A Case-Study using in vitro DNT NAMs as part of a Weight-of-Evidence (WoE) approach to decision-making for DNT

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The author of this presentation (TJ Shafer) has no conflicts of interest to declare

IATA Problem Formulation

EPA's Office of Pesticide Programs (OPP) is responsible for pesticide regulation.

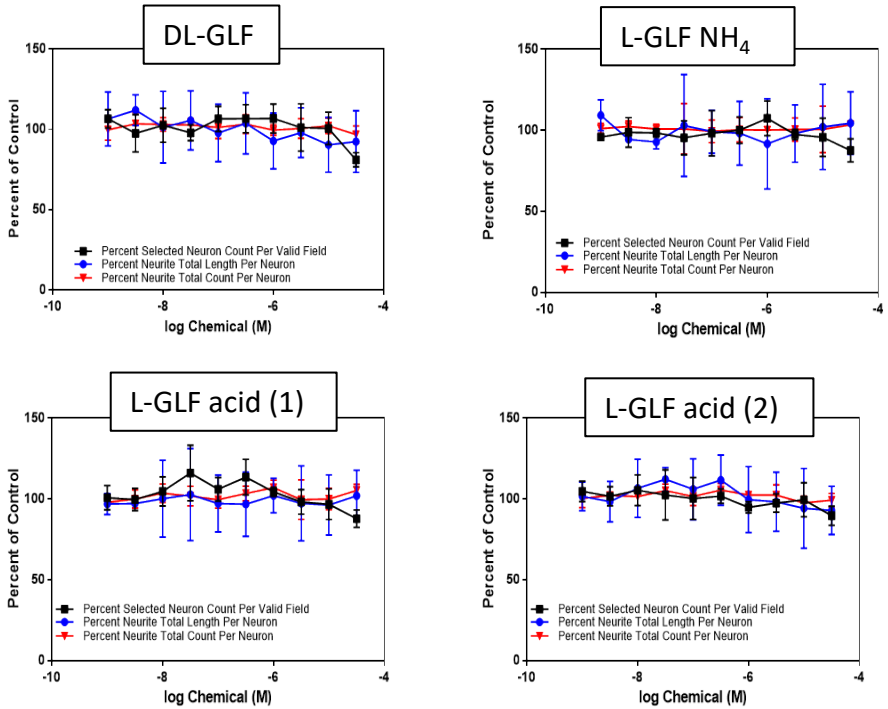
- OPP received notification that different parties intended to register L-glufosinate ammonium and L-glufosinate acid as pesticides (herbicides)
- DL-glufosinate ammonium was already registered as a pesticide, and a Guideline DNT study had been submitted to OPP
    - Decreased pup weight, morphometry changes in hippocampus, motor activity changes were reported
- DL-glufosinate also has acute neurotoxicity and in vitro, had been reported to alter network activity following acute exposure

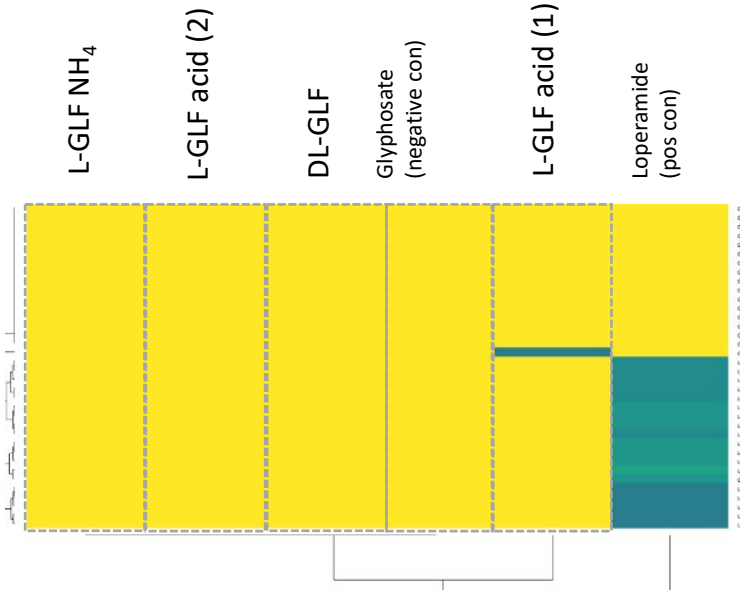
Problem: Is the Guideline DNT for DL-glufosinate sufficient to inform decisions for L-glufosinate isomers?
Need: Comparative bioactivity data for DL- vs L-Glufosinate isomers

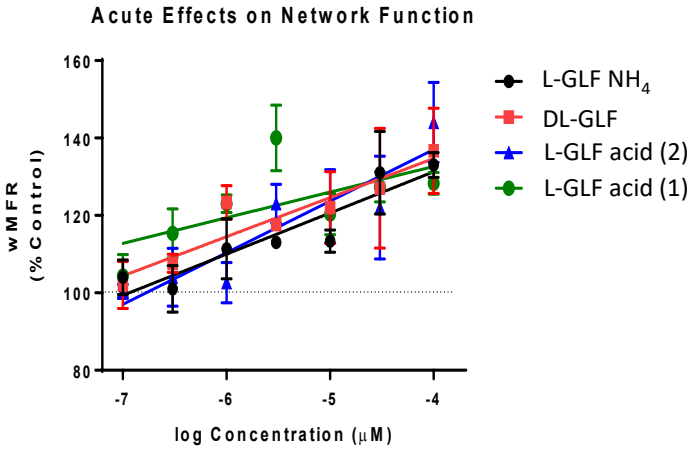
OPP asked EPA's Office of Research and Development to provide data to inform their decision on L-glufosinate compounds.

- **Neurite Outgrowth** and **Network Formation** assays were selected based on the activity of DL-glufosinate in Guideline Study and in vitro, respectively.
- Compounds DL-glufosinate, L-glufosinate acid and L-glufosinate ammonium were tested in these assays, + assay controls

Using WoE and DNT NAMs for Guideline DNT waiver decisions







From Guideline study, NOAEL of DL-GLF = **14 mg/kg/day**

Using HTTK and IVIVE

- 1 mg/kg/day = Css values of 0.66 and 2.21 µM in rats and humans, respectively
- 30 µM DL-GLF = AED of **45 mg/kg/day** (rats) and 13.5 mg/kg/day (humans)

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Weight of Evidence for Decision on DNT Waiver for L-glufosinate acid and ammonium

In vitro evidence

- Lack of effect on neurite outgrowth in human cells
- Lack of effect on network formation in rat cortical networks
- Positive effects on acute network activity demonstrate biological activity and add confidence to the lack of effects in DNT-related assays (neurite outgrowth and network formation)
- Similar effects of DL- and L-isoforms in all in vitro assays

In vitro to in vivo extrapolation (IVIVE)

- Tested concentrations in vitro > PODs selected for L-glufosinate risk assessment

In vivo evidence

- Existing guideline DNT study for DL-glufosinate showing effects on morphometry, motor activity and pup weight
- Non-guideline DNT for L-glufosinate showing increased motor activity, decreased body wt in pups (morphometrics not conducted)
- Comparable toxicity profiles for both DL- and L-glufosinate.

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Weight of Evidence for Decision on DNT Waiver for L-glufosinate acid and ammonium

Risk Calculations

- Point of Departure (POD) was 30x lower than calculated AED from in vitro studies (which were without effect)
- %Population adjusted doses (%PAD) < 100% (for dietary exposures)
- Margin of exposure (MOE) > Level of concern (LOC) for non-dietary exposures

CONCLUSION: Additional in vivo data would not likely identify a lower POD or more sensitive endpoint for isomer risk assessments

DECISION: Waivers granted for guideline DNT studies for L-glufosinate acid and L-glufosinate ammonium

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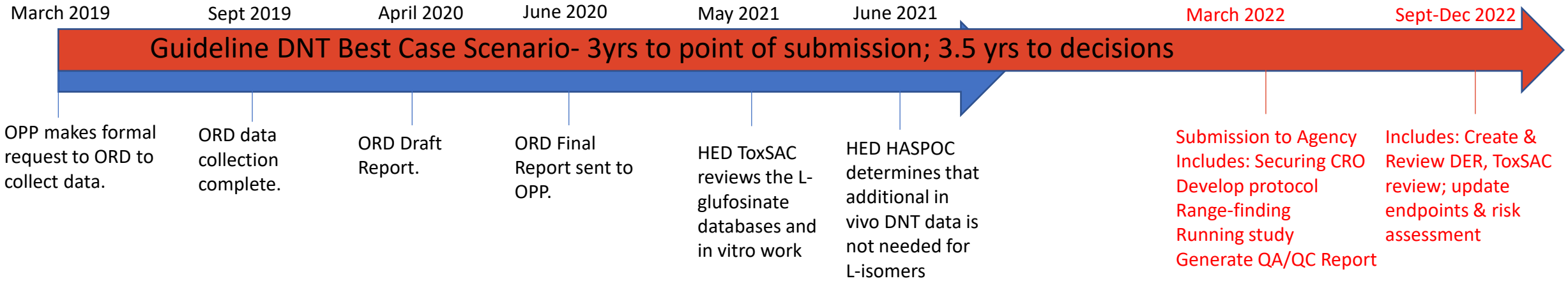
Comparison to a DNT Guideline study- Impacts of the Decision



Animals Used:

- In vitro study- 3 Pregnant Dams (~12-15pups)
- Guideline study- 160 Pregnant Dams (2 compounds X 3 doses + control @20/dose (recommended))
  - ~1600 pups

Cost:

- In vitro study- $1000 for Assays + $96,000 labor = $97,000
- Guideline study- $2,000,000 (2 compounds x $1M each)

Other Examples of the use of DNT NAMs at EPA

I. **Screening Level information**
   - Accelerating the Pace of Chemical Risk Assessment (APCRA),
   - Toxic Substance Control Act (TSCA) chemicals,
   - Perfluoroalkyl Substances (PFAS)
   - 6 PPD and 6-PPD quinone

II. **Weight of Evidence approach**
   - Organophosphates
     - Are PoDs based on AChE inhibition health protective for organophosphates?