



# *NDI GUIDANCE SEMINAR:*

*Understanding the  
New Safety Paradigm*

*July 26-27, 2011*

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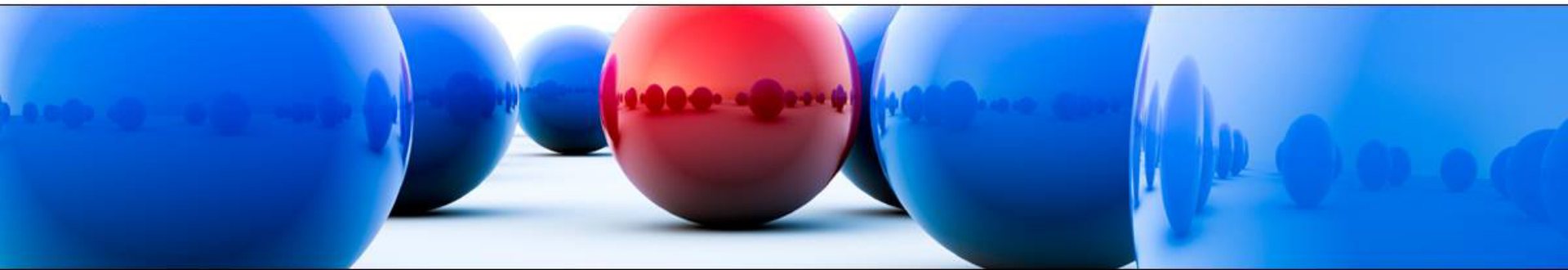
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# Toxicology and Safety Studies:

## What Does FDA Really Expect, and Do You Need to Do It?

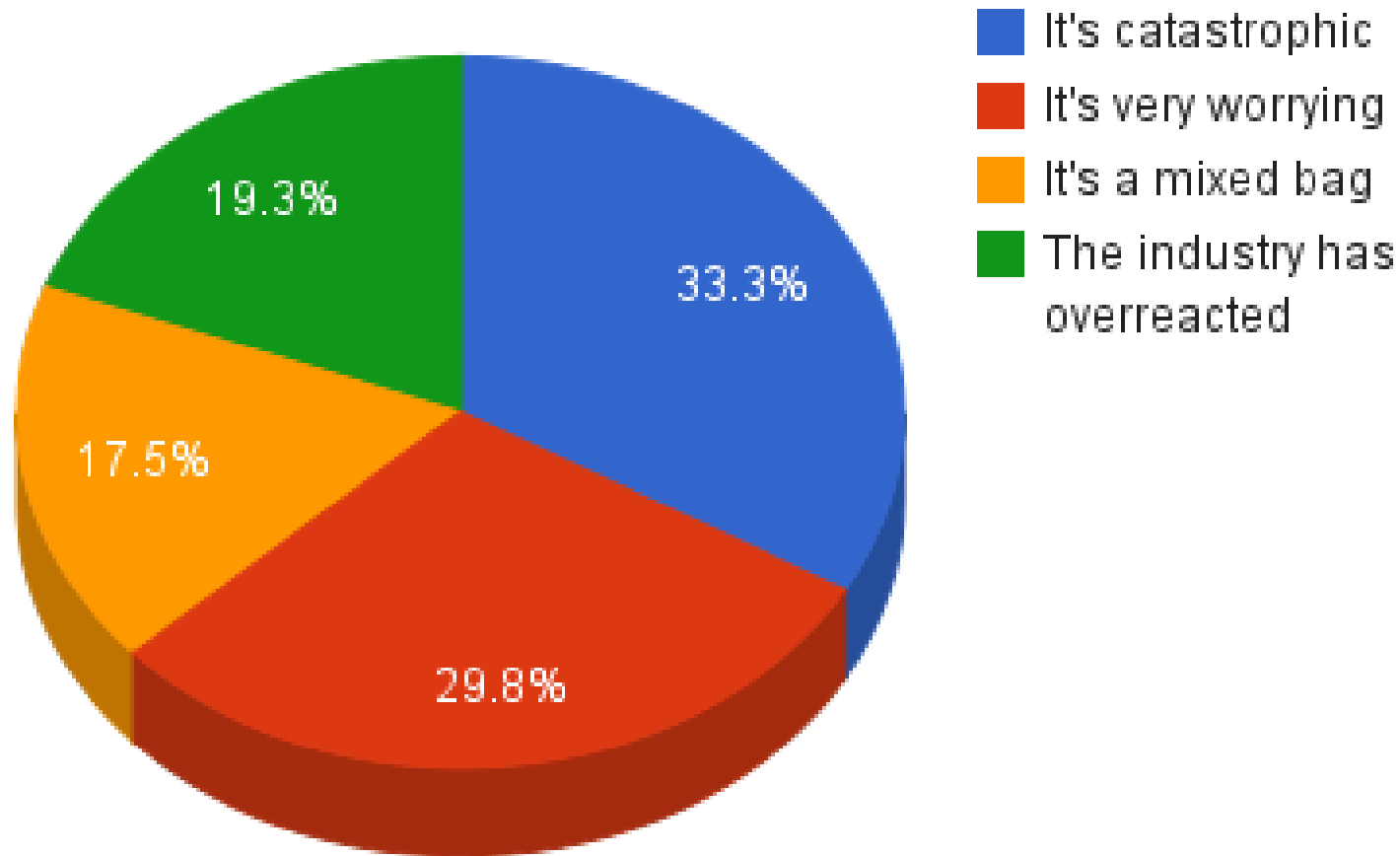


## Toxicology and Safety Studies:

What Does FDA Really Expect and Do You Need to Do It?

- **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?**

# Industry Reaction to NDI Guidelines

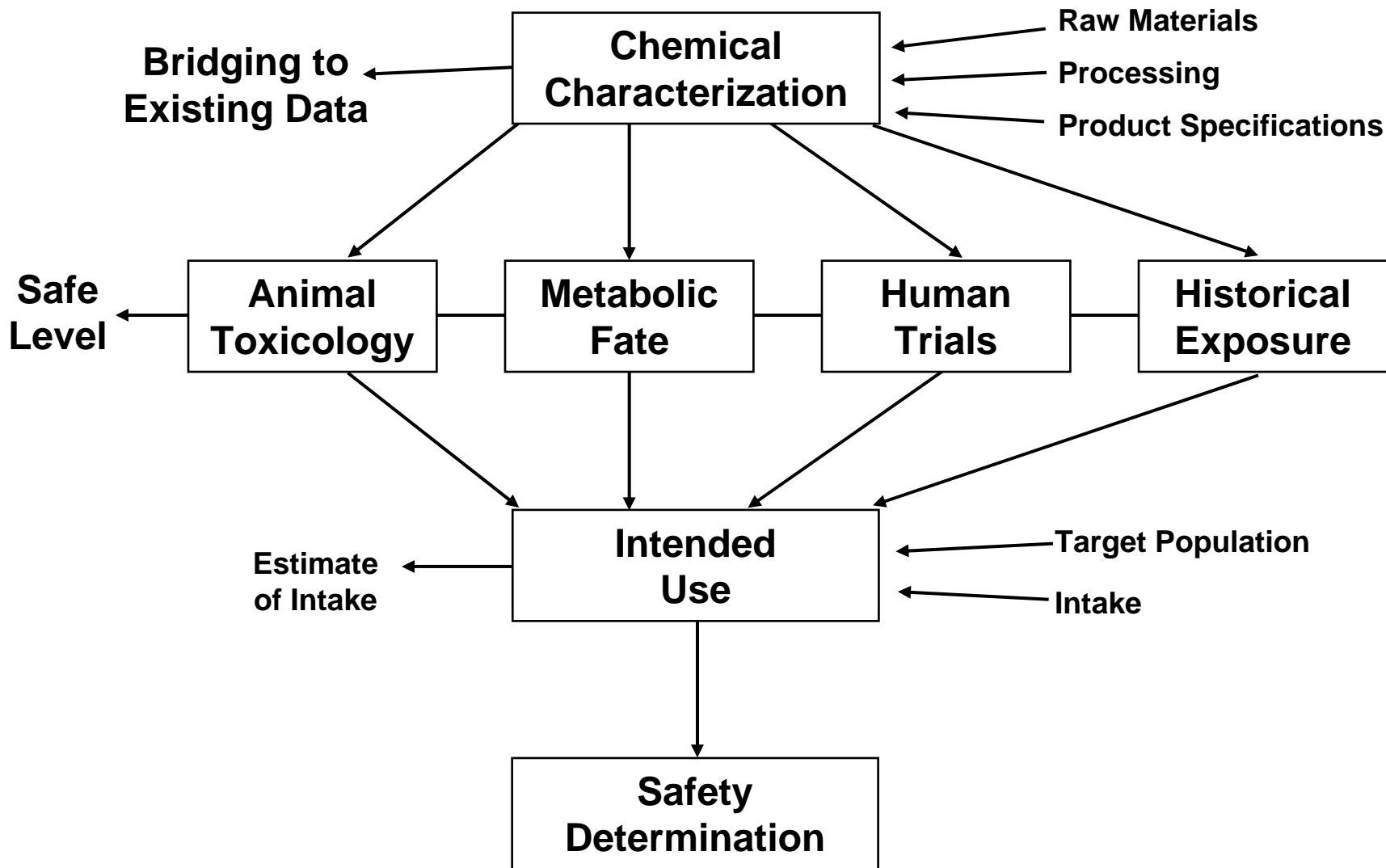


Source: [http://www.nutraingredients-usa.com/Regulation/NDI-guidance-poll-verdict-It-s-catastrophic/?c=maklFAHyk7ntZPwjrzctzA%3D%3D&utm\\_source=newsletter\\_weekly&utm\\_medium=email&utm\\_campaign=Newsletter%2BWeekly](http://www.nutraingredients-usa.com/Regulation/NDI-guidance-poll-verdict-It-s-catastrophic/?c=maklFAHyk7ntZPwjrzctzA%3D%3D&utm_source=newsletter_weekly&utm_medium=email&utm_campaign=Newsletter%2BWeekly)



"The Committee's decided to ban further research until it can be proven your 'wheel' poses no threat to the environment, Society or public health."

# Safety Analysis



- **Man in quest for food learned certain foods produced varying degrees of illness or death**
- **Soon recognized harmful & 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 & degrees of safeness for any chemical (the dose makes the poison)**
    - **All chemicals can cause toxic effects in large enough amounts**

- **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**

- **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**

- **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

- **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

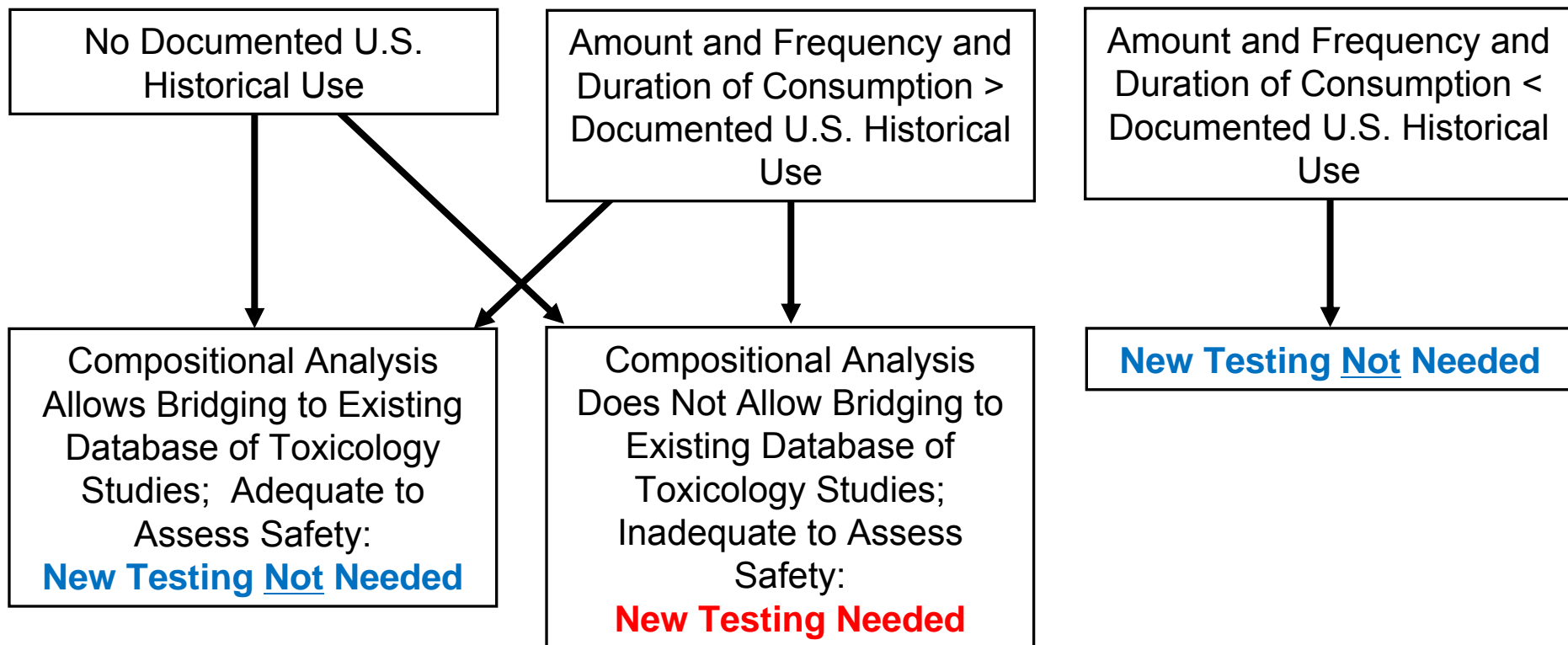
**“The NDI safety standard is different than the standard for food additives, drugs, pesticides, and other FDA-regulated products. Recommendations in guidance documents that are tailored to the safety assessment needs of other FDA-regulated products may not always be appropriate for dietary ingredients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

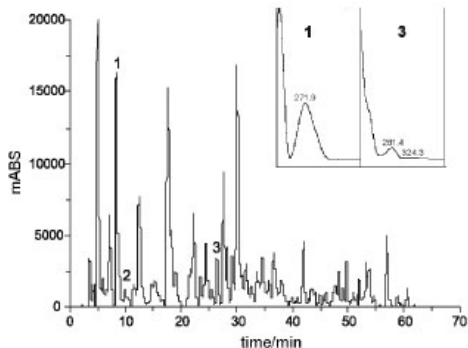
# What Necessitates Toxicology Testing for an NDI?

- **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?**

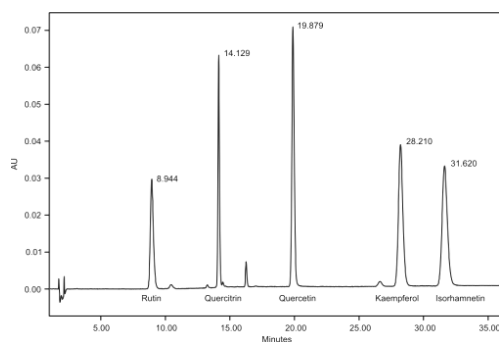
# Decision Tree Approach for Toxicology Testing New Dietary Ingredient



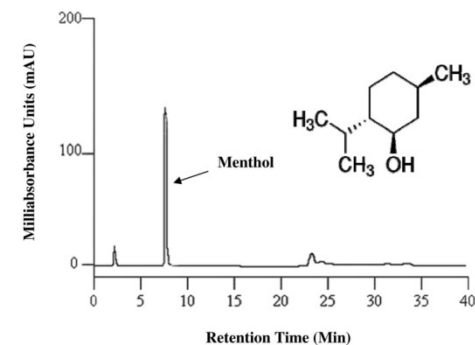
# Establishing a “Chemical Bridge” to Historical Use and Existing Toxicology Studies



◀ 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.

- **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)**

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

**“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.”**

**“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.”**

**“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**

## Plant Source Material

### ➤ Species and Variety

- Any known adulterant or frequently substituted species?

### ➤ Plant part

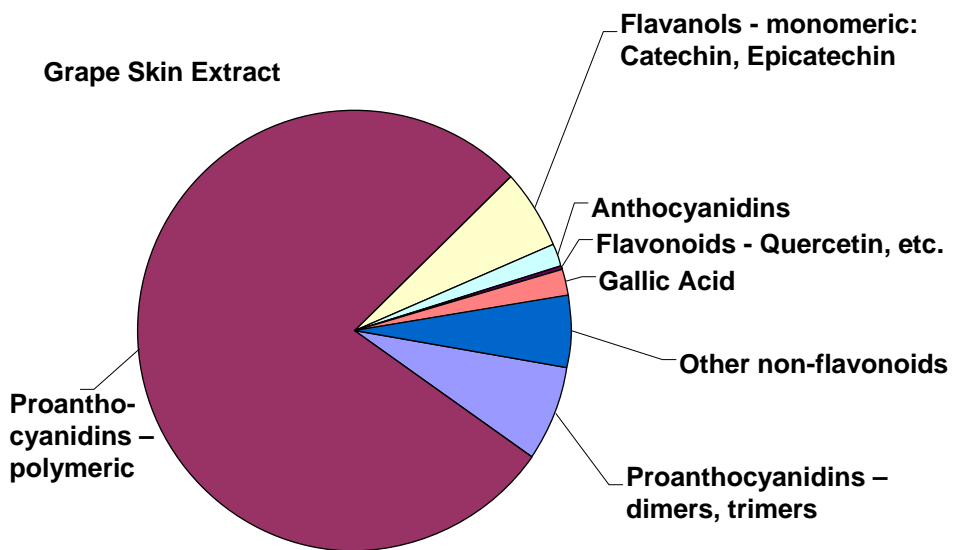
- Leaf, fruit, flowers, seed, stem, root, rhizome, total above ground parts

### ➤ Agricultural conditions, including country of origin

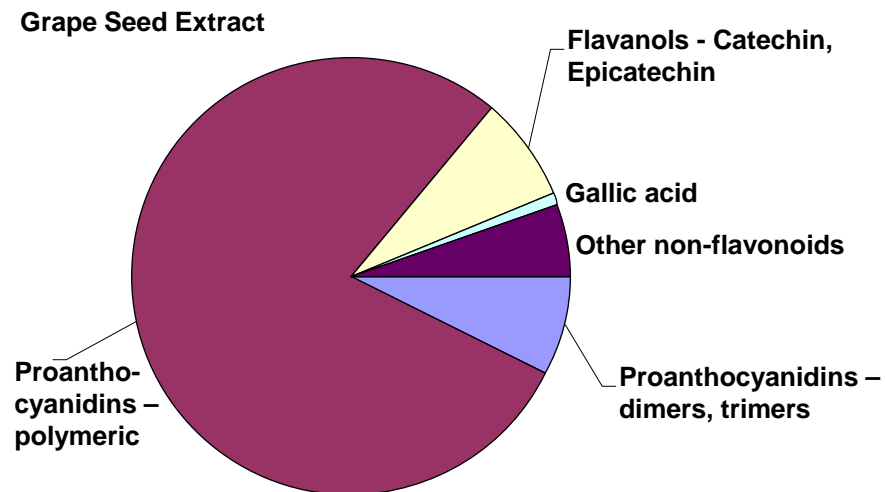
- Growing conditions: stressed plants produce more defense molecules
- Time of year to harvest: content of active(s), markers
- Pesticide/herbicide/insecticide application: chemical contamination
- Pollution: heavy metal content, etc.
- Harvesting and handling practices: mycotoxin content, mold, microbes, moisture

### ➤ Processing/Extraction Procedure

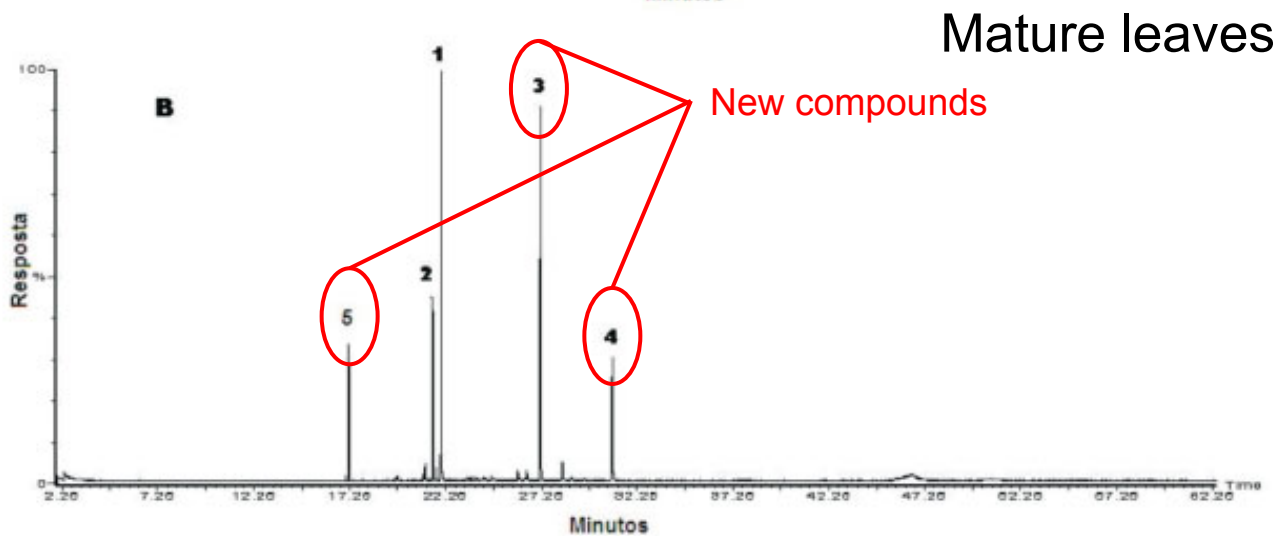
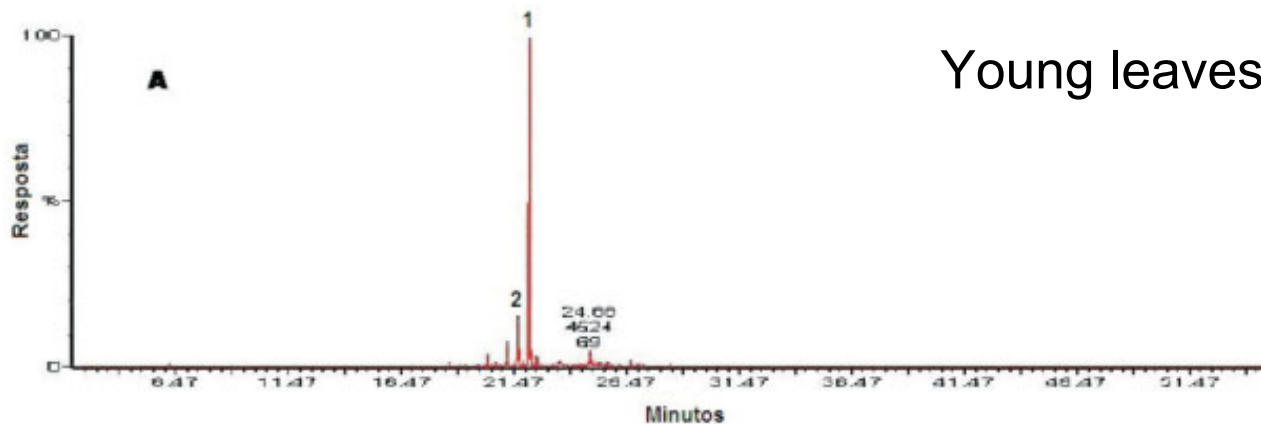
## Polyphenols from Grape Skins



## Polyphenols from Grape Seeds



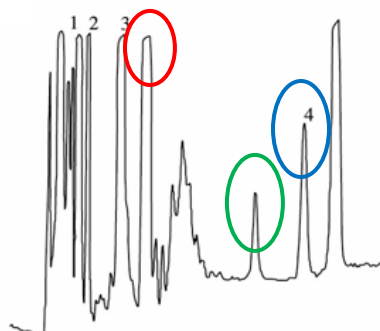
# Change in Time to Harvest: Young vs. Old Leaves



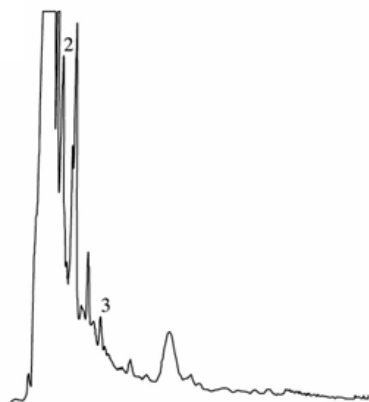
- **Extract vs. semi-purified fraction vs. pure compound?**
  - **Aqueous extract (tea or decoction)**
  - **Alcoholic extract (ethanol, isopropanol)**
  - **Oleoresin (hexanes, halogenated solvents, supercritical CO<sub>2</sub> extraction)**
  - **Essential oil (also present in oleoresins)**
  - **Semi-purified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



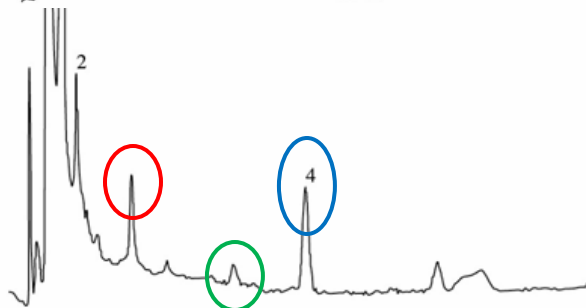
# Extraction Method Produces Compositional Change



**Fresh garlic**



**Ethanolic extract**



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

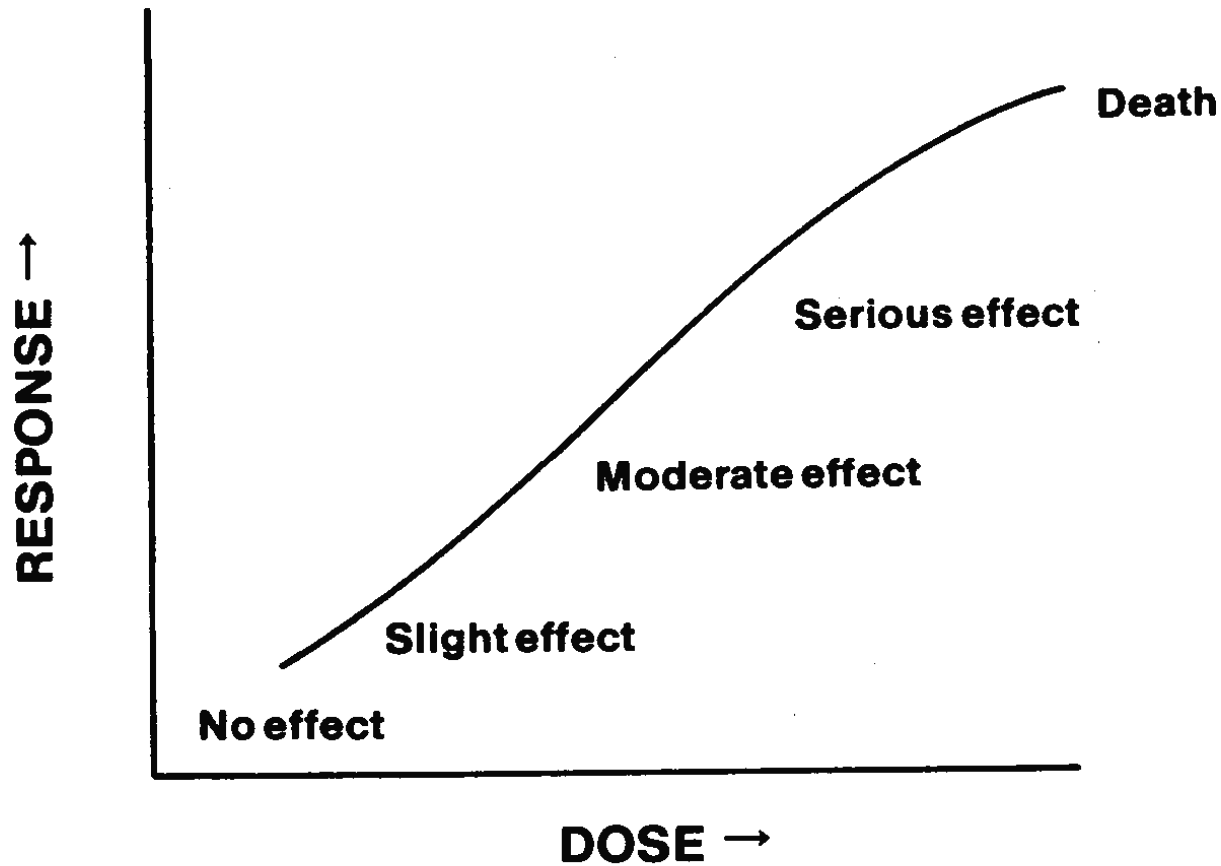
**We can't predict toxicity of the NDI for its intended use compared to historical use because of a change in:**

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

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

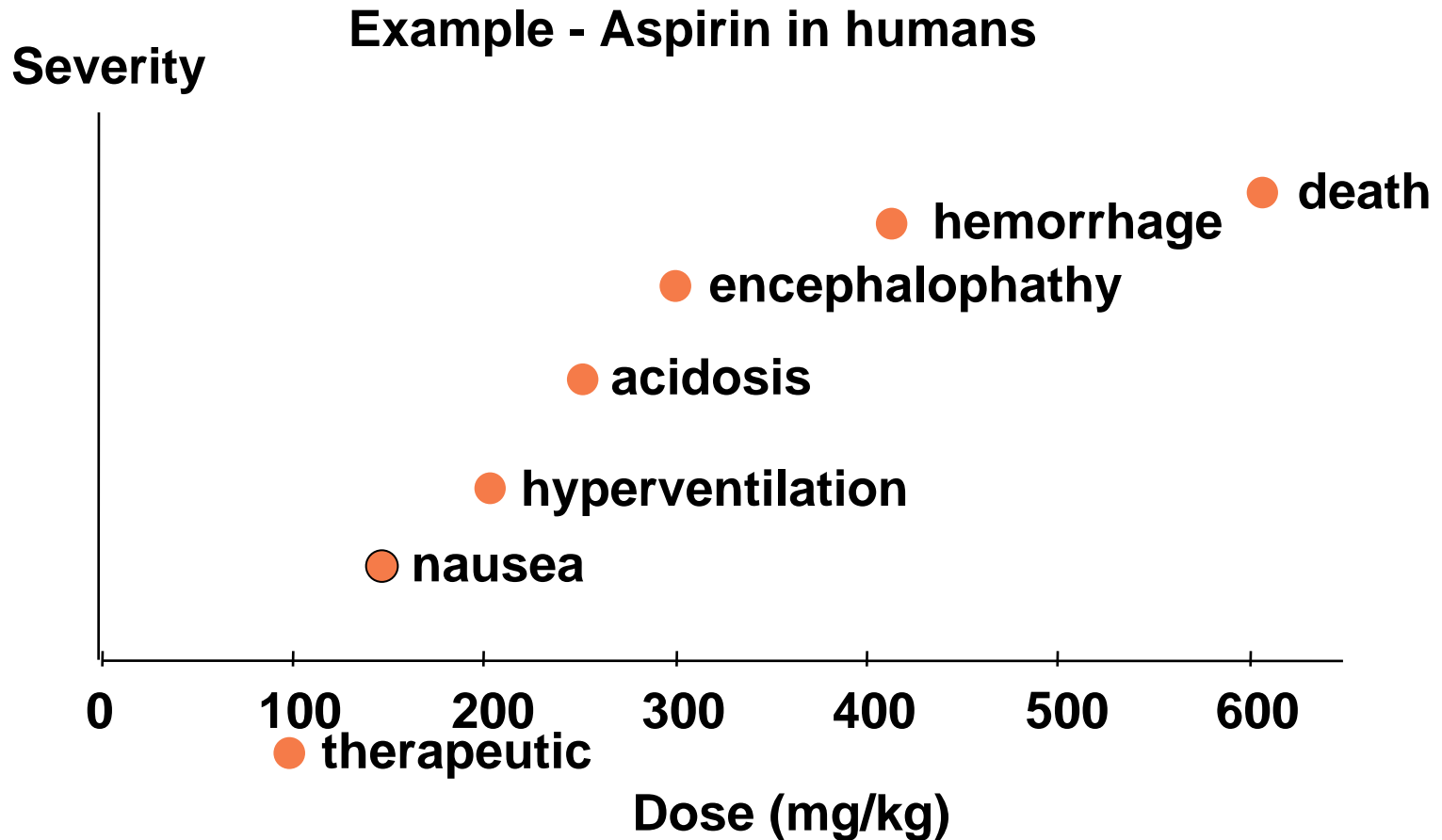
**The Central Tenet of Toxicology:**

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



Dose-response relationship for a typical chemical.

# Individual Dose-Response Function (Dose-Effect)



# 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**

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**Arch Pediat & Adol Med (1997) 8**

# 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         |

**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?

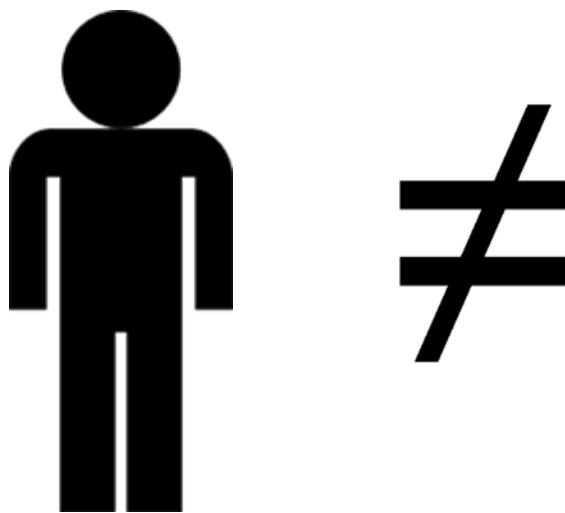
- **Dose Amount: How much?**
- **Dose Frequency: How often?**
- **Dose Duration: How long?**

- **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**

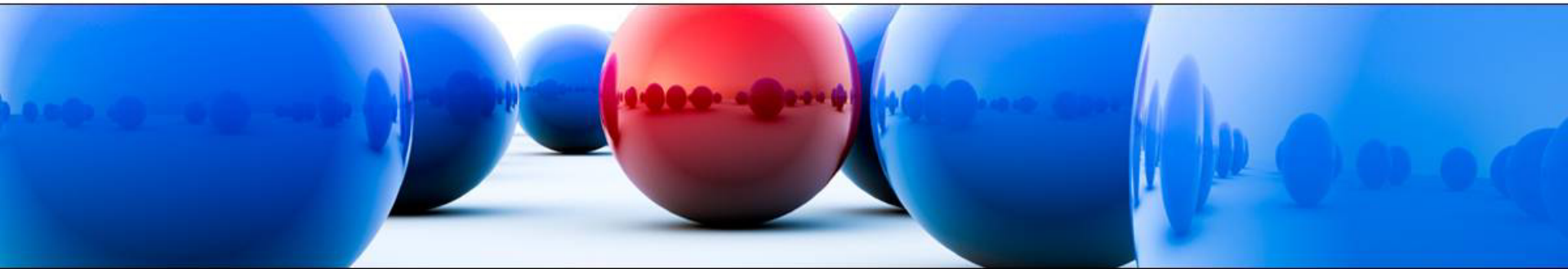
- **Species: (interspecies variation)**
  - **Not all organisms have same sensitivity to chemicals**
  - **Man is not a big rat**



- **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 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**

- **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**

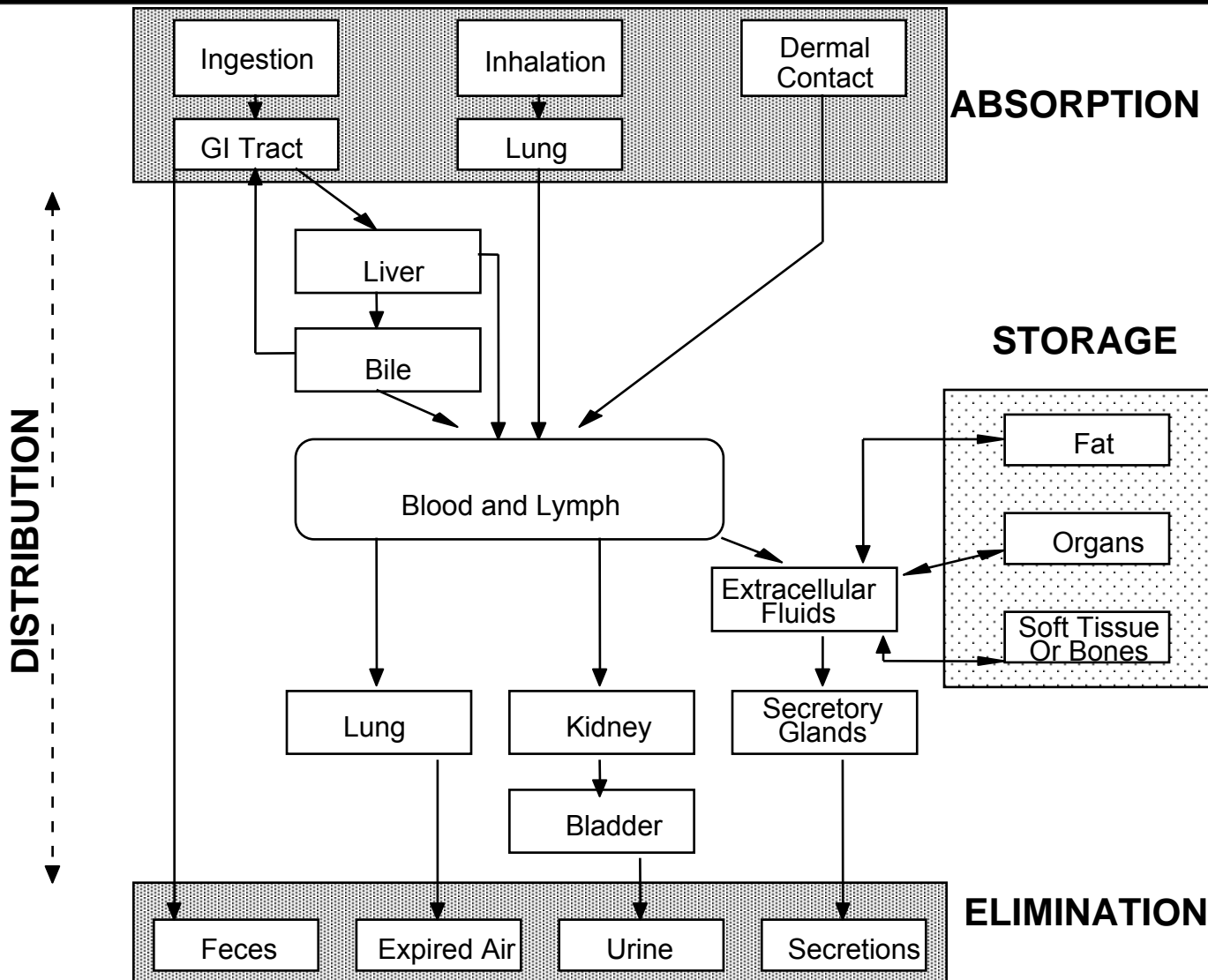
# Toxicology Testing Can Inform Us About:

- **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**

- **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)

- **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



- **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**

## **Risk Assessment**

### **Terms Defined**

- **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

- **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”**

- **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**

**Protective of daily, lifetime exposure**

- **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

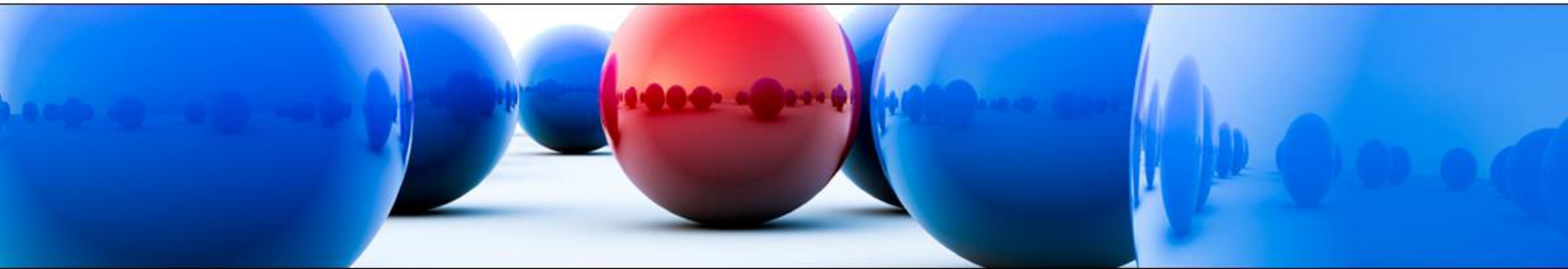
**0.2 mg/day < 0.35 mg/day**

**EDI < ADI**

**Ratio EDI:ADI is 0.57**

**$\leq 1$**

# 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<br/>Bacterial Mutagenesis (Ames)<br/><i>In vitro</i> cytogenetic</b> |              | √            |               | √            | \$4,310-\$7,800<br>\$26,000-\$34,000  |
| <b>14-Day Range-Finding Oral Study in<br/>Animals</b>  |              | √            |               | √            | \$50,000-\$75,000                     |
| <b>90-Day Sub-Chronic Oral Study in<br/>Animals</b>  |              | √            |               | √            | \$125,000-\$179,800                   |
| <b>One-Generation Rodent Reproductive<br/>Study</b>  |              |              |               | √            | \$220,000                             |
| <b>Teratology Study</b>  |              | √            |               | √            | \$138,000 (Rat)<br>\$164,000 (Rabbit) |
| <b>One-Year Chronic Toxicity or Two-Year<br/>Carcinogenesis Study</b>                                      |              |              |               | √            | \$1,500,000-<br>\$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

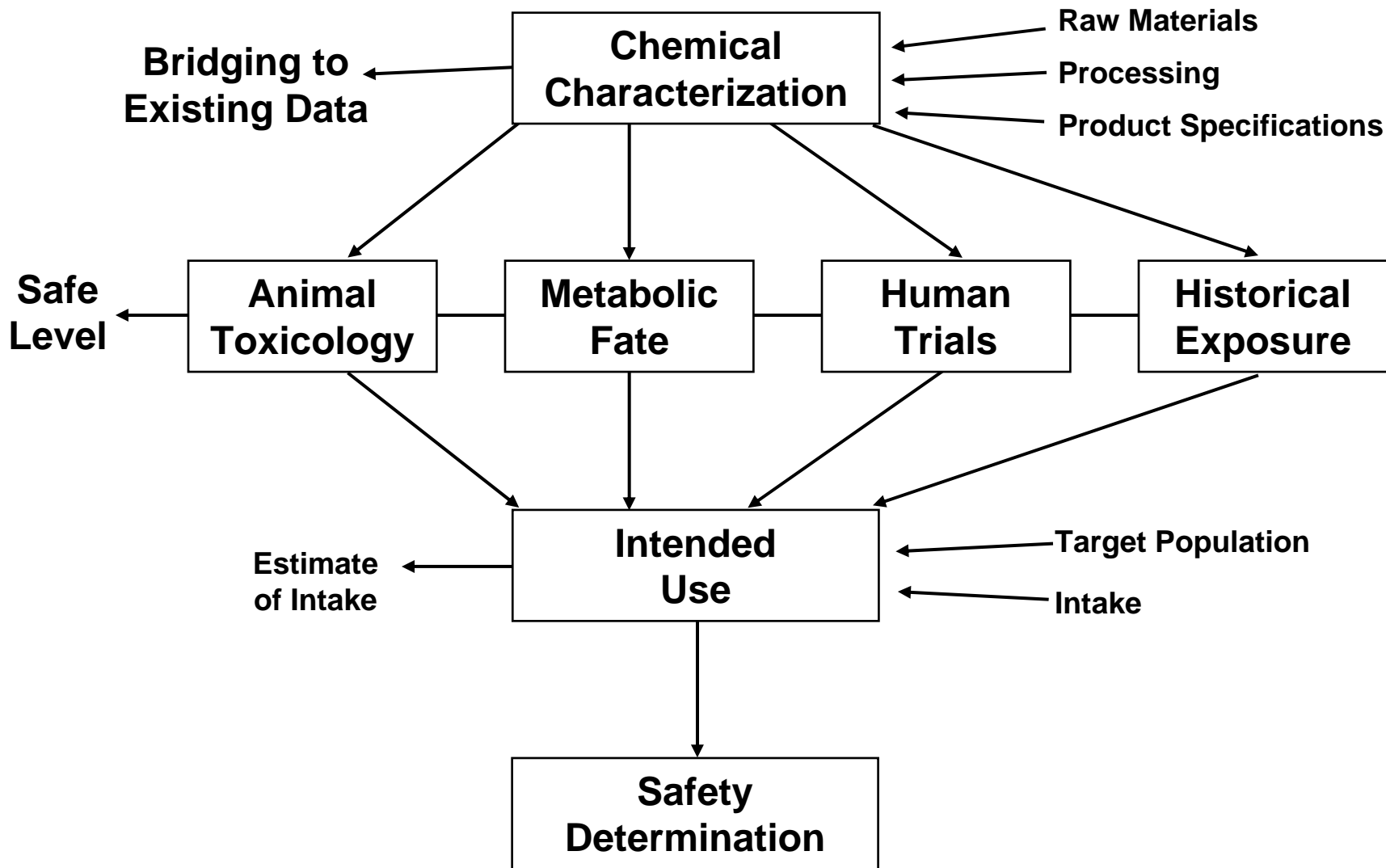
## Safety Testing Recommendations Intermittent Documented Historical Use

|   | Intermittent |              | Daily Chronic |              | Cost  |
|---|--------------|--------------|---------------|--------------|---|
|   | Less Than    | Greater Than | Less Than     | Greater Than |   |
| <b>Two-Study Genetic Toxicity Battery<br/>Bacterial Mutagenesis (Ames)<br/><i>In vitro</i> cytogenetic</b>  |              | √            |               |              | \$4,310-\$7,800<br>\$26,000-\$34,000                      |
| <b>Three-Study Genetic Toxicity Battery<br/>Bacterial Mutagenesis (Ames)<br/><i>In vitro</i> cytogenetic<br/><i>In vivo</i> mammalian test (micronucleus)</b> |              |              | √             | √            | \$4,310-\$7,800<br>\$26,000-\$34,000<br>\$25,000-\$31,900 |
| <b>14-Day Range-Finding Oral Study in<br/>Animals</b>   |              | √            | √             | √            | \$50,000-\$75,000   |
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| <b>One-Generation Rodent Reproductive<br/>Study</b>   |              | √            |               |              | \$220,000   |
| <b>Multi-Generation Rodent Reproductive<br/>Study</b>   |              |              | √             | √            | \$525,000   |
| <b>Teratology Study</b>   |              | √            | √             | √            | \$138,000 (Rat)<br>\$164,000 (Rabbit)                     |
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**Safety Testing Recommendations**  
**No History**  
**Documented Historical Use**

|   | Daily Chronic | Intermittent | Cost  |
|---|---------------|--------------|---|
| <b>Three-Study Genetic Toxicity Battery</b><br><b>Bacterial Mutagenesis (Ames)</b><br><i>In vitro</i> cytogenetic<br><i>In vivo</i> mammalian test (micronucleus) | √             | √            | \$4,310-\$7,800<br>\$26,000-\$34,000<br>\$25,000-\$31,900 |
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| <b>Repeat-Dose ADME Study in Animals</b>  | √             | √            | \$135,000 (Rat)   |

# Safety Analysis



# Thank You From the Spherix Team

- **US Based:**

- **Claire Kruger, PhD, DABT**
- **A. Wallace Hayes, PhD, DABT**
- **Nancy Booth, PhD**
- **Ronald Slesinski, PhD, DABT**
- **Susan Phillips, MS**
- **Yongming Lu, PhD**
- **Roger Clemens, PhD, CNS**
- **Dietrich Conze, PhD**
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- **International:**

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