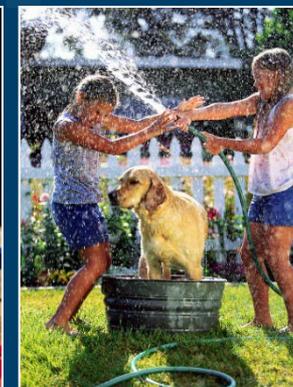


How Toxicology Research Is Used in Human Health Risk Assessment: Study Design and Evaluation



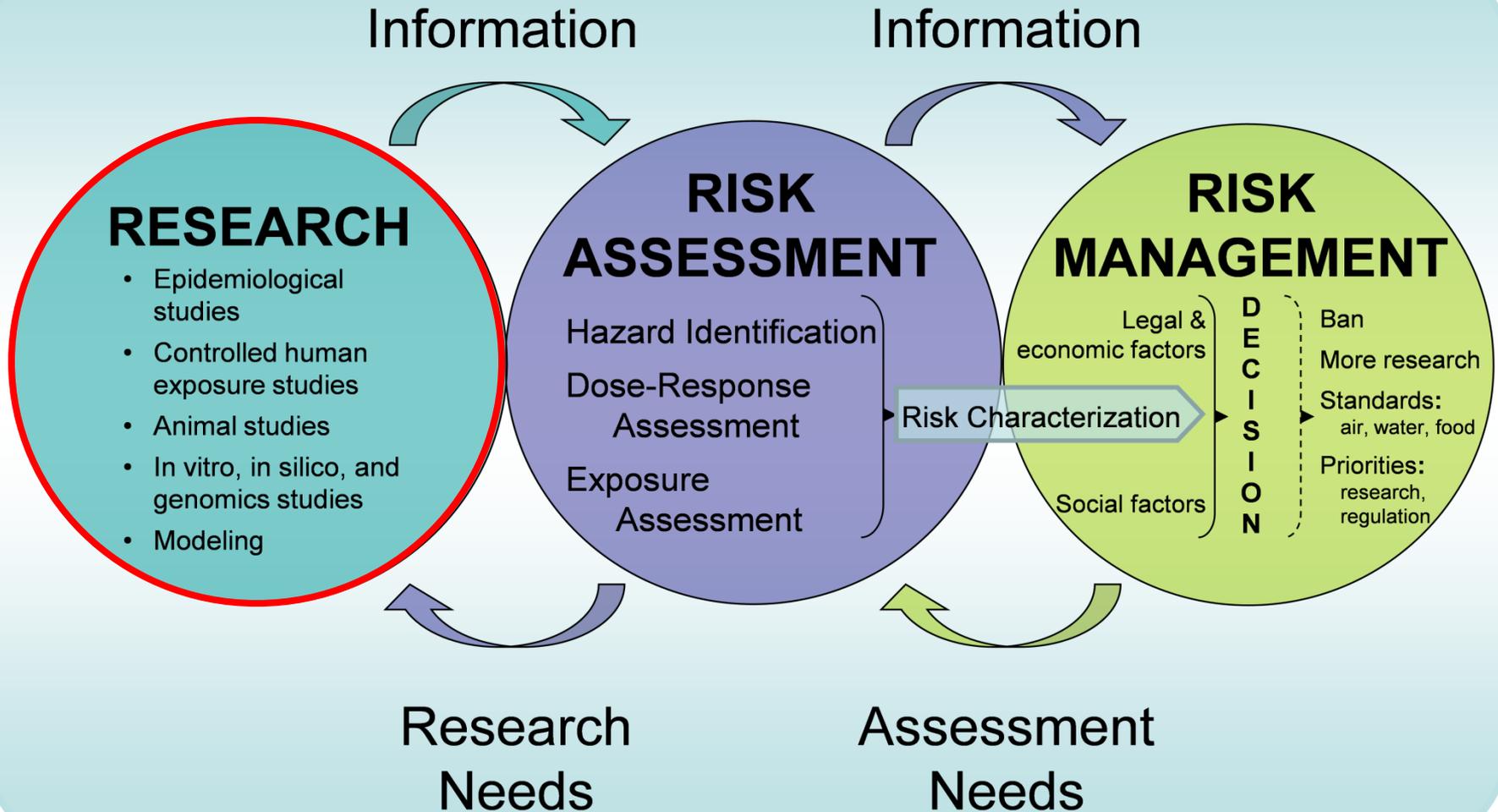
- Introduction to animal toxicology and human health risk assessment (HHRA)
 - Hazard identification
 - Dose-response assessment
- Animal study design considerations important in HHRA
- Types of animal toxicity studies and how they are used in HHRA

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

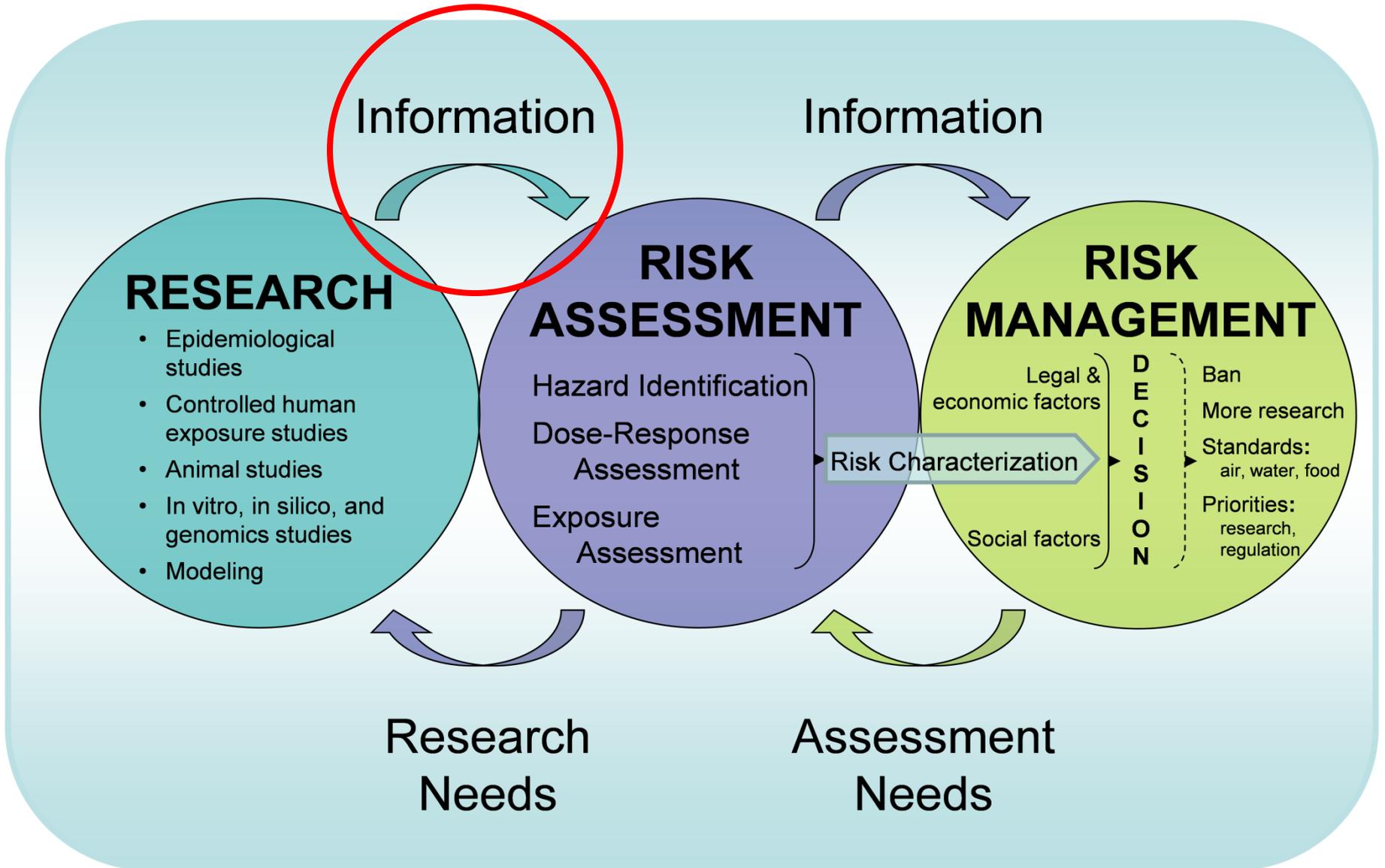


INTRODUCTION

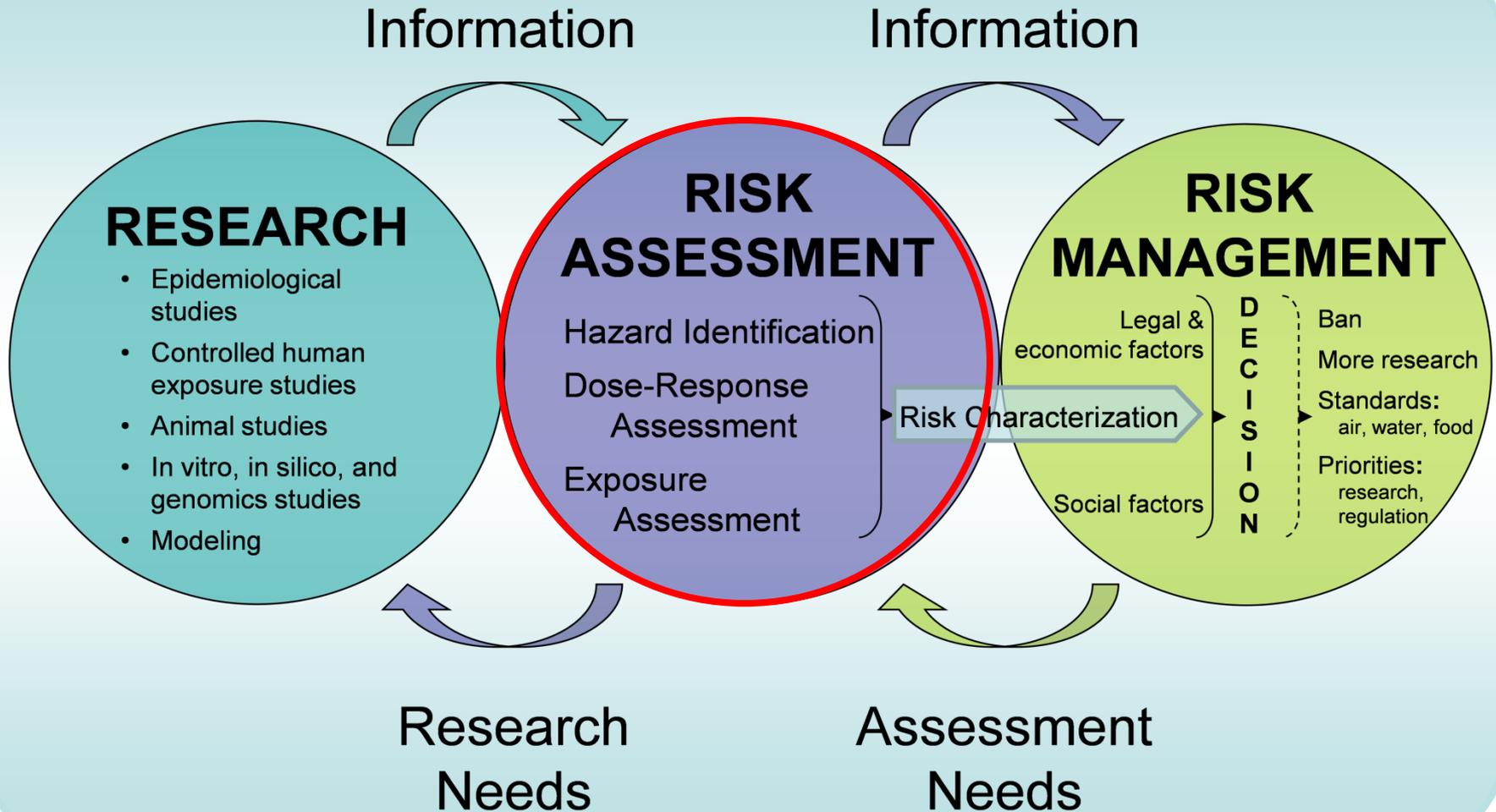
Risk Assessment Paradigm



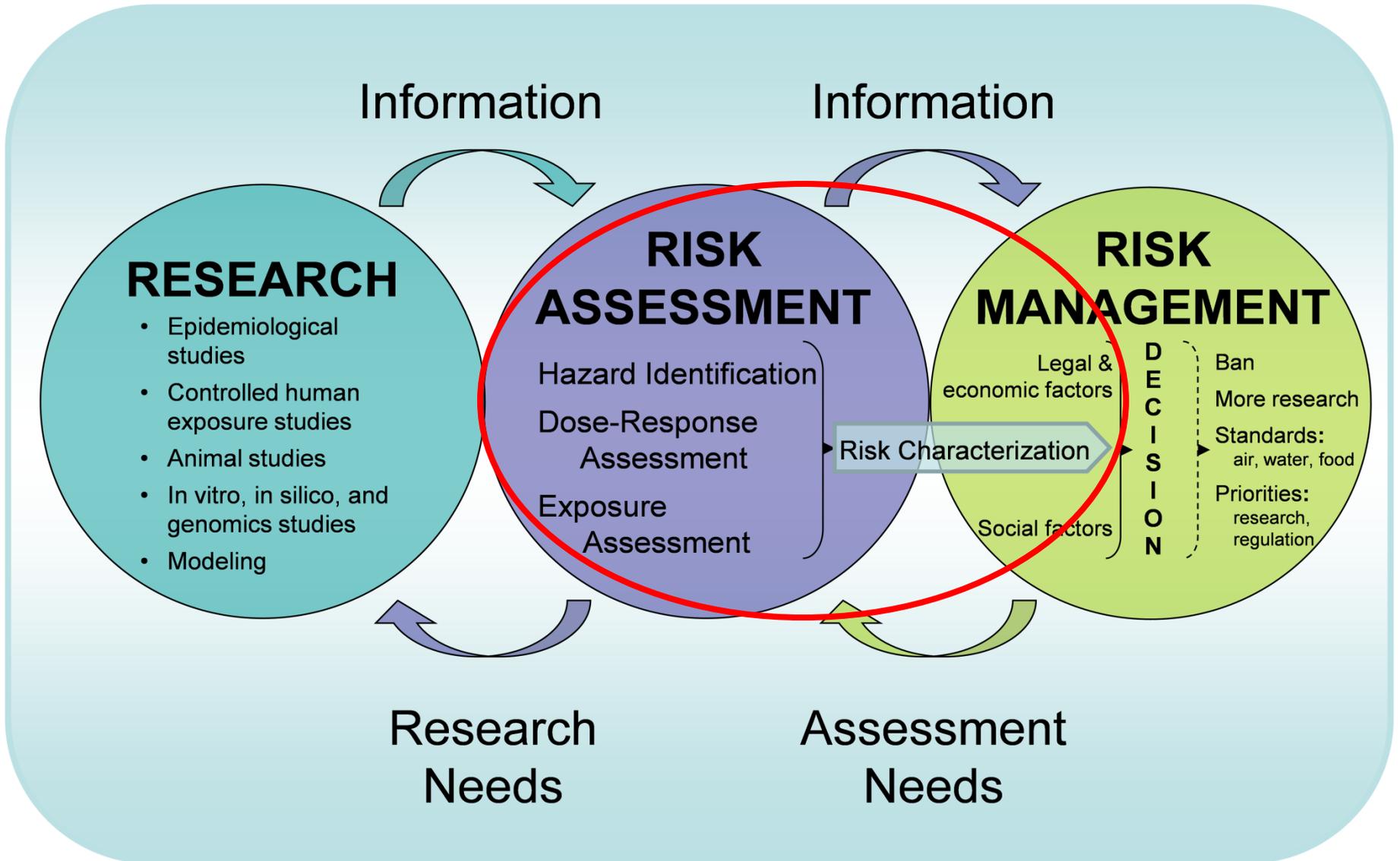
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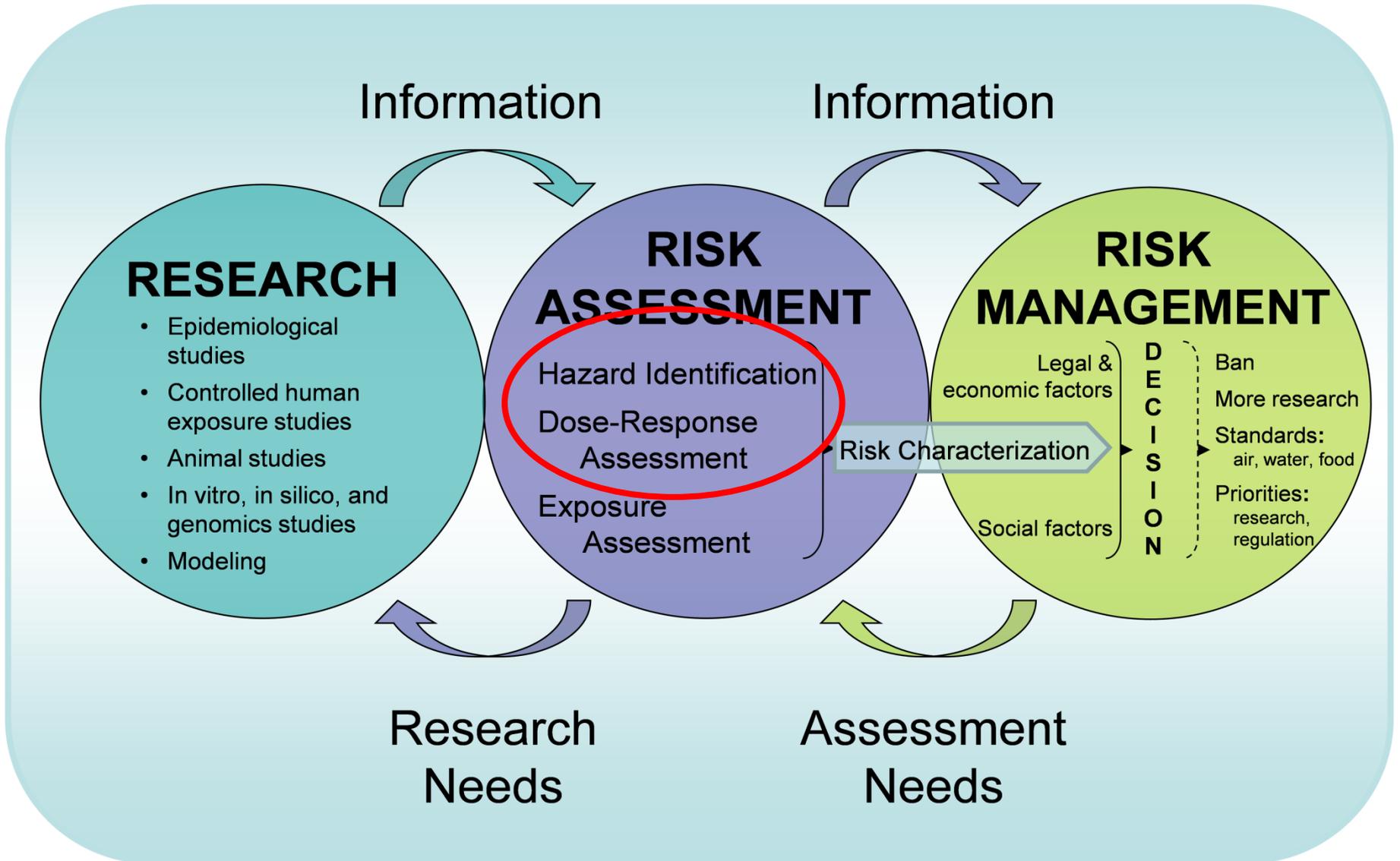
Risk Assessment Paradigm



Risk Assessment Paradigm

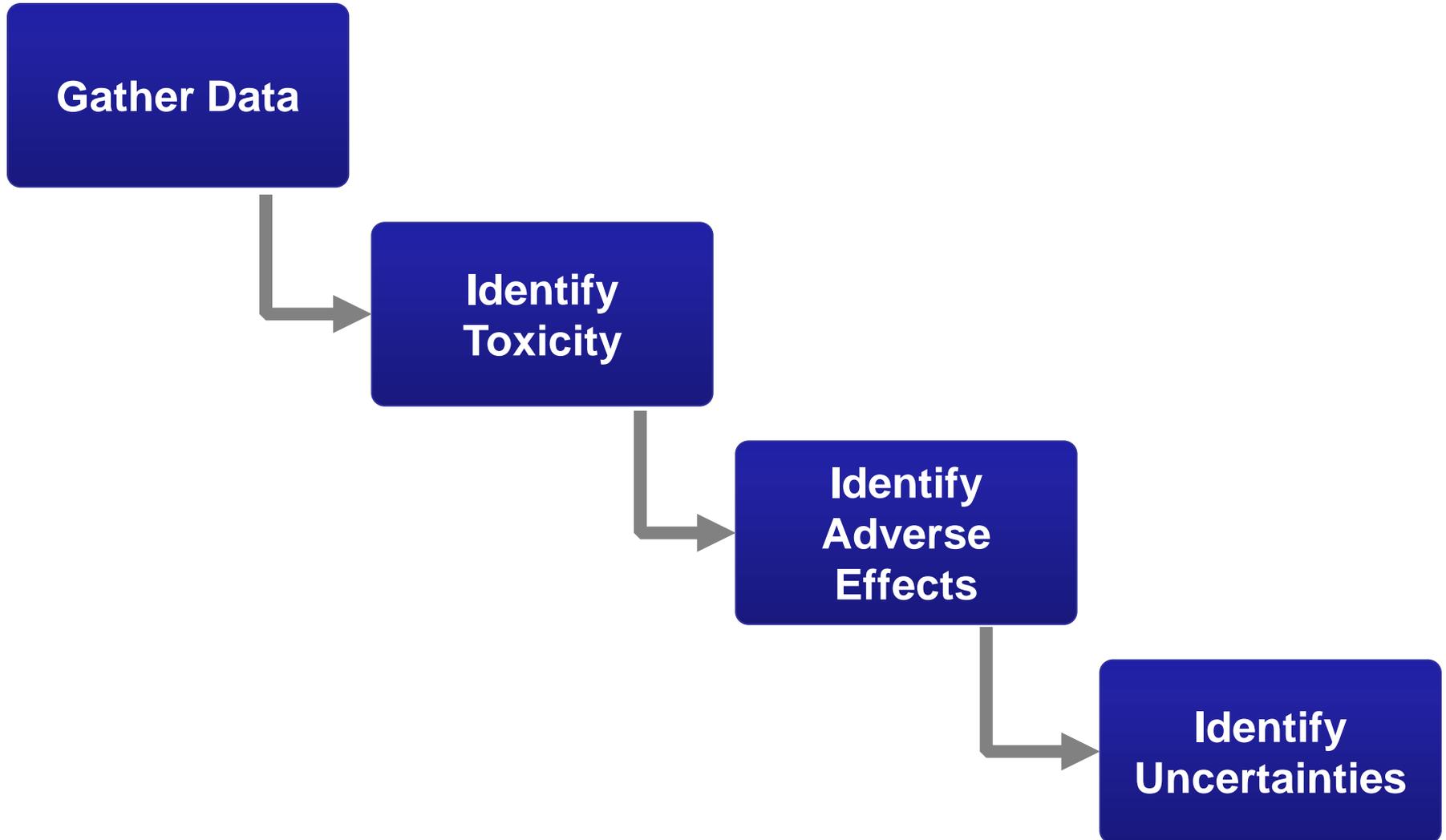


Risk Assessment Paradigm



HAZARD IDENTIFICATION

Hazard Identification



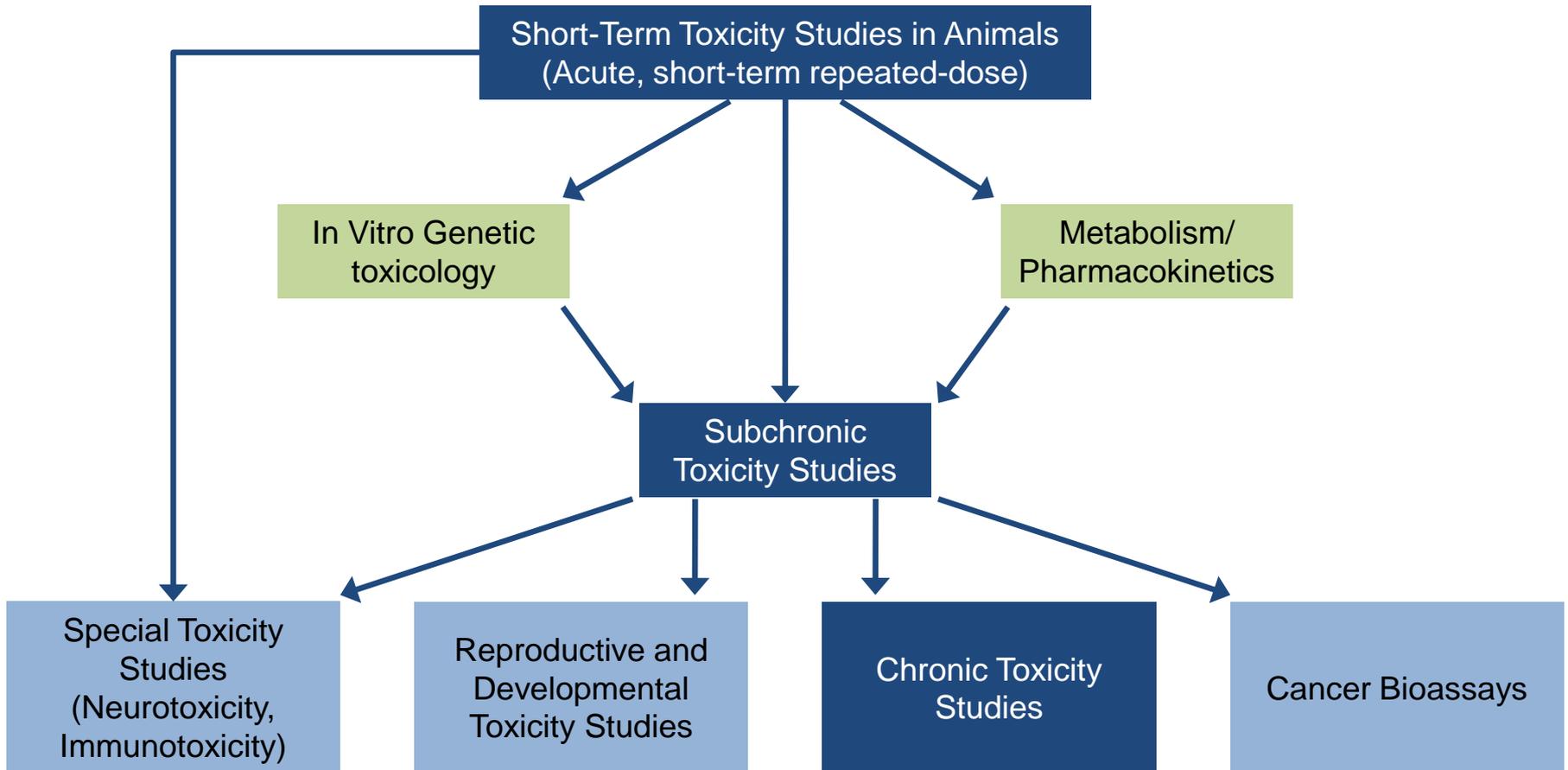
Gather Data

- Which populations might be affected?
- What toxicity data are available?
- Human Data
 - Epidemiology studies
 - ~~Controlled human exposure studies~~
- **Animal Bioassay Data**
- Other Data
 - In Vitro Data
 - Structure-activity relationships
 - Metabolic data
 - Genomics

Advantages and Limitations of Using Animal Toxicity Data

Advantages	Limitations
Enable proactive regulation and behavior	Variability in results
Toxic effects assumed to be similar in humans and animals	Results in animals sometimes different from humans
Allow for control of exposure and negative and vehicle controls	Anatomical differences between humans and animals
Allow for time course	Some serious chronic effects missed by standard toxicity studies
Control/knowledge of genetically associated responses	Traditionally only study exposure to individual chemicals
Allow for invasive endpoints such as necropsy	
Less costly and timely than human exposure or epidemiology studies	

Tiered Testing Approaches



DOSE-RESPONSE ASSESSMENT

Dose-Response Assessment

Characterize Dose-Response Relationship

Identify a NOAEL or LOAEL (for nonlinear) or an LED₁₀ (for linear)
Conduct dose-response modeling and BMD Modeling



Identify critical effect(s) and level(s)



Identify point of departure

NOAEL

No-Observed-Adverse-Effect Level. Highest dose at which no significant adverse effects are observed.

LOAEL

Lowest-Observed-Adverse-Effect Level. Lowest dose at which significant effects are observed.

LED₁₀

Dose that produces an adverse effect in 10% of exposed, relative to control.

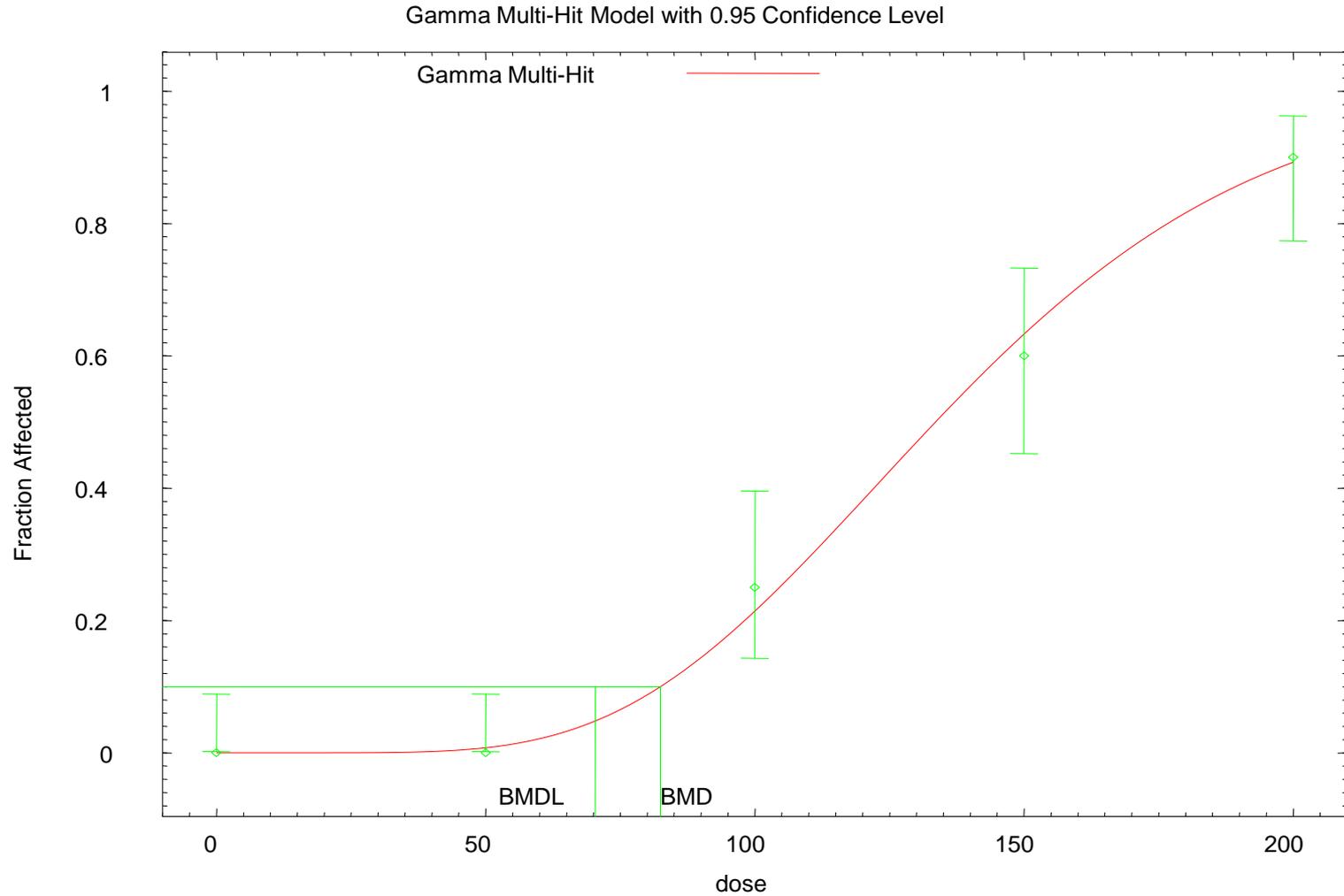
BMD

Benchmark Dose. An exposure to a low dose of a substance that is linked with a low (1-10%) risk of adverse health effects, or the dose associated with a specific biological effect.

BMDL

A lower, one-sided confidence limit on the BMD.

What is a Benchmark Dose?



Advantages of BMD Modeling over NOAEL/LOAEL Approach

Subject	NOAEL/LOAEL Approach	BMD Approach
Dose selection for point of departure	Doses in study only	More independent of study dose levels. BMD can correspond to the dose at any predetermined response level (BMR)

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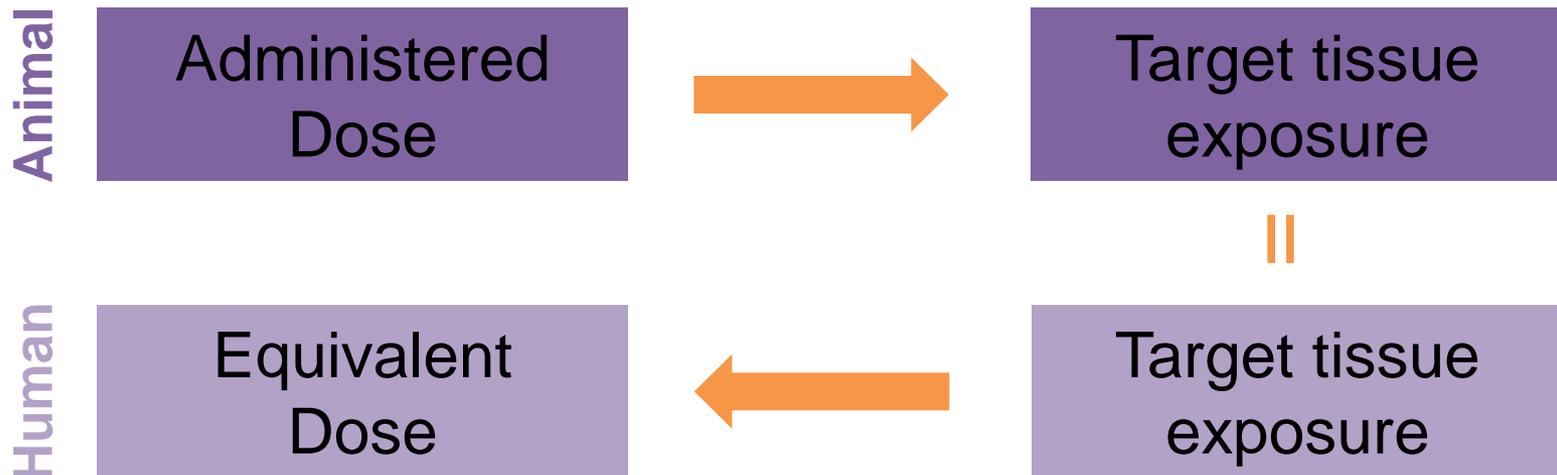
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Dose variability's impact on the point of departure	Does consider variability in significance tests, but more uncertainty can result in higher NOAEL	BMD accounts for sample size, background response rate, and response variability when using the BMDL value. More uncertainty, lower BMDL

Disadvantages over NOAEL/LOAEL Approach

- Some expertise is required to conduct the modeling and interpret results
- Sometimes the dose-response data cannot be adequately fit by available models
- In some cases, more data are required to model benchmark dose than to derive a LOAEL/NOAEL
 - Continuous data require standard deviation of each dose group's mean response
 - Individual animal-level data are required for some models

Dosimetry and Human Equivalent Dose and Concentration

- **Dosimetry:** Estimation of target tissue exposure
- Human equivalent doses and concentrations are estimated by equating the target tissue exposures of animals and humans



ANIMAL TOXICOLOGY STUDY DESIGN

Design Aspects of Animal Toxicology Studies

	To Support Hazard Identification	To Support Dose-Response Assessment
Test material purity	Analyze and report	

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Animal model	<ul style="list-style-type: none"> • Consider conducting studies in both sexes and > 1 species • Identify susceptible lifestage(s) by conducting studies in animals of various ages • Some specific animal models can be used to identify sensitive populations 	

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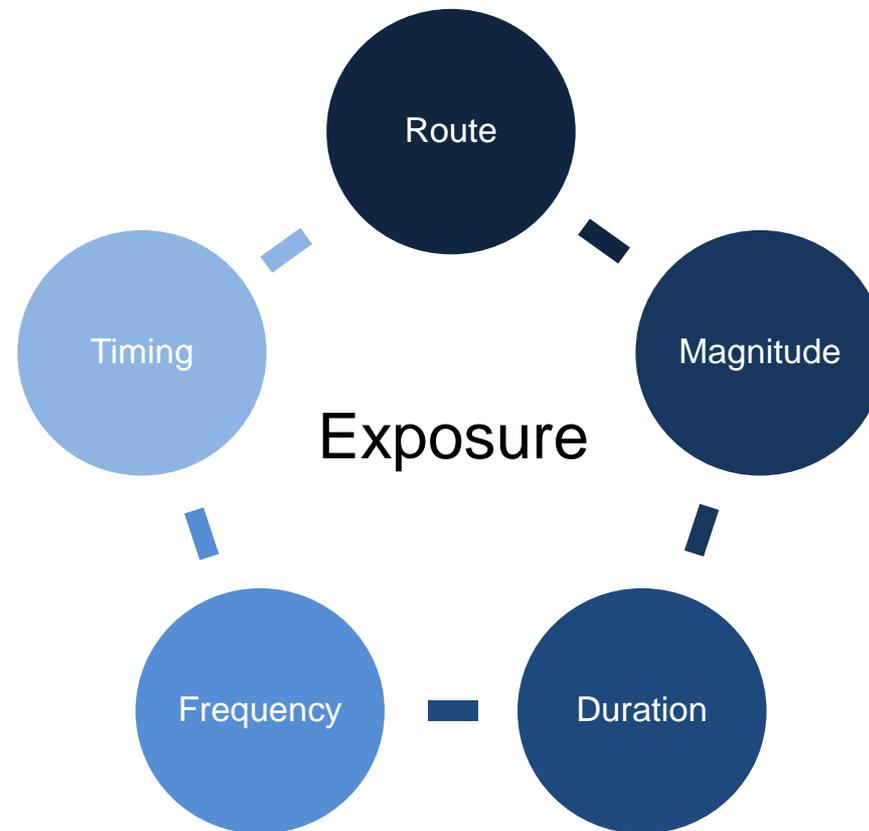
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Exposure Levels	Dose range should include a low-effect level (LOEL) for ≥ 1 health effect	<ul style="list-style-type: none"> • Use ≥ 3 chemical doses in addition to controls • Logarithmic spacing • Dose range should include a no-effect level (NOEL)

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Statistics	Sufficient statistical power	Clearly describe statistical methods

Importance of the Exposure Scenario

Exposures in animal toxicology studies are characterized by:



- Route affects toxicity
- Should represent potential human exposure
- Common routes:
 - Oral – feed, drinking water, gavage, or capsule
 - Inhalation – via chamber exposure (whole body, head-only, or nose-only)
 - Dermal – applied to the skin of the animal

Route of Exposure Considerations Supporting Dose-Response Assessment

ORAL:

- Monitor food or water consumption
- Gavage/capsule vs. dietary/drinking water?

DERMAL:

- Occlusive vs. semi-occlusive vs. non-occlusive?
- Monitor secondary oral exposure

INHALATION:

- Monitor secondary oral exposure
- Monitor reflex bradypnea
- Report target, nominal and actual concentrations, including information on variability over time

ALL ROUTES:

- Consider measuring chemical concentrations in tissues

Endpoints Examined

- Broad array of endpoints examined in general toxicity studies, including:
 - Organ weights
 - Organ and tissue histopathology
 - Hematology
 - Clinical chemistry
 - Urinalysis
- More specific endpoints used in targeted toxicity studies

General study:

↓ thymus weight



Targeted study:

Disease resistance
Antibody response
NK cell activity

Identify Types of Effects

What is an adverse effect?

A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge

Adverse? Maybe.

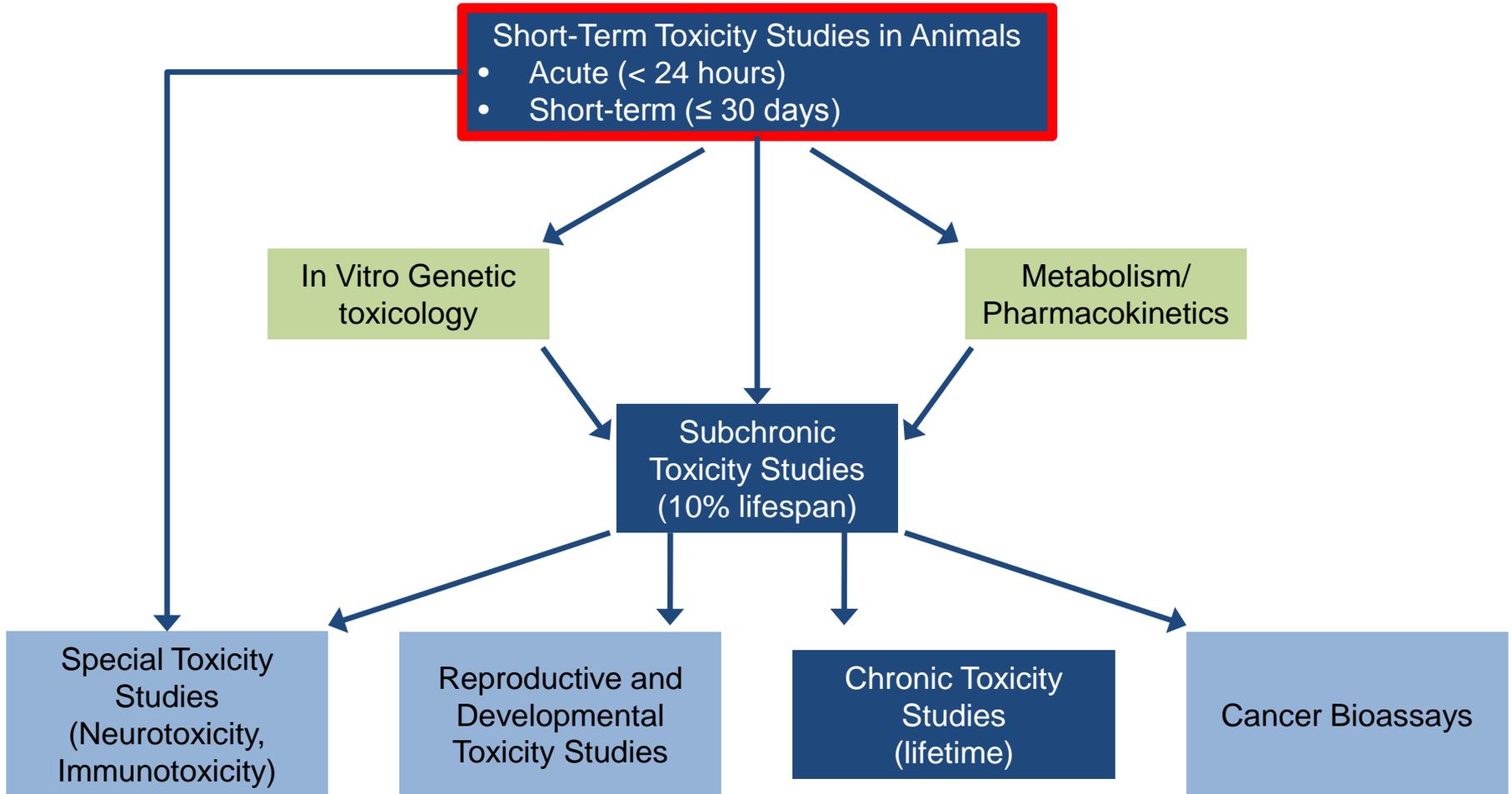
- Cytochrome P-450 induction
- Biochemical changes
- Altered immune cell phenotypes or cytokine profiles

Types of Effects

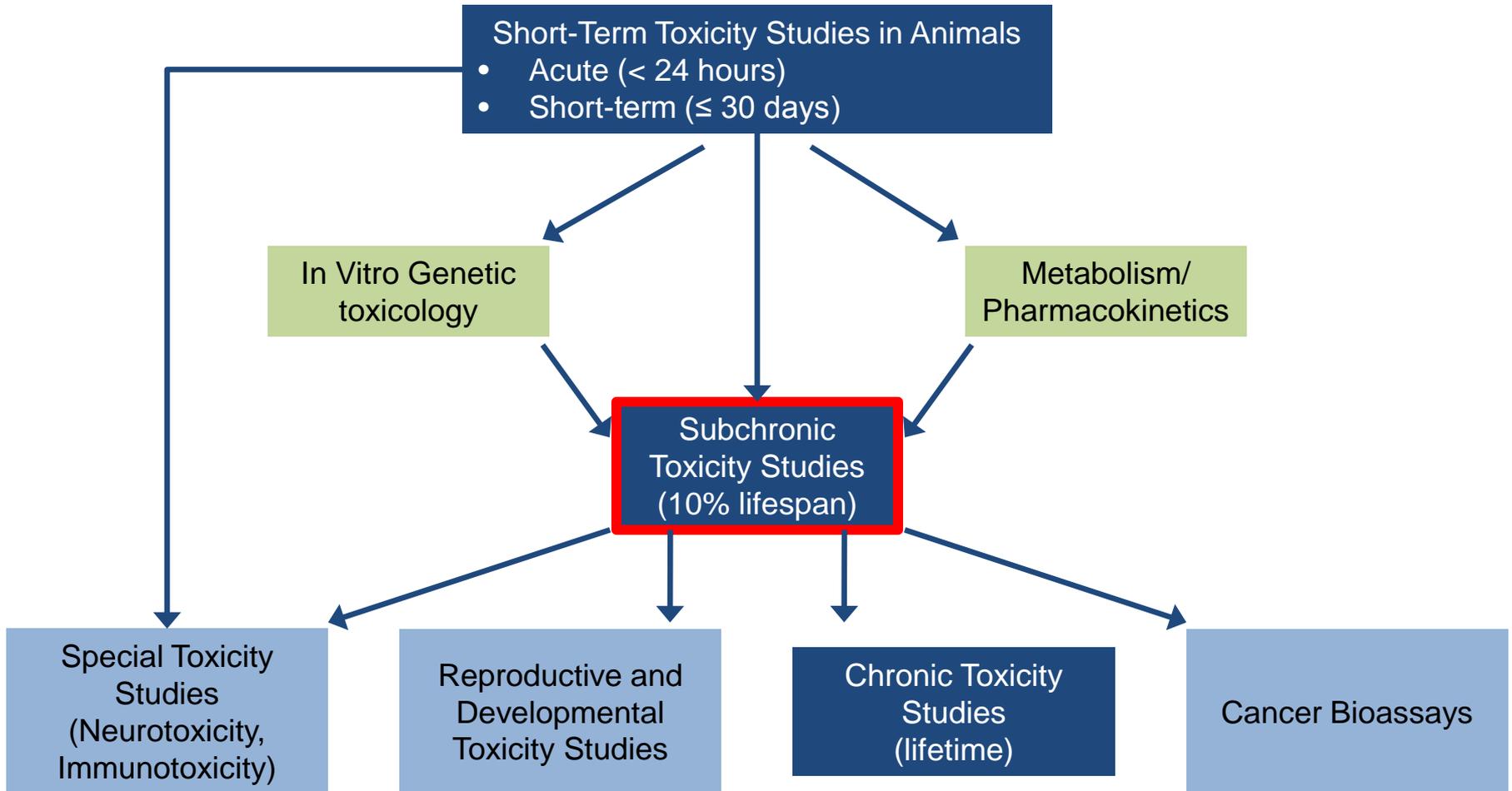
- Adaptive
- Compensatory
- Adverse
- Frank

TYPES OF ANIMAL TOXICITY STUDIES

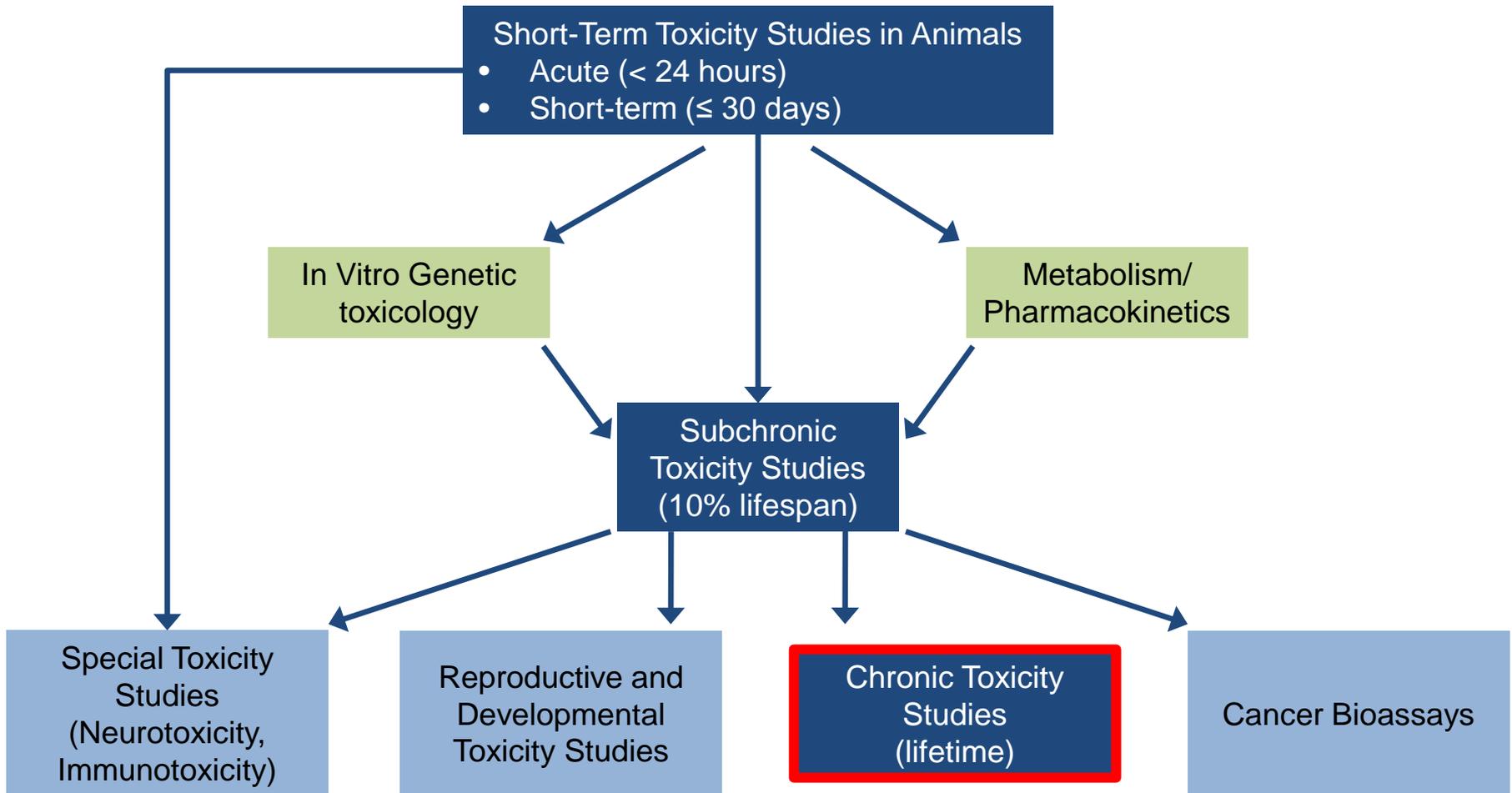
Tiered Testing Approaches



Tiered Testing Approaches



Tiered Testing Approaches



- **Exposure duration:** average lifespan of the rodent
- **Typical test animals:** young female and male weanlings (groups of ~60), rat/mouse (any route)
 - Should exposures be initiated at earlier lifestages?
Walker and Ho. 2012. Developmental reprogramming of cancer susceptibility. *Nat Rev Cancer* 12:479-86
- **Dose/concentration selection:** *sufficient for statistical analysis and gradation of effect*
- **Carcinogenic potential:** measured by comparison of tumor incidences
 - More confidence with *better dose-response data*
 - *Consider avoiding animals with high background tumor incidence*

Lifestage Susceptibility

Lifestage susceptibility: Sensitivity to chemical insult at certain periods in life

Prenatal and
postnatal windows
of susceptibility



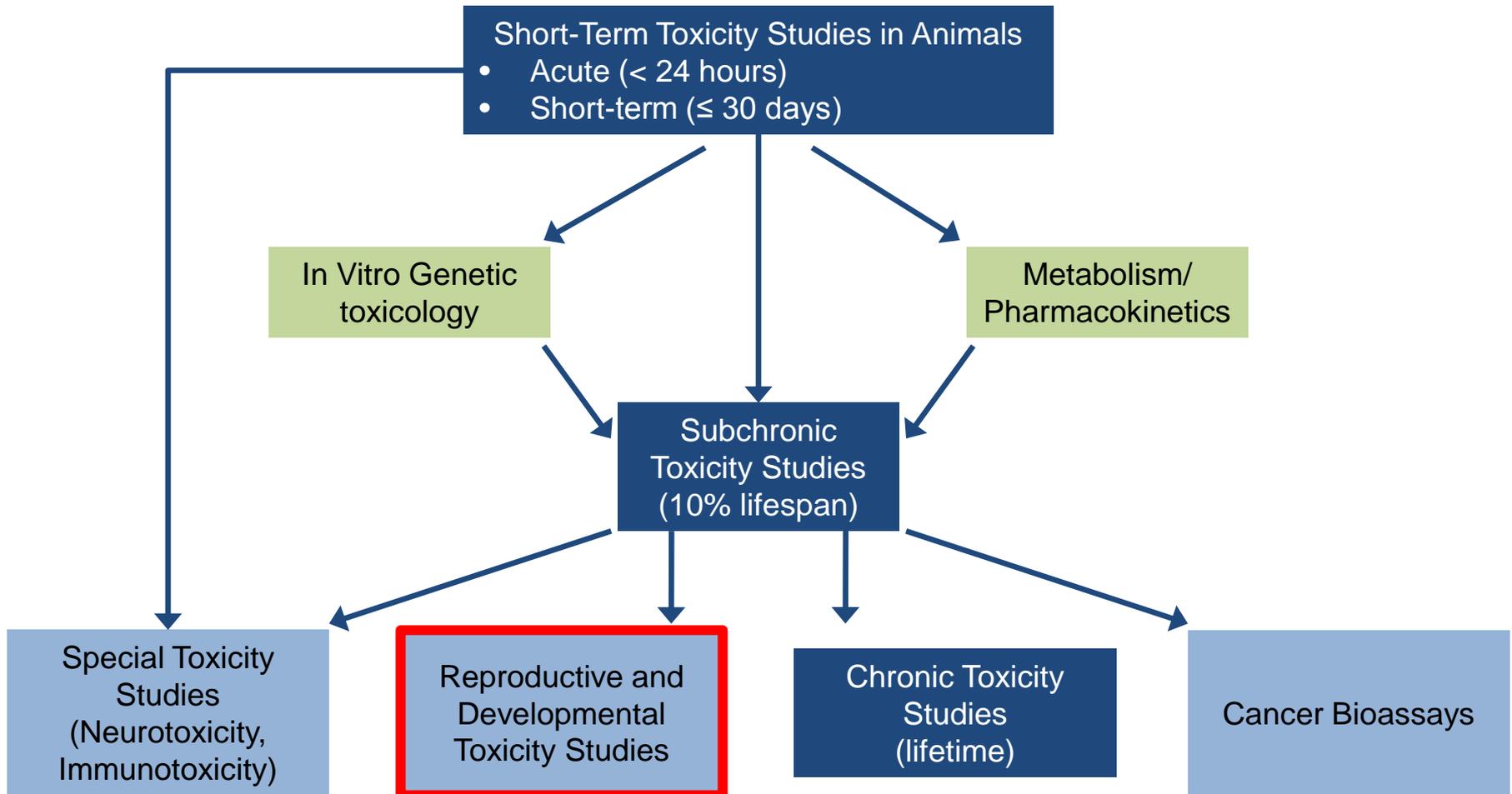
Early childhood



Endstages of life



Tiered Testing Approaches



Reproductive & Developmental Toxicity Studies

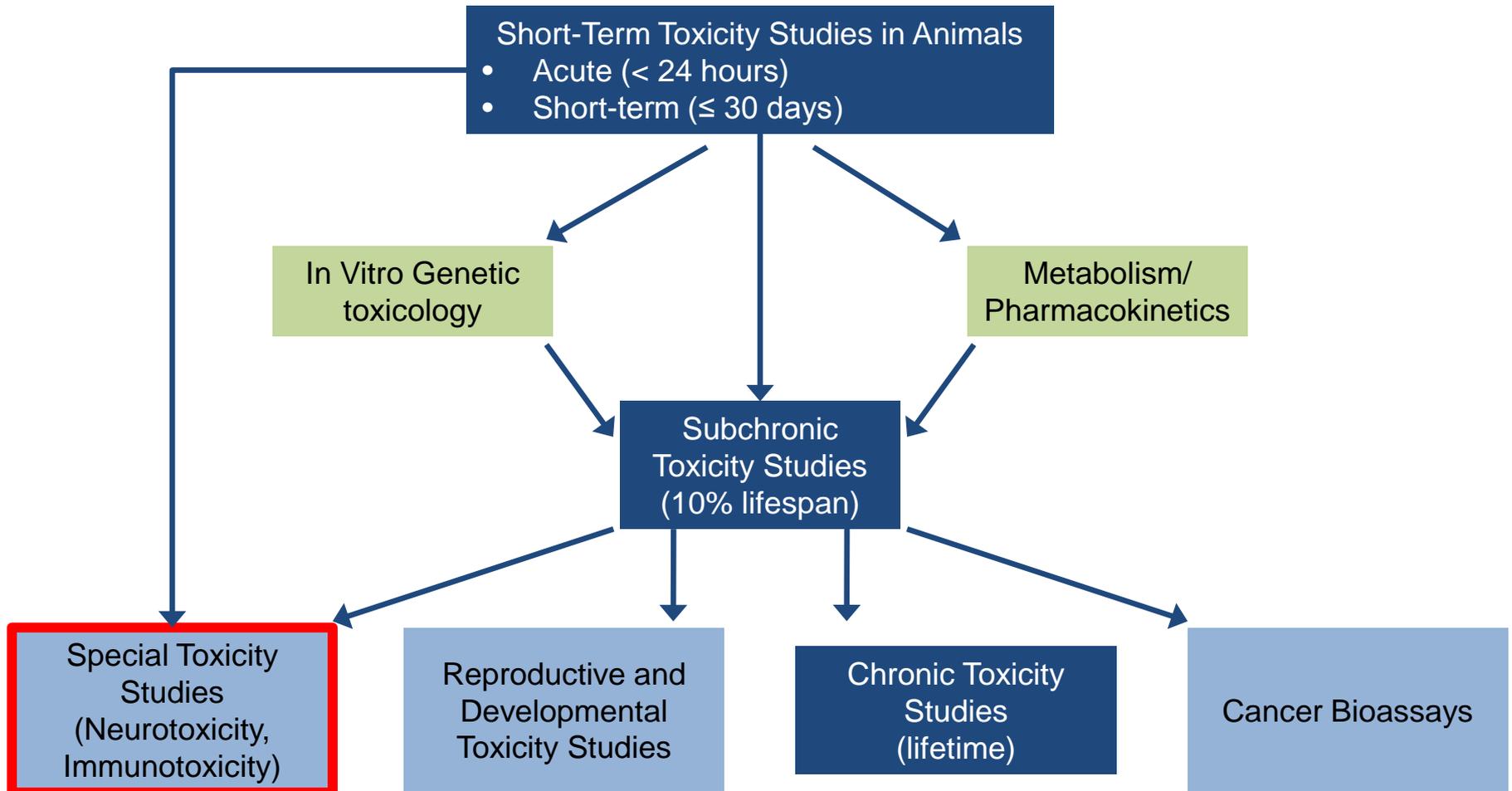
To support hazard identification:

- Developmental deficits may be observed in neonates, adolescents, or adults
- Multigenerational studies capture exposure during all relevant lifestages

To support dose-response assessment:

- Consider measuring chemical concentrations in fetal, neonatal, juvenile, and/or adult offspring tissues
- Report individual offspring data sorted by litter to support dose-response assessment

Tiered Testing Approaches



Guidelines For Toxicity Testing

- **EPA/OCSP** – Office of Chemical Safety and Pollution Prevention Harmonized Test Guidelines

- (<http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm>)



- **OECD** – Organization for Economic Co-operation and Development

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



- **NTP** – National Toxicology Program

- (<http://ntp.niehs.nih.gov/testing/types/index.html>)



Future Directions for HHRA

Methodology	Current Approach	Future Directions
Hazard Identification	Based largely on animal toxicity testing, mainly in rodent species	Based primarily on <i>in vitro</i> testing in human cells, and computational methods in biology and toxicology

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Dose and species extrapolation	Dose and species extrapolation translate animal test results to humans	<ul style="list-style-type: none"> • Cellular assays provide direct measures of toxicity pathway perturbations in humans. • <i>In vitro</i> to <i>in vivo</i> extrapolation techniques and pathway modeling calibrate <i>in vitro</i> and <i>in vivo</i> exposures. • Sensitive <i>in vitro</i> tests are used to evaluate risk directly at environmental exposure levels.

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- **Animal Toxicity Studies**

- Can provide data relevant to human exposure scenarios and useful for HHRA

- **Elements Important for Study Use in HHRA**

- Study design and **reporting**
- Test substance characterization
- Dose selection
- Exposure characterization
- Applicability to humans

Contact Information

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