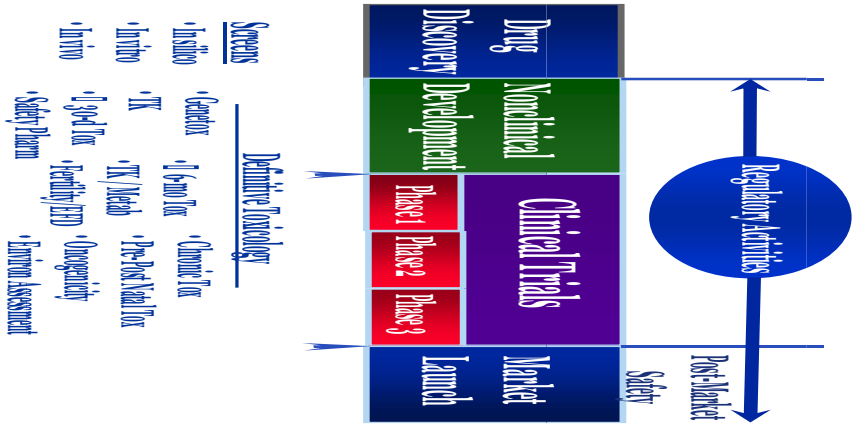
Animal model	Lifespan (years)	Comment
Rodent (Rat, Mouse)	1.5-2	Commonly used, small/inexpensive Large historical database & well-characterized biology Highly inbred and outbred strains available Rat is preferred rodent model for general toxicology
Beagle Dog	10-15 (6-8 kg)	Preferred nonrodent model for general and cardiovascular toxicology
Cynomolgus monkey	15 (3-5 kg)	Phylogenetic and physiologic homology with humans Costly and limited resource Often employed in biotherapeutic development
Rabbit	5-10 (2-3 kg)	Developmental & ocular toxicology, vascular irritation
MiniPig (www.eathink-eu.dk)	15-20 (<13 kg)	Dermal toxicology, cardiology
Transgenic Mice	Variable	Animal models of disease, Biopharmaceuticals

Design of Toxicology Studies: General Considerations on Species

- Two mammalian species (one nonrodent) expected (no 1 perfect model)…most of the time
- Both genders are expected, even for drugs designed to treat one sex clinically (reproductive health/urology)
- Possible exceptions where one species may be sufficient:
  - Biopharmaceuticals
  - Drug is pharmacologically active in only one species
  - Similar profile in 2 species allows chronic study in one species only due to highly targeted nature of drug (ICH S6(R1))
  - Genotoxic anticancer drugs (ICH S9) – one species (rodent) may be considered sufficient for an agent targeting rapidly dividing cells
  - New route of administration for an “old” drug
    - e.g., dermal formulation for oral pain medication - 1 species to assess local effects knowing systemic safety already established



Getting to IND: Which studies?
Test Case: small molecule vs. MAB



- Gene Tox (GLP, more in a bit): required small molecule/ not biologic
  - AMES
  - Sister Chromatid Exchange (in vitro)
  - Mouse lymphoma/ micronucleus
- Safety Pharmacology (GLP): required small molecule/ not biologic
  - hERG/ ion channel in vitro
  - Acute dog/ rodent studies designed to investigate major organ functions
  - Telemeter“ized” dogs (respiratory, cardiovascular; kidney)
  - Telemetered rats (respiratory; kidney; neuro)
- 2-4 week repeat dose toxicology in (rat and dog: small molecule): GLP, required
- 4 week repeat dose in NHP (MAB, “loose biologic example”): GLP, required

But lets get back to general principles……

Design of Acute and Repeat Dose Toxicology Studies: General Considerations of Species

Basic study designs are standardized but do differ for rodents and nonrodents (same but different!)

- Practicalities of handling & cost drive smaller numbers of nonrodents
- Both young adult at initiation, but lifespan differences over treatment periods
- Able to more liberally sample large animals
  - e.g., Toxicokinetics
- Clinical pathology
- Pre-dose samples allow within animal evaluations

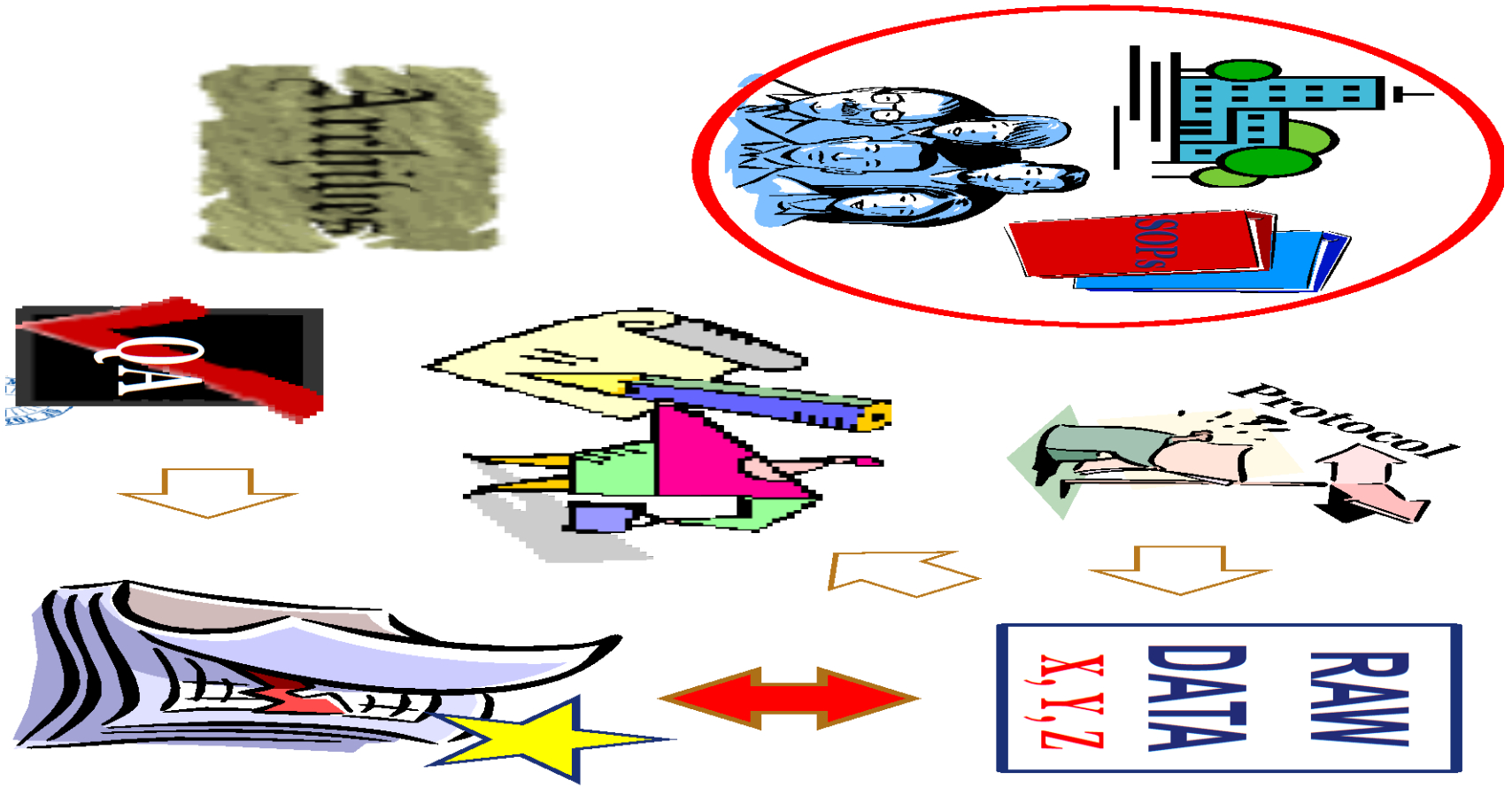
	Rodent	Nonrodent
Number of animals	10–15/sex/group	3–4/sex/group
Number of PK satellites	3–25/sex/group	Not needed
Age at initiation	~6 weeks	~4–6 months
Common dosing methods	Oral gavage, diet, iv, inhalation	Oral capsule, oral intubation, iv
Common blood sampling methods	Retro-orbital, cardiac puncture, lateral tail vein, jugular, posterior vena cava	Posterior vena cava, saphenous, cephalic and femoral vein
Parameters measured		
Clinical observations	At least weekly	At least weekly
Body weight	At least weekly	At least weekly
Food consumption	At least weekly for the 1st month	At least weekly for the 1st month
Ophthalmoscopic exam	Pretest, end of treatment	Pretest, end of treatment
Electrocardiographic exam	Not routine	Pretest, end of treatment
Hematology	At least once	Pretest, at least once during treatment
Clinical chemistry	At least once	Pretest, at least once during treatment
Urinalysis	Metabolism cage, at least once	Metabolism cage or catherization, pretest, at least once during treatment or at necropsy
Gross necropsy	Standard	Standard
Organ weight	At least liver and kidney	At least liver and kidney
Histopathology	At least high dose and control, target organs	At least high dose and control, target organs
Statistics	Various methods applicable	Very limited due to small group size

Keller and Banks (2006)

Conduct of Toxicology Studies: Laboratory Operations – Animal Care

- Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accreditation
  - Non-profit organization that promotes the humane treatment of animals
  - Global standard for assurance of quality animal care and facility operations
- Institutional Animal Care and Use Committee (IACUC)
  - Assure that study designs and study conduct adhere to standards
  - Reviews each protocol for its individual merits with an eye to responsible animal care and use
  - Independent oversight with outside member
  - Facility review and reporting to Management

Conduct of Toxicology Studies: Laboratory Operations - GLPs



Conduct of Toxicology Studies: Laboratory Operations - GLPs

- Good Laboratory Practice (GLP) regulations ensure quality and integrity of the data which must be:
- Well-documented - every action and step is documented in detail, signed, and dated on the day collected
- Signature = you agree data is correct & you are accountable
- Data must stand on its own without need for someone to explain what occurred
- Replicable - Scientists should be able to repeat the study • New study should produce the same results as original
- Reconstructable - Reviewers can recreate study from the data / documentation available

CFR Title 21 Part 58; www.fda.gov/ora/compliance;

Design of Toxicology Studies: General Considerations

General design of a repeated dose rodent toxicity study (e.g., 28-Day)

Group	Dose (mg/kg/day)	Main	Interim	Recovery	TK
		Males	Females		
1	0	10	10	5	3
2	10	10	10	5	3
3	30	10	10	5	3
4	100	10	10	5	3

- Chronic studies (i.e. 6-9 mo)
  - 20/sex/group (Main)
  - Interim necropsy(ies): ≥10/sex/group (e.g., 13-wk “peel-off”)
  - Recovery: ≥5/sex/group at necropsy (duration variable)
  - TK: # animals dependent on bioanalytical method, # time points
  - Sentinel animals for contamination monitoring

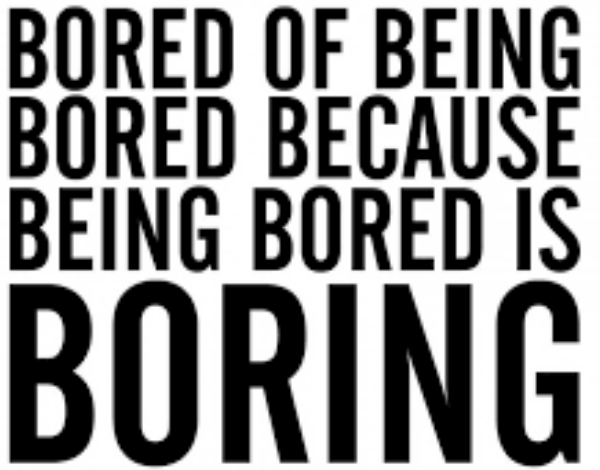
Don’t read this slide……

Design of Toxicology Studies: General Considerations

General design of a repeated dose nonrodent toxicity study (e.g., 28-Day)

Group	Main	Interim	Recovery	Total
1	4	3	2	9
2	4	3	2	9
3	4	3	2	9
4	4	3	2	9
5	4	3	2	9
6	4	3	2	9
7	4	3	2	9
8	4	3	2	9
9	4	3	2	9
10	4	3	2	9
Total	40	30	20	90

- Chronic studies (6-9mo)
  - ≥ 4/sex/group (Main)
  - Interim necropsy(ies): ≥ 3/sex/group (e.g., 13-wk “peel-off”)
  - Recovery: ≥ 2/sex/group at necropsy
  - duration may be substantial, eg, 3-4 mo for mAb clearance)
  - TK animals not required

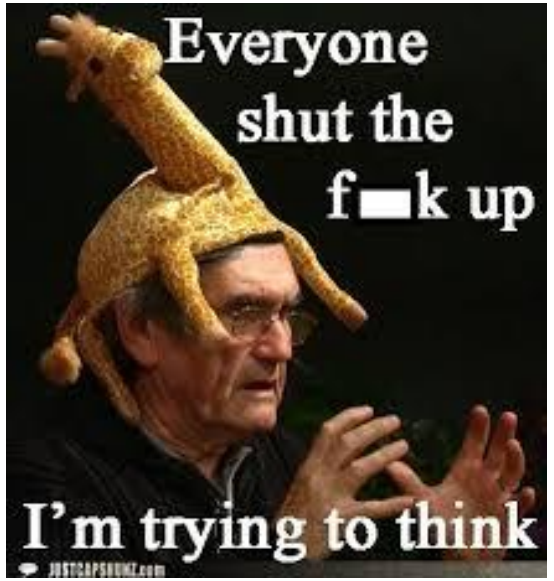


Lots to consider…. Developing a Nonclinical Safety Evaluation Plan Objectives: Early Studies to IND (2-Week, 1-Month tox studies in one-two species)

- Identify the target organs/systems of the drug Gender differences?
  - Target organs? Monitorable (is there a biomarker or “surface” sign of tox)?
  - If recovery groups: Reversible? Progressive?
  - Expected? (based on pharmacology / MoA)
- Characterize the dose-response relationship of toxicity
  - Linear? Supra / sublinear?
  - Identify No Observed Adverse Effect Level (NOAEL)
    - NOEL more appropriate for food additives, color additives, etc.
  - Characterize plasma exposure parameters (e.g., Cmax, AUC, t1/2)
- Aid in selection of doses for first-in-human (FIH) studies
  - NOAEL = typical push-off point for clinical dose selection
    - Anticancer drugs start higher: rodent STD10 (severely toxic dose in 10%) & nonrodent HNSTD (highest non-severely toxic dose)
- Assist in selection of doses for longer term toxicology studies

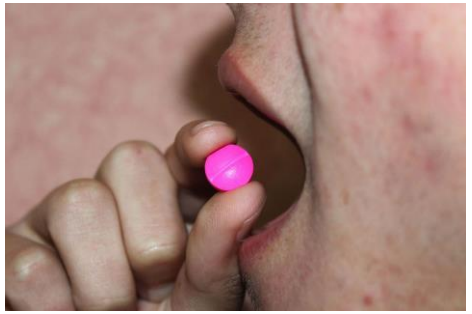
Developing a Nonclinical Safety Evaluation Plan IND and Beyond: Other Things to Consider

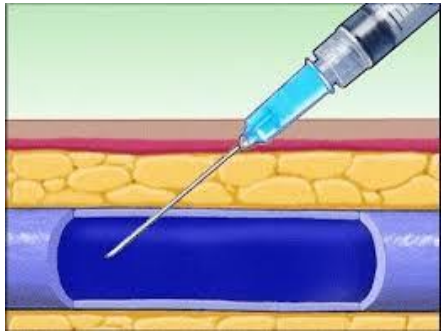
- Small Molecule vs. Biologic
  - Same basic principles, but implementation can be very different
- Acute vs. Chronic Therapeutic Indication
- The impact of Therapeutic Indication
  - Risk / Benefit
  - Life-threatening treatment vs Long-term maintenance
  - Alternative treatments?
- The impact of Route of Administration
- Medical device?
- The impact of Geography
  - ICH guidelines promote uniformity , BUT ... FDA inter-Division differences
- The significance of Rodent vs. Non-Rodent effects



Design of Toxicology Studies: Route of Administration

- Route should be same as that intended for use in humans
  - Other routes may be employed if human route cannot be duplicated in animal; must be supported by data such as:
    - Limited systemic exposure
      - e.g., topical NME programs often include oral (or IV) rodent (rat) + topical nonrodent (minipig) to assess both local & systemic tox
    - Model limitations due to route of administration
      - e.g., sublingual dosing (use IV tox + mucosal local tolerance)
    - Size/physiology of the animal species may limit options
  - Need to understand toxicokinetic behavior of clinical vs nonclinical route
- Frequency of dosing should match or exceed clinical dosing paradigm
  - May increase nonclinical dosing to “cover” more rapid clearance or presence of anti-drug antibodies in animals





Design of Toxicology Studies Route of Administration

- Different routes (and species) have different volume limitations
- Many CROs have their own limits based on experience and animal care and use policies

Table 1. Administration volumes considered good practice (and possible maximal dose volumes)[a]

Species	Route and volumes (ml kg^{-1})					
	Oral	s.c.	i.p.	i.m.	i.v. (bolus)	i.v. (slow inj.)
Mouse	10 (20)	10 (40)	20 (80)	0.05 (0.1)[b]	5	(25)
Rat	10 (40)	5 (10)	10 (20)	0.1[b] (0.2)[b]	5	(20)
Rabbit	10 (15)	1–2	5 (20)	0.25 (0.5)	2	(10)
Dog	5 (15)	1–2	1 (20)	0.25 (0.5)	2.5	(6)
Marmoset	5 (15)	2–5	– (10)	0.25 (0.5)	2	–
Marmoset	10 (15)	2–5	– (20)	0.25 (0.5)	2.5	(10)
Minipig	10 (15)	1–2	1 (20)	0.25 (0.5)	2.5	(5)

[a]For non-aqueous injectables, consideration must be given to time of absorption before re-dosing. No more than two intramuscular sites should be used per day. Subcutaneous sites should be limited to two or three sites per day. The subcutaneous site does not include Freund's adjuvant administration.

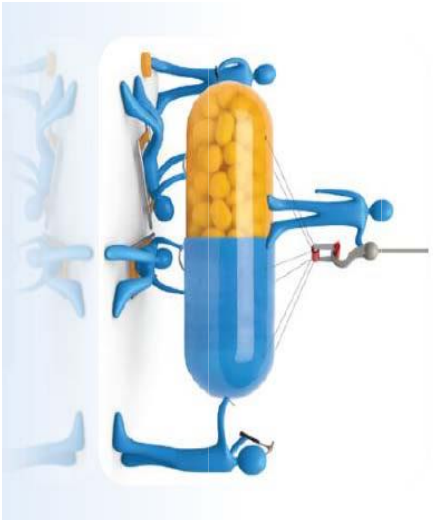
[b]Values in millilitres per site.

–Data not available.

Diehl et al., J. Appl. Toxicol. 21. 15-23 (2001)

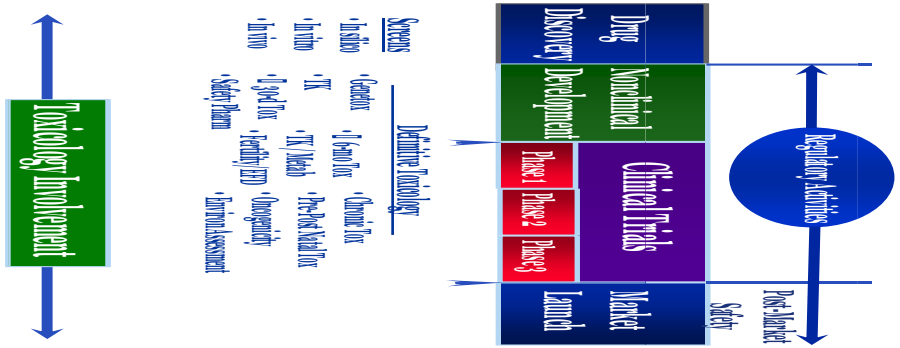
Design of Toxicology Studies: General Considerations

- ***The test article (active pharmaceutical ingredient; API) should be representative of what will be used clinically***
  - Qualification of impurities (ICH Q3; M7)
  - Characterization of test and control articles
    - Identity, strength, purity, and composition
    - Stability, homogeneity
- Considerations for inactive ingredients (excipients) and final formulation used nonclinically e.g., IV polysorbate 80 causes histamine release in dogs
- "novel" excipients may require stand-alone data

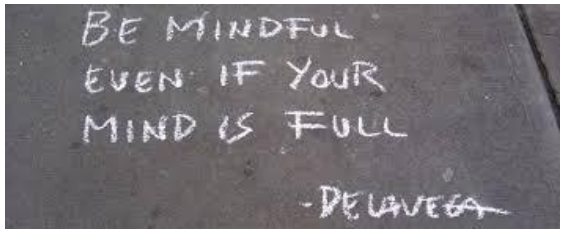


Design of Toxicology Studies: Duration of Treatment

- Duration (and timing) of general toxicity studies usually related to human exposure
- e.g., for drugs, duration/scope of proposed clinical trials for drugs



Clinical Trial Duration	Toxicology duration to support Ph 1, 2 (EU) & Ph 1, 2, 3 (Japan)		Toxicology duration to support Ph 3 (EU) & Marketing (All Regions)	
	Rodent	Non-Rodent	Rodent	Non-Rodent
Single dose	2 weeks	2 weeks	-	-
< 2 weeks	2 weeks	2 weeks	1 month	1 month
1 month	1 month	1 month	3 months	3 months
< 3 months	3 months	3 months	6 months	Chronic
> 3 months	-	-	6 months	6 months
< 6 months	6 months	6 months	-	-
> 6 months	6 months	Chronic	-	-



ICH M3(R2) Guidance

Design of Toxicology Studies: Dose Selection

- One of the most challenging aspects of study design!
  - Should be intentional and explicit in justification
- Typically, doses selected based on pilot work, dose range finding (DRF)studies, anc shorter-term GLP studies
  - 7-to10-dayDRF (with no or limited histopathology) guides dose selection for 14-day or 28-day IND-enabling GLP studies
    - 28-day study to select doses for 13-week study
      - 13-week study to select doses for chronic study (& rodent carcinogenicity)
- Number of dose groups dependent on objectives & degree of uncertainty
- Typically, 3 dose levels plus control(s)
  - High Dose = MTD (define target organs, reversibility, etc.)
    May need to reduce or stop dosing if unexpectedly excessive toxicity
  - Low Dose = NOAEL and systemic exposure at clinical efficacious dose
  - Mid Dose = some toxicity but <MTD (understand dose-response)

  - May have untreated (saline) control group in addition to a vehicle control

Design of Toxicology Studies: Dose Selection

- Five general criteria for defining the High Dose (ICHM3(R2))
    1. Maximum tolerated dose (MTD)
        - Dose where target organ toxicity is likely to be observed but where the dose is not so high that the study, or the interpretation of results, is jeopardized by morbidity or mortality
        - MTD is usually determined by parameters such as clinical signs and reductions in body weight and food consumption

    2. Limit dose
        - If cannot define sufficient toxicity (can not achieve MTD)
        - Generally accepted as 1000 mg/kg (both rodents & nonrodents)
        - If 1000 mg/kg/day < 10-fold margin of exposure to clinical exposure, drive high dose to achieve 10-fold exposure margin OR employ high dose of 2000 mg/kg/day OR the Maximum Feasible Dose (MFD)

Design of Toxicology Studies: Dose Selection

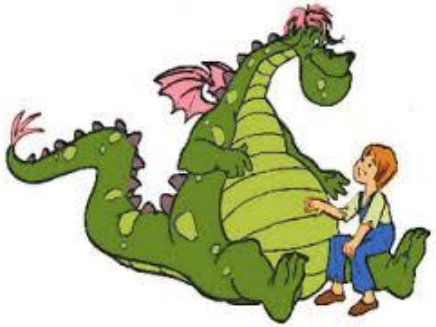
3. Top dose based on saturation of exposure
    - Toxicokinetic data indicate that absorption limits systemic exposure to the drug or its metabolite(s)
    - Acceptable to use the lowest dose which achieves maximum exposure in the absence of other dose limiting constraints
4. Top dose based on Maximum feasible dose (MFD)
    - Bound by limits of technically feasibility:
        - Highly relevant for IV formulations
        - Must justify due diligence efforts to achieve best formulation
5. Top dose based on 50-fold margin of exposure
    - Relative to maximum clinical exposure (AUC)

Maximum possible concentration of formulated test article

X

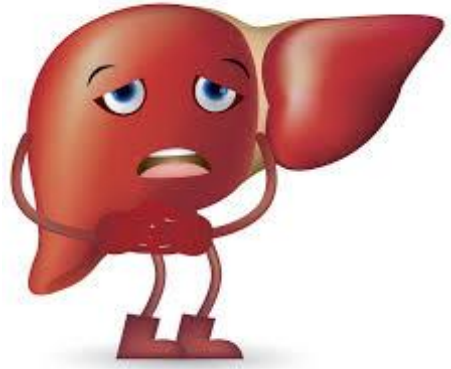
Maximum dose volume administer-able over study duration

Design of Toxicology Studies: Parameters Assessed



- ***In-life assessments***

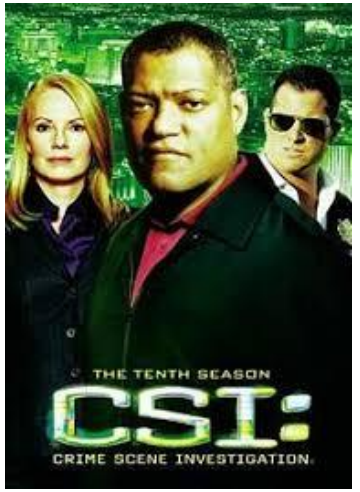
  - Toxicokinetics, Body weight, food consumption, clinical observations Safety pharmacology: cardiovascular, Behavior, Ophthalmology
  - Clinical pathology (hematology, clinical chemistry, urinalysis)
    - Can be sensitive markers of alterations
      - ↑ hepatic enzyme activity prior to morphologic changes
    - Can be very specific to organ system Troponins and myocardial alteration
    - Can assess functional impact of injury
      - Impact of bone marrow lesion on peripheral hematology
      - Can be evaluated over the study course (nonrodents)



Design of Toxicology Studies: Parameters Assessed

- ***Postmortem Evaluations***

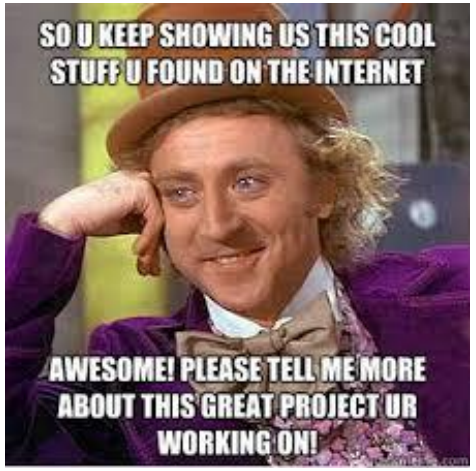
  - Complete necropsy exams on all animals
    - Dying spontaneously or killed in extremis
    - Euthanized at scheduled necropsies (not TK animals)
  - Organs/tissues weighed, preserved, & microscopically examined:
    - Comprehensive list of tissues*
  - Rodents: typically, histopathology performed for control and high dose animals initially
    - "Read down" of identified target organs from low & mid dose subsequently evaluated
  - Nonrodents: all animals and tissues examined
  - Special stains and electron microscopy, if warranted

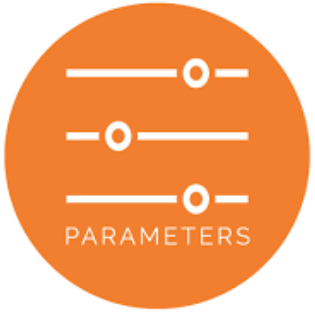


* Society Toxicologic Pathology: Bregman et al., Toxicol Path 31:252-253 (2003); FDA response (Jacobs et al., Toxicol Path 31:571 (2003)

Design of Toxicology Studies: Parameters Assessed

- Incorporate specific assessment endpoints to address expected (based on pharmacology or compound class) or previously observed “signals”
  - e.g.,: Serum testosterone, estradiol, LH, FSH for a hormone agonist
  - Liver toxicity (EMA’s “Reflection Paper on Non-Clinical Evaluation of Drug-Induced Liver Injury (DILI)”)
- Markers of cell proliferation (anticipating carcinogenicity concern)
- Immunotoxicity assessments (ICHS8;FDA2002)
- Standard toxicology studies(STS) to detect immune suppression/activation
- Hematology (e.g., cytopenias, leukocytosis)
- Organ weight and histopathologic examination of immune system organs (lymph nodes, spleen, thymus)
  - 2nd Tier testing based on results of STS (e.g., functional assays)
    - T-cell dependent antibody response, lymphocyte immunophenotyping, inflammatory biomarkers ...



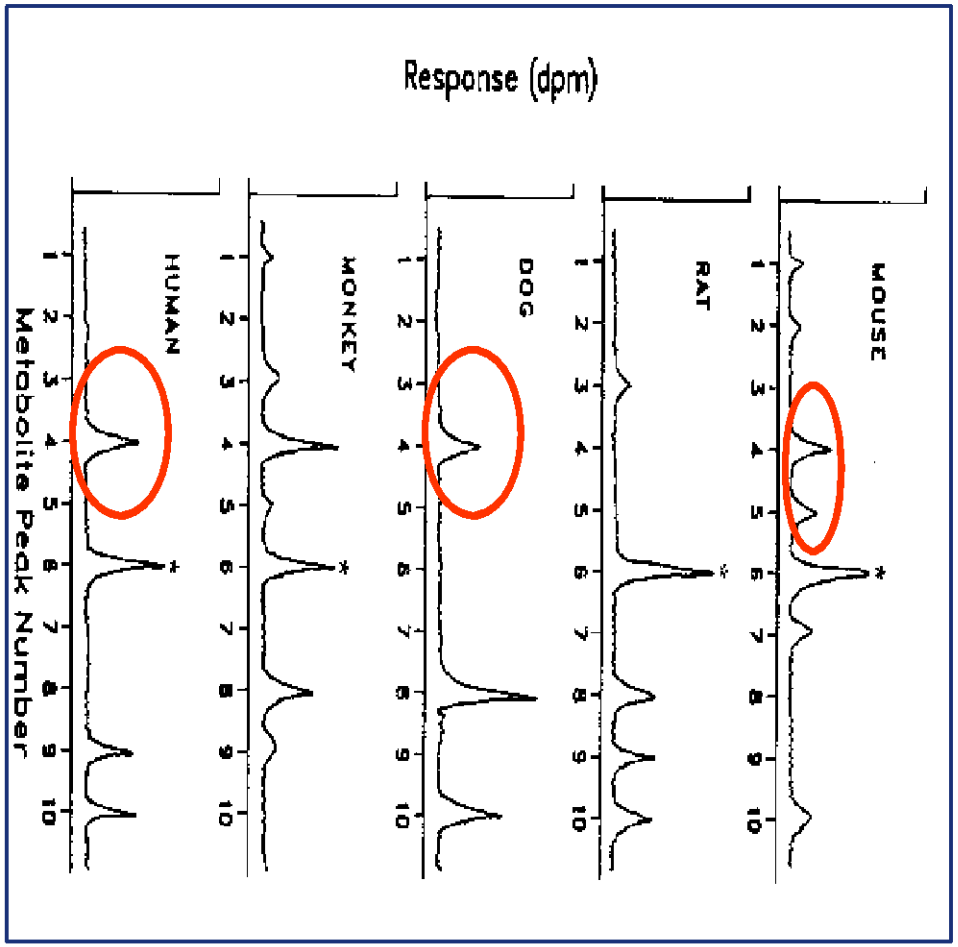


Some Data with Drug A

Drug A

- Small molecule inhibitor of Target A
- Clinical use: Daily oral dosing
- Chronic indication: a non-life-threatening disease for which other, partially-effective treatments exist

- Species selection
  - Rat and dogs selected as toxicology species based on similarities in:
    - Metabolic profile: human metabolites of Drug A present in rats & dogs (in vitro)
    - Target expression: Target A is highly conserved: mouse, rat, dog, monkey, human; although, in vitro data indicate that human Target A is ~3x more sensitive than mouse Target A
- 1-month (28-day) studies will support First-in-Human clinical trials (<1-month in duration) Include 1-month reversibility groups



1-Month Rat Gavage Study with Drug A:
Main Study but Recovery Group (5/5) Used in Overall Assessment

Dose (mg/kg)	0		0.1		1		10	
N=10/sex	M	F	M	F	M	F	M	F
Mortality*	0	0	0	1	0	0	1	0
Clinical observations – mostly reversible								
Soft feces*	4	2	2	2	2	6	8	12
Diarrhea*	1	2	1	2	1	3	4	6
Rough haircoat*	1	0	2	1	2	5	3	6
Body weight (% change vs control) Wk 12	N/A	N/A	--	↓6	↓4	↓10	↓10 NR	↓15 NR
Clinical pathology (change relative to control) – all reversible								
Altered electrolytes	--	--	--	--	↑	↑	↑	↑
↑ALT	N/A	N/A	--	↑	↑	↑	↑	↑
↑AST	N/A	N/A	--	--	--	--	↑	↑
↑Total cytochrome P450	N/A	N/A	--	↑	↑	↑	↑	↑
Morphologic pathology								
Stomach: necrosis & atrophy	--	--	--	--	--	--	↑ NR	↑ NR
Liver: Hepatocellular vacuolation	--	--	--	--	↑	↑	↑ NR	↑ NR
Liver: Hepatocellular necrosis	--	--	--	--	--	--	↑	↑
Heart: Myocardial necrosis	--	--	--	--	--	↑ NR	↑ NR	↑ NR

*# of rats affected N/A = Not Applicable -- No important changes NR = Not Reversible

1-Month Rat Gavage Study with Drug A:
How do we systematically interpret the data?

NOAEL?

Dose (mg/kg)	0		0.1		1		10	
N=10/sex	M	F	M	F	M	F	M	F
Mortality*	0	0	0	0	0	0	1	0
Clinical observations – mostly reversible								
Soft feces*	4	2	2	2	2	6	8	12
Diarrhea*	1	2	1	2	1	3	4	6
Rough haircoat*	1	0	2	1	2	5	3	6
Body weight (% change vs control) Wk 12	N/A	N/A	--	↓6	↓4	↓10	↓10 NR	↓15 NR
Clinical pathology (change relative to control) – all reversible								
Altered electrolytes	--	--	--	--	↑	↑	↑	↑
↑ALT	N/A	N/A	--	↑	↑	↑	↑	↑
↑AST	N/A	N/A	--	--	--	--	↑	↑
↑Total cytochrome P450	N/A	N/A	--	↑	↑	↑	↑	↑
Morphologic pathology								
Stomach: necrosis & atrophy	--	--	--	--	--	↑	↑ NR	↑ NR
Liver: Hepatocellular vacuolation	--	--	--	--	↑	↑	↑ NR	↑ NR
Liver: Hepatocellular necrosis	--	--	--	--	--	↑	↑	↑
Heart: Myocardial necrosis	--	--	--	--	--	↑ NR	↑ NR	↑ NR

* # of rats affected N/A = Not Applicable -- No important changes NR = Not Reversible

Oh S#%!: Low dose
Likely not drug related,
High dose at MTD

Background:
monitorable

Adverse related to
other findings, MTD

Monitorable:
Non-adverse along,
Target organ predictive

Adverse

Non-adverse

Adverse

Adverse and NOT
MONITORABLE

More data: 1-Month Dog Study with Drug A

NOAEL?

Dose (mg/kg)	0		0.1		1		10	
	M	F	M	F	M	F	M	F
Mortality*	0	0	0	0	0	0	0	0
Clinical observations - all parameters reversible								
Soft feces*	1	1	2	1	2	3	3	4
Lacrimation*	--	--	1	--	2	3	3	4
Body weight (% change vs control) Week 12	N/A	N/A	--	--	--	--	D6	D7
Clinical pathology (change relative to control) - all parameters reversible								
↑ALT	N/A	N/A	--	--	--	--	D	D
↑Total cytochrome P450	N/A	N/A	--	--	--	--	D	D
Morphologic pathology - all parameters reversible								
Liver: Hepatocellular vacuolation	--	--	--	--	--	--	D	D
Lacrimal gland: atrophy	--	--	--	--	--	--	D	D
NOAEL	D							

MS: M3 Rec 2/2 Vehicle and High

of dogs affected N/A = Not Applicable -- = No important changes

Do we have a path forward with Drug A?

- NOAEL 0.1 mg/kg in rat
- NOAEL 10mg/kg in dog
- Overall our path to the clinic is being able to support dosing of 0.1 mg/kg (converted to body surface area equivalent [HEC] in clinic)
- Rats currently the “most sensitive species”
- If our translational pharmacology/ PD indicators won’t work at 0.1mg/kg, then the drug dies a hard death!!!!!
  - But wait, both options can actually be good depending on who you are working for!!!!!

What we did not cover??????????

Carcinogenicity

- Prior to approval
  - 2-year carcinogenicity assay in rodents and/ or genetically modified mouse

What we did not cover?????????

Developmental and Reproductive Toxicology; Juvenile toxicology

- Supports neonatal-juvenile patient populations and those women of child bearing age/ who could become pregnant; must be performed prior to clinical administrations in these populations
- Rats and rabbits used most often
- Clinical route of administration
- Endpoints generally focus on sex organs or the fetus

What we did not cover????????

Vaccines

- Immunogenicity generally is the leading cause of adverse events/ tox
- N (clinical administration); N + 1 administration in nonclinical studies
- 3m/3f (rabbits generally used as main species supporting safety and IND-approvals)
- Many of same standard endpoints for toxicity assessments
- Novel excipients may need additional toxicology packages

What we did not cover????????
Cell and Gene Therapy????? Things to consider?

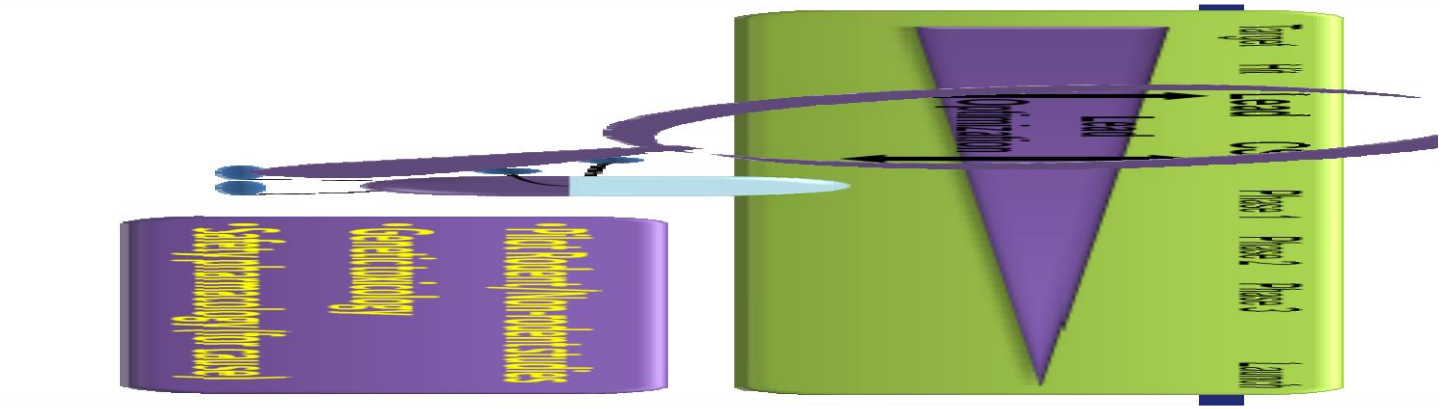
All bets are off!!!!

- Safety studies performed by clinical route of administration
- Ex vivo-In Vivo
- Viral vectors and tropism of cell type important (insertion)
- Crisp-CAS; Zinc Finger, etc.
- Cell source
- Lipid nanoparticles and gene delivery; excipients (liver targets?); IV
- Pharmacology models used to assess safety
- Many times only one animal species (most relevant to human condition); NHPs and mouse used in many cases
- Endpoints are similar
- Special endpoints (biodistribution of gene or viral genes); near a promoter region for tumor suppressors / promoters?

Application of General Toxicology Studies to Support Regulatory Requirements for Development of Therapeutics

Courtesy of Michael Dorato

Early Drug Development: Lead Optimization



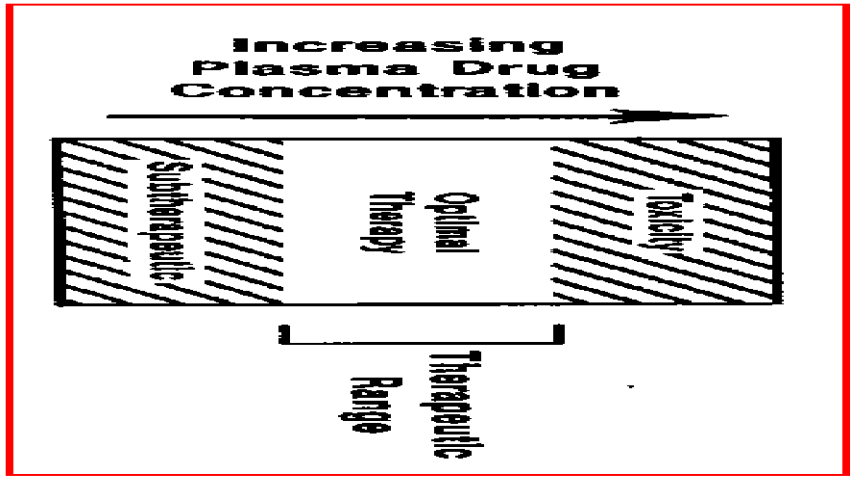
- Provide initial characterization of target organ toxicities
- Estimate therapeutic margins based on efficacious/ toxic exposures
- ID issues to monitor/ manage in Development
- Provide pilot data to aid in design of definitive tox studies supporting FIH
- Includes investigative mechanisms of toxicity to…….
  - Develop better screening paradigms
  - Evaluate human relevance of adverse effect
  - Identify clinically useful biomarker of toxicity

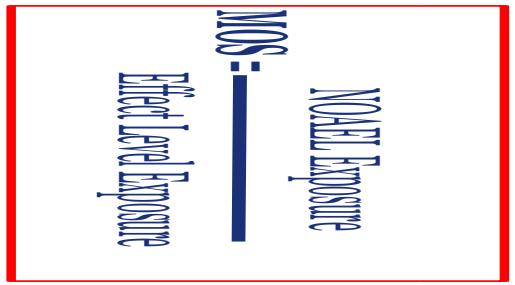
Courtesy of Anja Stauber

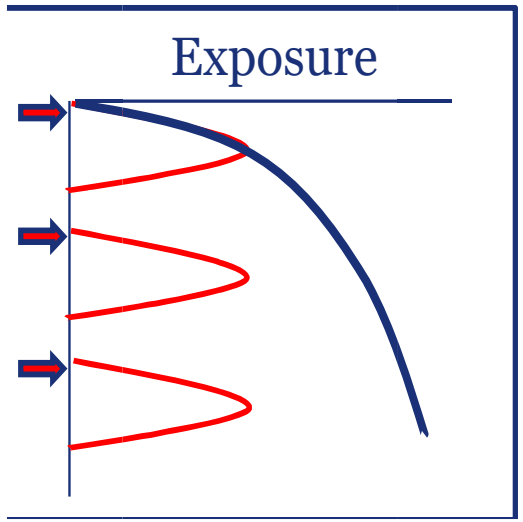
Therapeutic Margin for Drugs

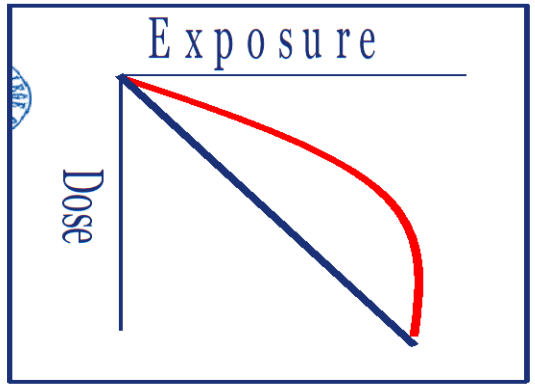
ADME/PK Data Supports Nonclinical Safety

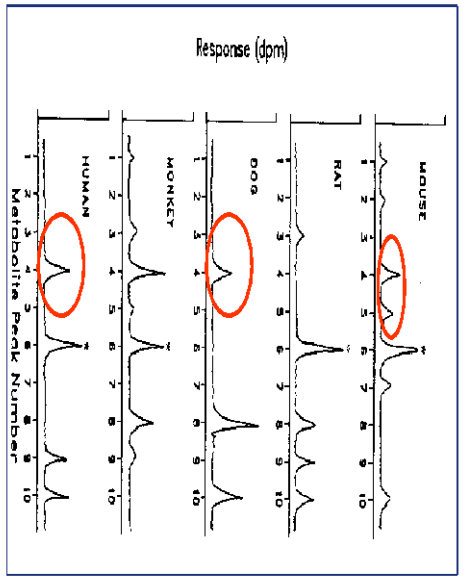
- Evaluation of exposure to parent drug in toxicology species
  - Establish dose-exposure relationship
  - Species or sex differences
  - Insight into enzyme induction
  - Cross-species metabolism
- Extent of accumulation
  - MOS based on exposure



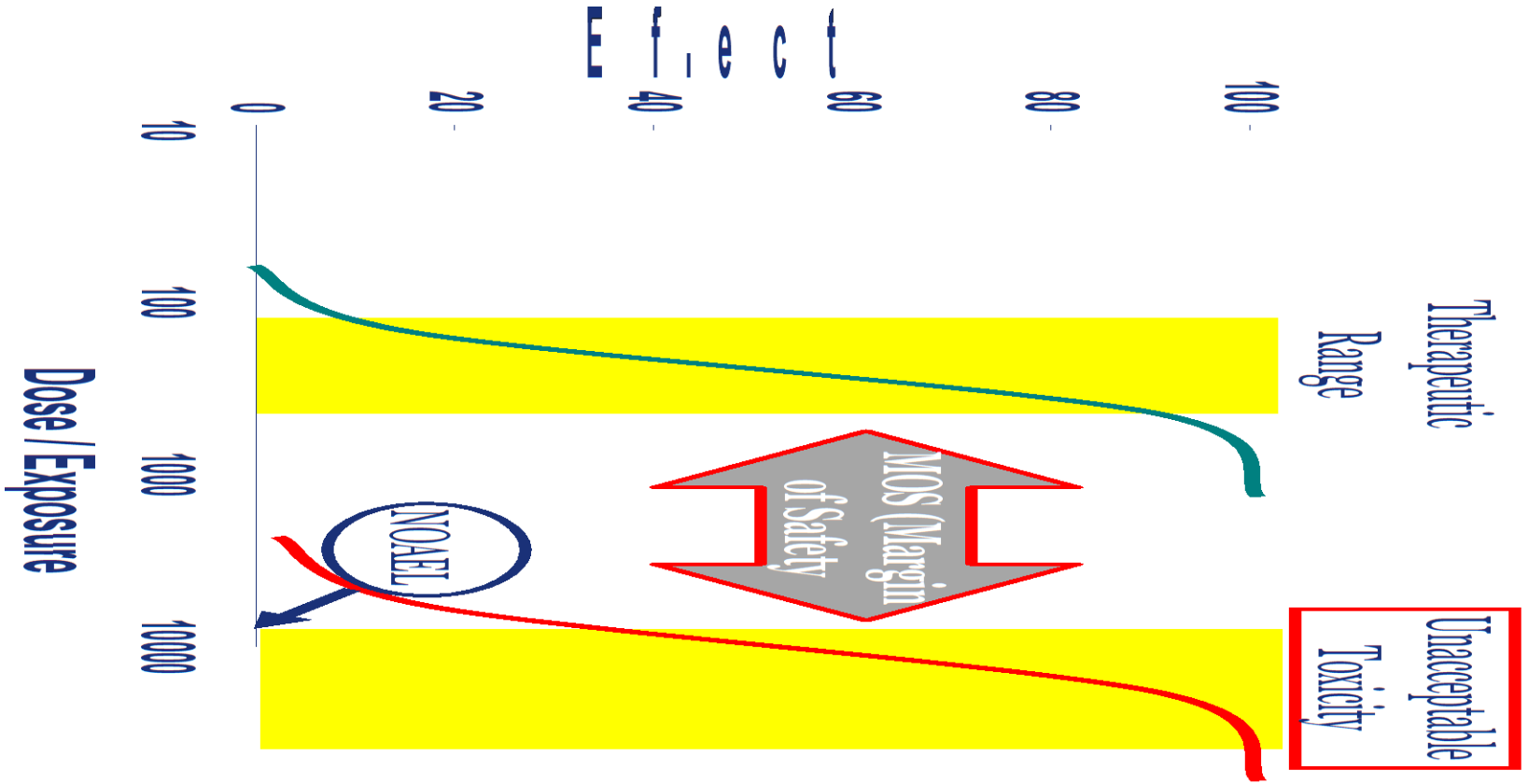








Clinical Dose Selection:
Estimating MAXIMUM Recommended Starting Dose



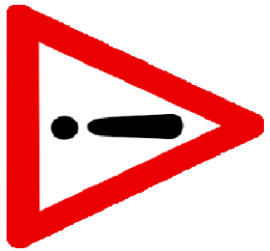
Clinical Dose Selection of an Estimated Maximum Recommended Starting Dose (MRSD) using NOAEL

Defining NOAEL for Pharmaceuticals

Highest dose/exposure that does not cause biologically important increases in the frequency or severity of effects between the exposed population and the appropriate control. While minimal toxic effects may be observed at this level, they are not considered to endanger human health, or be precursors of serious events.

The NOAEL is not Risk Free

Variability in species and individual response, and existence of sensitive populations, increases the probability of undesired effects



M. A. Dorato, J. A. Engelhardt. The no-observed-adverse-effect-level in drug safety evaluations: Use, issues, and definition(s). Reg. Tox. Pharm., 42(2005) 265-274.

The “Facts” About the NOAEL

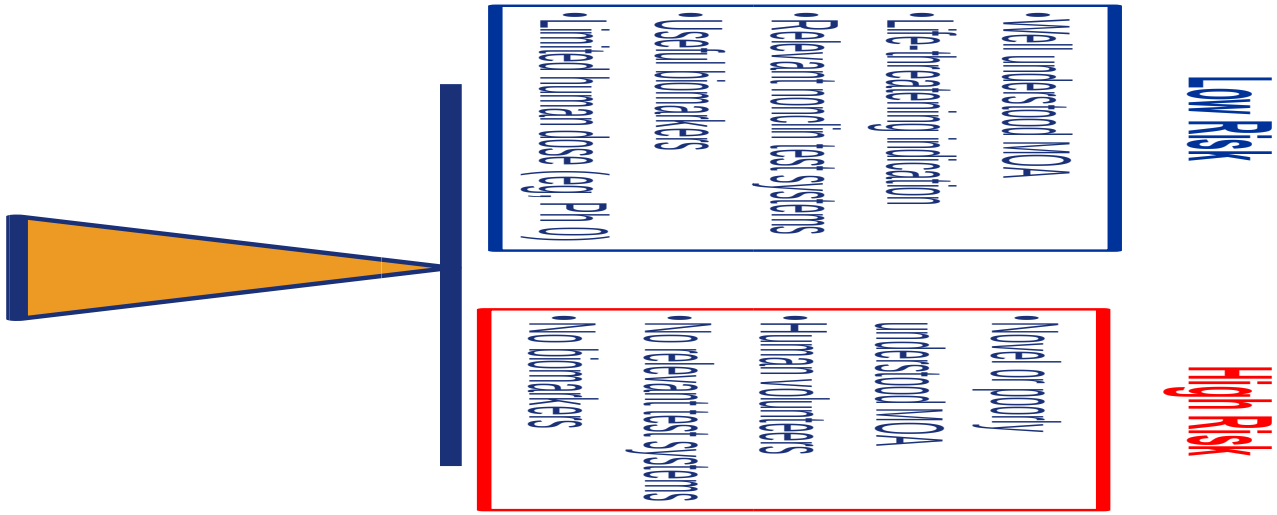
- The NOAEL must be one of the NOAEL experimental doses tested and, thus, is influenced by the study design
- The NOAEL does not consider the slope of the dose response curve or the nature of the effects at the next highest dose
- The NOAEL is sensitive to sample size with a tendency to be higher with fewer animals/dose
- The NOAEL may vary from experiment to experiment
- Risk levels may be higher than estimated by the NOAEL reflecting variability in species and/or individual responses as well as the possibility of sensitive populations (i.e., toxicology studies are conducted using “healthy” animals)
- Definition of “adverse” can vary between reviewers

Clinical Dose Selection: Other Considerations (e.g., Safety Factor)

Default SF = 10

- Factors which may justify need for ↑↓ MOS
  - Increase SF non-monitorable
    - steep dose/response
    - variable bioavailability or PK
    - novel therapeutic targets
  - Decrease SF
    - well-characterized therapeutic class
    - monitorable/ reversible effects
    - predictable toxicity

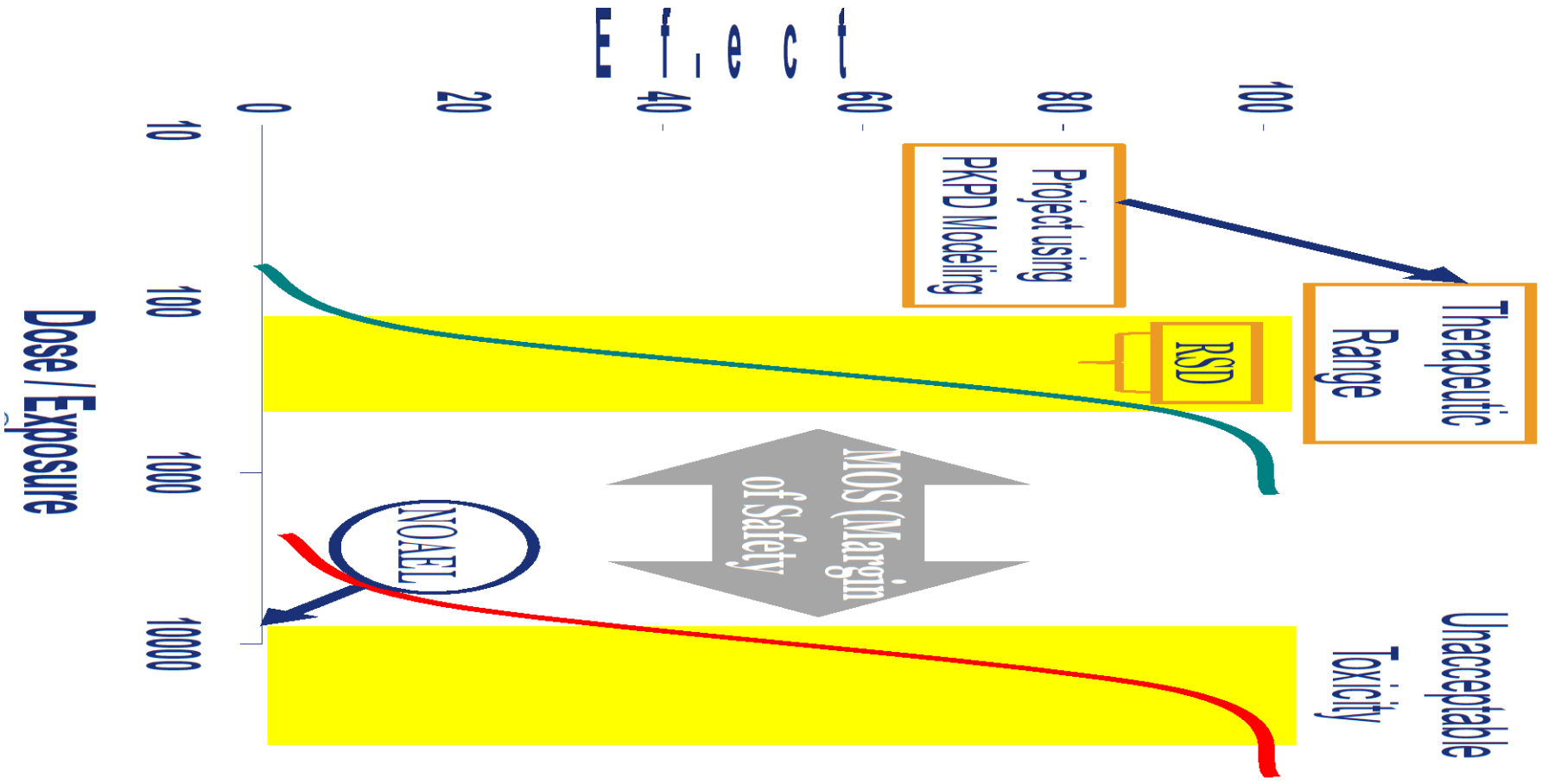
Benefit – Risk Considerations



Goal: Minimize risk and escalate dose accordingly

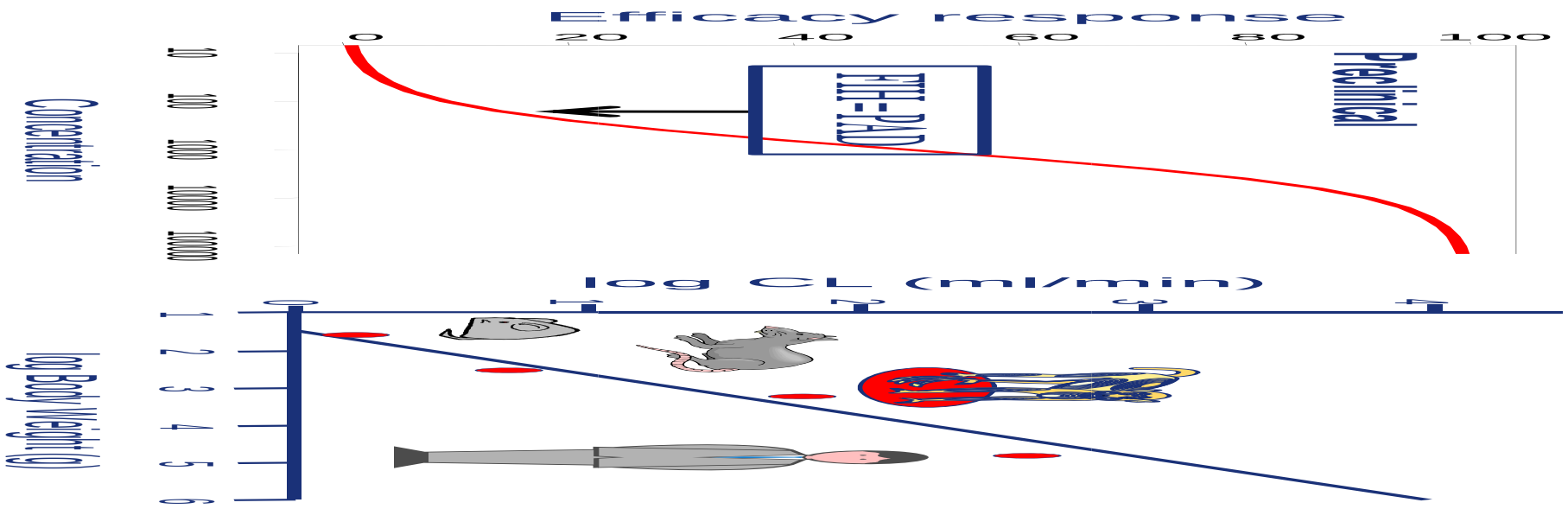
Tibbets 2011

Clinical Dose Selection: Estimating Clinically EFFICACIOUS Dose



Clinical Dose Selection: Modeling Human PK-PD from Nonclinical Data

Pharmacodynamics (PD) **Pharmacokinetics (PK)**



Assume concentration x efficacy response in animal model predicts that for humans (First In Human dose = Pharmacologically Active Dose)

Interspecies allometric scaling based on anatomical, physiological & bio-chemical similarities among animals; proportional to body mass/size

Benefit – Risk Considerations

Application of General Toxicology Studies to Support First Safe Dilutions

Following Slides Courtesy Eric Foxman

Attenuation (Medication) Level Changes

The Toxicity and Safety Committee considers up to *seven categories* of information when calculating the OTC Attenuation Level (First Safe Dilution):

- Single Dose Toxicity
- Repeat Dose Toxicity
- Genotoxicity, Carcinogenicty, Reproductive and Developmental toxicity
- Local Tolerance
- Antigenicity
- Immunotoxicity
- Addiction Potential

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- Homeopathic Drug Stability Guidelines
- Expanded Labeling Guidelines
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Table of Alcohol Strength, Manufacturing Class and Dispensing Potencies for Monographs recognized by the HPCUS

The following table has been compiled by the committees of the Homœopathic Pharmacopœia Convention of the United States to establish standards for the preparation and dispensing of official homeopathic drug products. The table lists 1,286 monographs that have been approved by the HPCUS Board of Directors and published in the HPUS Revision Service (HPRS). Products claiming to be official homeopathic drug products and/or bearing the appellation "HPUS" on their labels must be manufactured in accordance with the standards of the Homœopathic Pharmacopœia of the United States as referenced in the General Pharmacy and Good Manufacturing Practices sections, and as specified in their respective monographs. Such products must be labeled as OTC, External Use, or Rx products in accordance with the designations appearing in the table. These designations are defined as follows:

NAME
The official name for the homeopathic drug product.

LIQUID CLASS
The appropriate class reference in the General Pharmacy section of the HPRS under which the product should be prepared in liquid form, if such form is possible.

SOLID CLASS
The appropriate class reference in the General Pharmacy section of the HPRS under which the product should be prepared in solid form, if such form is possible.

Note to interpreting this table: This table was developed using *acute toxicity data* from the literature. Those data were used to determine the *100-fold margin of safety* associated with the *accidental ingestion* by a *10 kg child* of a maximum dose of 30 ml or 16.2 g (250 1-grain size tablets), whichever provides a greater dose, of the noted substance. *The potencies listed are the finished concentration equivalent, defined as the actual concentration of the starting material delivered in the finished product.*

Accidental ingestion

Assumption: child accidentally ingests entire usual consumer package.

Parameters for calculation of the First Safe Dilution (FSD):

Child's body weight = 10 kg (Adult = 60 Kg)

Consumer Package: 16.2 g trit. (equivalent to 250 tablets of 1 grain size [64.8 mg]) or 30 ml dilution (drops)

This calculation is the current *primary* approach of HPUS to determine the FSD.

Attenuation (Medication) Level Changes

Accidental ingestion

Max. Accidental Ingestion Amt.	Entire Consumer Package 16.2 g or 30 ml

Attenuation (Medication) Level Changes

Accidental ingestion

Max. Accidental Ingestion Amt.

Entire Consumer Package
16.2 g or 30 ml

% Content
(in Starting material)
Of Subst. of Concern

Found in Literature
(note need for analyses GMP)

(e.g. Belladonna up to @ 0.5%) Alkaloids
or
Nux Vomica up to 1.5% Strychnine
or
Asclepias curr. up to 0.6% Cymarin

Attenuation (Medication) Level Changes

Accidental ingestion

Max. Accidental Ingestion Amt.	Entire Consumer Package 16.2 g or 30 ml
% Content (in Starting material) Of Subst. of Concern	Found in Literature
Attenuation Deconcentration Basis	1X = "$1x10^{1}$" 2X = "$1x10^{2}$" 3X = "$1x10^{3}$" 4X = "$1x10^{4}$" Etc. 3X = "$1x10^{3}$" = 1x10x10x10

Attenuation (Medication) Level Changes

Accidental ingestion

Max. Accidental Ingestion Amt.	Entire Consumer Package 16.2 g or 30 ml
% Content (in Starting material) Of Subst. of Concern	Found in Literature
Attenuation Deconcentration Basis	1X = "$1x10^{1}$" 2X = "$1x10^{2}$" 3X = "$1x10^{3}$" 4X = "$1x10^{4}$" Etc.
Qty Ingested of Subst. of Concern	Actual Amt. Accidentally Ingested by consuming entire consumer Package of homeopathic product

Attenuation (Medication) Level Changes

Accidental ingestion

$$\left(\frac{\text{Max. Accidental Ingestion Amt.} \times \text{\% Content (in Starting material) Of Subst. of Concern}}{\text{Attenuation Deconcentration Basis}} \right) = \text{Qty Ingested of Subst. of Concern}$$

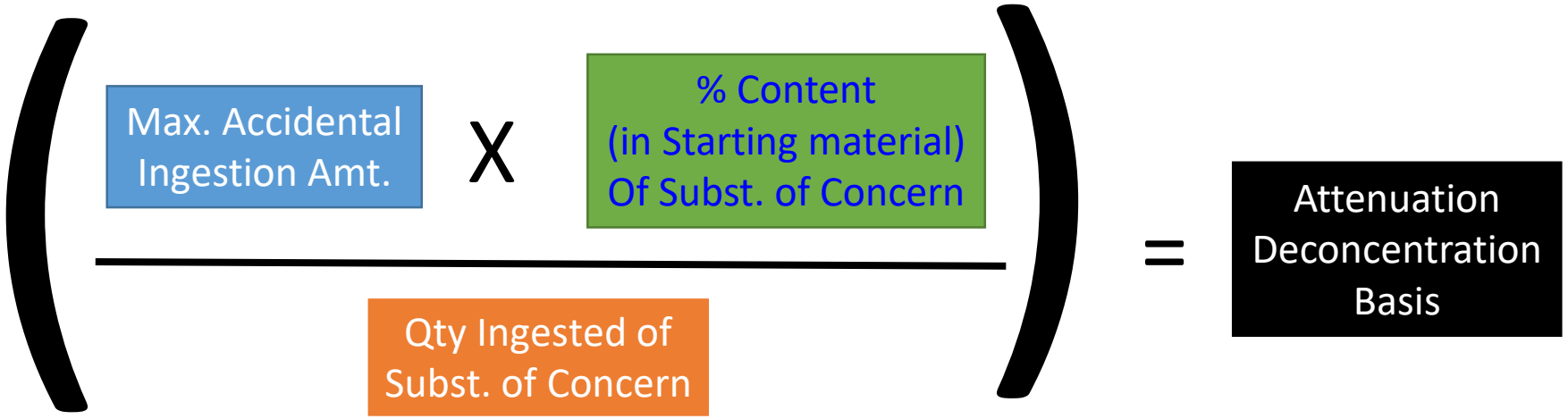
Attenuation (Medication) Level Changes

Accidental ingestion -- Formula for calculating

Max. Accidental Ingestion Amt.	Entire Consumer Package 16.2 g or 30 ml
% Content (in Starting material) Of Subst. of Concern	Found in Literature
Qty Ingested of Subst. of Concern	What if we know the Min. Qty that Triggers an Adverse Event? Again, taking into account child's weight!)
Attenuation Deconcentration Basis	**Can use the *Formula* to Calculate FSD**

Attenuation (Medication) Level Changes

Accidental ingestion -- Formula for calculating



Attenuation Deconcentration Basis

→ *Round Exponent up to the next integer.*

The Exponent is the calculated FSD.

Attenuation (Medication) Level Changes

Accidental ingestion -- Formula for calculating

$$\left(\frac{\text{Max. Accidental Ingestion Amt.} \times \text{\% Content (in Starting material) Of Subst. of Concern}}{\text{Qty Ingested of Subst. of Concern}} \right) = \text{Attenuation Deconcentration Basis}$$

Attenuation Deconcentration Basis

→ *Round Exponent up to the next integer.*

The Exponent is the calculated FSD.

Add 2 additional Decimal Attenuation levels as Additional Safety Factor (100-fold more dilute)

Attenuation (Medication) Level Changes

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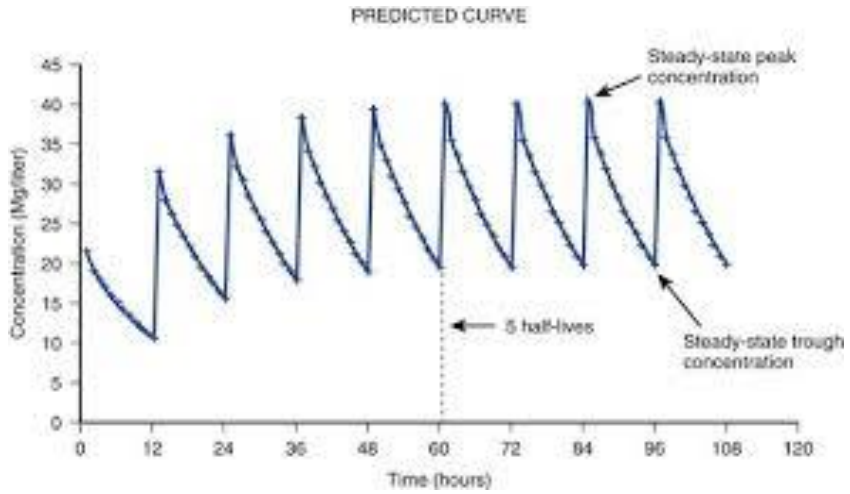
But…

What about

Repeated Doses or Chronic Exposure?



Attenuation (Medication) Level Changes

Permissible Daily Exposure (PDE) [recall we discussed in sessions 1?]

Method for calculating:

Appendix 3 ICH "Impurities: Guideline for Residual Solvents Q3C(R5)"

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Attenuation (Medication) Level Changes

Permissible Daily Exposure (PDE)

$$PDE = \frac{(NOAEL \times Weight\ Adjustment)}{(F1 \times F2 \times F3 \times F4 \times F5)}$$

NOAEL = No Observable Adverse Event Level

F1 = factor used for the extrapolation from animal to humans.
F2 = factor used for variability between individuals.
F3 = factor used for different study period times.
F4 = factor used for severe toxicity (but non-carcinogenic, non-teratogenic, non-genotoxic)
F5 = factor used if no NOAEL data available but LO(A)EL data is

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Attenuation (Medication) Level Changes

But…

What about

Carcinogenic, Teratogenic, Genotoxic substances?

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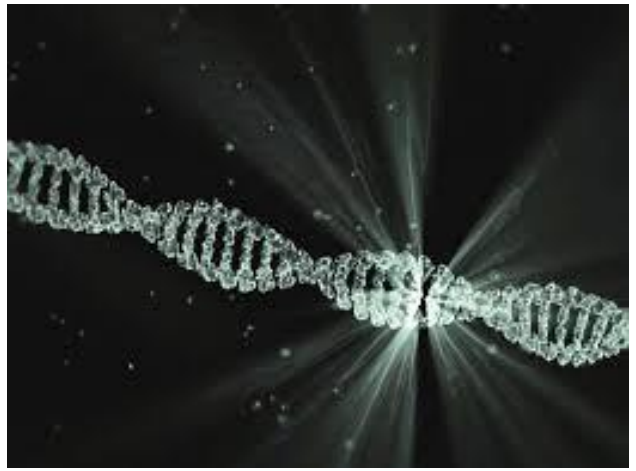
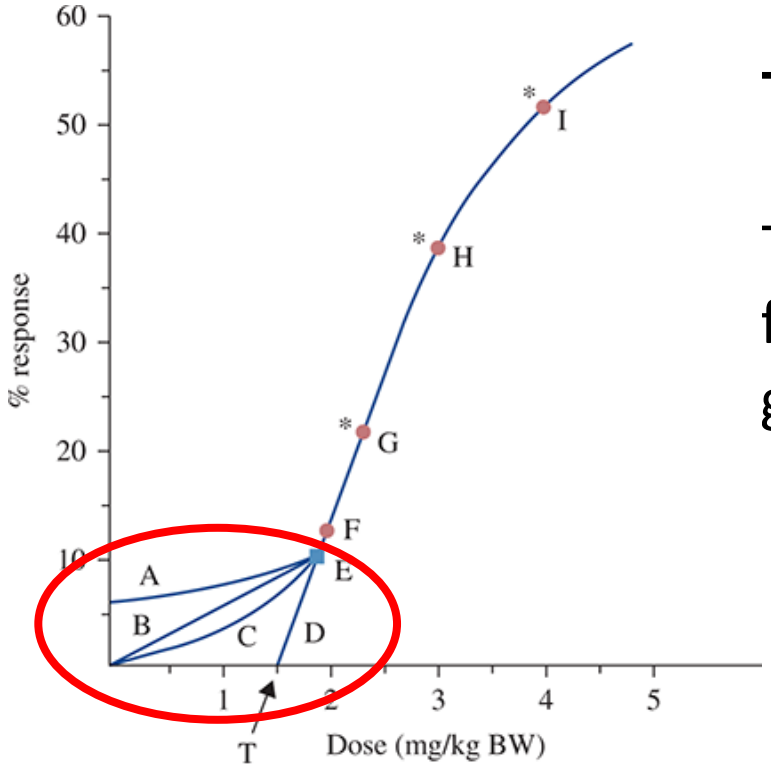


Attenuation (Medication) Level Changes



Source: Curtis D. Klaassen, PhD, DABT, ATS, FAASLD: *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 9e

Threshold of Toxicological Concern (TTC)

TTC allows safety evaluation of substances
for which insufficient toxicological data is available; provides a general threshold value, below which there is no concern.

Review of where we have been today?

- Practicing Risk Assessment
- Basic Tenets of Toxicology Testing
- Program Design Considerations
  - General Regulations and Guidelines
  - Species Selection
  - Specific chemical/product class/use
- Study Design Considerations
  - Specific Design Parameters
- Regulatory application of nonclinical data
  - Nonclinical safety assessment in drug development
  - Assessment of FSD for homeopathy

Key Toxicology References (general)

- Klaassen, C.D., Ed.: Casarett and Doull's Toxicology: The Basic Science of Poisons. 8th Edition, McGraw-Hill, (2013)
- Hayes, A.W., Ed.: Principles and Methods of Toxicology. 6th Edition, CRC Press, (2014)
- ICH Guidelines - Safety (S) and Multidisciplinary M3, M4 and M7. (http://www.ich.org/products/guidelines.html)
- OECD/OPPTS guidelines for the Testing of Chemicals – Human Health Effects (http://www.oilibrary.org/environment/oecd-guidelines-for-the-testing-of- chemicals-section-4-health-effects_20745788)
- U.S. EPA Risk Assessment Guidelines - Human Health (https://www.epa.gov/risk/human-health-risk-assessment), and Ecological (https://www.epa.gov/risk/guidelines-ecological-risk-assessment)
- USFDA Guidance for Industry documents (https://www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ ucm065014.htm)
- National Research Council (NRC). 2009. Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, D.C. https://www.nap.edu/catalog/12209/science-and-decisions-advancing-risk- assessment
- National Academies of Sciences, Engineering, and Medicine. 2017. Using 21st Century Science to Improve Risk- Related Evaluations. Washington, DC: The National Academies Press. https://doi.org/10.17226/24635.
- Torres, J. and Bobst, S. (eds.). Toxicological Risk Assessment for Beginners, Springer, (2015)
- Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P., Eds: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th Edition (2011)

End Presentation