Current and Emerging Chemical Exposure Science

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TWO MAJOR QUESTIONS

- What does science say about human exposure to chemicals in the environment?
- How are chemical toxicity standards derived?

My charge —

1. Risk assessment in your practice
2. The science behind the curtain–toxicology
   - –Concepts
     - –Toxicity
     - –Adversity
     - –Dose-response
     - –Absorption, distribution, metabolism, excretion (ADME)
   - –Experimental methods
   - –Epidemiologic methods
2. Risk assessment re-visited
3. Hot topics
   - –New approaches to toxicity testing
   - –Clustering of cases
   - –Mixed exposures

New Scientist, Bijal Trivedi

Toxic Cocktail - 1 September 2007

Today, and every day, you can expect to be exposed to some 75,000 artificial chemicals. All day long you will be breathing them in, absorbing them through your skin and swallowing them in your food. Throughout the night they will seep out of carpets, pillows and curtains, and drift into your lungs. Living in this chemical soup is an inescapable side effect of 21st-century living……..

Table 7.1 Major Legislation Dealing with Regulation of Toxic Substances

Legislation	Types of Regulations
Clean Air Act	air pollution standards
Comprehensive Environmental Response, Compensation and Liability Act	remediation of hazardous waste sites
Dangerous Cargo Act	regulation of water shipment of hazardous materials
Federal Coal Mining Safety & Health Act	mine health and safety standards
Federal Food, Drug & Cosmetic Act	regulation of drugs, food additives, and cosmetics
Federal Hazardous Substances Act	regulation of hazardous household products
Federal Insecticide, Fungicide & Rodenticide Act	pesticide regulations
Federal Water Pollution Control Act	effluent and water quality standards
Hazardous Materials Transport Act	regulation of transport of hazardous materials
Lead-based Poisoning Prevention Act	regulations for lead control
Occupation Safety and Health Act	occupational health and safety standards
Resource Conservation & Recovery Act	hazardous waste management regulations
Safe Drinking Water Act	drinking water standards
Toxic Substance Control Act	hazardous substance regulations

Risk Assessment Paradigm — The "Red Book" Approach (1983)

- Hazard identification – animal studies
- Dose-response assessment – animal studies
- Exposure assessment – field studies
- Risk characterization – hazard x exposure, extrapolations across large dose range and from animals to humans, use of safety factors
- Risk Management – exposure standard depends on context, risk-benefit analysis

Risk Assessment for Non-Cancer Endpoints

- A threshold is assumed
- Determine No Observed Adverse Effect Level (NOAEL)–This is a single number, often chosen from the best animal study available (usually mg/kg/d, hopefully by a relevant route of exposure)
- Or use Benchmark Dose (BMD) modeling to identify the BMDL–the 95% lower confidence limit of the BMD
- Add a 10-fold safety factor for species extrapolation
- Add a 10-fold safety factor for vulnerable populations (children, the elderly)
- Calculate the acceptable safe exposure level and compare to measured levels of exposure

Major Toxicology Concept #1: Toxicity

What is the difference between a toxin and a toxicant?

Paracelsus: The "Father" of Toxicology

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."

Philippus Aureolus Theophrastus Bombastus von Hohenheim: 1493-1541

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How do we measure toxicity ?

- The classical measure of acute toxicity is the LD50
  - LD50: Lethal dose that kills 50% of the study population.
  - Dosage is measured in weight of toxicant per body weight of subject, often as mg/kg body weight.
- Drug action is measured by the ED50
  - ED50: Effective dose that produces the desired effect in 50% of the population.

Acute versus Subacute/Chronic Toxicity

- **Acute Toxicity arises from a single exposure to a toxicant.**
  - Poisoning
  - Usually life threatening effect
  - Difficult to diagnose
  - Treatments or antidotes may be available
- **Subacute/Chronic Toxicity results from prolonged exposure to a toxicant.**
  - No immediate effect but could be life-threatening in the long term
  - Organ system effects and cancer are endpoints
  - Usually difficult to diagnose and treat
  - Difficult to distinguish toxicant-specific effects from other influences

LD_{50} of Selected Chemicals

Chemical	LD_{50} (mg/kg)
Sugar	29,700
Polybrominated biphenyls (PBBs)	21,500
Alcohol	14,000
Methoxychlor	5,000
Vinegar	3,310
Salt	3,000
Malathion	1,200
Aspirin	1,000
Lindane (benzene hexachloride delta isomer)	1,000
2,4-D	375
Ammonia	350
DDT	100
Heptachlor	90
Arsenic	48
Dieldrin	40
Strychnine	2
Nicotine	1
Dioxin (TCDD)	0.001
Botulinus toxin	0.00001

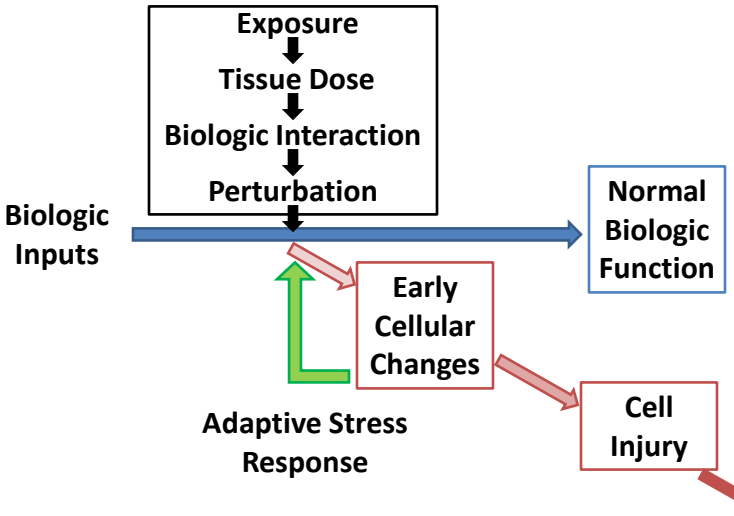
Note — LD_{50} is the dose that kills 50% of those who are exposed

Table 6.5 Characteristics of Some Assays Used in Human Risk Assessment

	Usual Subjects	Endpoint	Dose Criterion	Extrapolation Method Used
Acute Assay	Rodents	Death	50% lethality	Not generally done
Subacute Assay	Rodents	Multiple	Safe level	Safety factor
Chronic-Carcinogenesis Bioassay	Rodents	Cancer	Acceptable risk level	Mathematical modeling
Chronic-Epidemiology	Humans	Multiple	Variable	Not applicable

Important endpoints used in risk assessment:
NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level

Major Toxicology Concept #2: Adversity



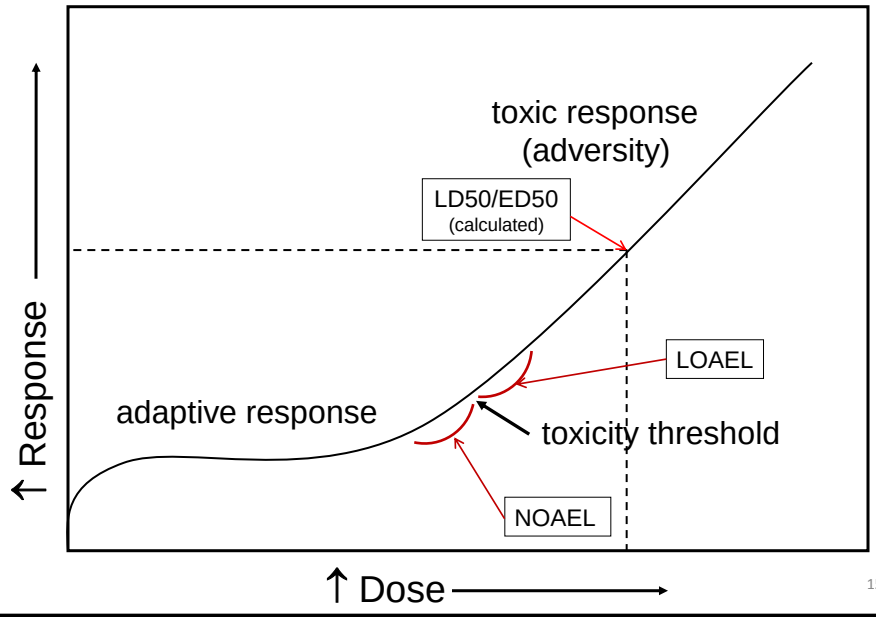
Adapted from: NRC. Toxicity Testing in the 21st Century: A Vision and a Strategy. NRC Press: 2007. Washington, DC

Adversity has been defined by what we know at the phenotypic level

Definition of an adverse effect (Lewis, 2002):

"A biochemical, morphological, or physiological change….that….adversely affects the performance of the whole organism or reduces the organism's ability to respond to an additional environmental challenge."

Major Toxicology Concept #3: Dose-Response



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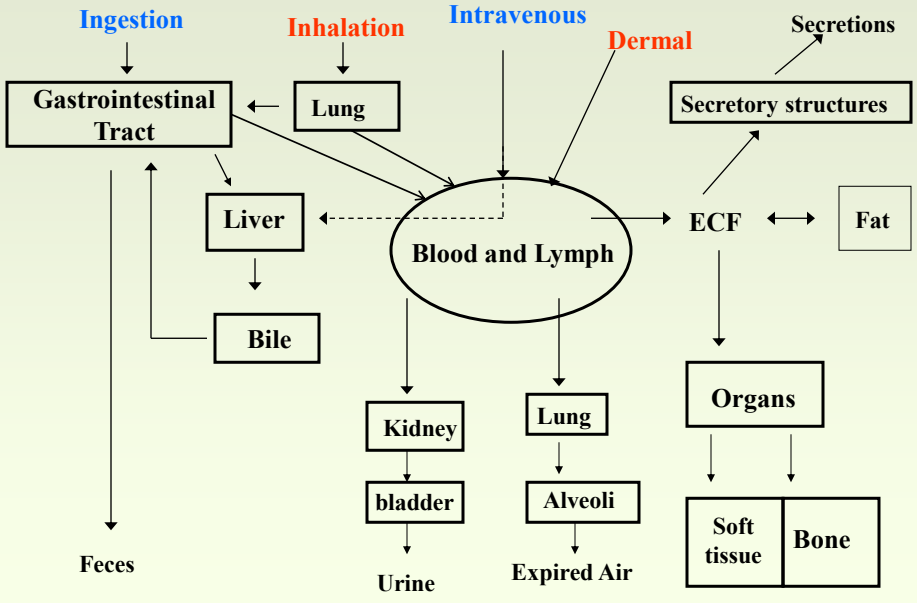
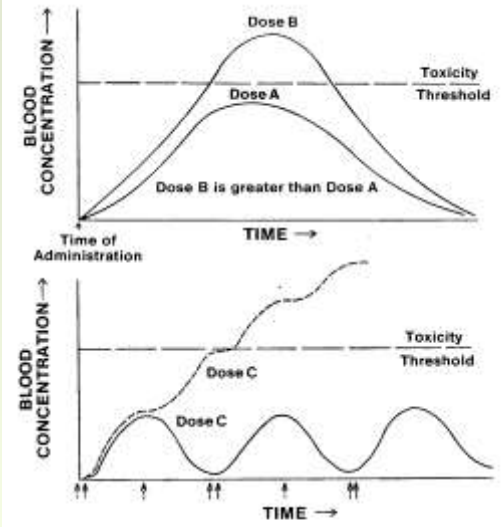
Major Toxicology Concept #4: Absorption, Distribution, Metabolism, Excretion (ADME)

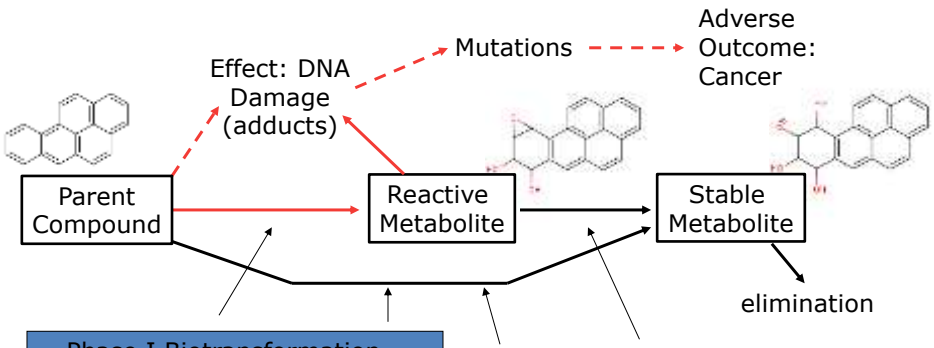




Times of administration indicated by ↑ for solid curve and ↓ for dashed curve. The same dose is administered at each time.

Figure 4.4 Relationship between time course of exposure and toxic expression.

Metabolism: How a Xenobiotic Is Detoxified or Activated



Phase I Biotransformation

- Oxidation/reduction reactions make the compound more polar
- Sometimes produce a more biologically active compound

Phase II Biotransformation

- Conjugation: Add on a more polar (water soluble) structure
- Increase urinary elimination

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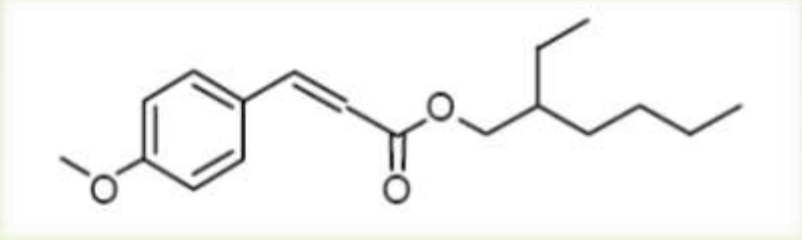
How do we know if an exposure is related to a toxic outcome?

- **Experimental Methods:** Highly controlled experiments, usually in a laboratory setting, typically using animals.
- **Epidemiological Methods:** Observations on uncontrolled populations, usually in the natural environment.

An Example — Toxicity Testing in Practice



Ethylhexyl methoxycinnamate (EHMC)

A very common UV filter in sunscreen

Reviewed by the National Toxicology Program (NTP) as a "proposed research project."

The Concern

- **Widespread use**
- **Lifelong exposure**
- **Potential for endocrine disruption**
- **Potential for increased absorption in children**
- **Lack of information on the effects of *in utero* exposure**

The Limited Information Generates Questions

- Industry says it has a study that clears EHMC of concerns as an endocrine disruptor, but the data are not public
- Reasonably strong evidence that absorption through the skin is most often very limited (~1%)
- Sunlight causes a large amount of EHMC isomerization
- Metabolism generates 2-ethylhexanol and 2-ethylhexanoic acid, known developmental toxicants
- Nanoparticles now widely used in sunscreens have unknown effects on transdermal transport
- Young age and some common skin conditions (eczema) may enhance transdermal absorption

The NTP Testing Proposal

- Evaluate toxicokinetics and absorption, distribution, metabolism, and excretion (ADME), comparing dermal and oral routes of exposure
- Conduct a large **ORAL** multigenerational study

The proposed high dose is the maximally tolerated dose (MTD), and the low dose is many orders of magnitude above anticipated exposure levels

With our current approach, this is what we do, but does it make sense?

Proposed Animal Tests

- Adult rats or mice
- 3 doses plus a control
  - –High dose = maximally tolerated dose
  - –Medium dose: want a dose that produces adverse effects without systemic toxicity (LOAEL dose)
    - Often ¼ - ½ of high dose
  - –Low dose: want a dose that is not adverse (NOAEL dose)
    - Often ¼ - ½ of medium dose
- Usually a 28 day, 90 day, or 2 year study
- Goal is to identify one number: the NOAEL
- The NOAEL changes with more experiments

Epidemiological methods: Dr. Needleman, a public health hero

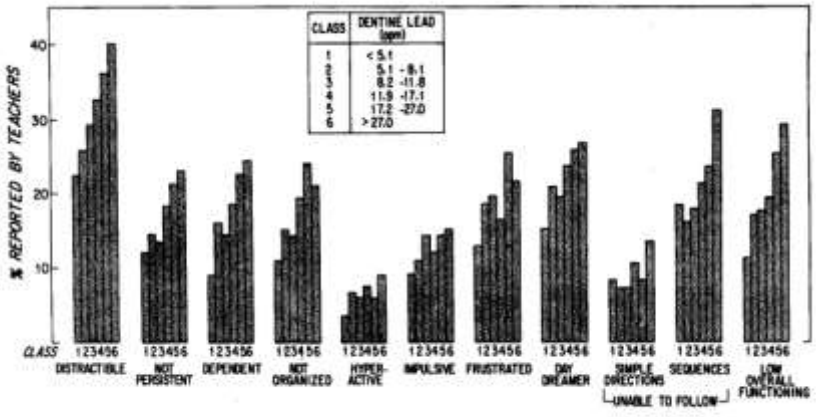
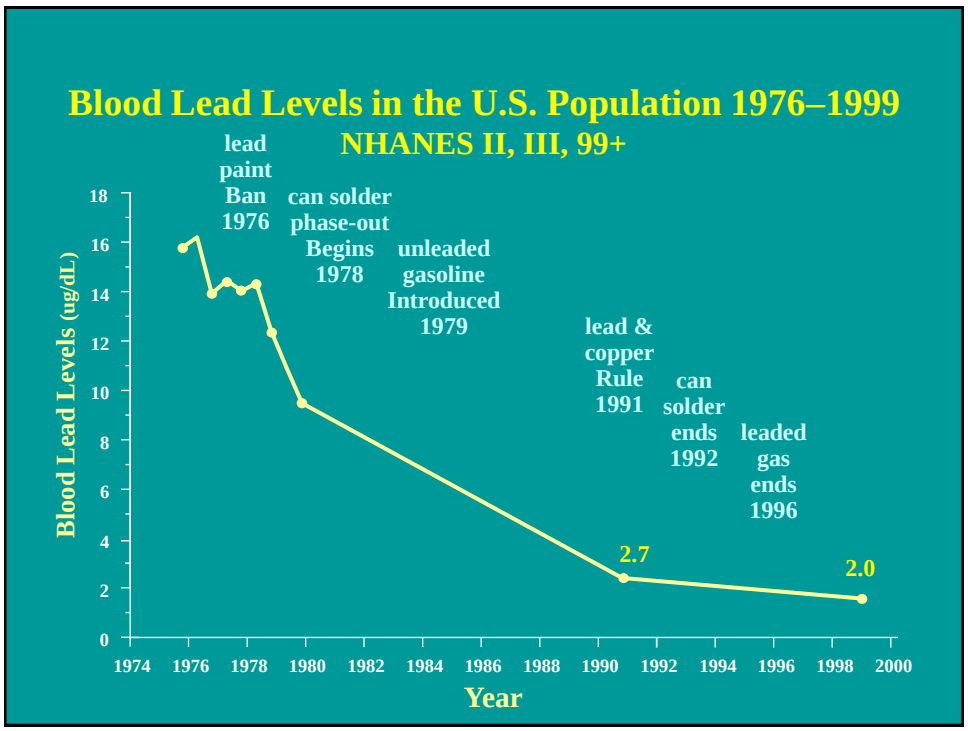
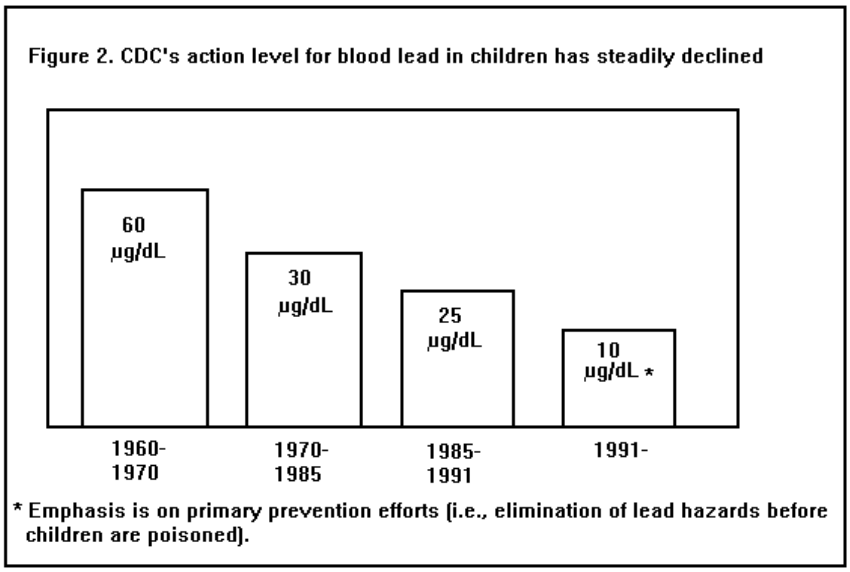


FIGURE 1. Teachers' ratings of classroom behavior in relation to dentine lead level. Teachers were blind to lead levels and had known students for at least 2 months. $n = 2146$. From Needleman et al. (1).





Risk assessment Re-visited

All substances at some level of exposure are toxic; therefore, the determination of the level of exposure to a substance of concern is critical to the understanding of toxicity.

The classical approach to toxicity testing uses the maximally tolerated dose (MTD) as the highest level of exposure. The MTD is expected to produce noticeable changes in the animal, such as weight loss, meaning that classical toxicity testing is anchored by a dose selection process that requires that adverse effects be produced.

People are most often exposed to doses as little as one-trillionth as high as the MTD. The difficulties in extrapolating from classical animal studies of toxicity testing to human risk are a real problem.

Risk Assessment for Cancer Endpoints

- Determine the cancer incidence in a 2-year rat or mouse carcinogenicity study
  - –This is often an incidence of cancer in a given organ, often at the MTD alone, or MTD plus middle dose
  - –Usually mg/kg/d, hopefully by a relevant route of exposure
- Add a 10-fold safety factor for species extrapolation
- Add a 10-fold safety factor for vulnerable populations (children, the elderly)
- Do modeling to extrapolate with the assumption that there is no threshold for effect
  - –You likely only have 2 or 3 relevant doses: 0, MTD, middle dose
- Calculate the safe exposure level based on a lifetime exposure that produces a defined incidence of cancer considered acceptable, such as 1 cancer in 10^5 or 10^6 exposed

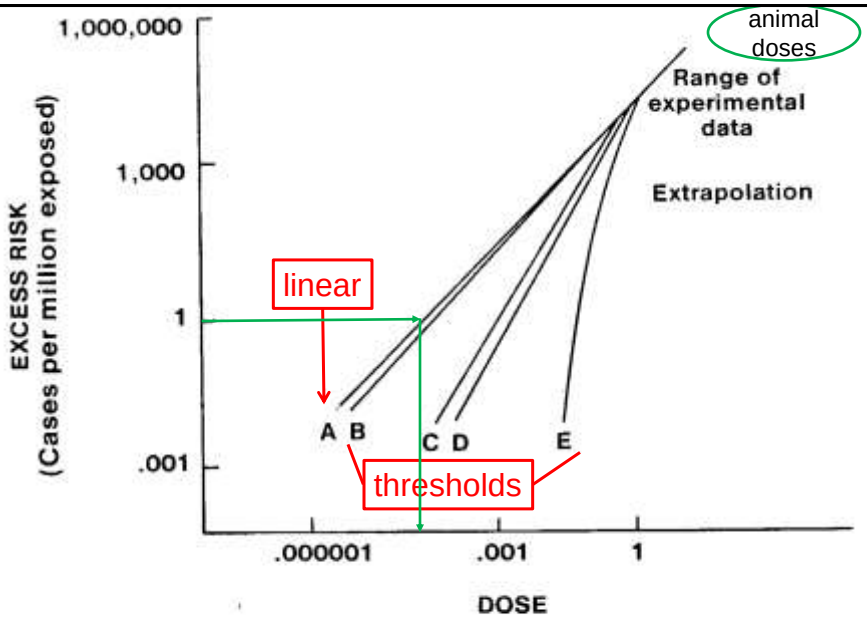


Figure 6.1 Low-dose extrapolation for a carcinogen under several mathematical models: A, B, C, D, and E.

Hot topics

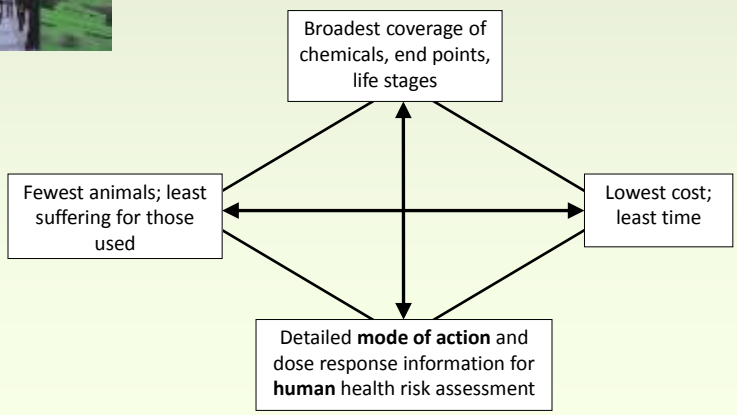
New approaches to toxicity testing

Clustering of cases

Mixed exposures

New Approaches to Toxicity Testing of Environmental Chemicals

Design Criteria

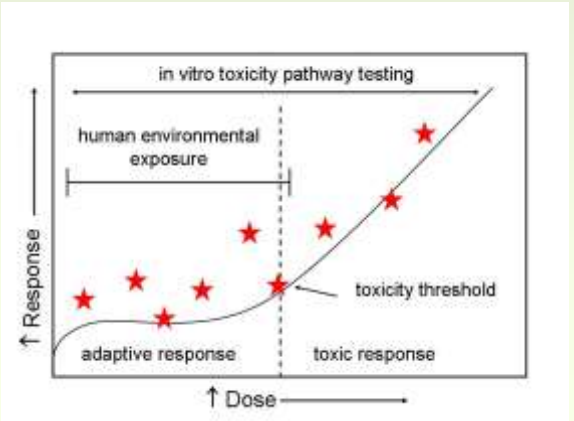


Options for Future Toxicity Testing Strategies Table 2-1

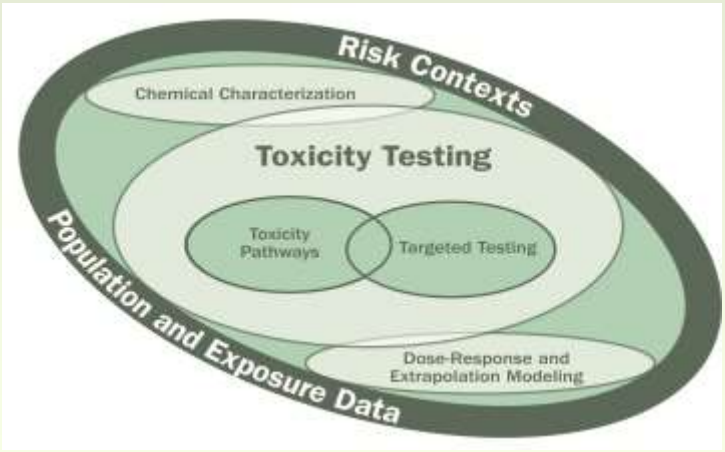
Option I In Vivo	Option II Tiered In Vivo	Option III In Vitro/In Vivo	Option IV In vitro
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

Vision of a future of toxicity testing based on a very different paradigm……

- Multiple doses in vitro
- Defined number of toxicity pathways
- High throughput
- Expensive to develop, cheap to do
- Fast
- Mechanistic endpoints
- In vitro-to-in vivo extrapolations of dose response
- Based on human biology



Components of the Vision

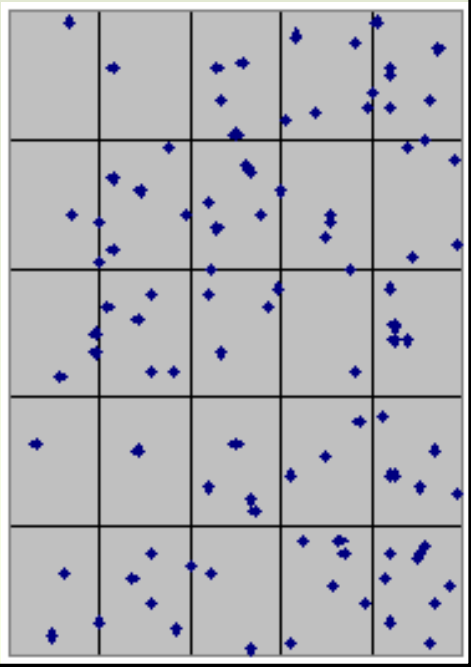


Adversity re-defined — Questions

What about dose?
What about time?
Is adversity defined by the testing system itself, or by extrapolation to a higher level of organization?
Is a change of state sufficient, or does the change need to be irreversible?

Clusters

In this diagram, 100 dots are randomly distributed on the grid. By chance, some of the boxes have only one dot, while some have many more. Clustering does occur by chance. Statistical approaches are used to determine the likelihood that a cluster is a real or chance occurrence.



Clusters are Difficult to Investigate

- Clusters of any disease may occur by chance. Investigators may be searching for a cause that doesn't exist.
- The time between exposure to a harmful substance and the development of disease can be decades.
- People move in and out of counties and states throughout their lives, making it even more difficult to measure a person's level of exposure.
- Diseases are often caused by a combination of factors not yet fully understood.

Mixtures and ‘‘toxicologic similarity’’

- Toxicologic similarity is required before the modeling (for risk assessment purposes) of mixed exposures affecting the same target can be performed from data generated from testing single chemicals alone.
- Toxicologic similarity means sharing the same mode of action, defined as ‘‘a series of key events and processes starting with the interaction of an agent with a cell and proceeding through operational and anatomical changes causing disease formation’’ which, in some cases, ‘‘may be relaxed to require that these chemicals act only on the same target organ’’ (EPA, 2000).
- Therefore, risk assessment of mixed exposures has defaulted to the testing of the mixtures themselves, which is very limited as an approach.

Summary

- We are constantly exposed to a mixture of potential toxins and toxicants.
- Toxicity is defined as an adverse outcome of exposure, and is dependent on:
  - **Dose and duration** of exposure
  - Absorption, distribution, metabolism, and elimination(**ADME**)
  - **Mechanism** at the site of action
- Toxic effects are assessed through experimental exposure studies and epidemiological data.
- **Risk = hazard x exposure**: the current challenge is to improve characterization of risk at low doses, in mixtures, for lots of compounds.