

Toxicology of Pesticides Used on Cannabis

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AGRICULTURE YOUTH & FAMILIES HEALTH ECONOMY ENVIRONMENT ENERGY COMMUNITIES



1



“A leading toxicologist, who reviewed The Oregonian/OregonLive’s analyses, said the high concentration of one chemical in particular suggested the cannabis plants used to make Dutch Treat had been “soaked” in that pesticide before harvest.”

A tainted high

LAX STATE RULES, INCONSISTENT LAB PRACTICES AND
INACCURATE TEST RESULTS PUT PESTICIDE-LACED POT ON
DISPENSARY SHELVES

Reported and written by NOELLE CROMBIE | ncrombie@oregonian.com

Photography by BETH NAKAMURA | bnakamura@oregonian.com

June 11, 2019

2

Pesticide Residues Have Been Analyzed After Artificial Smoking

Hindawi Publishing Corporation
Journal of Toxicology
Volume 2013, Article ID 378168, 6 pages
<http://dx.doi.org/10.1155/2013/378168>



Research Article

Determination of Pesticide Residues in Cannabis Smoke

Nicholas Sullivan, Sytze Elzinga, and Jeffrey C. Raber

The Werc Shop, Inc., Pasadena, CA 91107, USA

Correspondence should be addressed to Jeffrey C. Raber; jeff@thewercshop.com

Received 11 February 2013; Accepted 22 April 2013

“The lack of quality control results in patients potentially being exposed to cannabis contaminated with toxic levels of pesticides.”

3

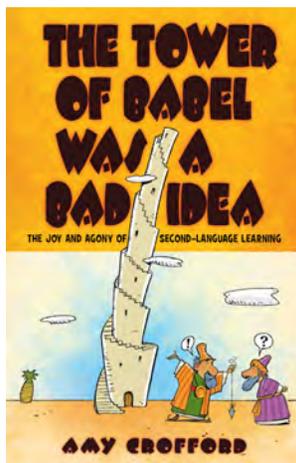
Objectives

- Super short course on toxicology
- Separating hazard and risk
- Understanding exposure
- If you wanted to get a pesticide registered on pot (‘good luck’), how does EPA play the risk game?

4

Speaking the Same Language--Tox Terms

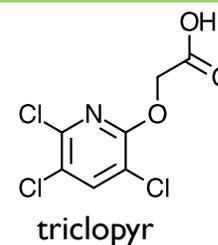
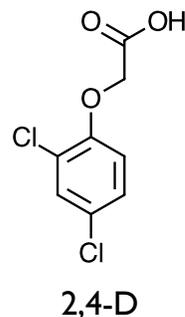
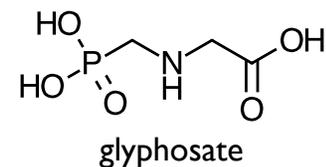
- **Toxicity:** innate potential of a substance to cause injury (attribute of specific 3-D chemical structure & appropriate receptor in organism)
- **Hazard:** potential to cause injury under specific set of circumstances
- **Risk:** probability (likelihood) of harm; function of the magnitude of exposure (or contamination) integrated with hazard
- **Safety:** subjective term and therefore not definitive, but in the context of risk management it refers to the practical certainty that injury will not result from use of a substance under specified conditions of quantity and manner of use.



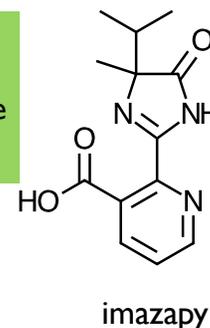
Do Not Confuse Toxicity, Hazard & Risk

5

Rule of Thumb: What Pesticide Are You Talking About?



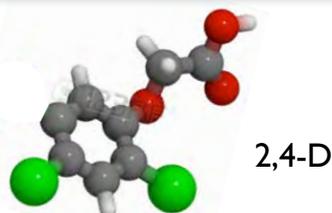
- Each individual type of pesticide has unique physicochemical & biochemical properties by virtue of its unique 3D structure



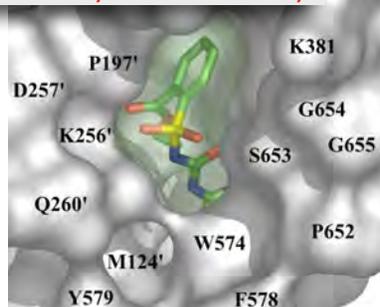
WASHINGTON STATE UNIVERSITY
EXTENSION

6

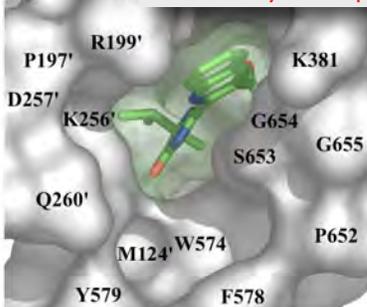
See the World of Chemistry in 3D



ALS Enzyme Active Site
Blocked by Chlorimuron-Ethyl



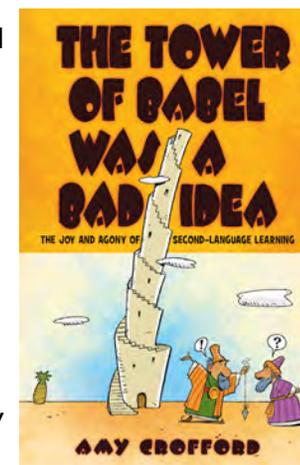
B) ALS Enzyme Active Site
Blocked by Imazaquin



7

Speaking the Same Language--Tox Terms

- **Toxicity:** innate potential of a substance to cause injury (attribute of specific 3-D chemical structure & appropriate receptor in organism)
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Do Not Confuse Toxicity, Hazard & Risk

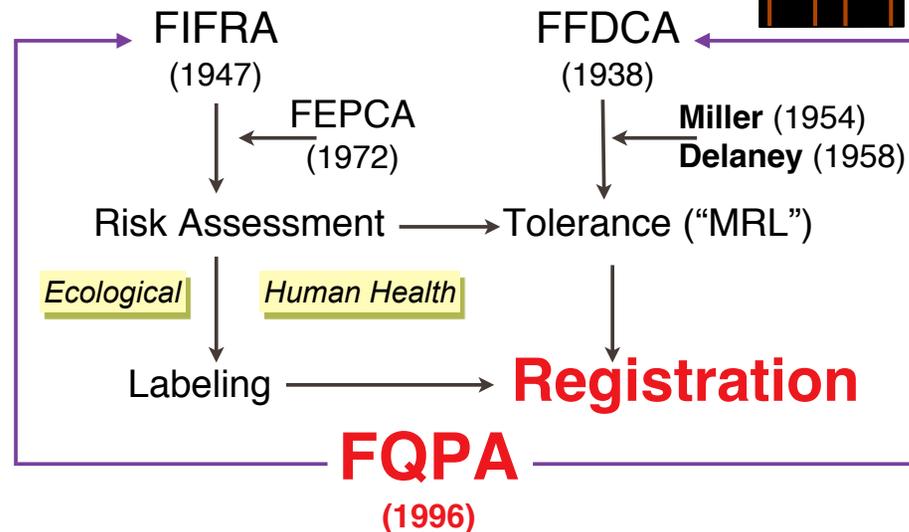
8

Separate But Not Equal

- **Risk assessment:** scientific endeavor for determining the hazards of a substance, potential exposures, and the likelihood of adverse effects
 - ✓ Mandated by statutory and administrative (regulatory) law
 - ✓ Mostly science based activity: hypothesis, prediction, experiment, observation
- **Risk management:** social endeavor for avoiding adverse effects
 - ✓ Mandated by statutory law
 - ✓ Implementation defined by administrative law
 - ✓ Influenced by politics, economics, social goals

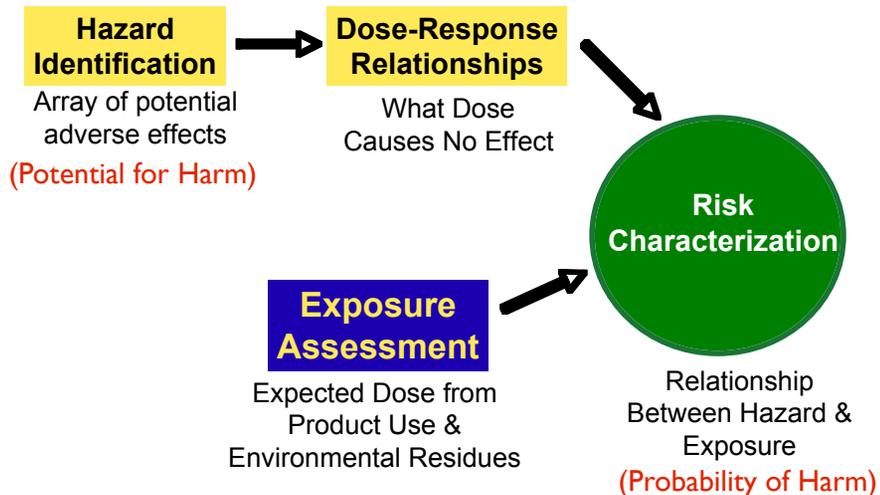
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U.S. Pesticide Law 101



10

Risk Assessment Estimating the Probability of Harm



11

Other Concepts of Toxicity

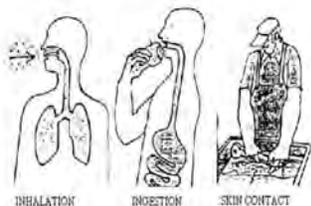
- “The accumulation of injury over short or long periods of times that renders an organism incapable of functioning within the limits of adaptation or other forms of recovery.” (Rozman et al. 2001)
- Or as Paracelsus over 500 years ago stated,
 - ✓ All things are poison, and nothing is without poison; only the dose permits something not to be poisonous
 - ✓ In other words “Dose Makes the Poison”
 - * Substances considered toxic are harmless in small doses, and conversely an ordinarily harmless substance can be deadly if over-consumed



12

Toxicity: Critical Variables

- Two variables are most important in determining the likelihood that exposure to a toxicant will result in an adverse response:
 - ✓ Amount of exposure (dose)
 - * Should be distinguished from dosage, the amount relative to body weight, and absorbed dose (the amount actually in the body)
 - ✓ Frequency and duration of exposure (time)
- Note that the influence of these two variables are often exposure route specific



13

Toxicological Focus: Individual vs Population

- If we're concerned about pesticides affecting human health, we are focused on individual responses
 - ✓ i.e., we try to protect the individual from any type of toxic response
- If we're concerned about other nontarget organisms, we are focused on ensuring their reproductive potential is unaffected
 - ✓ i.e., we accept the possibility of some loss of individuals but want to ensure population in any locale remains stable
- If we're concerned about pest control, we are focused on populations of pests
 - ✓ i.e., we're focused on lowering the population and the only toxic response we're interested in is death



14

Toxicological Endpoints

- Must have endpoints if we are to measure toxicity at either the individual or population level
 - ✓ An endpoint is the direct or indirect biochemical, cellular, physiological, or behavioral response following an exposure to a toxicant
- Death (often equated with acute toxicity, although severe injury rather than death may result)
 - ✓ In the context of non-occupational exposures to pesticides, this endpoint is only relevant to the pests we're trying to control
 - * However, mortality will still be measured to understand potency among chemicals and also to determine whether non-target organisms are likely to be harmed
 - ✓ Lethality can be expressed quantitatively as the median response in a population, i.e.,...
 - * LD₅₀: dose lethal to 50% of the test population
 - * LC₅₀: concentration lethal to 50% of the test population



15

Toxicological Endpoints

- Sublethal Effects
 - ✓ Biochemical
 - ✓ Genetic
 - ✓ Cellular
 - ✓ Physiological
 - ✓ Morphological
 - ✓ Functional
 - ✓ Behavioral

Although these endpoint can be caused by a single dose exposure, often these endpoints are studied in association with repeated exposures

Sublethal effects following repeated "small dose" exposures is often called chronic toxicity, which includes cancer, developmental effects, neurological deficits

16

Another Perspective of Toxicity: Integration with Exposure Time

- Acute vs Chronic Toxicity
 - ✓ Acute toxicity refers to signs & symptoms appearing immediately after a single exposure to a substance
 - * Most common endpoint is death
 - ✓ Chronic toxicity refers to adverse effects occurring from multiple exposures
 - * Common endpoints include blood, organ, developmental, reproductive, and carcinogenic effects
 - For compounds that work as neurotoxins, behavioral effects are examined
 - * Note that risk assessment decisions are based on studies of chronic exposure

17

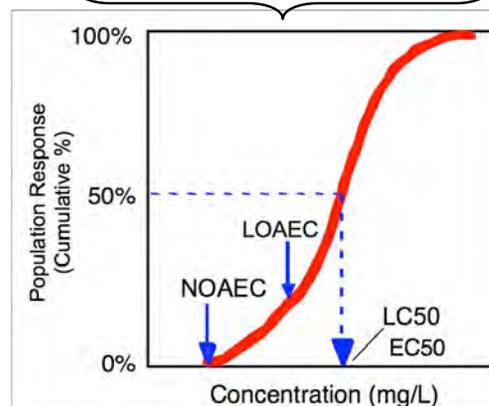
Risk Assessment: Estimating the Probability of harm

Hazard Identification

Array of potential adverse effects

Dose-Response Relationships

What Dose Causes No Effect



Risk Characterization

Relationship Between Hazard & Exposure

18

Hazard Testing

- Potential to do harm; manifestation is conditional
- Testing to characterize hazard

- ✓ In vitro tests



- ** ✓ In vivo tests



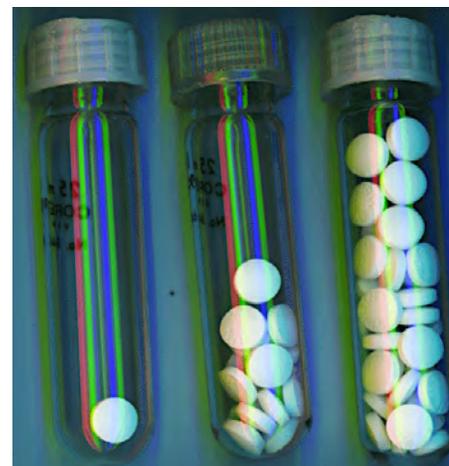
- ✓ Epidemiological studies



19

What Is Dose?

- Dose is the amount of substance per unit of body weight
 - ✓ Expressed as mg/kg/day



5 mg/kg/day

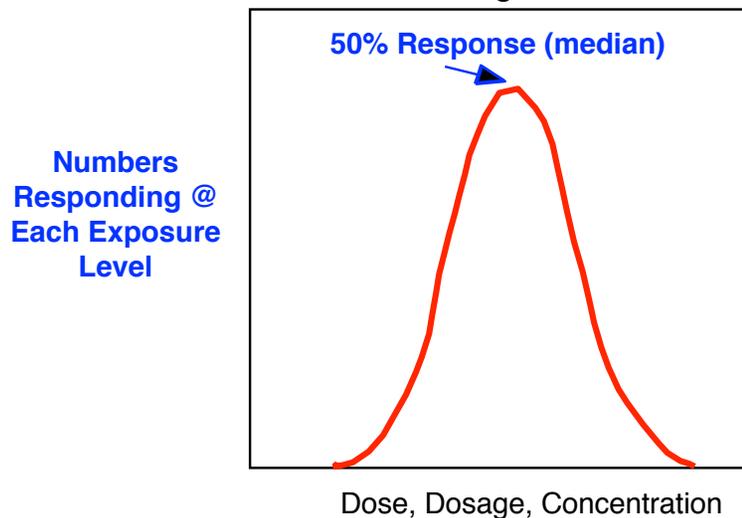
75 mg/kg/day

150 mg/kg/day

20

Basis for Quantitatively Expressing Toxicity within a Population A Quantal Response

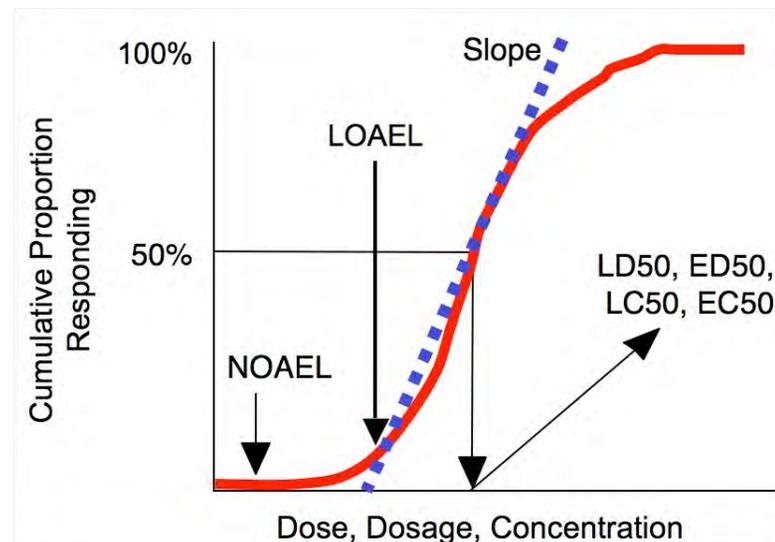
Distribution of Individual Responses to
Increasing Doses



21

Basis for Quantitatively Expressing Toxicity

Cumulative Proportion Responding



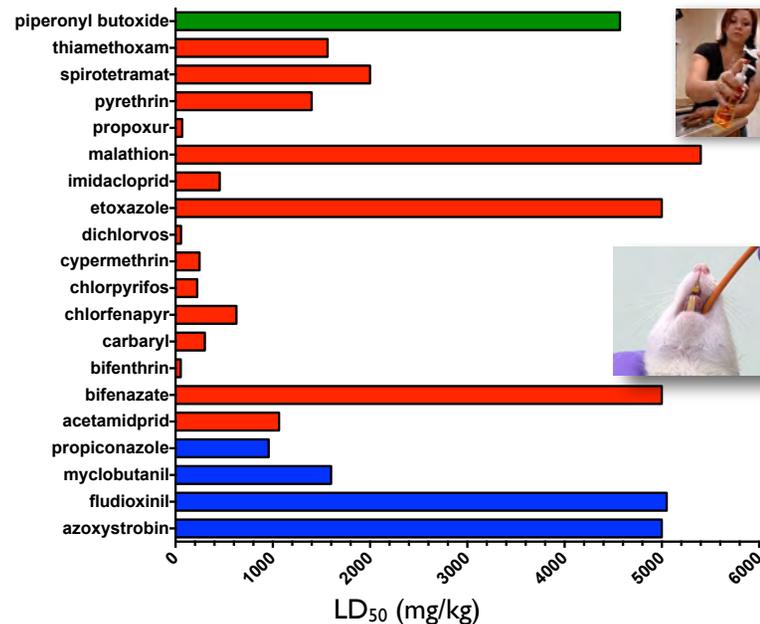
22

Expressing Toxicity

- LD/LC50 = dose or concentration causing 50% mortality (or other effect) in a test population
 - ✓ A measure of acute toxicity; occurs after single or short term exposure (acute exposure)
 - ✓ Often the measured effect is death
- NOAEL/NOEC = level (dose) or concentration not causing an adverse reaction
 - ✓ Usually refers to chronic exposures
 - ✓ Typically 90 days to 2 years for mammalian (rodent) testing
 - ✓ Effects often called chronic effects
 - * Examples: cancer; fertility impairment; birth defects

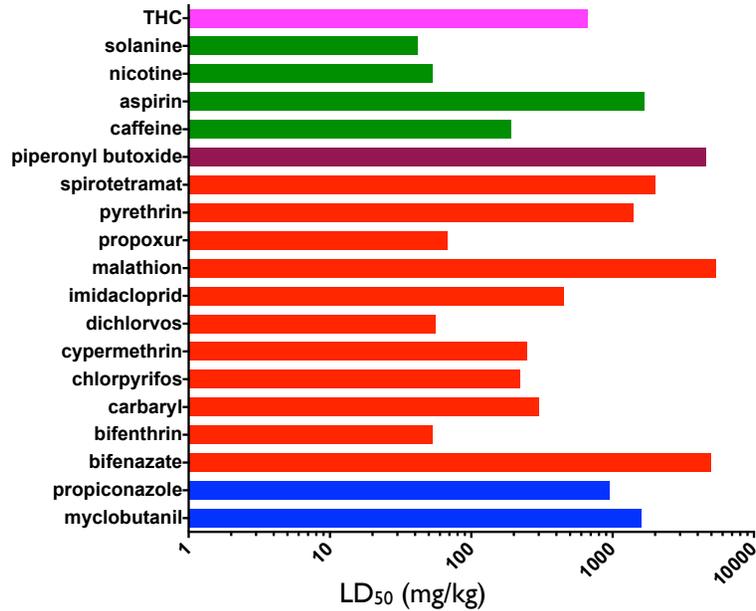
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Acute Oral Toxicity (LD₅₀) of Pesticides Reported on Cannabis Samples



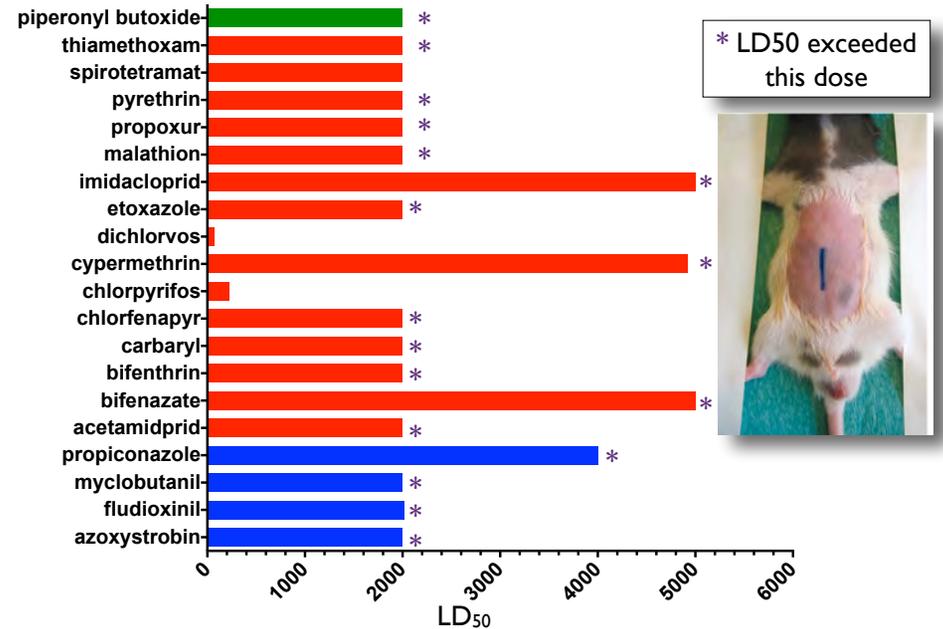
24

All Chemicals Have Biological Activities at Some Dose



25

Acute Dermal Toxicity (LD50) of Pesticides Reported on Cannabis Samples



26

So, Why Are there Differences Among Chemicals?

Why Are there Differences Among Different Species?

Bottom Line: Why Are Chemicals Selective & Without Effect at One Dose But Toxic at Another?

27

The Secrets of Pesticide Selectivity

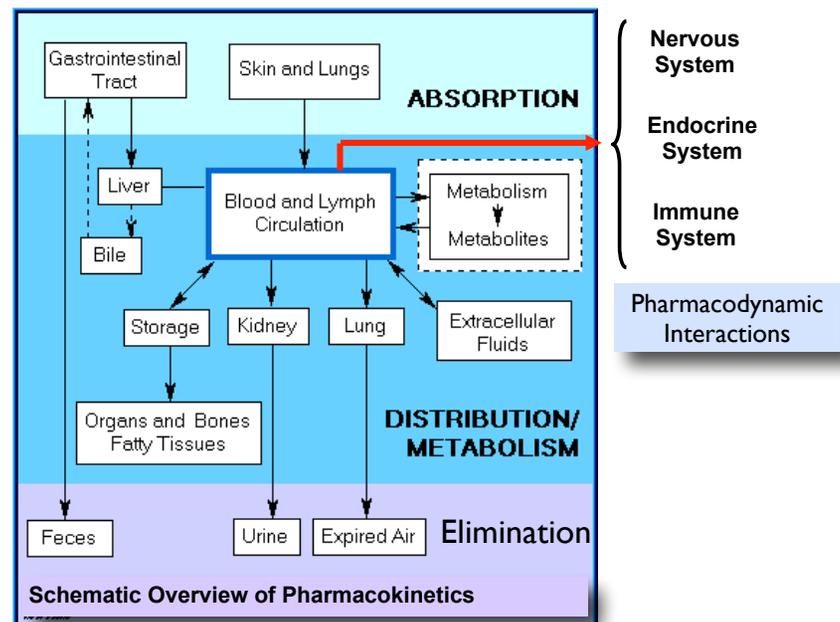
- Selectivity refers to the pesticidal activity of a chemical against pests with minimal biological activity against non-target organisms
 - ✓ For example, herbicides are toxic to plants at legal rates of application but lack effects on animals
 - ✓ For example, 2,4-D products are toxic to most broadleaf plants but lack effects on grasses when applied according to label instructions
- Selectivity is what allows you to take therapeutic doses of pharmaceutical drugs that are known poisons at higher doses
- Selectivity is what allows us to eat many plant products, even though those plants produce biochemical toxins against herbivorous pests

28

Selectivity Is Due to Two Major Physiological Phenomena

- Pharmacokinetic (toxicokinetics)
 - ✓ Processes controlling absorption, distribution, metabolism, and excretion (ADME) of pharmaceutical drugs and toxicants by an organism
 - ✓ The differential toxicity of a compound between a pest organism and a nontarget organism may be due to...
 - * Differences in penetration through integument
 - * Conferred by extent (reactivity) and/or rate of metabolism (toxicokinetics)
 - ➔ The nontarget organism very quickly detoxifies the chemical (but in the pest it is more persistent)

29



30

Selectivity Is Due to Two Major Physiological Phenomena

- Pharmacodynamics (toxicodynamics)
 - ✓ Interactions of pharmaceutical drugs and toxicants with specific biochemical receptors or enzymes in an organism
 - ✓ Selectivity occurs when...
 - * Nontarget organisms lack the appropriate biochemical receptor or enzyme target
 - * The pesticide poorly binds (or interacts) with the biochemical target

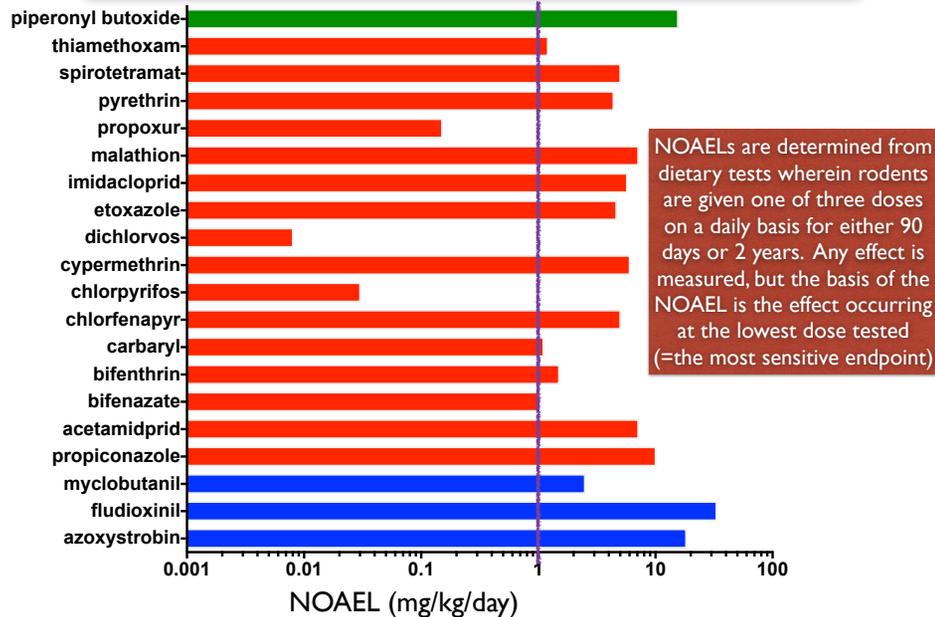
31

Pharmacodynamics of Pesticides Detected on Cannabis

Active Ingredient	Pesticide Class & Mechanism of Action
myclobutanil propiconazole	Triazole fungicides; inhibit sterol biosynthesis in fungi; membranes rely on cholesterol for flexibility
pyrethrin, bifenthrin, cypermethrin	Pyrethroid Insecticides; neurotoxins— block closing of sodium channel along nerve axon membrane; abnormal firing of nerves
chlorpyrifos, malathion carbaryl, propoxur	Organophosphate & carbamate insecticides ; inhibit acetylcholinesterase on post synaptic nerve membrane thereby causing nerves to excessively fire
imidacloprid, acetamiprid, thiamethoxam	Insecticide agonist of the nicotinic acetylcholine receptor on the post synaptic nerve membrane, ultimately causing a block of its function
piperonyl butoxide	Synergist for pyrethrins insecticide; inhibits metabolism of the insecticide by blocking access to P450 enzymes

32

Most Important Data for Risk Assessment: NOAEL (No Observable Adverse Effect Level)



33

To Resolve Uncertainty, Use Safety Factors

- EPA will apply safety factors to the NOAELs to hedge their bets
 - ✓ Alleviate concerns that different age groups (kids vs adults) have widely different susceptibilities (10X factor)
 - ✓ Alleviate concerns that humans are more susceptible to chemical effects than rodents (10X factor)
- When EPA does its risk assessment, it applies an uncertainty factor (=safety factor) to the NOAEL to create a new parameter called the Reference Dose (RfD)



34

Playing the Risk Game: How EPA Might Do It

- Once EPA has established the NOAEL (No Observable Adverse Effect Level) for a pesticide, the next step is to establish an RfD (Reference Dose)
- If there are no concerns for hormonally active agents (a.k.a. endocrine disrupters), for child sensitivity greater than an adult, or for cancer, then EPA would divide the NOAEL by 100 to create a new benchmark for safety called the RfD

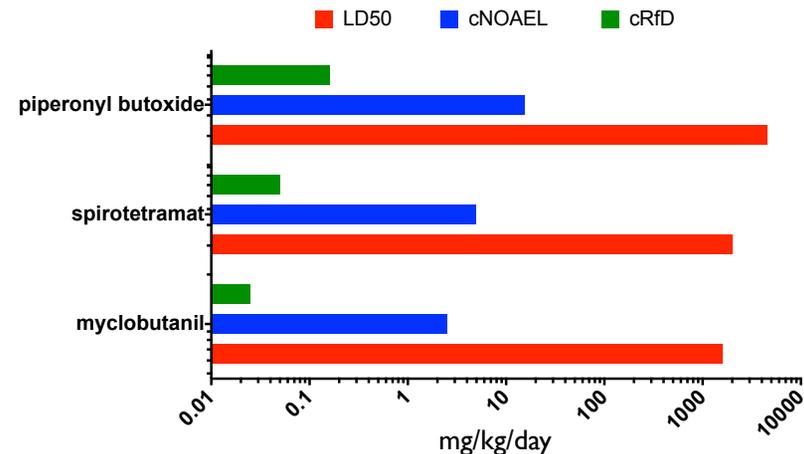
✓ $RfD = NOAEL/100$

* If any of the above concerns are true, then a new parameter called the PAD (Population Adjusted Dose) is used, adding another factor of 10 to the uncertainty factor of 100

* $PAD = RfD/10$

35

Pulling Back the Curtain: LD50 vs NOAEL vs RfD

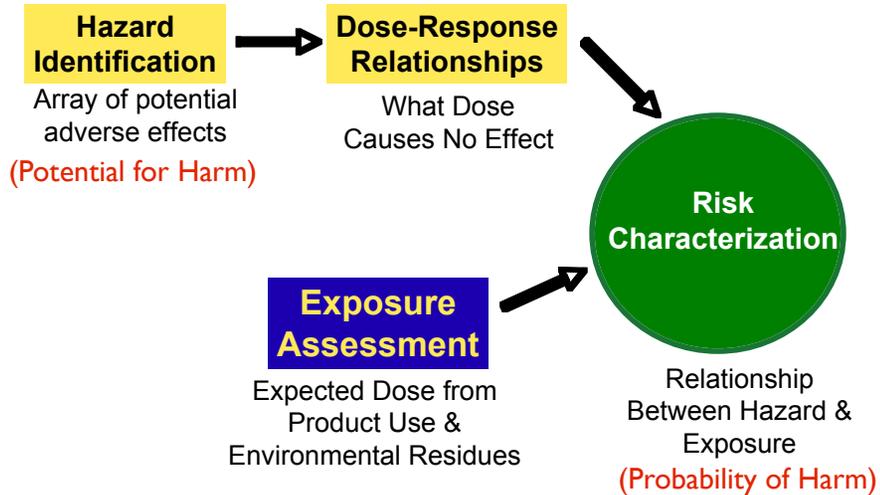


$RfD = NOAEL/100$

The Reference Dose (RfD) may be interpreted as the dose "reasonably certain" not to cause harm

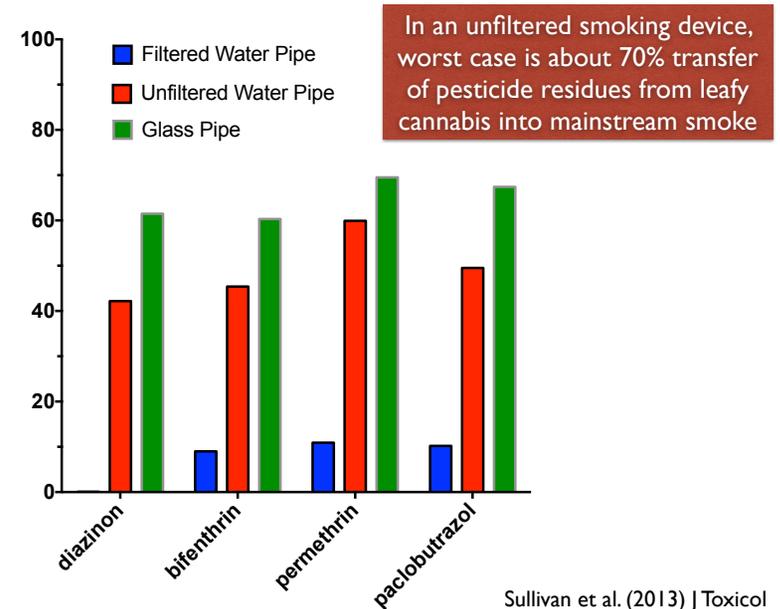
36

Risk Assessment Estimating the Probability of Harm



37

Percentage Recovery of Pesticides in Mainstream Pot Smoke



Sullivan et al. (2013) J Toxicol

38

Playing the Risk Game: Estimating Exposure How Much Matrix Is Consumed?

- Problem set up for concentrates
 - ✓ Lachenmeier & Rehm (2015) estimated a maximum intake of 60 mg THC per day
 - * If a concentrate has ~50% THCA, then there are ~0.5 g in 1 gram, so the consumption is ~0.12 grams per day
 - * I estimated about 100 hits in a one gram concentrate, and a medical marijuana user dabs (tokens) 1.33x per hour for 24 hrs straight; thus, the total intake is ~0.23 gram per day
- Problem set up for flowers
 - ✓ High Times estimated about 4 joints per day
 - * Thus, if each joint with about 1 gram, then 4 grams per day
 - ✓ Use this estimate to estimate risk from exposure to any pesticide because it is the highest estimated exposure

39

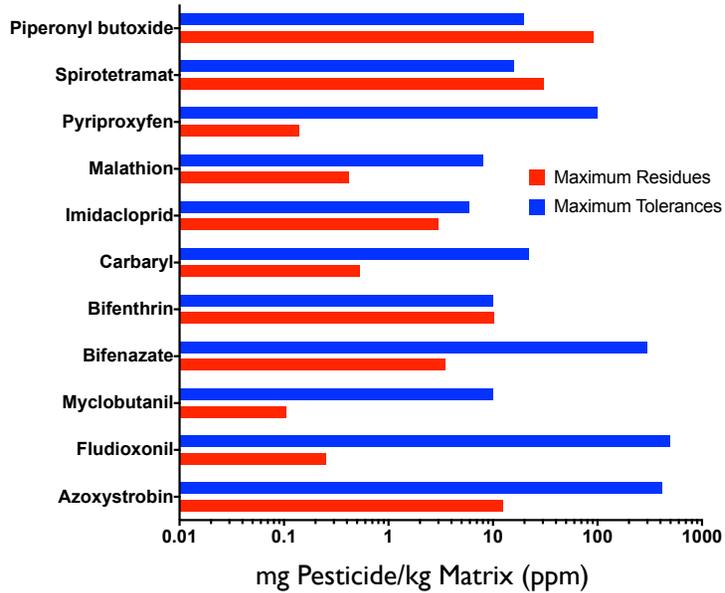
Playing the Risk Game: Estimating Exposure What Residues to Use

- For dietary consumption, EPA uses a probabilistic method and a statistical technique called Monte Carlo sampling
 - ✓ This method presumes a lot of residue data are available
- To be super conservative, one can take the highest residue found, and use this number...such an analysis is called deterministic as opposed to probabilistic
- Another possibility, is to check the EPA tolerance database, and use the highest tolerance

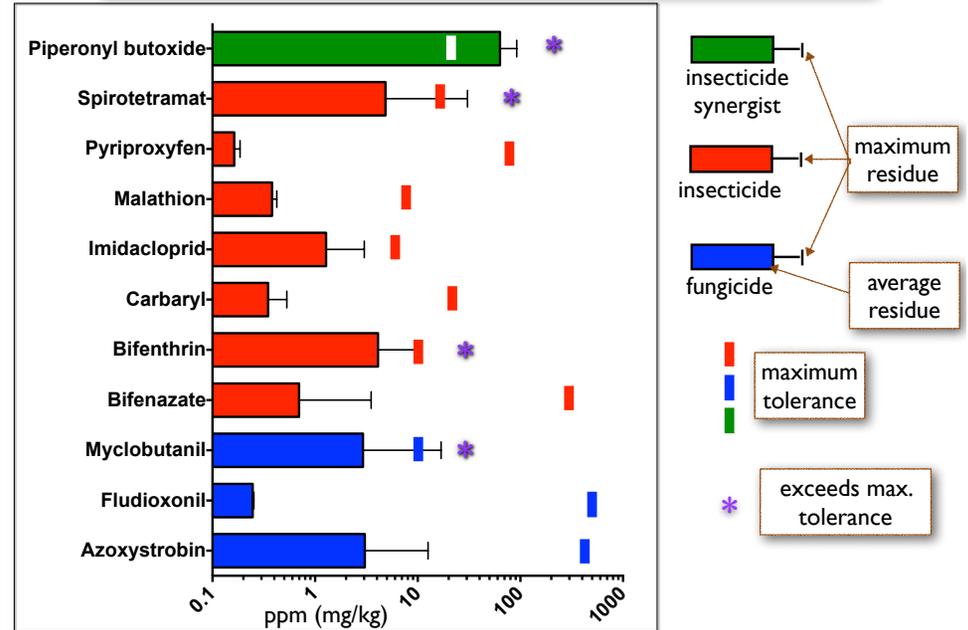
Caveat: A tolerance is a legal residue not a safety standard; i.e., it is the highest residue allowable in a commodity and still be allowed for commercial sale

40

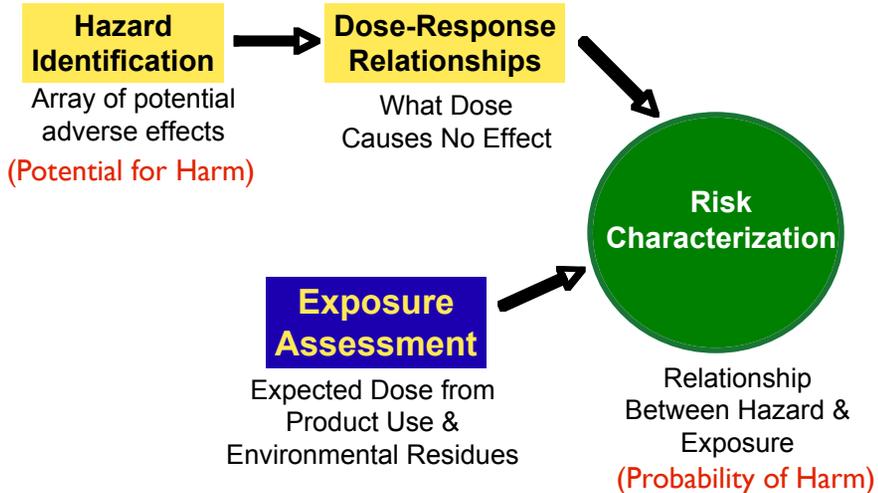
Based on Data Received from One Analytical Lab,
In Most Cases Tolerances are Higher



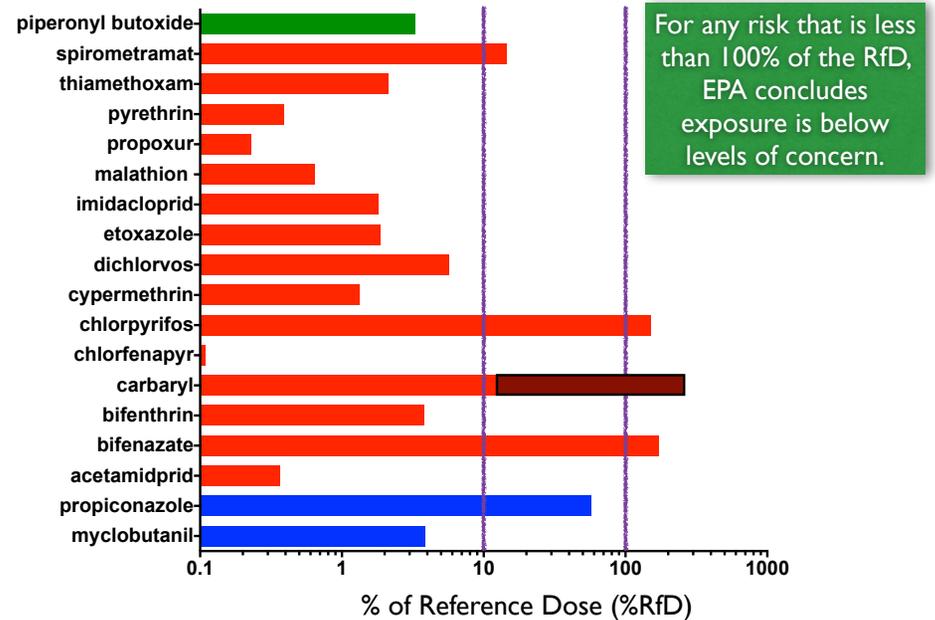
Pesticide Residues on Cannabis Matrices from
One Analytical Lab vs. Maximum Tolerances



Risk Assessment
Estimating the Probability of Harm

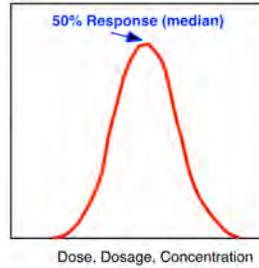


% of Reference Doses from Estimated High Consumption

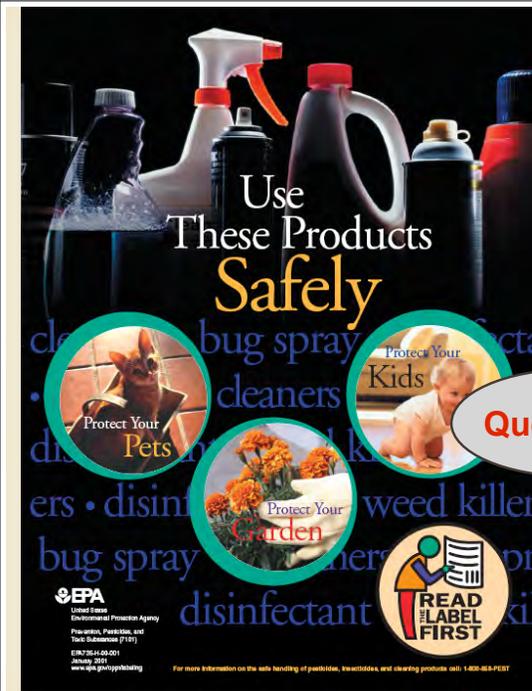


Risk Estimates Have Uncertainty

- Insufficient data on consumption
- Recall that population response is actually a distribution of responses, and we don't really know the variability of susceptibility
- Using a conservative high end exposure estimate, two of the detected pesticides are >100% of the RfD, and thus EPA would conclude, "exposure exceeds levels of concern"
- The residues tell a tale
 - ✓ Which pests
 - ✓ "Bad practices"
 - * Leads to excessive exposure of workers and environment
 - * Wasting money if using products prophylactically
 - * May be causing pest resistance, especially indoors



45



Questions?

46

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