Understanding Herbicide Risks to Fish & Wildlife

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- Herbicides are routinely used in invasive weed control and habitat restoration.
- There's a common perception that herbicide use is incompatible with protecting wildlife.
- But this perception is based largely on notions that are not science-based.

Factors associated with declining populations of the California red-legged frog …*

- habitat loss or degradation
- non-native plants
- impoundments
- water diversions
- degraded water quality
- introduced predators
- **use of pesticides**

* from USFWS profile for the CRLF April 2011

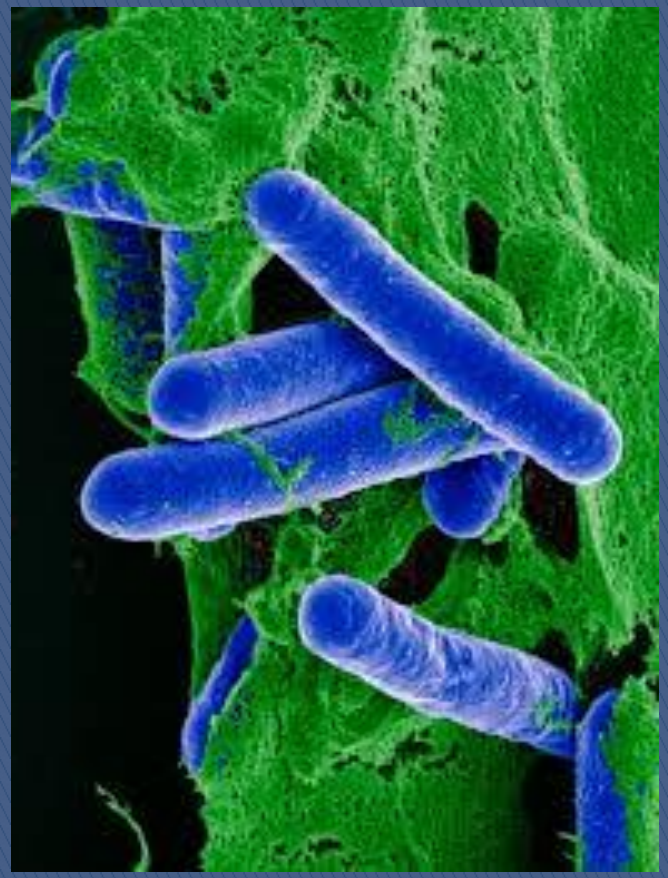
These statements …

- are often made without referencing any particular study or research;
- do not take in to account the wide variation in the toxicities of different pesticides;
- assume that all pesticides behave similarly in the environment; and
- rarely incorporate exposure estimates in to their assumptions.

Risk = toxicity x exposure

- You cannot assess toxicological risk without considering both toxicity and exposure.
- If exposure is insignificant, hazard is likely to be insignificant as well… but that depends on toxicity…and visa versa.

Botulism toxin from *C. botulidum*



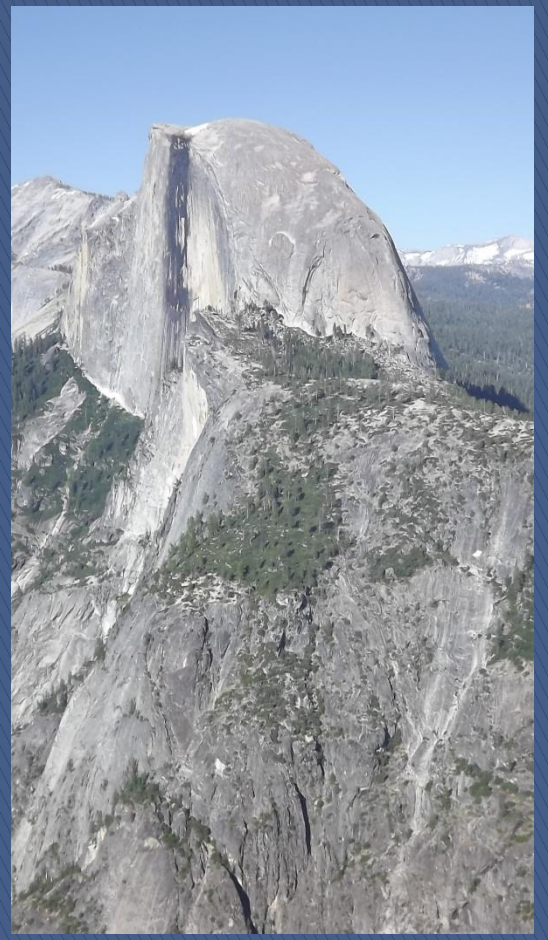
- When injected, the fatal dose is 1.3 to 2.1 nanograms per kilogram.
- For an 80 kg adult that's 104 to 168 ng (0.000000006 oz)
- ≈ one million times more toxic than Sarin nerve gas

- How do we determine if herbicides used in habitat restoration are safe for wildlife?
- By recognizing the relationship between risk, toxicity and exposure.

Some terminology…

- **Risk** – the risk the chemical poses
- **Exposure** – the amount, duration and type of contact a subject has to a toxicant
- **Toxicity** – a measurement of the "poisonousness" of the toxicant

RISK = TOXICITY x EXPOSURE



LD_{50}... Lethal Dose 50%

The concentration that will kill 50% of the test organisms.

Expressed in mg/kg. The weight of the chemical (mg) per kg of body weight of the lab rat.

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Small LD_{50} values mean higher toxicity.

USEPA Pesticide Toxicity Categories

	Mammal/Avian LD_{50} (mg/Kg)	Fish LC_{50} (mg/L)
very highly toxic	<10	<0.1
highly toxic	10–50	0.1 – 1
moderately toxic	51–500	>1–10
slightly toxic	501–2000	>10 –100
practically non-toxic	>2000	>100

NOAEL and NOAEC

- no observable adverse effect level or concentration
- the highest concentration that doesn't cause an adverse effect
- based on sub-lethal impacts
- More conservative than using the LD_{50} or LC_{50}

Common Observable Effects

- changes in body weight
- changes in organ weight
- organ damage
- changes in blood chemistry
- skin lesions
- tremors
- coordination difficulties
- swimming difficulties

Relative Toxicity: mammals

	Product example	Mode of action	Mammal toxicity
imazapyr	Habitat	Herbicide amino acid inhibitor	Practically non-toxic
glyphosate + NIS	Roundup Pro	Herbicide amino acid inhibitor	Practically non-toxic
triclopyr BEE	Garlon 4	Herbicide auxin mimic	Slightly toxic
carbaryl	Sevin	Insecticide CNS poison	Moderately toxic

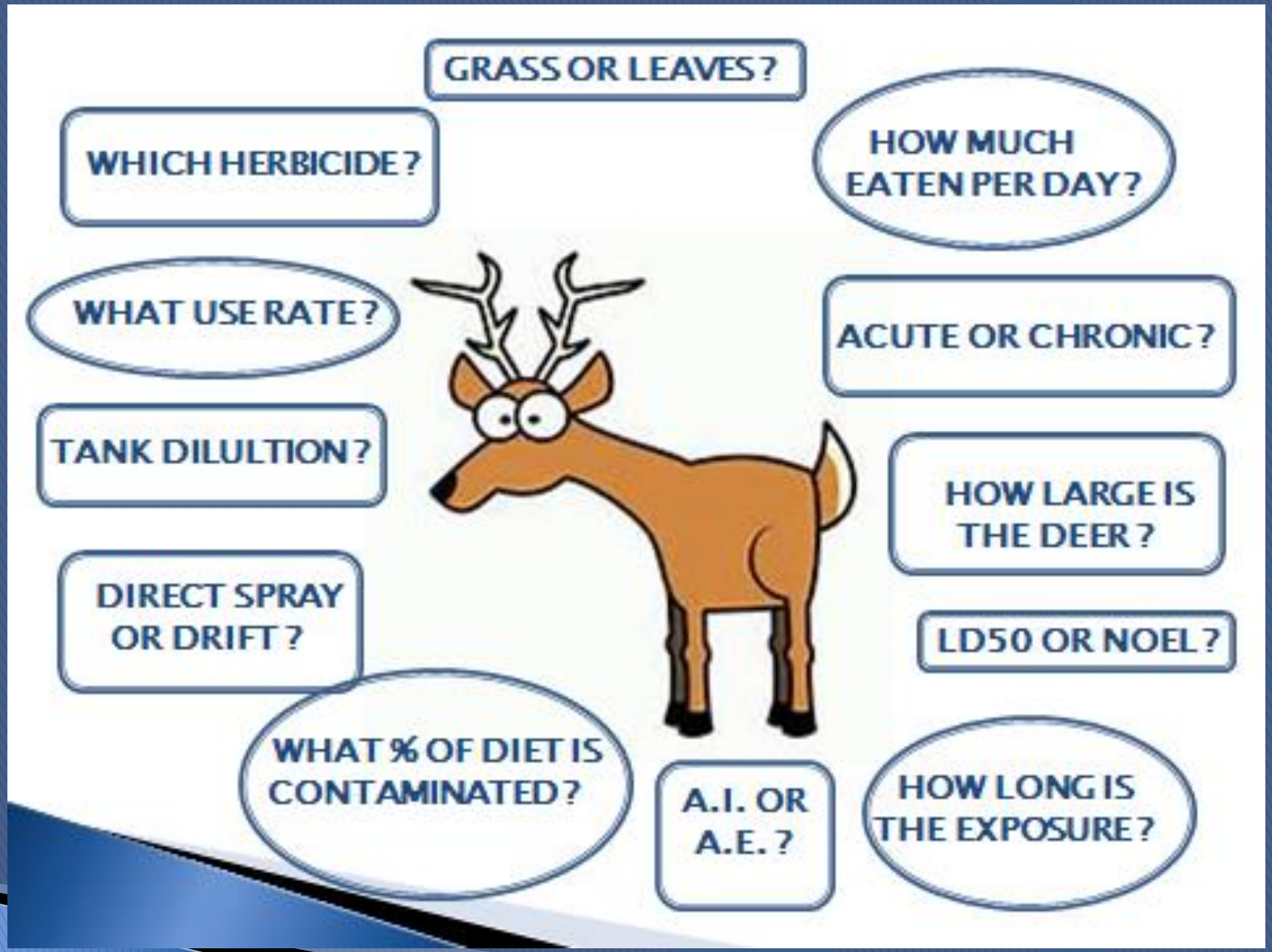
LD_{50} vs NOAEL Values Mammals

	LD_{50} (mg/kg)	NOAEL (mg/kg)
imazapyr	>5,000	738
glyphosate + NIS	>5,000	175
triclopyr BEE	630	440
carbaryl	250	4

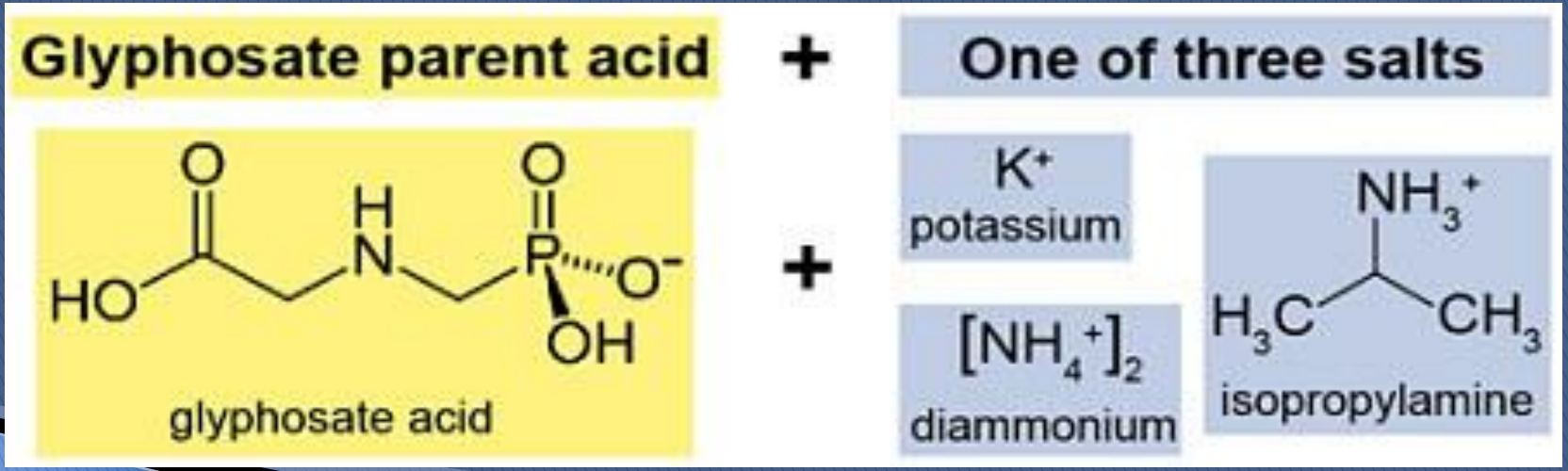
Meanwhile, back to our formula…
Risk = Toxicity x Exposure

Exposure is determined by…

- field monitoring
- computer modeling


GRASS OR LEAVES?
WHICH HERBICIDE?
HOW MUCH EATEN PER DAY?
WHAT USE RATE?
ACUTE OR CHRONIC?
TANK DILULTION?
HOW LARGE IS THE DEER?
DIRECT SPRAY OR DRIFT?
LD50 OR NOEL?
WHAT % OF DIET IS CONTAMINATED?
A.I. OR A.E.?
HOW LONG IS THE EXPOSURE?

- formulated product?
- active ingredient?
- acid equivalent?



Modeling Exposure

- Foliar applications
- Broadcast, ground-based
- Typical application rates
- Accepted dietary requirements for a typical, small mammal (20 g) were used.
- Assume 100% of the animal's diet is contaminated.
- No herbicide breakdown is assumed.

Triclopyr BEE – Garlon 4

- 24-h LD_{50} = 630 mg/kg
- Acute NOAEL = 440 mg/kg
- Expected environmental concentration = 144 mg/kg body weight-day

Risk Quotients (RQ)

$$RQ = \frac{EEC}{LD_{50} \text{ or } NOAEL}$$

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RQ – Triclopyr BEE using the NOAEL: 440 ppm

$$RQ = \frac{144 \text{ mg/kg}}{440 \text{ mg/kg}} = 0.3$$

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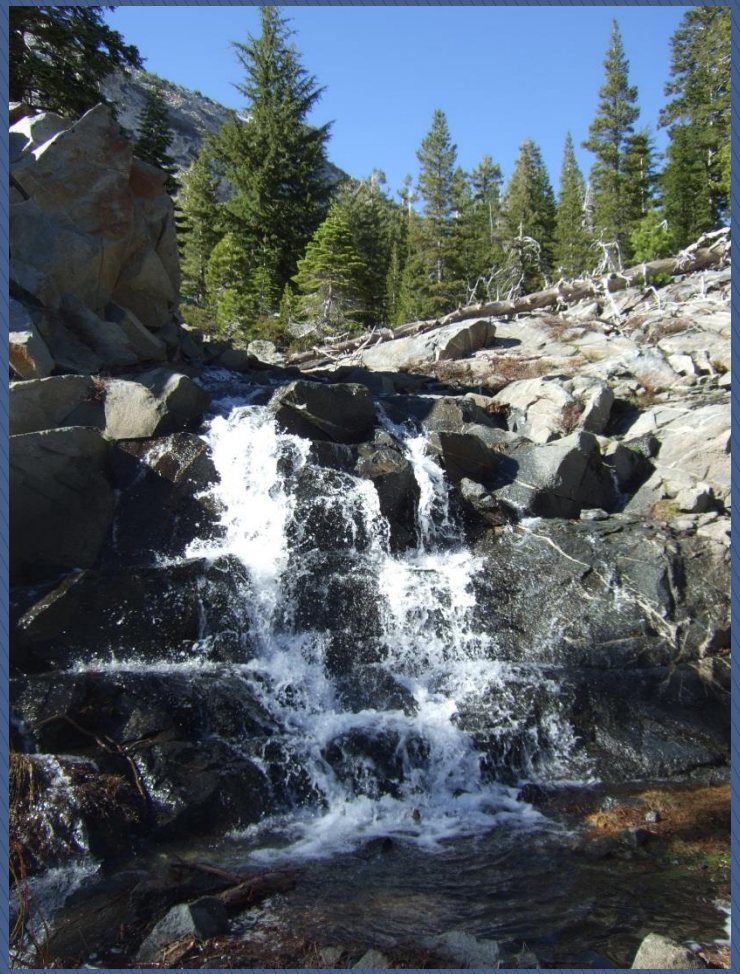
What does "0.3" actually mean?

Level of Concern (LOC)



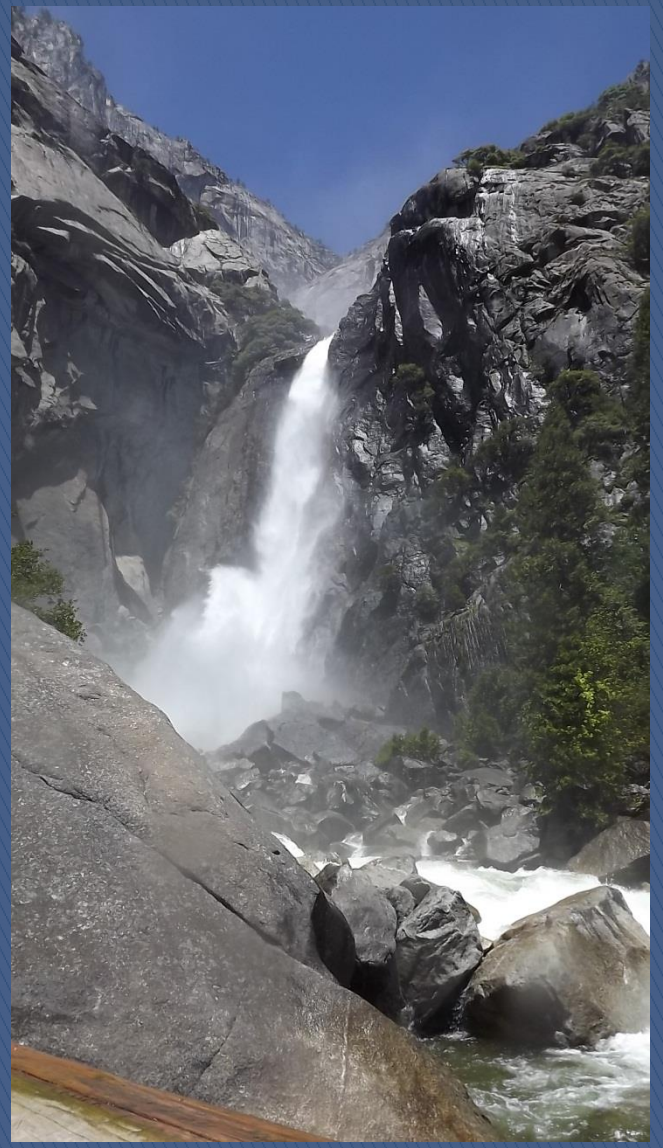
- The RQ needs to be compared to something.
- The Level of Concern (LOC)
- When RQ>LOC , adverse effects are plausible.
- Plausible **not** assured.

The Level of Concern

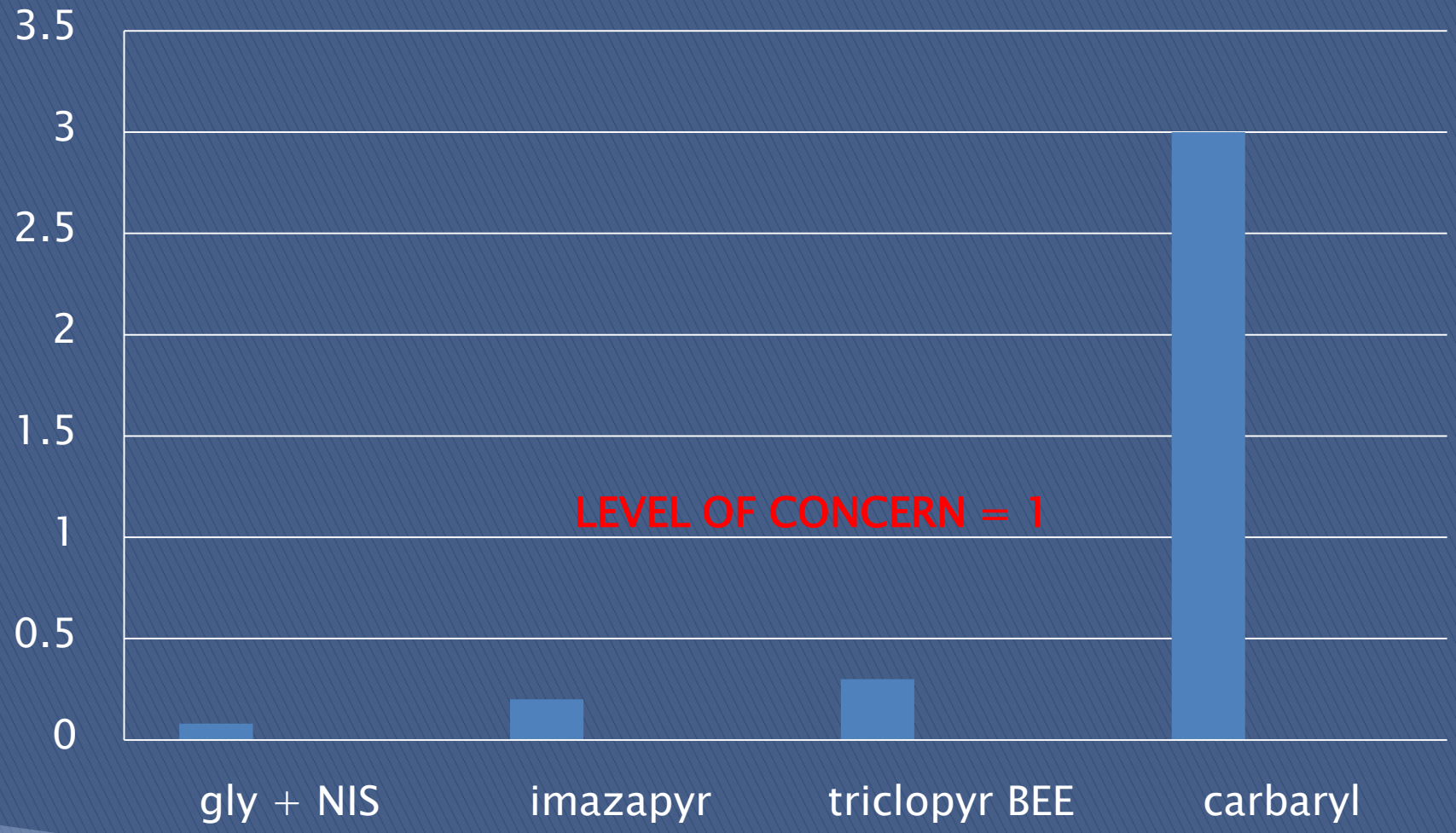
An RQ of 1 means the exposure value is equal to the toxicity value.

This being the case, having the LOC = 1 seems reasonable.

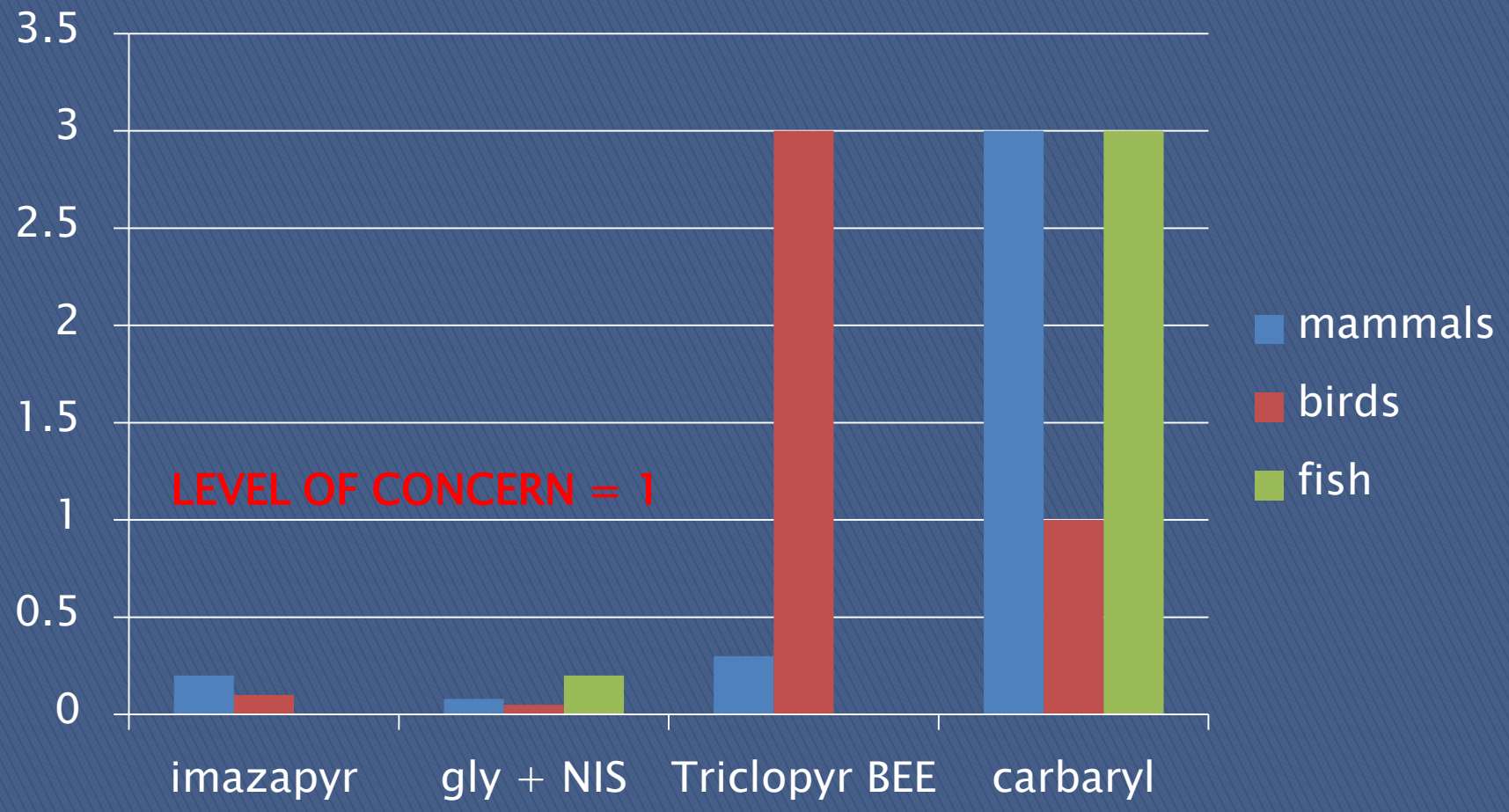
In some cases, (i.e. T/E species) the LOC may be < 1



RQ Values: Acute Small Mammals



RQ Values: Mammals, Birds & Fish



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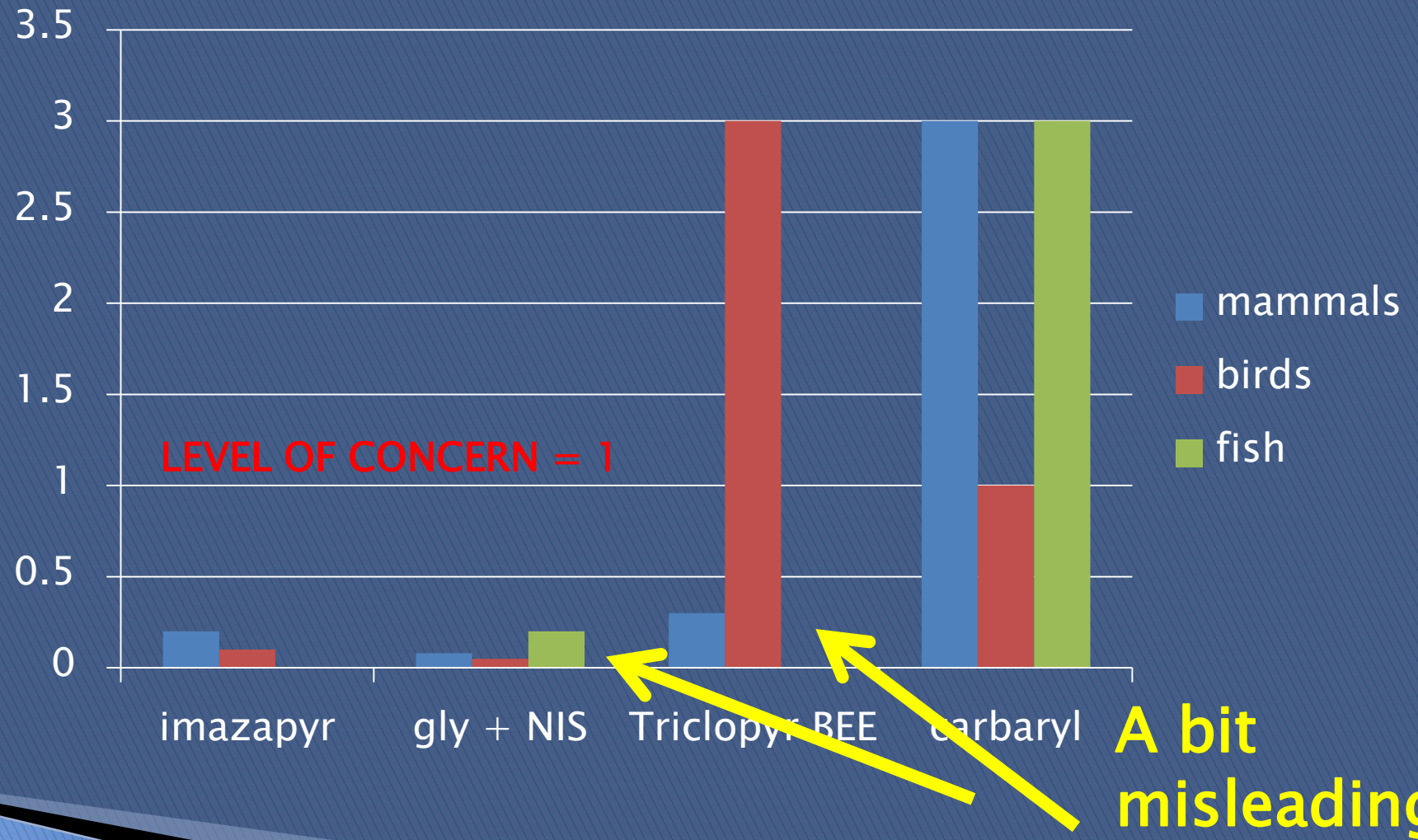


A Conservative Approach

- Modeled exposure values are conservative.
- The NOAEL vs the LD_{50}
- Some studies have shown that wildlife may avoid herbicide-treated foliage as food sources

OVERESTIMATIONS

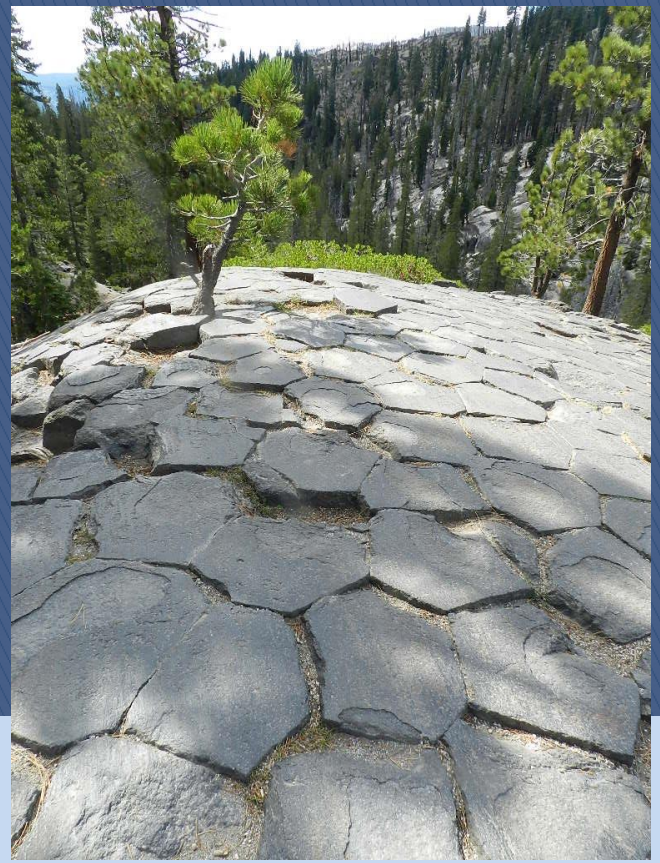
- Assumption that 100% of the animal's diet is contaminated with the herbicide.
- Spot treatment vs broadcast treatments

OTHER CONSIDERATIONS

- **Synergy between environmental chemicals**
  - other pesticides
  - fertilizers
- **Toxicity values are from surrogate species**
  - rats for mammals
  - bluegill sunfish or rainbow trout for fish
  - Japanese quail for birds
  - larval fish for juvenile frogs
  - birds for snakes
  - honeybees for terrestrial invertebrates

OTHER CONSIDERATIONS

- **Other herbicide-caused impacts…**
  - changes in habitat quality/quantity
  - changes in behavior (i.e. prey avoidance)
- **Multiple environmental stressors**
  - disease
  - predation
  - weather



US FOREST SERVICE Herbicide Risk Assessments

http://www.fs.fed.us/foresthealth/pesticide/risk.shtml

Glyphosate's a carcinogen?

In March 2015, the International Agency for Research on Cancer (IARC) concluded that glyphosate is a probable human carcinogen.

- IARC placed the herbicide in its 2A group…probable human carcinogens
- …along with malathion, red meat and working the night shift

Who is IARC?

- International Agency for Research on Cancer
- An intergovernmental agency forming part of the World Health Organization (WHO)

IARC Carcinogen Groups

Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

Group 1: Known Carcinogens

International Agency for Research on Cancer

Centre International de Recherche sur le Cancer

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A little history…

- 1985 USEPA…
  possible human carcinogen
- 1986 FIFRA SAP…
  not classifiable as to human carcinogenicity
- 1991 USEPA…
  evidence of non-carcinogenicity for humans

A little bit more history…

- 2015 March, IARC…
  probable human carcinogen
- 2015 September, USEPA…
  not likely to be carcinogenic to humans
- 2015 November, European Food Safety Authority…
  unlikely to pose a carcinogenic risk to humans
- 2016 May, FAO/WHO…
  unlikely to pose a carcinogenic risk to humans
  from exposure through the diet

Just a tad bit more history…

- 2017 March, OEHHA…glyphosate proposed for listing in CA as a Prop 65 carcinogen
- 2017 March, OEHHA…a No Significant Risk Level (NSRL) of 1,100 µg/kg is proposed. (1.1 ppb)
- 2017, June, OEHHA…glyphosate added to Prop 65 list

IARC's 3 Areas of Evidence…

- **Limited Evidence** from human epidemiological studies that demonstrated a positive association for non-Hodgkin lymphoma.
- **Sufficient Evidence** from laboratory toxicity tests based on significant positive trends for kidney tumors in rats and for hemangiosarcomas in mice.
- **Strong Evidence** for genotoxicity based on DNA and chromosomal damage in human cells (in vitro)

Epidemiological Studies

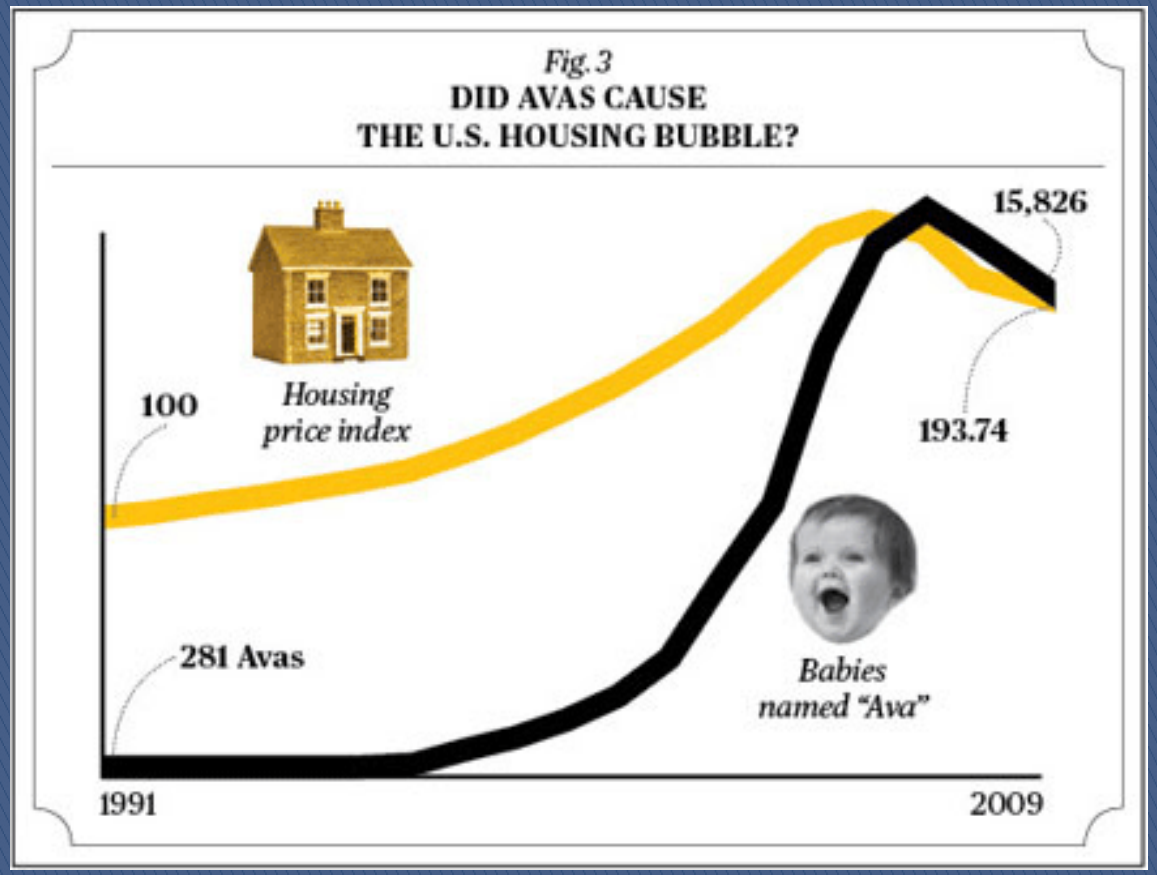
- Observational studies of large groups of people that look at the relationship between exposure and illness.

- These studies can reveal if there's a positive association…or correlation… between exposure to the agent and cancer, but they can't be used to determine the cause of the cancers.

Epidemiological Studies

- Can't completely rule out other explanations such as chance or bias.
- Additionally, these studies have limitations such as the accuracy of self-reported information and the effect that exposure to other substances…including other pesticides…might have on cancer incidence.

Correlation ≠ Causation



Animal Studies

- Feeding studies using either rats or mice.
- Generally 18-24-months (lifetime)
- 3 or more doses are used
- A statistical evaluation to determine whether exposure to the test agent is associated with an increase in tumor development, rather than due to chance alone.

Genotoxic Studies

- Damage to the genetic information within a cell causing mutations, which **may** lead to cancer.
- *In vitro* tests (cultured bacteria or mammalian cells)
- *In vivo* tests (animal feeding studies or i.p. injection)

- A “weight of evidence” approach is used.
- Permanent (inheritable) DNA damage is given more weight than damage that is reversible.
- *In vivo* tests are given more weight than *in vitro* tests.
- The greatest weight is given to *in vivo* tests that use doses and routes of exposure that are relevant for human exposure.

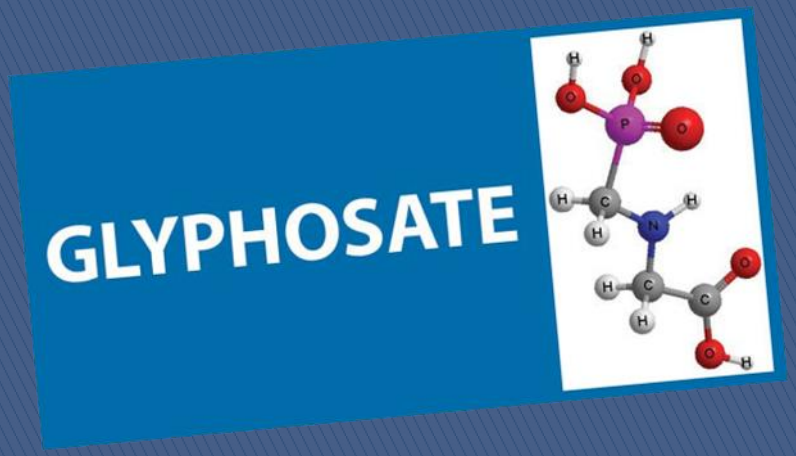
Why is there so much confusion?

- There is a lot of data.
- The science of assessing chronic risk to humans is complicated.
- Especially when you compare acute risk to chronic risk.

Let's focus on the science

- Class action lawsuits
- GMOs
- pollinators
- Glyphosate bans
- EPA corruption
- IARC's agenda

Some personal disclaimers

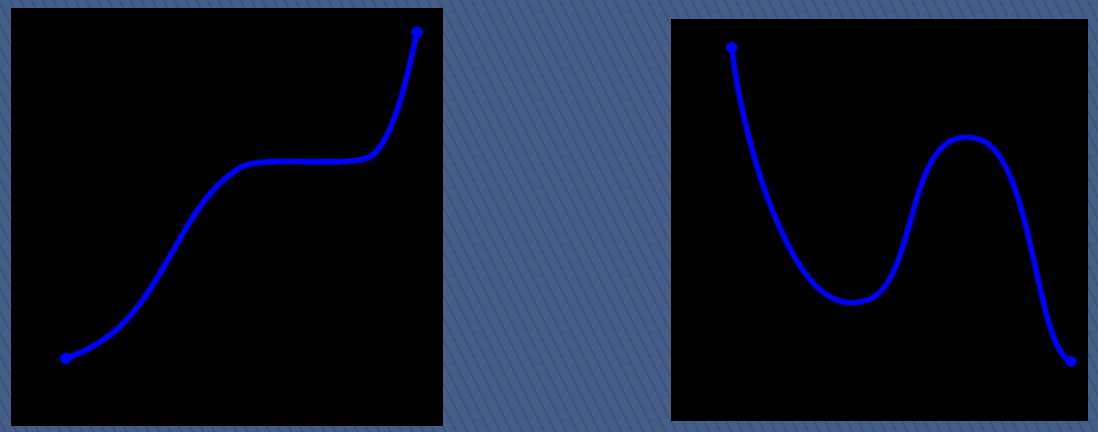


- No criticism of the science
- Ignoring real or perceived agendas
- A serious concern…the precedent that is set when regulatory decisions are made without understanding the science…or worse…**ignoring** the science.

Let's make a comparison…

- Acute tests of lethality are…
  - Straight-forward
  - Not subjective
  - No need for interpretation

Animal Studies

- Statistical significance of tumor incidence
  - Significance observed in unadjusted p-values but disappears after the values are corrected (multiple comparison problem)
- Lack of monotonic response
  - 

Some Examples: Animal Studies

- Is the increase in tumors statistical significant?
- Lack of monotonic response
- Lack of pre-neoplastic lesions or evidence of tumor progression
- Lack of consistency between studies in the data set (weight of evidence)
- Significance of high dose tumors (≈1,000 mg/kg)
  - Effect on homeostatic mechanisms

TUMOR TYPE	DOSE GROUP (mg/kg-day)			
Hemangiosacrcoma	0	100	300	1000
	0/50	0/50	0/50	4/50

- Two-year diet study in male mice
- Reviewed by IARC, Joint FAO/WHO,
- Used by OEHHA to set proposed Prop 65 NSRL

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USEPA Issue Paper Sept 2016

- 23 epidemiological studies
- 15 animal studies
- ≈ 90 genotoxicity studies

USEPA Issue Paper Sept 2016

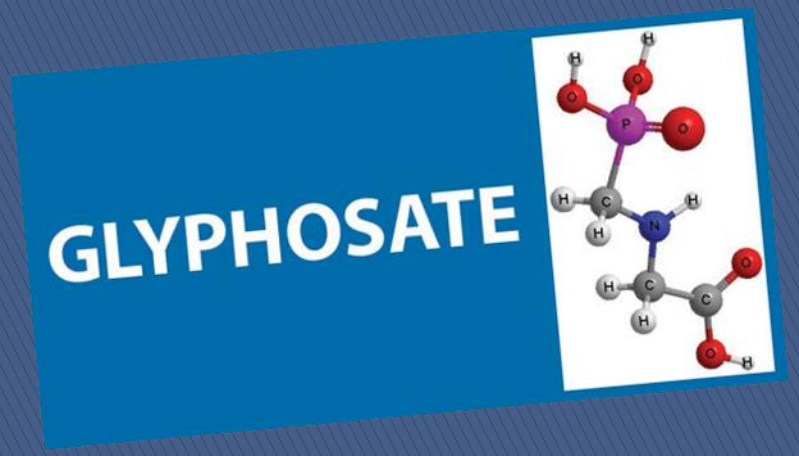
- **Overall, animal carcinogenicity and genotoxicity studies** did not demonstrate a clear association between glyphosate exposure and carcinogenic potential.
- **In epidemiological studies**, there was no evidence of an association between glyphosate exposure and numerous cancer outcomes.
- Due to conflicting results and various limitations, a conclusion regarding the association between glyphosate exposure and risk of NHL cannot be determined based on the available data.

USEPA Issue Paper Sept 2016

For cancer descriptors, the available data clearly do not support the descriptors…

- “carcinogenic to humans”,
- “likely to be carcinogenic to humans”, or
- “inadequate information to assess carcinogenic potential”…

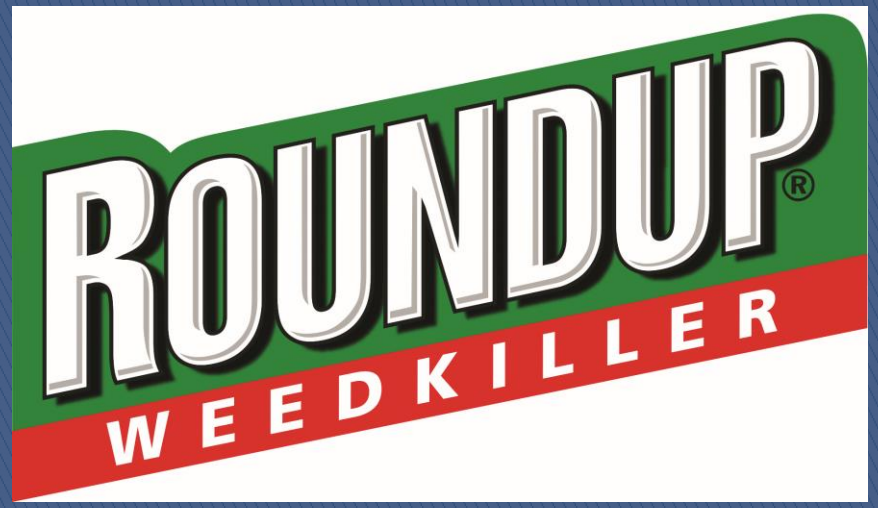
USEPA Issue Paper Sept 2016



The strongest support is for…

“not likely to be carcinogenic to humans ”at doses relevant to human health risk assessment

FIFRA SAP 2016 Final Report



- An ad hoc advisory committee comprised of independent scientists
- The SAP reviewed the USEPA's September 2016 Glyphosate Issue Paper.

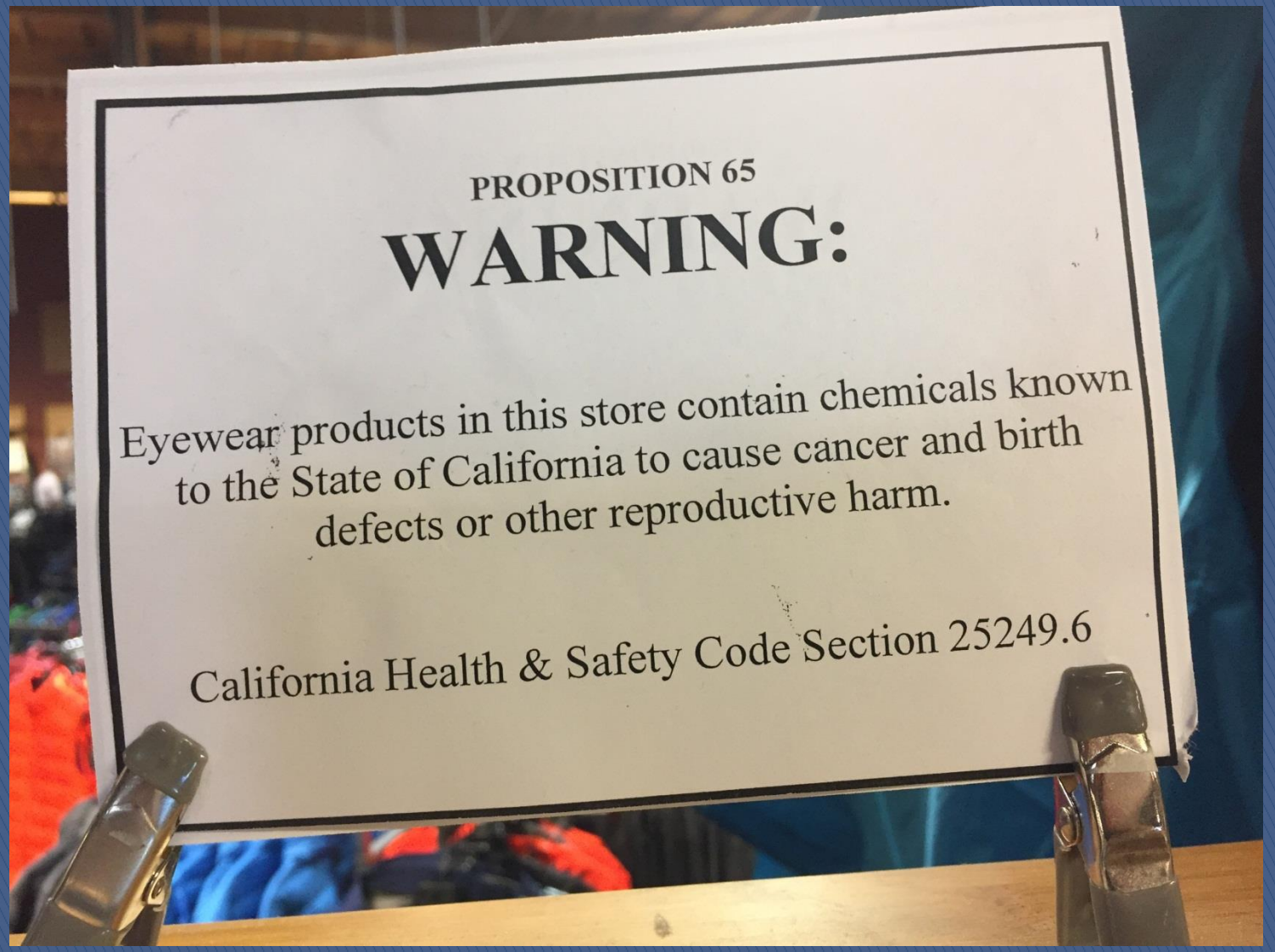
FIFRA SAP 2016 Final Report

The Panel was split between those members agreeing with the issue paper conclusions and those members who felt that the characterization of…“not likely to be carcinogenic to humans” should be replaced by the hazard descriptor of…

“suggestive evidence of carcinogenic potential”.

FIFRA SAP 2016 Final Report

- Most of the Panel's discussion centered on assessment of the potential for glyphosate to be a carcinogen, and less on the conditions under which glyphosate exposure would represent a significant human health risk.
- In other words, the FIFRA SAP focused mostly on hazard identification (is it a carcinogen?) rather than (what exposure routes and levels produce carcinogenicity in humans?)
- Just like IARC.


PROPOSITION 65
WARNING:
Eyewear products in this store contain chemicals known to the State of California to cause cancer and birth defects or other reproductive harm.
California Health & Safety Code Section 25249.6

When am I at risk?

- When I'm close enough to read the sign?
- When I put on the sunglasses?
- When I eat the sunglasses?
- If I'm wearing them on a hot day?

You cannot assess toxicological risk without considering both toxicity and exposure…

- Risk = Toxicity x Exposure

Back to Prop 65 NSRL

- NSRL is defined as the daily intake level calculated to result in one excess case of cancer in a population of 100,000 exposed individuals.
- Exposures <NSRL do not require warnings

Back to Prop 65 NSRL

- The proposed NSRL is 1,100 µg/day (1.1 mg/day).
- OEHHA's decision on the glyphosate NSRL is still pending.

How much is 1.1 mg/day?

- How do you compare laboratory dietary exposure levels to occupational exposure?
- Dietary exposures may not be directly comparable to occupational exposures.
  - Dermal absorption potential
  - Inhalation potential (volatility)

How much is 1.1 mg/day?

USFS backpack exposure estimate:

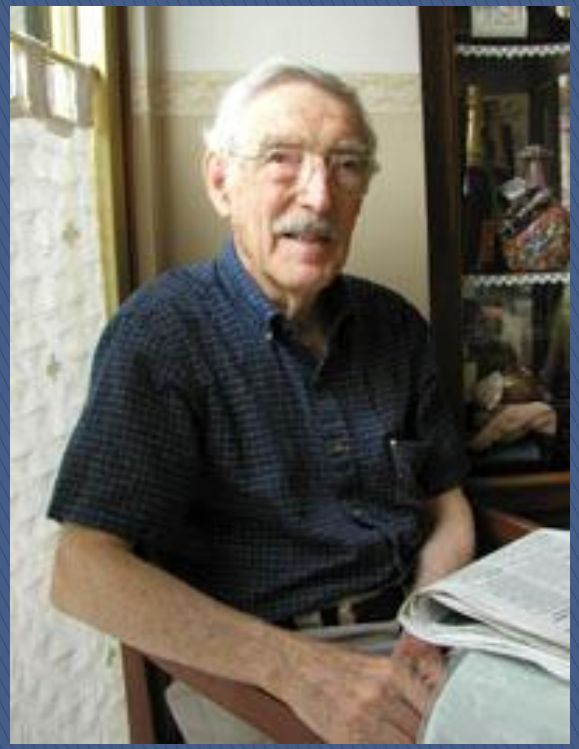
- An application rate of 1.2 glyphosate acid/acre may create a 1.1 mg/day exposure (70 kg person)
- 1.2 lbs glyphosate acid/acre = 1.2 gals RUPM/acre

How much is 1.1 mg/day?

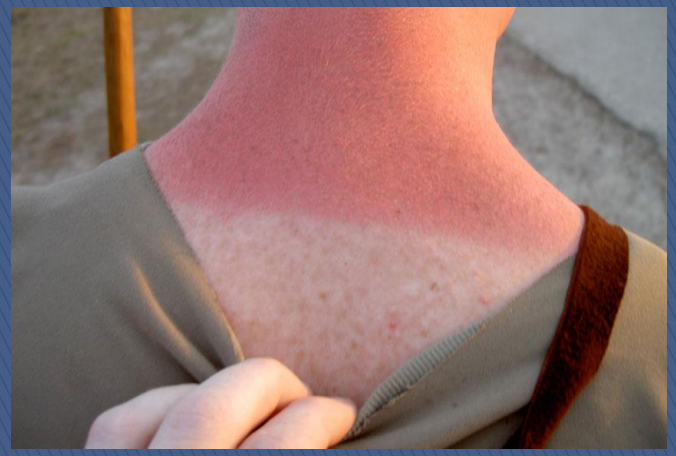
- Real-world applicator exposure in wildlands settings…
  - PPE?
  - Not lifetime
  - Infrequent?
  - Spot treatment

Bruce Ames

- "if you have thousands of hypothetical risks that you are supposed to pay attention to, that completely drives out the major risks you should be aware of."



Bruce Nathan Ames professor of Biochemistry and Molecular Biology Emeritus at the University of California, Berkeley, and a senior scientist at Children's Hospital Oakland Research Institute (CHORI).



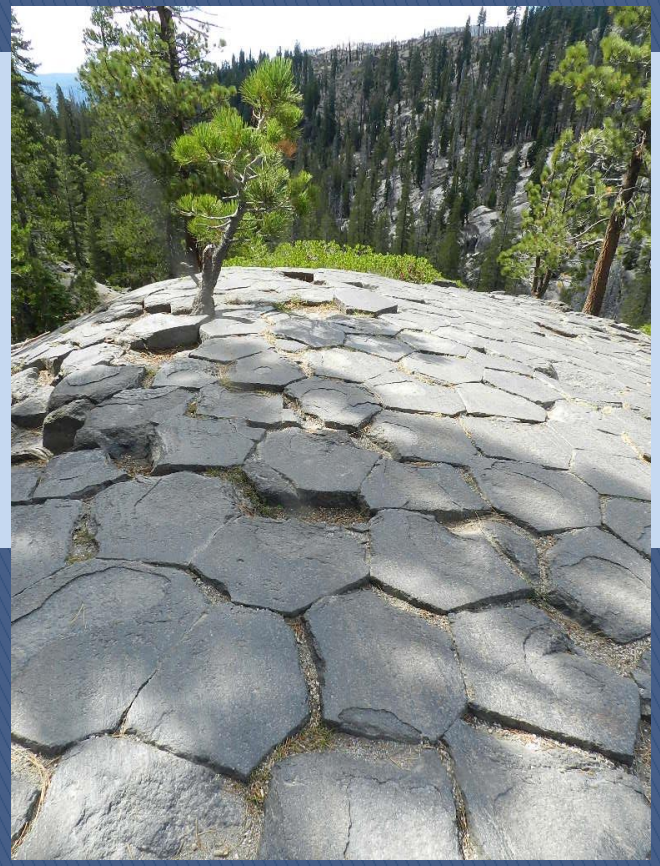
- "if you have thousands of hypothetical risks that you are supposed to pay attention to…

“Even if a substance or exposure is known or suspected to cause cancer, this does not necessarily mean that it can or should be avoided at all costs”.

American Cancer Society website

Final Thoughts

- Most regulatory agencies worldwide have not reached the same conclusion as IARC.
- The weight of evidence continues to point towards a lack of carcinogenic potential…certainly at exposure levels that are relevant to people.
- Science is a dynamic undertaking. This topic, like all scientific topics is subject to the findings of future investigations.



QUESTIONS?