

# **Evidence of Safety Needed to Support NDI Notification Part 2 – Toxicity Studies**

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# Draft Guidance for Industry

## Toxicology and Safety Studies:

- **Why do we need toxicology studies?**
- **How do we decide which studies to conduct?**
- **How do we use results from these studies to assess safety?**
- **What will this cost?**

# From Beginning of Civilization

- **Man in quest for food learned certain foods produced varying degrees of illness or death**
- **Soon recognized harmful and beneficial consequences associated with taking materials into his body**
- **Concept involving division of chemicals into two categories has persisted to the present day**
  - **Not possible, however, to describe a strict line of demarcation:**
    - **Beneficial chemicals**
    - **Harmful chemicals**
  - **Degrees of harmfulness and degrees of safeness for any chemical (the dose makes the poison)**
    - **All chemicals can cause toxic effects in large enough amounts**

# What is Toxicology

- **The Society of Toxicology defines toxicology as:**
  - **The study of the adverse physiochemical effects of a chemical, physical or biological agent on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects**
- **The goal of toxicology is to ensure the safety of products for human consumption**

# The Objective of Toxicology

- **To determine how an organism is affected by exposure to a substance**
  - **How the substance moves through the body**
  - **Metabolism of the substance**
  - **What organs or tissues are affected**
  - **The health outcomes of this exposure**
- **The more thorough this understanding, the more accurately we can predict what will happen when humans ingest the substance**

# Terms Defined

- **Dose**
  - The amount of a substance that enters the body
- **Toxic**
  - Injurious to health or dangerous to life
- **Hazard**
  - Types of toxic effects caused by the chemical
  - Manifestation depends on route, amount, duration and frequency of exposure

# Terms Defined

- **Dose-response**
  - Quantitative relationship between dose and the magnitude of toxic response in the range of doses that might be or have been encountered
- **Risk**
  - Likelihood that the toxic properties of a chemical will be produced in populations of individuals under their actual conditions of exposure; exposure must precede adverse event
- **Safety**
  - Little or no harm will result from chemical under given set of exposure circumstances
  - It is not the absolute absence of risk; it is the inverse of risk

# FDA Guidance

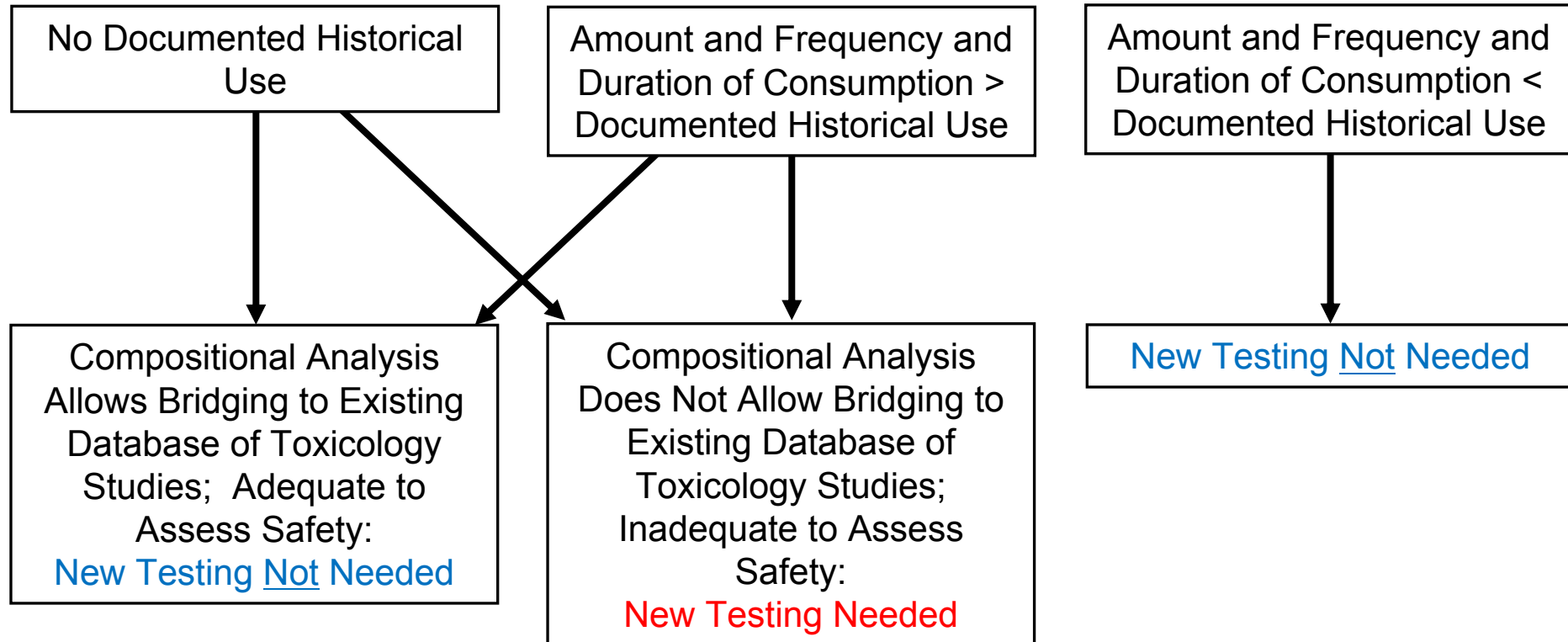
**“The NDI safety standard is different than the standard for food additives, drugs, pesticides, and other FDA-regulated products. Recommendations in guidance documents that are tailored to the safety assessment needs of other FDA-regulated products may not always be appropriate for dietary ingredients and dietary supplements.”**

**“You should use your own best judgment in compiling scientific evidence that provides a basis to conclude that the NDI that is the subject of your notification will reasonably be expected to be safe when used under the conditions recommended or suggested in the labeling of the dietary supplement described in the notification.”**

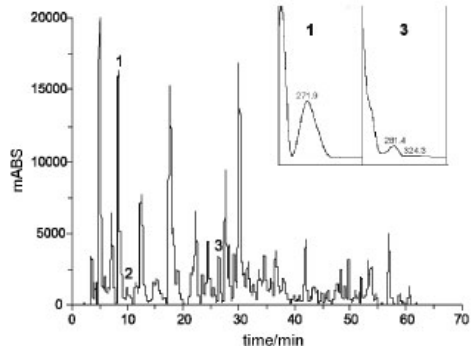
# What Necessitates Toxicology Testing for an NDI?

- **Is there any historical use in the U.S.?**
- **Are current intake levels or recommended intake levels different from historical use?**
- **Is the historical duration and frequency of use consistent with historical use?**
- **Is the historical indication consistent with historical use?**
- **Has the target population changed?**
- **Has the traditional delivery matrix been altered or eliminated? (chemical or compositional change)**
- **If there are traditional cautions in the use of the NDI, are these cautions communicated to the consumer?**
- **Are there other reasons to expect a different toxicity profile for the proposed formulation versus the traditional preparations?**

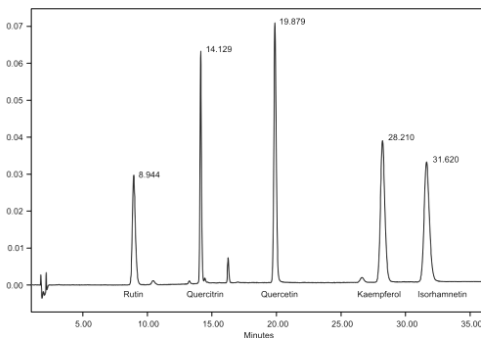
# Decision Tree Approach for Toxicology Testing New Dietary Ingredient



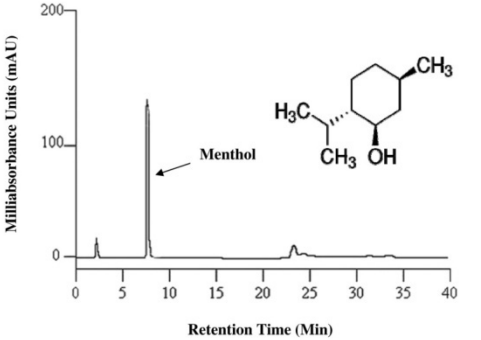
# Establishing a “Chemical Bridge” to Historical Use and Existing Toxicology Studies



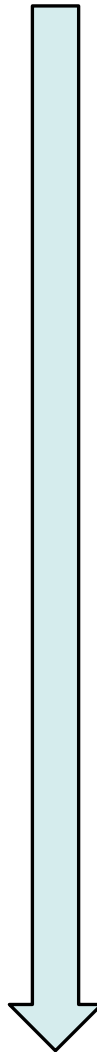
◀ Extract



◀ Semi-purified fraction



◀ Purified compound



- **Chemically complex:** May be possible to bridge the NDI to historically consumed preparations, but only if both are well characterized. Preparations used in published toxicology studies may not be sufficiently well-characterized to enable their use for establishing safety by bridging to the NDI.
- **Chemically simple:** May not be possible to bridge the chemistry of the simpler NDI to “historical” preparations more inherently complex; however, may be possible to bridge the NDI to other substances that have safety data.

# **Case Study: Glycerol Ester of Gum Rosin (GEGR)**

- **Food Additive Approval Obtained on the Basis of Chemical Equivalence to Glycerol Ester of Wood Rosin (GEWR)**
- **Federal Register: March 29, 2005 (Volume 70, Number 59) (Page 15756-15758)**

# **Case Study: Glycerol Ester of Gum Rosin (GEGR)**

- **The FDA reviewed:**
  - **Comparative chemical composition**
  - **Manufacturing process**
  - **Physicochemical properties**
  - **Conformance with specifications**
  - **Functional equivalence**
  - **Relevant safety information**

# **Case Study: Glycerol Ester of Gum Rosin (GEGR)**

**“While FDA agrees that there are differences in raw material sourcing and processing for GEGR and GEWR, FDA has concluded that the compositions of these two substances are so similar that any differences are not of toxicological concern for the petitioned use.”**

# Case Study: Glycerol Ester of Gum Rosin (GEGR)

**“FDA also agrees there will be variability in the composition of the rosins depending on the source and even from the same source due to differences in climate and soil conditions. However, this natural variability does not result in a qualitatively different composition of the rosin but rather a typical range of values for the individual components of the rosin.”**

# Case Study: Glycerol Ester of Gum Rosin (GEGR)

**“Because the agency has determined that GEGR and GEWR are similar with respect to the identity of their chemical components and that any difference in the ranges for the components of GEGR and GEWR are not significantly different and would be of no toxicological concern, there is no need for toxicological testing of GEGR to demonstrate that the petitioned use is safe.”**

# When Toxicology Studies Are Needed

When we can't bridge the safety of the NDI for its intended use to documentation of historical use because of a change in:

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

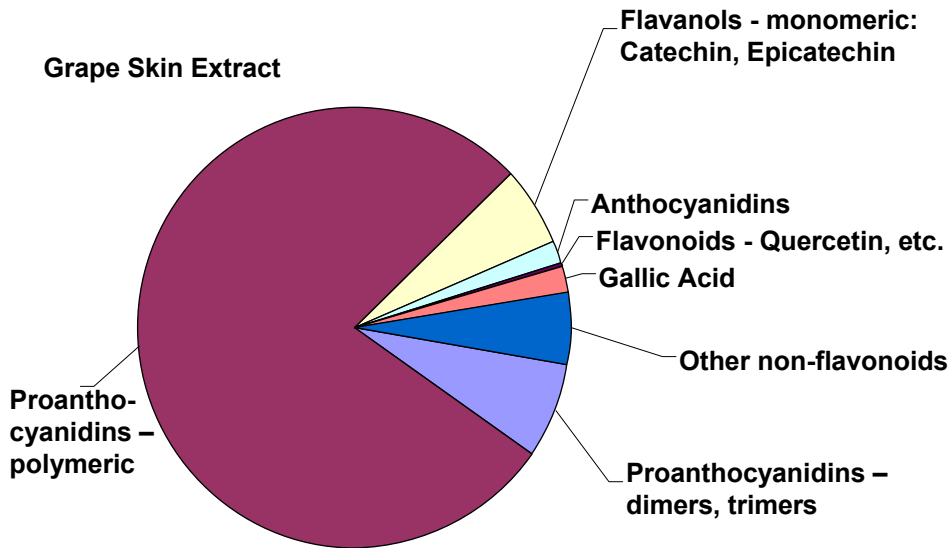
# Chemical Composition Varies

## Plant Source Material

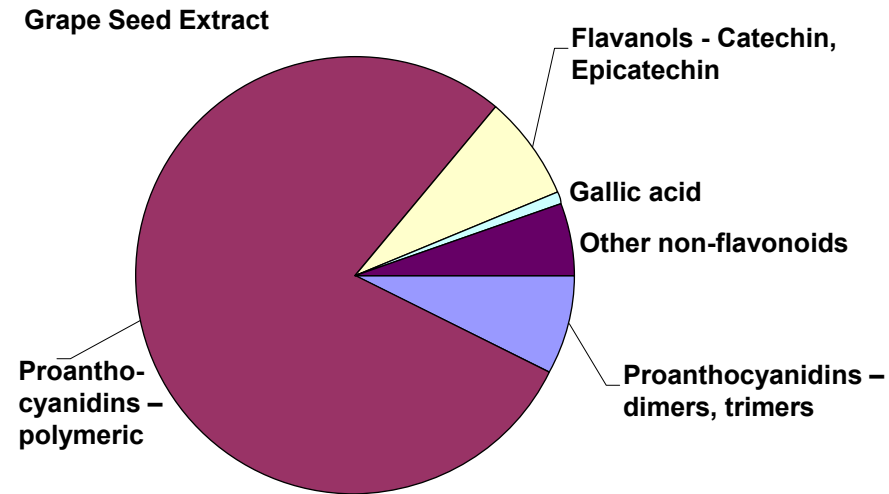
- **Species and Variety**
  - Any known adulterant or frequently substituted species?
- **Plant part**
  - Leaf, fruit, flowers, seed, stem, root, rhizome, total above ground parts
- **Agricultural conditions, including country of origin**
  - Growing conditions: stressed plants produce more defense molecules
  - Time of year to harvest: content of active(s), markers
  - Pesticide/herbicide/insecticide application: chemical contamination
  - Pollution: heavy metal content, etc.
  - Harvesting and handling practices: mycotoxin content, mold, microbes, moisture
- **Processing/Extraction Procedure**

# Change in Plant Part

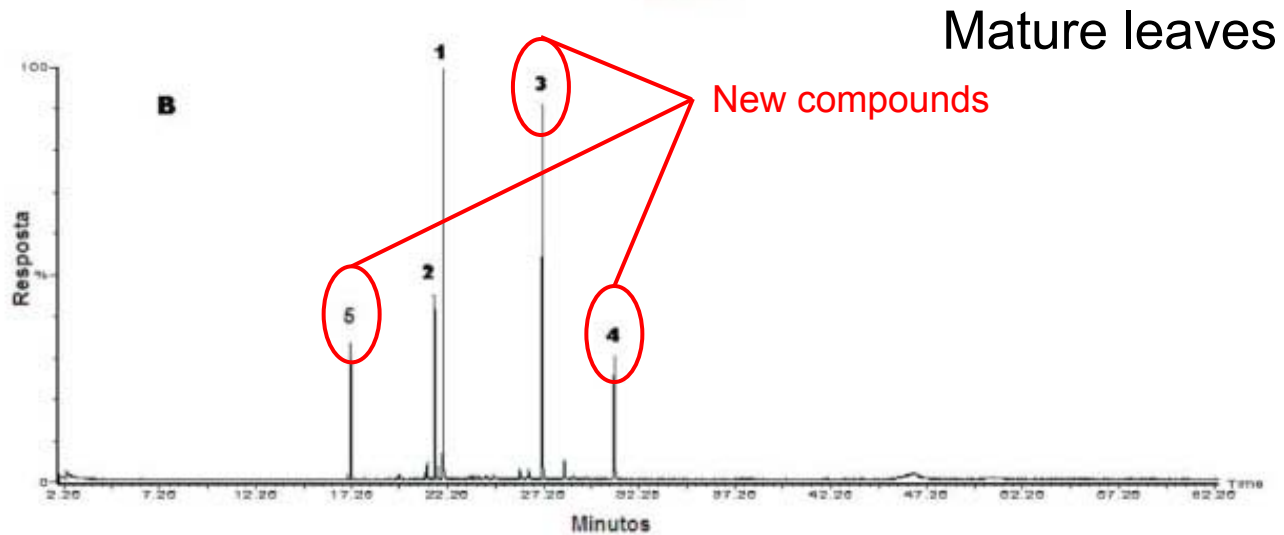
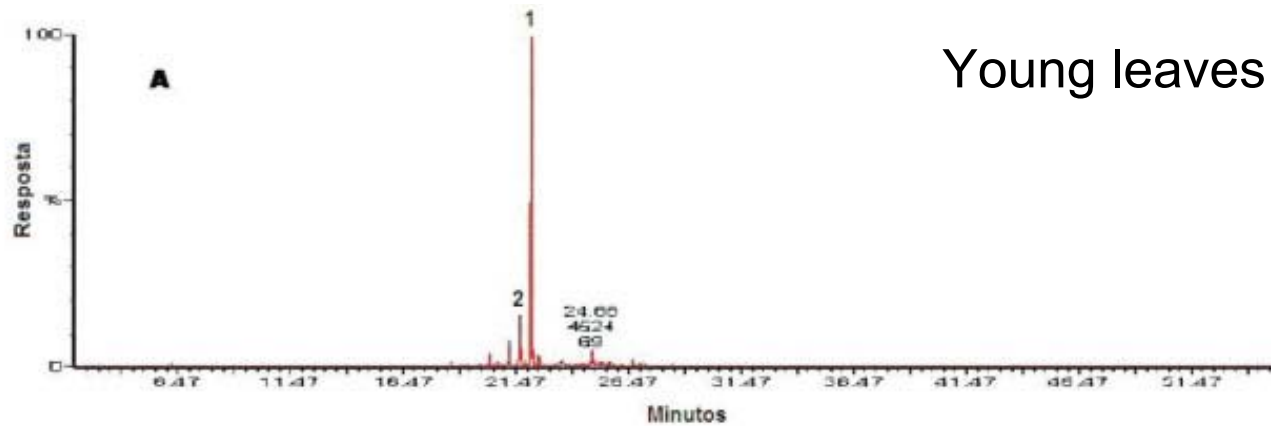
## Polyphenols from Grape Skins



## Polyphenols from Grape Seeds



# Change in Time to Harvest: Young vs. Old Leaves

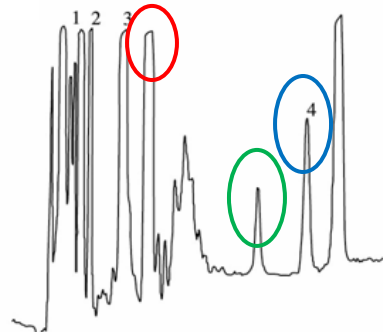


# Extraction Method

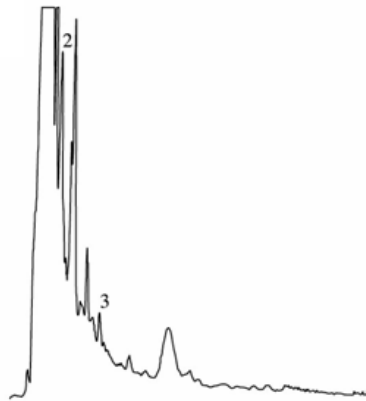
- **Extract vs. semi-purified fraction vs. pure compound?**
  - **Aqueous extract (tea or decoction)**
  - **Alcoholic extract (ethanol, isopropanol)**
  - **Oleoresin (hexanes, halogenated solvents, supercritical CO<sub>2</sub> extraction)**
  - **Essential oil (also present in oleoresins)**
  - **Semi-purified chromatographic fraction (“cleaner” than a crude extract but still contains multiple compounds)**
  - **Purified compound (single entity or racemic mixture of one molecule)**



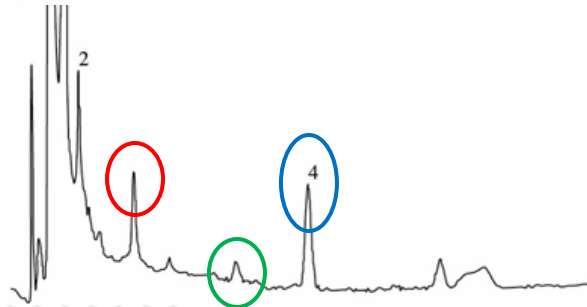
# Extraction Method Produces Compositional Change



**Fresh garlic**



**Ethanolic extract**



**Supercritical CO<sub>2</sub>**  
(More like fresh garlic)

# Why We Need Toxicology Studies

**We can't predict toxicity of the NDI for its intended use compared to historical use because of a change in:**

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

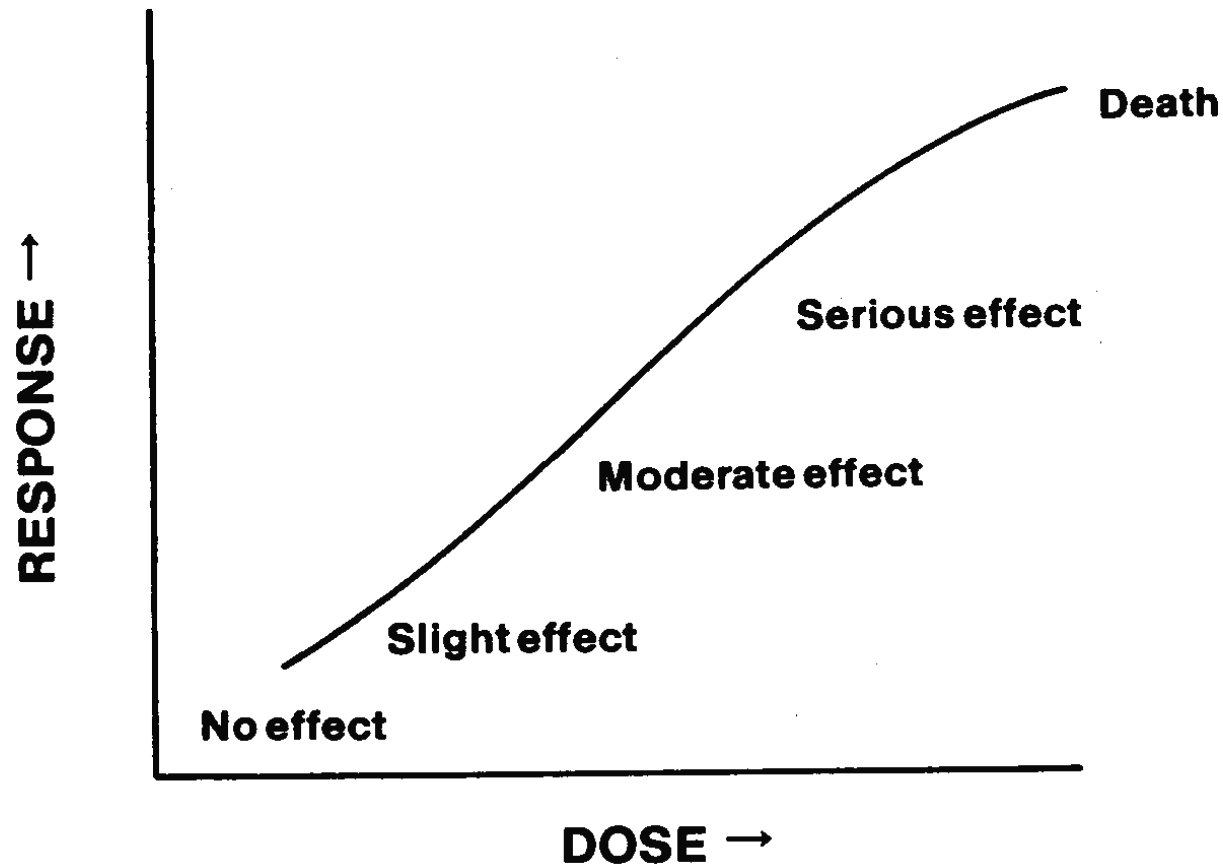
# Paracelsus' Notion of Dose

**“All things are poison and nothing is without poison, only the dose permits something not to be poisonous.”**

**The Central Tenet of Toxicology:**

**It Is the Dose That Makes the Poison**

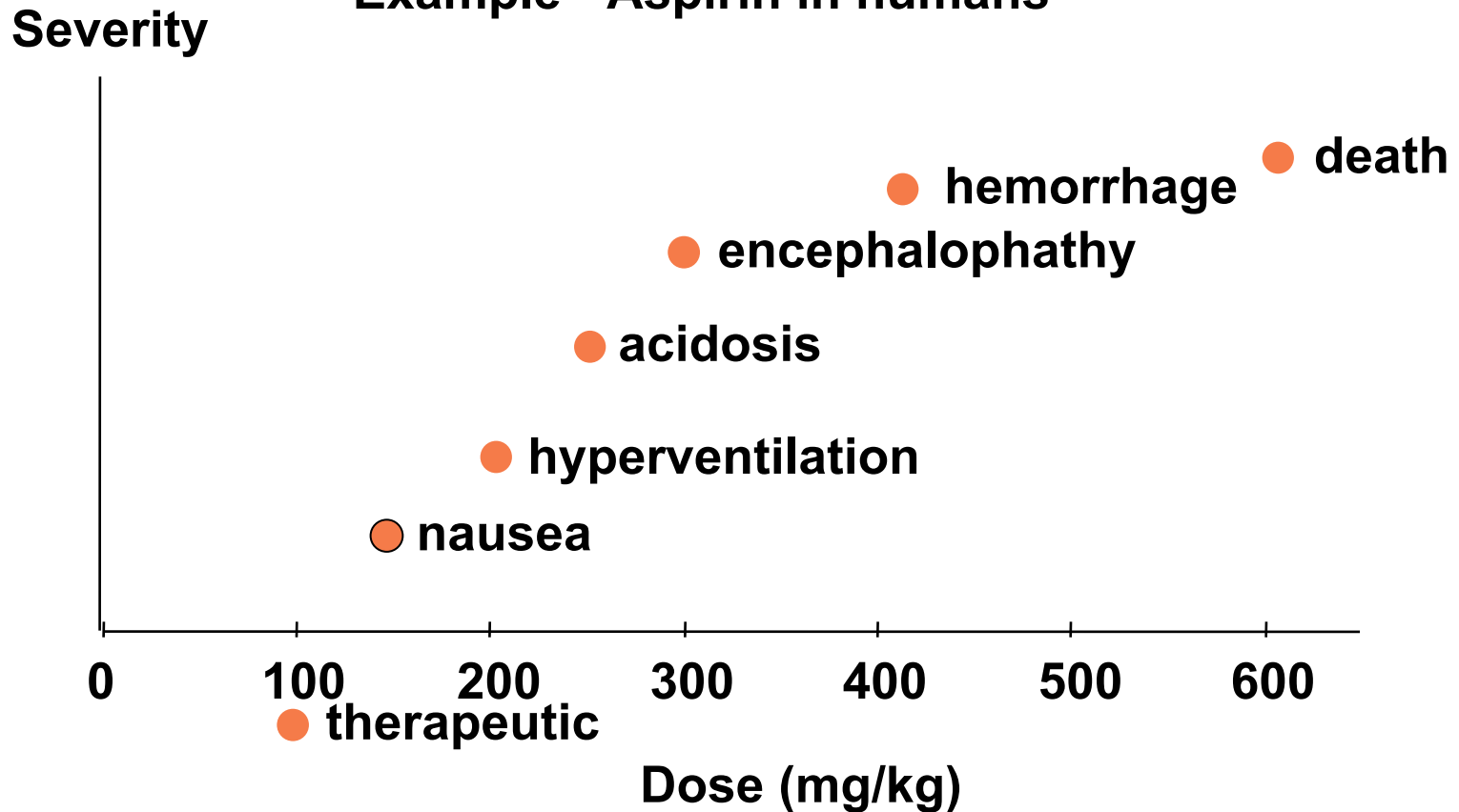
# Dose Response



Dose-response relationship for a typical chemical.

# Individual Dose-Response Function (Dose-Effect)

Example - Aspirin in humans



# Even Water Can Be Toxic At A High Enough Dose

- **Infant (< 1 month)**
- **Excess water**
  - **Dilutes sodium in the blood**
- **Results in untoward effects including**
  - **Bloating**
  - **Low body temperature**
  - **Altered mental state**
  - **Seizures**

# Toxicity of Common Chemicals

<u>Chemical</u>	<u>Daily use</u>	<u>Lethal dose</u>
Water	1.5 quarts	15 quarts
Salt	1/3 ounce	7 ounces
Caffeine	2 cups coffee	75 cups
Ethanol	2 ounces	64 ounces
Sugar	2 ounces	80 ounces
Aspirin	2 tablets	90 tablets

# Why We Need Toxicology Studies

**We can't predict toxicity of the NDI for its intended use compared to historical use because of a change in:**

- **Chemical composition**
- **Dose or amount ingested**
- **Duration of administration**
- **Frequency of administration**

# What Affects The Response to A Dose?

- **Dose Amount: How much?**
- **Dose Frequency: How often?**
- **Dose Duration: How long?**

# Effect of Duration and Frequency

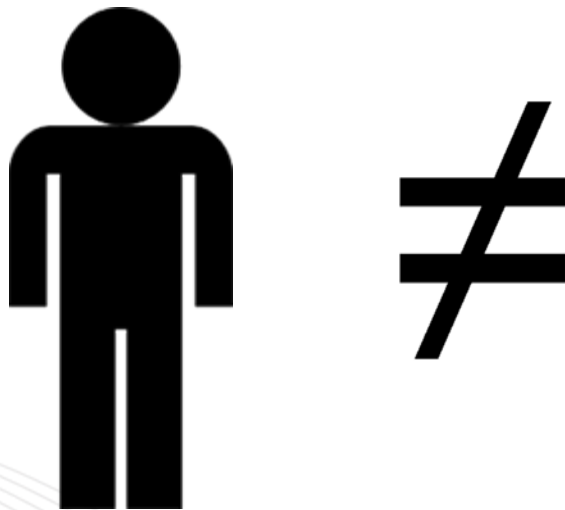
- **Efficiency of repair is an important determinant of the dose-response relationship**
  - **Amount, frequency and duration of exposure are involved**
    - **For example, repair processes may not be overwhelmed at a dose given over a short period of time but the same dose given over a longer period of time may overwhelm these repair processes, resulting in toxicity**
    - **Similarly, frequency of exposure of the same dose may affect the efficiency of the repair processes, producing more toxicity at greater frequencies of administration**

# What Changes the Sensitivity of a Response to a Dose

- **Interspecies Variation**
  - **Animals → Humans**
- **Intraspecies Variation: Human variability**
  - **Health status**
  - **Body weight**
  - **Age**
  - **Sensitivity**

# Differences In Sensitivity

- **Species: (interspecies variation)**
  - Not all organisms have same sensitivity to chemicals
  - Man is not a big rat



# Differences In Sensitivity

- **Individual: (intraspecies)**
  - **Genetic makeup (polymorphism)**
  - **Age (differences in metabolism, immune status)**
  - **Body weight (relative dose on a mg/kg basis higher for children than adults)**
  - **Gender (pregnant females; males vs. females)**
  - **Life style (smoking, alcohol, food, previous exposure)**
  - **Health status (underlying health conditions)**

# **Animal Toxicology**

**How do we decide which studies to carry out?**

# Animal Toxicology Studies

- **Animal toxicology is a tool: classic rodent studies evaluate toxicity**
- **Animal models must be chosen appropriately to extrapolate to the human, including consideration of:**
  - **Bioavailability**
  - **Nutritional requirements/limitations**
  - **Metabolic parameters**
  - **Developmental stage**
- **Study must be designed to prevent differences in pharmacokinetic handling or dietary imbalance from confounding toxicology results**
- **Strengths**
  - **Well controlled experiments, controlled doses, no confounding exposures issues**

# Safety Testing Recommendations

## Draft Guidance

- **Genotoxicity Battery**
  - Bacterial mutagenesis, *in vitro* cytogenetics, *in vivo* mammalian test
- **Repeat dose toxicity**
  - 14-day Range-Finding
  - 90-Day Subchronic
- **Chronic/Carcinogenicity**
- **Reproductive**
  - One generation; Multi-generation
- **Developmental/Teratology**
- **ADME**

# Toxicology Testing Can Inform Us About:

- **Hepatotoxicity**
- **Nephrotoxicity**
- **Cardiovascular toxicity**
- **Pulmonary toxicity**
- **Dermal toxicity**
- **Ocular toxicity**
- **Developmental toxicity**
- **Neurotoxicity**
- **Behavioral toxicity**
- **Immunotoxicity**
- **Hematopoietic toxicity**
- **Reproductive toxicity**
- **Endocrine organ toxicity**
- **Gastrointestinal toxicity**

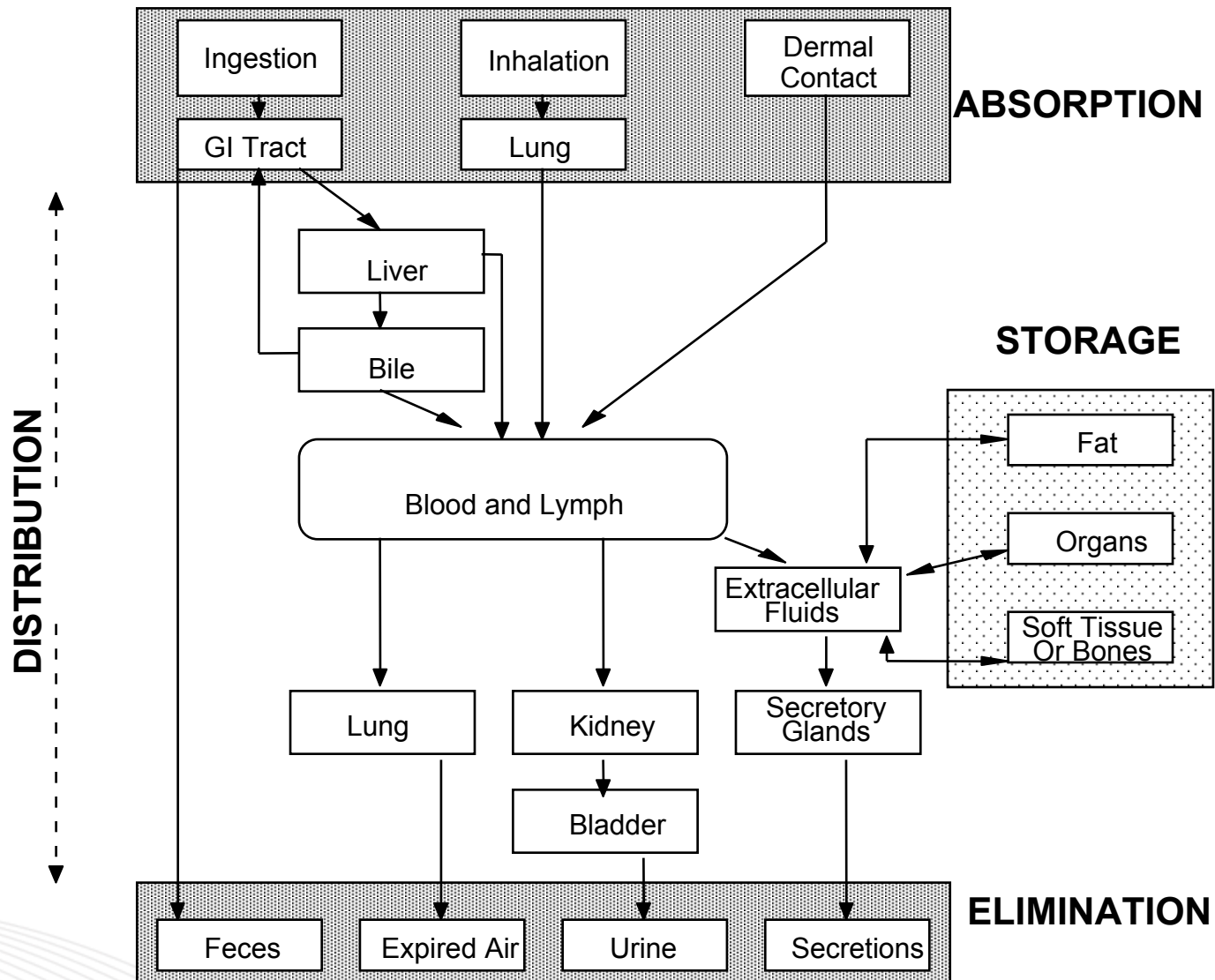
# Spectrum of Toxic Effects

- **Local and systemic effects**
  - **At site of first contact (gastrointestinal)**
  - **At site(s) distal to point absorbed (internal organ damage)**
- **Reversible and irreversible effects**
  - **Disappear following cessation of exposure (enzyme changes, respiratory depression)**
  - **Persist or even progress after exposure is discontinued (cancer, genetic alterations, birth defects, death)**
- **Immediate and delayed effects**
  - **Develop shortly after single exposure (cyanide poisoning)**
  - **Occur after a lapse of time (10-20 years for cancer)**

# Purpose of ADME Studies

- **The delivered dose to a tissue or organ is determined**
- **Toxicity studies are more easily interpreted, likely to achieve target doses, and avoid excessive toxicity if data from these studies are available**
- **Determination of metabolic pathways and the rates of metabolism in different test species may provide explanations for species differences**

# Fate of Chemicals in the Body



# ADME for Complex NDIs

- **Issues involved in the application of ADME in the context of complex, multi-component NDIs:**
  - **ADME follows the fate of a single chemical not complex mixtures;**
  - **Identification of the correct marker compound(s) to use in an ADME study may not be feasible;**
  - **ADME studies for one component of a complex mixture may not represent the fate of unidentified components;**
  - **May not be relevant for the safety determination on NDIs**

# How Do We Use These Studies To Assess Safety

## Risk Assessment Terms Defined

# Terms

- **NOAEL**: the No-Observable-Adverse-Effect Level which is the highest dose that did not elicit an adverse effect in a properly designed and executed toxicology study
- **NOEL**: the No-Observable-Effect Level which is the highest dose at which no effects (beneficial, neutral or adverse) are observed in a properly designed and executed toxicology study

# Terms

- **Safety Factor or Uncertainty Factor:**
  - a multiplier used to account for differences between animals and humans, between differences in humans, and limitations in animal studies that allows us to deal with the uncertainty about the predictive value of the animal data to extrapolate to humans in the context of safety

# Safety Factors

- **Intraspecies (10 X):**
  - **May be smaller when there is a long history of food use by a large, diverse population. Factor should be large when toxicity is severe or studies have limited duration or small populations**
- **Interspecies (10 X)**
- **Subchronic to chronic (10 X):**
  - **If only one subchronic study is available an additional factor of 2 may be used**

# Frequency and Duration of Exposure: Terms Defined

- **Chronic Use:** long-term use, assumed to be consumption every day throughout life
  - **Daily Use:** ingestion at least once a day, every day, for at least three months in a row or for more than 90 days in a year
- **Intermittent Use:** any use that is less frequent than daily use
  - **Subchronic Use**
    - Daily and finite
    - Non-daily and lifetime

**“USE DAILY MEANS LIFETIME”**

# Terms

- **Acceptable Daily Intake (ADI) is defined as the daily intake of the NDI that during the human lifetime appears to be without appreciable risk.**
- **Risk is the likelihood that toxicity will be produced under the conditions of exposure.**
- **Safety is the inverse of risk.**
- **Safety for an NDI is defined as the reasonable expectation of safety under the conditions of use.**

# Derivation of the ADI

$$\text{ADI} = \frac{\text{NOAEL (mg/kg/day)}}{\text{Safety Factors}}$$

# Case Study:

## 90-day Subchronic Rat Study

- Tested at doses of
  - 10, 100, 1000 mg/kg/day
- Outcome
  - Frank liver toxicity identified at 1000 mg/kg/day
  - Substantial liver enzyme changes at 100 mg/kg/day
  - No Observed Adverse Effects at 10 mg/kg/day
- Conclusions
  - Hazard: liver toxicity
  - NOAEL: 10 mg/kg/day

# Calculation of the ADI

$$\begin{aligned} \text{ADI} &= \frac{10 \text{ mg/kg/day}}{10 \times 10 \times 10 \times 2} \\ &= 0.005 \text{ mg/kg/day} \end{aligned}$$

**For 70 kg human = 0.35  
mg/day**

# EDI

- **Estimated Daily Intake (EDI): the highest total intake level determined from the proposed conditions of use (mg/kg/day or mg/day).**
- **Label states: 2 pills per day (0.1 mg/pill)**
- **EDI = 0.1 mg/day x 2 = 0.2 mg/day**

# Determination of Safety

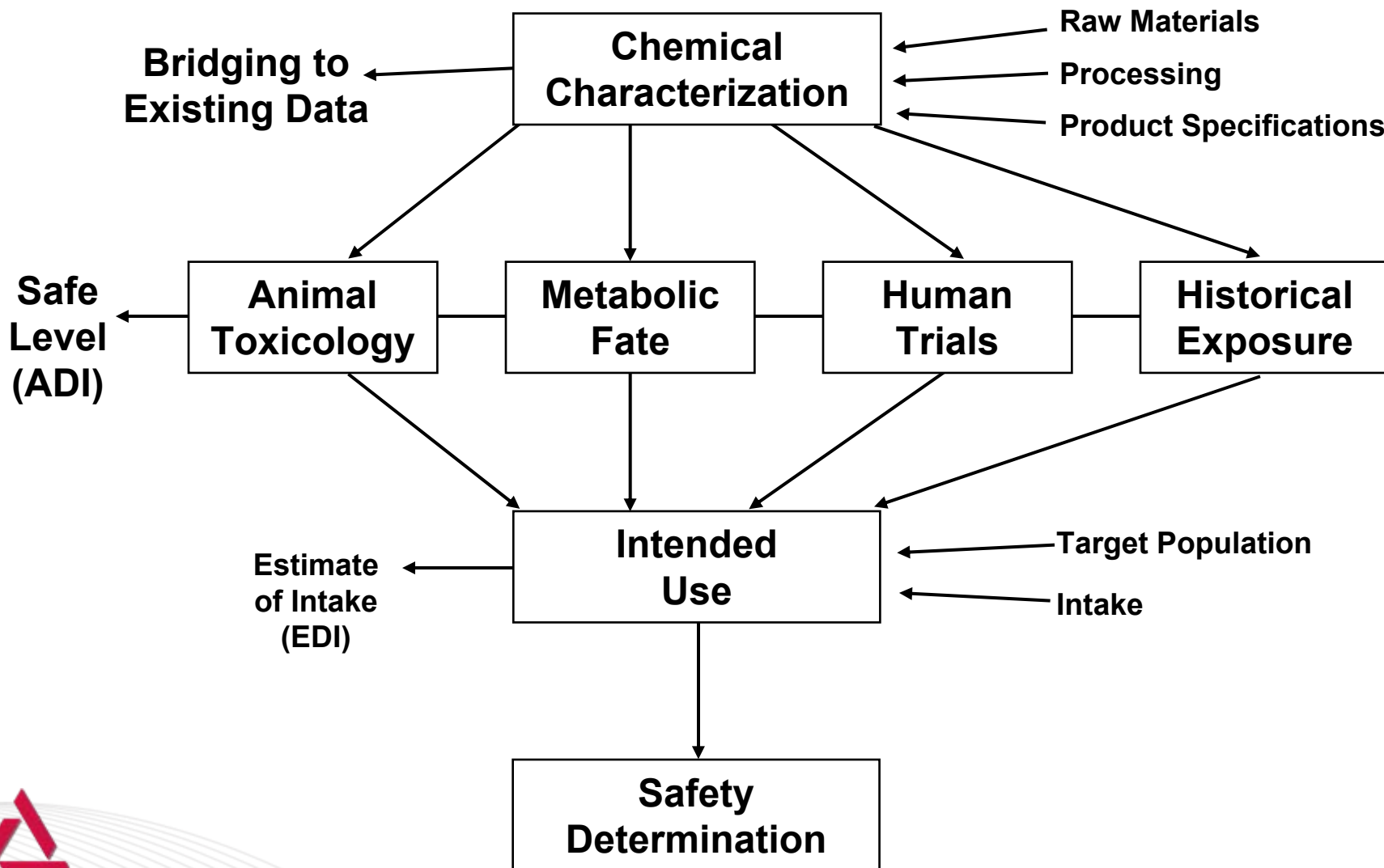
**0.2 mg/day < 0.35 mg/day**

**EDI < ADI**

**Ratio EDI:ADI is 0.57**

**≤ 1**

# Safety Analysis



# What will this cost?

# Safety Testing Recommendations

## Safety Testing Recommendations Daily Chronic Documented Historical Use

	Intermittent		Daily Chronic		Cost
	Less Than	Greater Than	Less Than	Greater Than	
<b>Two-Study Genetic Toxicity Battery</b> <b>Bacterial Mutagenesis (Ames)</b> <i>In vitro</i> cytogenetic		√		√	\$4,310-\$7,800 \$26,000-\$34,000
<b>14-Day Range-Finding Oral Study in Animals</b>		√		√	\$50,000-\$75,000
<b>90-Day Sub-Chronic Oral Study in Animals</b>		√		√	\$125,000-\$179,800
<b>One-Generation Rodent Reproductive Study</b>				√	\$220,000
<b>Teratology Study</b>		√		√	\$138,000 (Rat) \$164,000 (Rabbit)
<b>One-Year Chronic Toxicity or Two-Year Carcinogenesis Study</b>				√	\$1,500,000- \$2,000,000 (Rat)
<b>Single-Dose ADME Study in Animals</b>		√			\$230,000 (Rat)
<b>Repeat-Dose ADME Study in Animals</b>				√	\$135,000 (Rat)

# Safety Testing Recommendations

## Safety Testing Recommendations Intermittent Documented Historical Use

	Intermittent		Daily Chronic		Cost
	Less Than	Greater Than	Less Than	Greater Than	
<b>Two-Study Genetic Toxicity Battery</b> <b>Bacterial Mutagenesis (Ames)</b> <i>In vitro</i> cytogenetic		√			\$4,310-\$7,800 \$26,000-\$34,000
<b>Three-Study Genetic Toxicity Battery</b> <b>Bacterial Mutagenesis (Ames)</b> <i>In vitro</i> cytogenetic <i>In vivo</i> mammalian test (micronucleus)			√	√	\$4,310-\$7,800 \$26,000-\$34,000 \$25,000-\$31,900
<b>14-Day Range-Finding Oral Study in Animals</b>		√	√	√	\$50,000-\$75,000
<b>90-Day Sub-Chronic Oral Study in Animals</b>		√	√	√	\$125,000-\$179,800
<b>One-Generation Rodent Reproductive Study</b>		√			\$220,000
<b>Multi-Generation Rodent Reproductive Study</b>			√	√	\$525,000
<b>Teratology Study</b>		√	√	√	\$138,000 (Rat) \$164,000 (Rabbit)
<b>One-Year Chronic Toxicity or Two-Year Carcinogenesis Study</b>				√	\$1,500,000- \$2,000,000 (Rat)
<b>Single-Dose ADME Study in Animals</b>		√			\$230,000 (Rat)
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# Safety Testing Recommendations

## Safety Testing Recommendations No History Documented Historical Use

	Daily Chronic	Intermittent	Cost
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<b>Repeat-Dose ADME Study in Animals</b>	√	√	\$135,000 (Rat)

# Thank You From the Spherix Team

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