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With support from the Society of Toxicologic Pathology and the Teratology Society

Early Toxicology Studies – From Design to Dose Selection: What You Need to Know

Michael V. Templin, PhD, DABT
Director, Scientific Advisory Services
Charles River Laboratories

Scott E. Boley, PhD, DABT
Vice President of Toxicology
Sinclair Research Center

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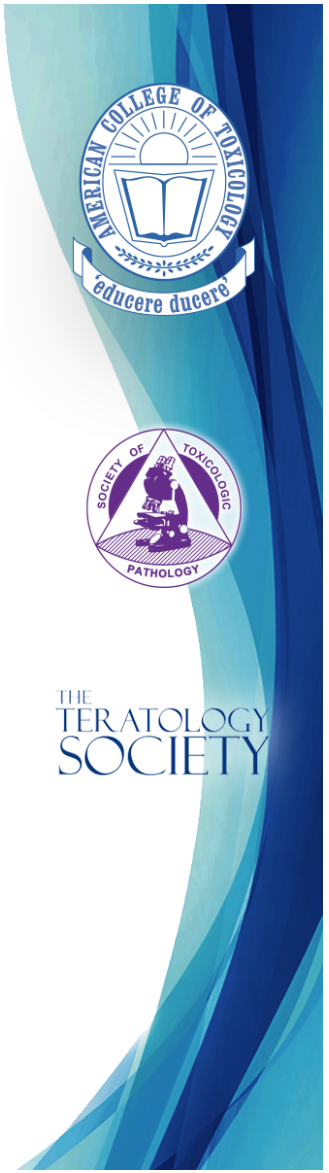


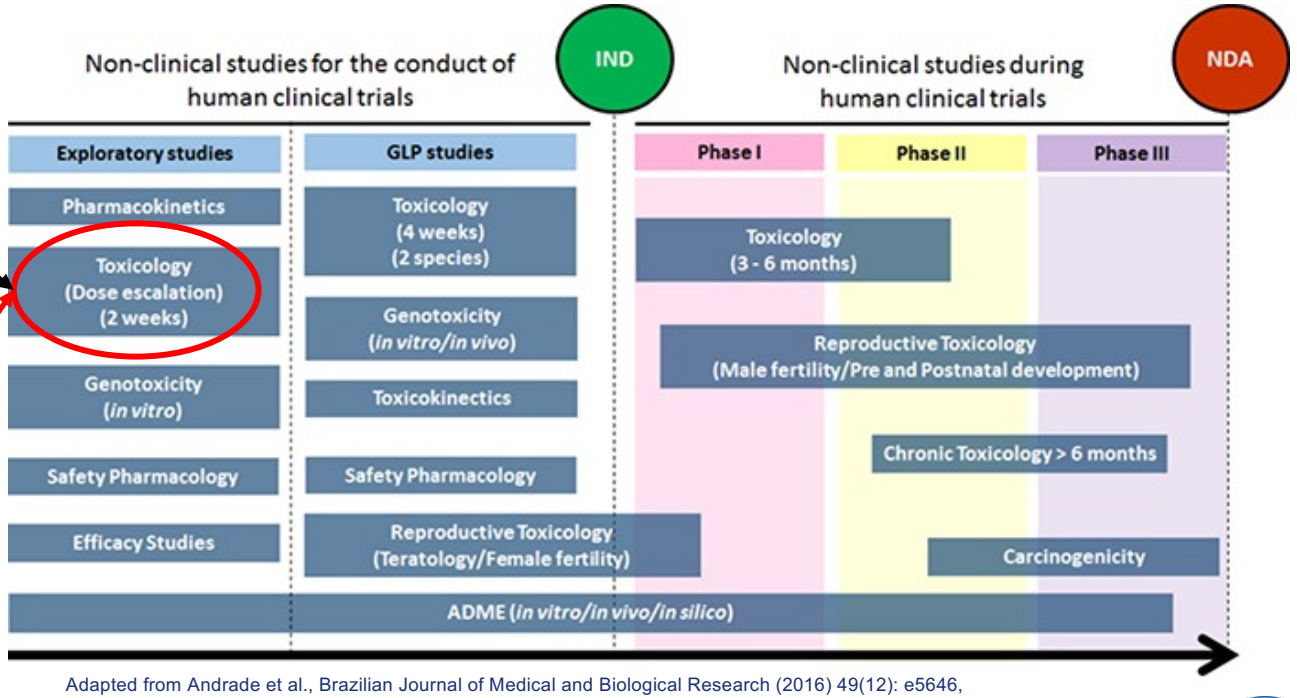
Acute/Dose Range Finding (DRF) Studies

Developing a Nonclinical Program

Drug Development is often represented as defined steps in a process

Acute and Dose Range Finding (DRF) studies are early stage and often considered “exploratory” in nature

Results can have a meaningful impact on the overall nonclinical program



Adapted from Andrade et al., Brazilian Journal of Medical and Biological Research (2016) 49(12): e5646, http://dx.doi.org/10.1590/1414-431X20165646

Objectives for Acute/DRF Studies

1. Primary objective to establish *dose-response* relationship
   - Intended pharmacology
   - Unintended/exaggerated pharmacology
   - Adverse effect/toxicity
2. Primary intent to enable dose selection and design of "regulatory" toxicology studies
   - Goal: expose the lowest number of animals to the highest severity of toxic effects
   - Insufficient data early in the program may require higher animal numbers later in the program

General Points for Consideration

1. Rodent first and non-rodent second vs. reality
   - Common practice to dose rodents prior to non-rodents
     - Scientific and ethical concerns
     - Rodent data can aid in dose selection and design of the non-rodent study
   - Rodent may not predict toxicity in a non-rodent species
   - Rodent may not be a relevant/appropriate species
2. Staggered approach for dose escalation
   - Dosing of one group followed by observation period before next dose level
     - Minimizes the number of animals “at risk”
     - Time interval between dosing can range from minutes, to hours, to days
       - Impacted by drug class, dose route, formulation, and pharmacokinetics

Study Design Considerations

1. Regulatory guidelines and recommendations
   - No guidelines specific to Acute/DRF studies
     - Multiple documents outline the importance of such studies
     - Descriptions of study design are vague…..
       - *"appropriate animal numbers"*
       - *"appropriate species"*
       - *"appropriate dose range and sufficient study duration"*
   - Ultimately it is the responsibility of the individual/development team to determine what is appropriate and sufficient
     - Unwritten rule – *data should provide a path forward, and not more data collection*

Starting Dose and Dose Intervals

1. Selection of a starting (low) dose
   - No “one dose levels fits all” approach to dose selection
   - Starting dose not intended to result in overt toxicity
     - Balance starting too low with dose range to be covered
   - Review all available information to select a starting dose
     - Pharmacology studies (highest therapeutic dose)
     - Pharmacokinetic/metabolism studies
     - Literature/published information of similar compounds
2. Dose escalation (or decrease) plan
   - Half-log interval is a common default approach
     - 1, 3, 10, 30, 100, ……. mg/kg

Formulations and “Inactive Ingredients”

1. Optimal for the acute/DRF study formulation to be the intended clinical formulation
   - Move forward with the best available
     - Utilize previous programs and common formulations
       - E.g., hydroxypropyl methylcellulose with or without a surfactant
     - Work closely with formulators to develop a near and long-term plan
   - Modest changes in formulation can have meaningful impact
     - Increased/decreased systemic exposure
     - Local tolerability can be influenced
   - “inactive ingredient” may not be silent components
     - Co-solvents and solubility agents in early discovery may not transfer into later stages
     - Published information may be available, but often needs a case-by-case review

Additional Considerations

1. Strain, age, and source of the animals
   - When possible match previous studies
     - Mouse pharmacology in specific strains or transgenic animals
2. Inclusion of clinical and anatomical pathology
   - Information provided can be essential for data interpretation
     - For rodent studies, this has a meaningful impact on animal numbers
3. Pharmaco- / Toxicokinetics
   - Standalone (dedicated) studies or include in Acute/DRF studies?
   - Don't forget the bioanalytical assays!

Example Designs

Rat Acute/DRF – Example 1

1. Dose escalation followed by repeat dose
   - Males and females included in data collection

Group	Dose (mg/kg)	Main Study Animals	
		Male	Female
1 (escalating)	Level 1	3	3
2 (escalating)	Level 2	3	3
3 (escalating)	Level 3	3	3

Dose levels needed to identify a maximal dose?

Number of animals needed for evaluation?

Rat Acute/DRF – Example 1

1. Dose escalation followed by repeat dose
   - Males and females included in data collection
   - Repeat dose to further establish MTD (or similar)

Group	Dose (mg/kg)	Main Study Animals	
		Male	Female
1 (escalating)	Level 1	3	3
2 (escalating)	Level 2	3	3
3 (escalating)	Level 3	3	3
4 (repeat dose)	From escalating phase	5	5

Higher N to increase confidence in the results

Single dose level for repeat dose tolerability?

Rat Acute/DRF – Example 1

1. Dose escalation followed by repeat dose
   - Males and females included in data collection
   - Repeat dose to further establish MTD (or similar)

Group	Dose (mg/kg)	Main Study Animals	
		Male	Female
1 (escalating)	Level 1	3	3
2 (escalating)	Level 2	3	3
3 (escalating)	Level 3	3	3
4 (repeat dose)	From escalating phase	5	5
5 (repeat dose)	TBD	5	5

Two dose levels, e.g., single dose MTD and lower dose

Total main study animals = 38

Rat Acute/DRF – Example 1

1. Dose escalation followed by repeat dose
   - Males and females included in data collection
   - Repeat dose to further establish MTD (or similar)
   - Include toxicokinetics to define the exposure-response relationship

Group	Dose (mg/kg)	Main Study Animals		Toxicokinetics	
		Male	Female	Male	Female
1 (escalating)	Level 1	3	3		
2 (escalating)	Level 2	3	3		
3 (escalating)	Level 3	3	3		
4 (repeat dose)	From escalating phase	5	5	[3 – 9]	[3 – 9]
5 (repeat dose)	TBD	5	5	[3 – 9]	[3 – 9]

Total animals = 50 to 74

Opportunity for micro-sampling or alternatives to decrease animal numbers

Rat Acute/DRF – Example 2

1. Single sex dose escalation approach
   - Males or females for dose escalation

Group	Dose (mg/kg)	Main study Animals	
		Male	Female
1 (escalating)	Level 1	3 (male or female)	
2 (escalating)	Level 2	3 (male or female)	
3 (escalating)	Level 3	3 (male or female)	

Confirm maximal dose in three animals of the opposite sex

Approach involves 9 to 12 animals (vs 18 for Example 1)

Rat Acute/DRF – Example 2

1. Single sex dose escalation approach
   - Males or females for dose escalation
   - Multiple doe groups for repeat-dose phase

Group	Dose (mg/kg)	Main study Animals	
		Male	Female
1 (escalating)	Level 1	3 (male or female)	
2 (escalating)	Level 2	3 (male or female)	
3 (escalating)	Level 3	3 (male or female)	
3 (escalating)	Level 3	Dose opposite sex at Level 3	
4 (repeat dose)	From escalating phase	3	3
5 (repeat dose)	From escalating phase	3	3
6 (repeat dose)	From escalating phase	3	3

Higher number of animals and/or groups in the repeat-dose phase (when range better defined)

Rat Acute/DRF – Example 2

1. Single sex dose escalation approach
   - Males or females for dose escalation
   - Multiple dose groups for repeat-dose phase
   - Include toxicokinetic evaluation

Group	Dose (mg/kg)	Main study Animals		Toxicokinetics	
		Male	Female	Male	Female
1 (escalating)	Level 1	3 (male or female)			
2 (escalating)	Level 2	3 (male or female)			
3 (escalating)	Level 3	3 (male or female)			
3 (escalating)	Level 3	Dose opposite sex at Level 3			
4 (repeat dose)	From escalating phase	3	3	[3 – 9]	[3 – 9]
5 (repeat dose)	From escalating phase	3	3	[3 – 9]	[3 – 9]
6 (repeat dose)	From escalating phase	3	3	[3 – 9]	[3 – 9]

Opportunity to decrease animal numbers?

Total animals = 48 to 102 (without third group = 42 to 78)

Dog Acute/DRF – Example 1

1. Escalating dose involves 1 male and 1 female
   - Time interval between dose events ranges from hours to several days
2. Repeat-dose phase in an additional 1 male and 1 female
   - Option for a second dose group

Group	Dose (mg/kg)	Animals	
		Male	Female
1 (escalating)	Level 1	1 male and 1 female	
	Level 2		
	Level 3		

May allow for better dose selection for longer-term and/or definitive studies

4 to 6 animals total

Dog Acute/DRF – Example 2

1. Re-use of animals in repeat-dose phase
   - Escalating phase conducted as previously described
   - Transfer animals to repeat-dose phase; include an additional 1 male and 1 female
   - Option to include a second dose group

Group	Dose (mg/kg)	Animals	
		Male	Female
1 (escalating)	Level 1	1 male and 1 female	
	Level 2		
	Level 3		

Washout/recovery before animals assigned to repeat dose

4 to 6 animals total

NHP Acute/DRF – Example 1

1. Escalating dose in 1 male and 1 female
   - Time interval between dose events ranges from 1 to several days
     - Design used for small molecules and specific large molecules

Group	Dose (mg/kg)	Animals	
		Male	Female
1 (escalating)	Level 1	1 male and 1 female	
	Level 2		
	Level 3		
2 (repeat dose)	From escalating phase	1	1
3 (repeat dose)	From escalating phase	1	1

Similar questions and points for consideration as those outlined for rat and dog

1) Dose levels for escalating phase?
2) Feasibility of using escalating dose animals in repeat-dose phase?
3) One or two dose levels in repeat-dose phase?

NHP Acute/DRF – Example 2

1. Dose escalation and range finding for biologics (or similar compounds)
   - Escalating phase completed with dedicated animals in each group/dose level

Group	Dose (mg/kg)	Animals	
		Male	Female
1 (escalating)	Level 1	1	1
2 (escalating)	Level 2	1	1
3 (escalating)	Level 3	1	1

May require 6+ animals to achieve dose escalation

With biologics, escalation in the same animals may not be feasible/appropriate

1) Long half-life of drug (PK)
2) Extended duration of effect (PD)
3) Potential for anti-drug antibodies

NHP Acute/DRF – Example 2

1. Dose escalation and range finding for biologics (or similar compounds)
   - Escalating phase completed with dedicated animals in each group/dose level
   - Repeat dose in dedicated animals for each group/dose level

Group	Dose (mg/kg)	Animals	
		Male	Female
1 (escalating)	Level 1	1	1
2 (escalating)	Level 2	1	1
3 (escalating)	Level 3	1	1
4 (repeat dose)		1 - 2	1 - 2
5 (repeat dose)		1 - 2	1 - 2

Single or multiple dose level for repeat-dose phase?

Careful evaluation of animal use for robust data set with minimal numbers

Summary and Conclusions

1. Acute/DRF studies are a critical step in the nonclinical plan
   - Essential bridge between research and regulatory studies
   - Provide context for risk (toxicology) vs benefit (pharmacology)
2. No set guidelines/recommendations for design of Acute/DRF studies
   - Identify the key questions or concerns to select a “best design” or approach
   - The 3R’s principle is relevant for specific studies and the program overall
3. Primary intent is dose selection for regulatory studies
   - The dose **range** is an important consideration
     - Low, middle, and high are relative terms with specific values
     - Each dose level has an objective and function in the nonclinical program

Dose Level Selection and Justification

You Have Picked the Perfect Design

- How do I select a dose level?
  - Where do I start?
  - How high do I go?
  - If all I have is mouse data how do I pick a rat dose?
  - If all I have is rodent data, how do I pick a non-rodent dose?
- The universal answer of a regulatory toxicologist
  - It depends

Selecting/Changing Dose Levels

- Factors to be considered
  - What is the test article type?
    - Small molecule
    - Biopharmaceutical
  - Dose route
    - Oral, IV, SC, IM, …
  - Physical characteristics of the test article
    - Soluble in aqueous or organic

Everything Is a Poison

- *Poison is in everything, and no thing is without poison.*
- *The dosage makes it either a poison or a remedy.*
- - Paracelsus

https://upload.wikimedia.org/wikipedia/commons/4/4a/Paracelsus.jpg

Objective of a High Dose – Small Molecule

- In early studies
  - Start with a reasonably safe dose
  - Define the upper range of toxicity, and establish an MTD
  - Can be subjectivity around what constitutes an MTD
- In IND-enabling studies
  - Top dose should demonstrate toxicity and can be adverse
  - Define a No Observed Adverse Effect Level (NOAEL), Severely Toxic Dose (STD10 – rodents), or Highest Non Severely Toxic Dose (HNSTD – non-rodents)
- In Phase 2/3/registration studies
  - Same as for IND studies
  - Define a NOAEL

How do I Select a Dose between Species?

- How do you select a dose when going from rats to dogs
  - Use of body surface area – mg/kg to mg/m^2

	Conversion
Mouse	3
Rat	6
Dog	20
Cyno	12
Minipig	35

- Example – dose of 150 mg/kg in rats, want to dose dogs
  - 150 mg/kg * 6 = 900 mg/m^2; 900 mg/m^2 divided by 20 = 45 mg/kg

Reaching a High Dose – Small Molecule

- How do you get from a starting dose to the high dose
  - Various means are employed
    - Half log is commonly used
    - Other means are also used
      - What is observed at a given dose
      - What is the route of administration
      - What is known about class effects/pharmacology

High Dose Justification – Small Molecule

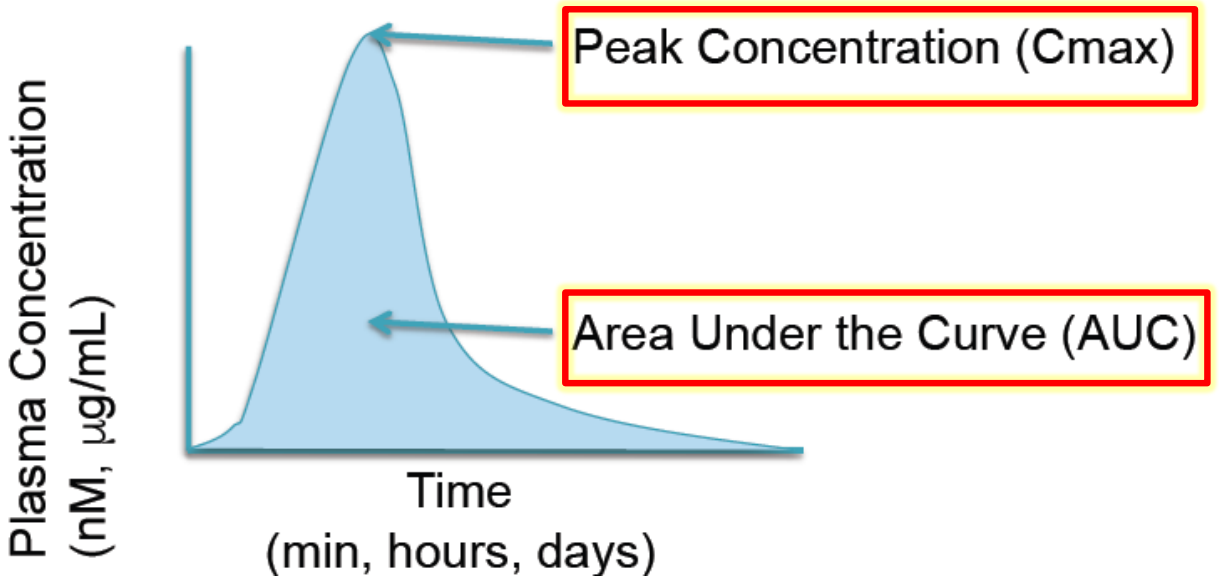
- For the initial dose range finding (DRF) studies, how do you know you have achieved a top dose?
  - Per the ICH guidelines – ICH M3(R2)
    - Maximum tolerated dose (MTD)
    - Limit dose
    - Maximum feasible dose (MFD)
    - Saturation of exposure
    - 50X clinical exposure

High Dose Justification – Small Molecule

- Maximum Tolerated Dose
  - Dose limiting toxicity which is significant toxicity up to and including mortality, prostration, convulsions etc.
- Limit dose (no MTD)
  - High dose of 1000 mg/kg
    - Provided you have 10X clinical exposure, or
    - Clinical dose is < 1g/day
  - High dose of 2000 mg/kg or MFD, whichever is lower
    - If clinical dose is > 1g/day and 1000 mg/kg does not generate 10X clinical exposure
  - High dose of MFD
    - If clinical dose is > 1g/day and you do not have 10X clinical exposure at 2000 mg/kg

High Dose Justification – Exposure

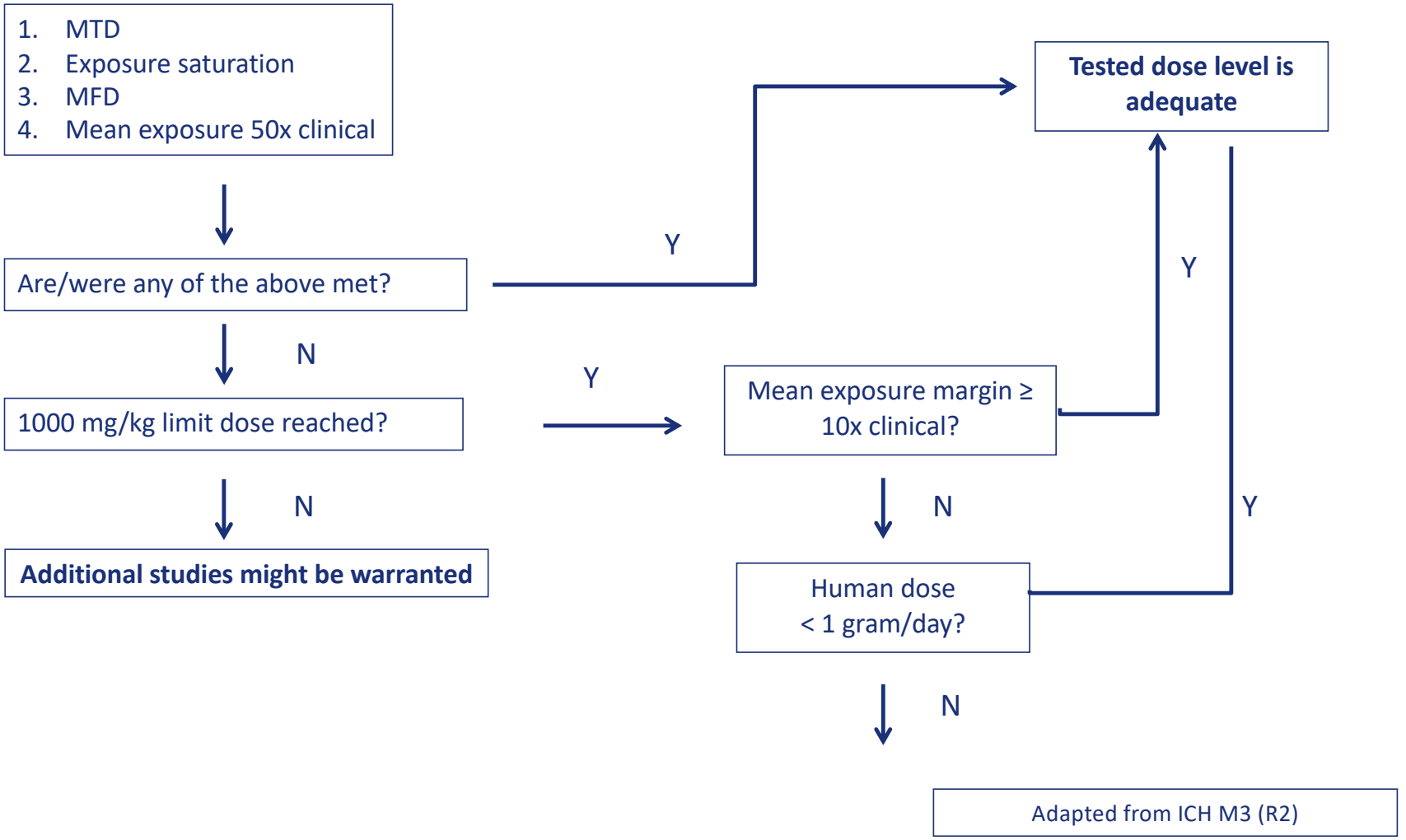
- Exposure is generally reported as a function of plasma concentration.

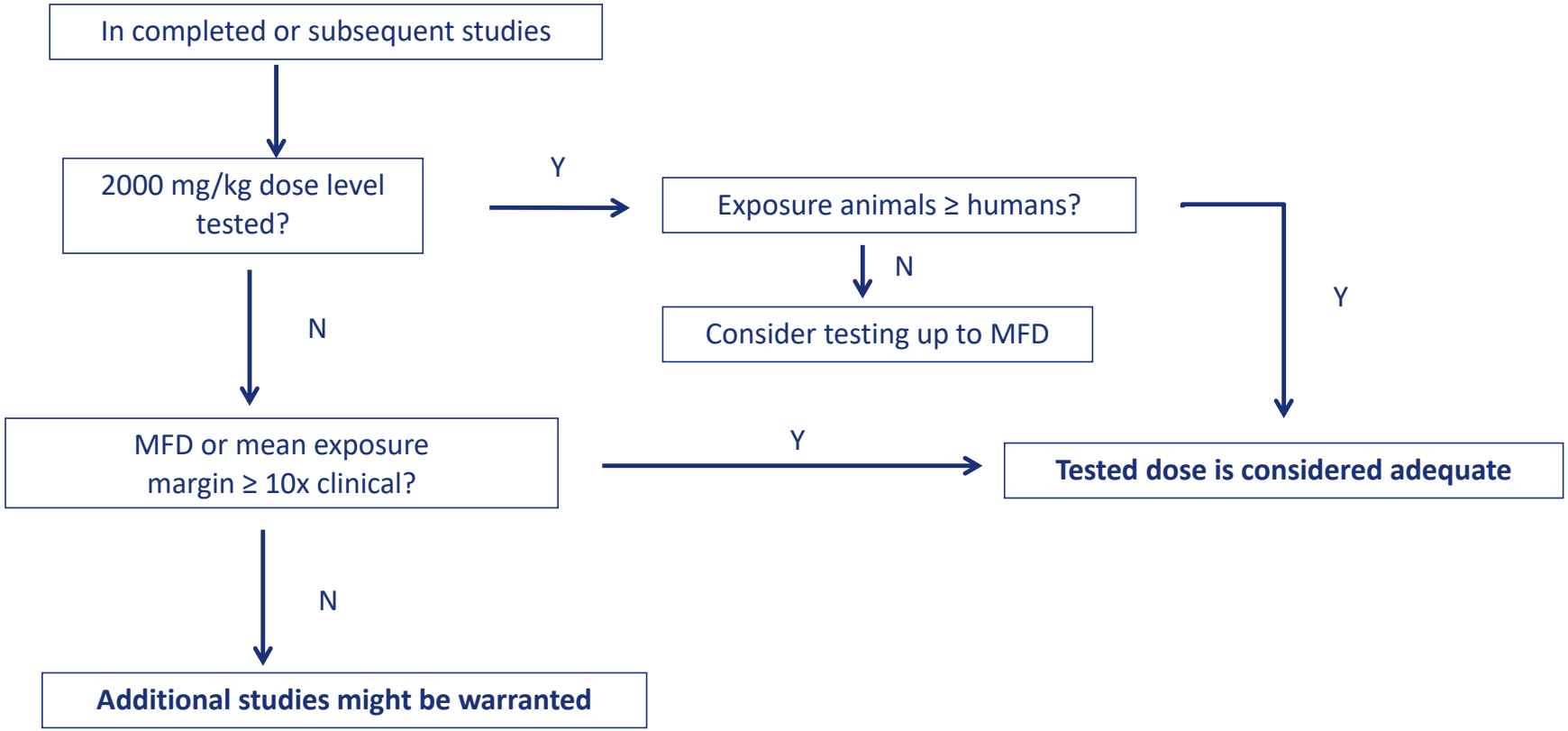


Bogdanffy MS. ACT Meeting 2016, Baltimore.

High Dose Justification – Small Molecule

- Other justifications
  - MFD
    - Need to explore at least 3 formulations
  - Saturation of exposure
    - Can be difficult to demonstrate
    - May need to look at BID/TID to support saturation
  - 50X clinical exposure
    - Need clinical data or robust modeling
    - Based on active entity
    - Applies to AUC at maximal clinical dose


1. MTD
2. Exposure saturation
3. MFD
4. Mean exposure 50x clinical
Are/were any of the above met?
Y
Tested dose level is adequate
N
Y
1000 mg/kg limit dose reached?
Mean exposure margin ≥ 10x clinical?
Y
N
Y
Additional studies might be warranted
Human dose < 1 gram/day?
N
Adapted from ICH M3 (R2)



Adapted from ICH M3 (R2)

High Dose Justification – Biopharmaceutical

- Per ICH guidance – ICH S6(R1)
  - Covers peptides, antibodies, antibody-drug conjugates, proteins, etc.
- Not necessarily targeting a MTD
  - Does not mean you can't have toxicity
  - Typically exaggerated pharmacology
- High dose – the greater of these
  - Dose that induces a maximum pharmacological effect
  - Doses providing a 10-fold margin over maximum clinical exposure

Summary

- Regulatory toxicology is not easy or straightforward
  - But it is interesting and challenging
- Guidance documents are available and should be familiar to SDs
  - Covers both small molecules and biopharmaceuticals
  - Dose level justification is contingent on a number of factors
  - Studies are conducted in a series and build upon each other
  - Ultimate goal is to understand the potential toxicity of the test article to the people involved in the clinical trials
- Institutional Animal Care and Use Committees should also be familiar with these guidance documents