Challenges with Guideline DNT Studies Facilitate the Application of DNT NAMs by the US EPA

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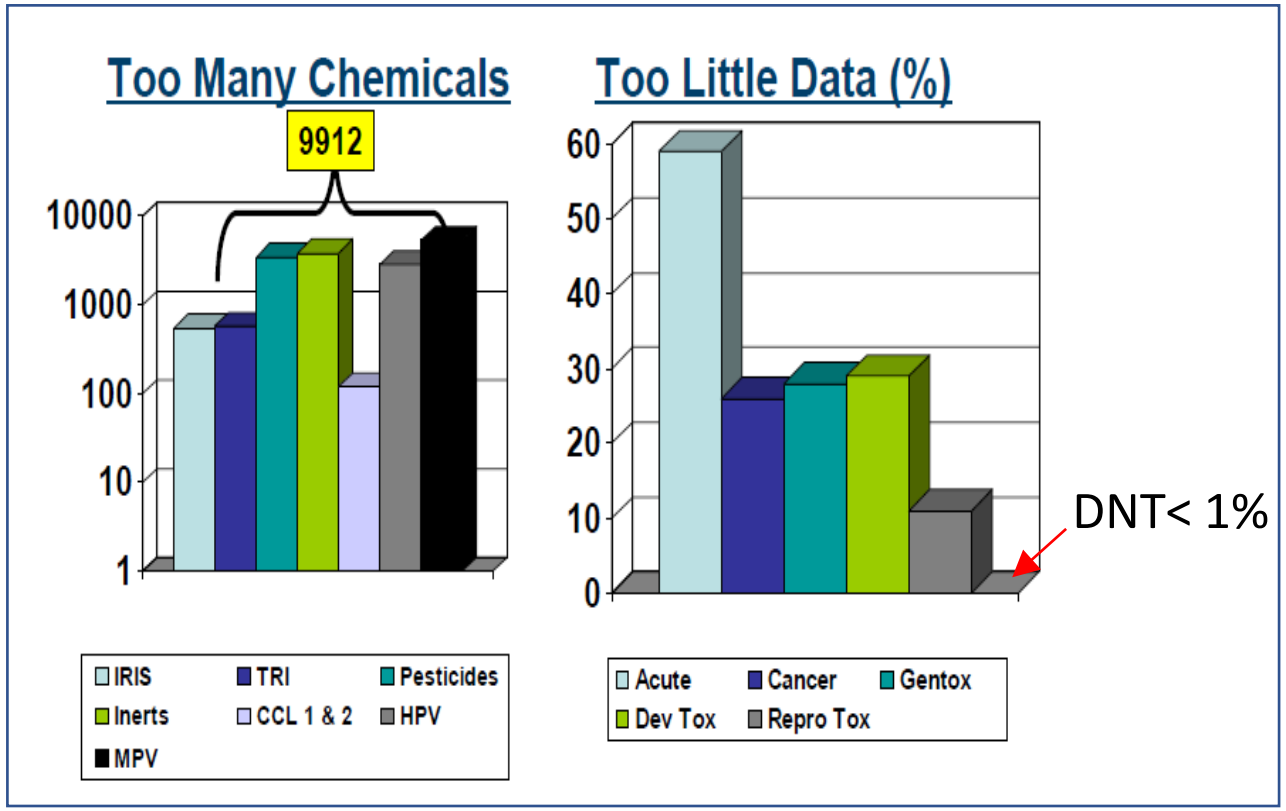
The Problem: Developmental Neurotoxicity (DNT) has been examined for too few chemicals



In the absence of DNT hazard data, it is not possible to:

a) Evaluate the role of environmental chemicals in neurodevelopmental disease
b) Evaluate potential DNT risk for individual chemicals
c) Consider DNT as an adverse outcome in clean-up decisions at contaminated sites (e.g. Superfund sites).

The Differences between TSCA and FIFRA

Toxic Substances Control Act (TSCA)

All New Chemicals
>60-80K "Grandfathered" Chemicals

Available Data
90 Day Premanufacture Notice

"Data Poor"- little or nothing may be known about toxicity hazard

Intended to Kill Something

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

All "Pesticides", including "antimicrobials"

Required Guideline Studies
Health and Environmental Effects

Data Rich- Toxicity hazard is well characterized

Lautenberg Chemical Safety Act 2016

- **Mandatory requirement for EPA to evaluate existing chemicals with clear and enforceable deadlines;**
- **Risk-based chemical assessments;**
- **Increased public transparency for chemical information; and**
- **Consistent source of funding for EPA to carry out the responsibilities under the new law.**
- **Directs EPA to utilize alternatives to animals**

Food Quality Protection Act of 1996

- **Mandates an extra 10x safety factor for children/infants**
- **Mandates Assessment of Cumulative Risk to Pesticides with the same mode of action**

Requirements of EPA 870.6300 (OECD TG 426/443)

- 6 Pregnant females/dose (20 litters/dose recommended)
- 10 pups/litter (5 male/5 female)
- Minimum 3 doses + control
- Dosing period GD6-PND10
- Assessments on PND 4, 11, 21, 35, 45, 60
- Signs of Maternal Toxicity
- Developmental landmarks
- Brain/body weights (4, 11, 17, 21 PND)
- Motor activity (13, 17, 21, 60 PND)
- Auditory Startle (weaning, PND 60)
- Learning and memory (weaning, PND 60)
- Neuropathology (PND 11 and termination)
  - Major brain regions

https://beta.regulations.gov/document/EPA-HQ-OPPT-2009-0156-0042
https://www.oecd-ilibrary.org/environment/test-no-426-developmental-neurotoxicity-study_9789264067394-en
https://www.oecd.org/chemicalsafety/test-no-443-extended-one-generation-reproductive-toxicity-study-9789264185371-en.htm

Issues with in vivo DNT studies

- "Triggered" test- Only requested if concern for neurotoxicity
- Expensive- ~$1,000,000/chemical
- Time-consuming- takes 1-2 years to complete
- Ethically questionable- Estimated ~1000 animals/test
- Value of Information*
  - Quality of data varies considerably
  - Not often used for point of departure values for risk assessment

*Crofton KM, A qualitative retrospective analysis of positive control data in developmental neurotoxicity studies. Neurotoxicol Teratol. 2004 May-Jun;26(3):345-52.
Tsuji R, Crofton KM. Developmental neurotoxicity guideline study: issues with methodology, evaluation and regulation. Congenit Anom (Kyoto). 2012 Sep;52(3):122-8.
Vorhees CV, Williams MT. Issues in the design, analysis, and application of rodent developmental neurotoxicology **studies**. Neurotoxicol Teratol. 2021 Sep-Oct;87:107018
Raffaele et al. The use of **developmental neurotoxicity** data in pesticide risk assessments. Neurotoxicol Teratol. 2010 Sep-Oct;32(5):563-72.

EPA DNT in vitro NAMs

Assays:

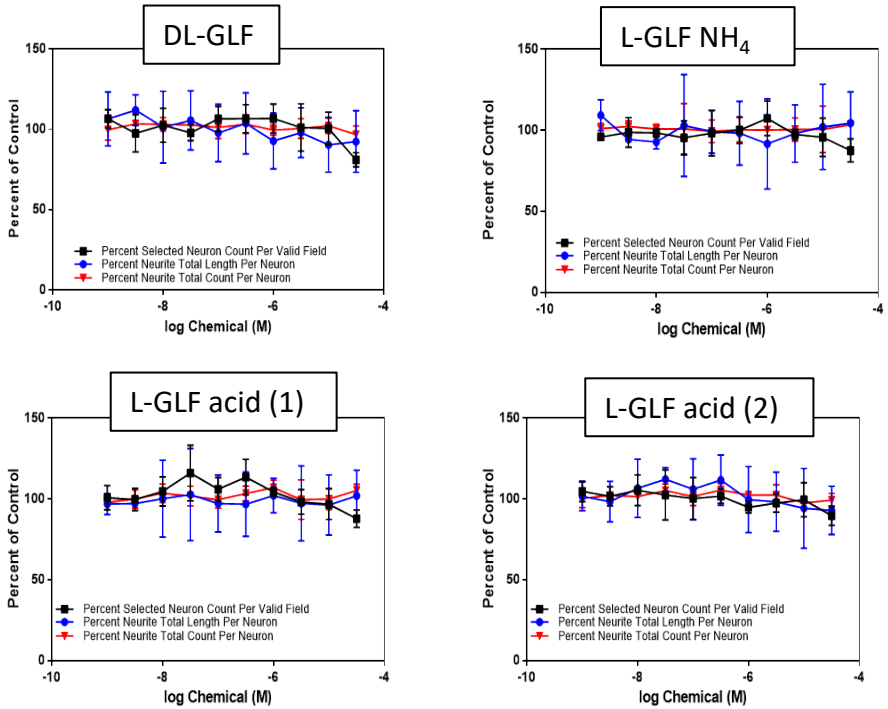
Proliferation	-	human neuroprogenitors (hNP1)
Apoptosis	-	human neuroprogenitors (hNP1)
Neurite initiation	-	human neurons (CDI I_{gluta})
Neurite initiation	-	rat primary neural culture
Neurite maturation	-	rat primary neural culture
Synaptogenesis	-	rat primary neural culture
Network formation (MEA)	-	rat primary neural culture

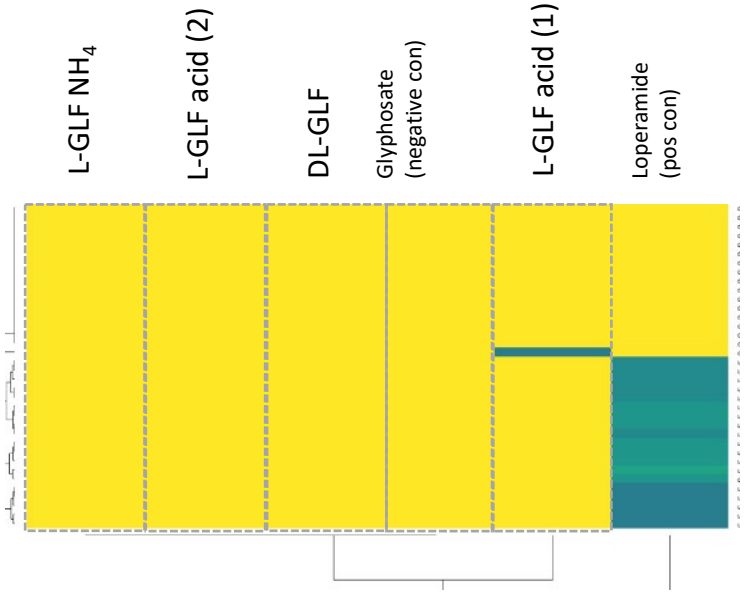
A Case-Study using in vitro DNT NAMS as part of a Weight-of-Evidence (WoE) approach to decision-making for DNT

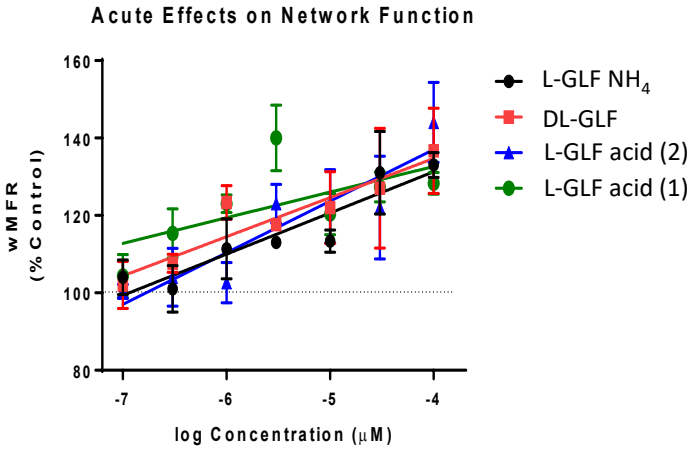
Using WoE and DNT NAMs for Guideline DNT waiver decisions

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







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 wt
- Non-guideline DNT for L-glufosinate showing increased motor activity, decreased body wt in pups (morphometrics not conducted)
    - Indicates 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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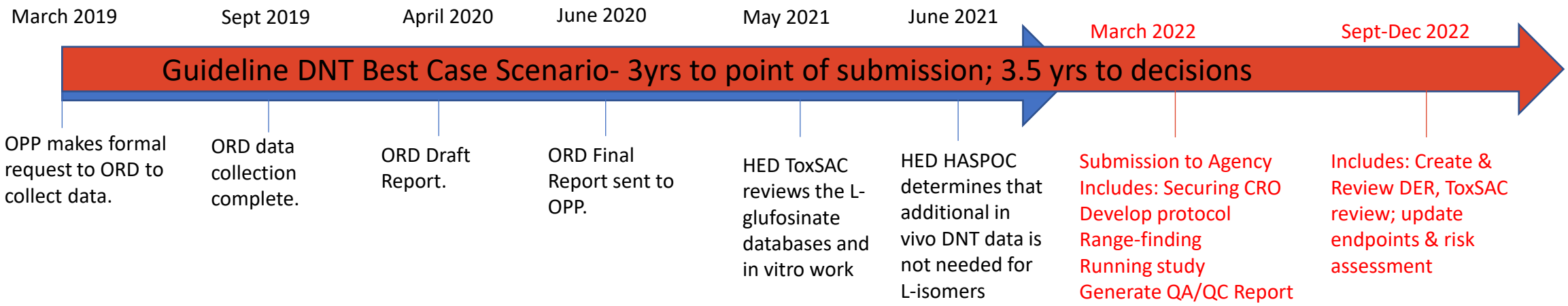
Comparison to a DNT Guideline study- Impacts of the Decision



Animals Used:

- In vitro study- 3 Pregnant Dams (~12-15pups)
- Guideline study- 160 Pregnant Dams (2 compounds X 3 dose + 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?

Summary and Conclusion

- Lack of data for DNT hazard characterization leads to uncertainty about the DNT risk for thousands of chemicals.
- High-resource commitments of in vivo studies, combined with interpretation challenges precludes the use of Guideline studies to address DNT concerns.
- Higher-throughput, biologically-based New Approach Methodologies (NAMs) have been developed to address this challenge.
- A case-study was presented that demonstrated how data from a subset of these DNT NAMs were used to reach a decision to waive DNT Guideline studies for L-glufosinate isomers.

DNT NAMs provide valuable information that can be informative to decision-making regarding DNT risks, and will be increasingly utilized in this process