Expert Panel Review of the Neurotoxicity of Glyphosate in Mammalian Systems for Risk Assessment Purposes

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Glyphosate and neurological outcomes: A systematic literature review of animal studies

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Disclosures

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- The sponsor did not review or comment on the manuscript at any stage during preparation or submission
- The conclusions presented in the paper and this presentation are those of the authors alone

Focus of Review

- Systematic review of glyphosate literature with emphasis on its usefulness in regulatory decisions
  - *In vivo* nervous system endpoints appropriate for current regulatory risk assessments
    - Laboratory mammalian models (rats, mice)
  - *In vitro* studies of mammalian cell systems were considered supportive data
- Critical evaluation of methods and results, independent interpretation of outcomes
  - Expert judgment
  - Publicly available guidance: regulatory agencies, scientific publications
- Data presented in terms of severity and nature of effect, overall pattern and consistency of effect, graded dose-response, replications across studies, biological relevance, in relation to general toxicity

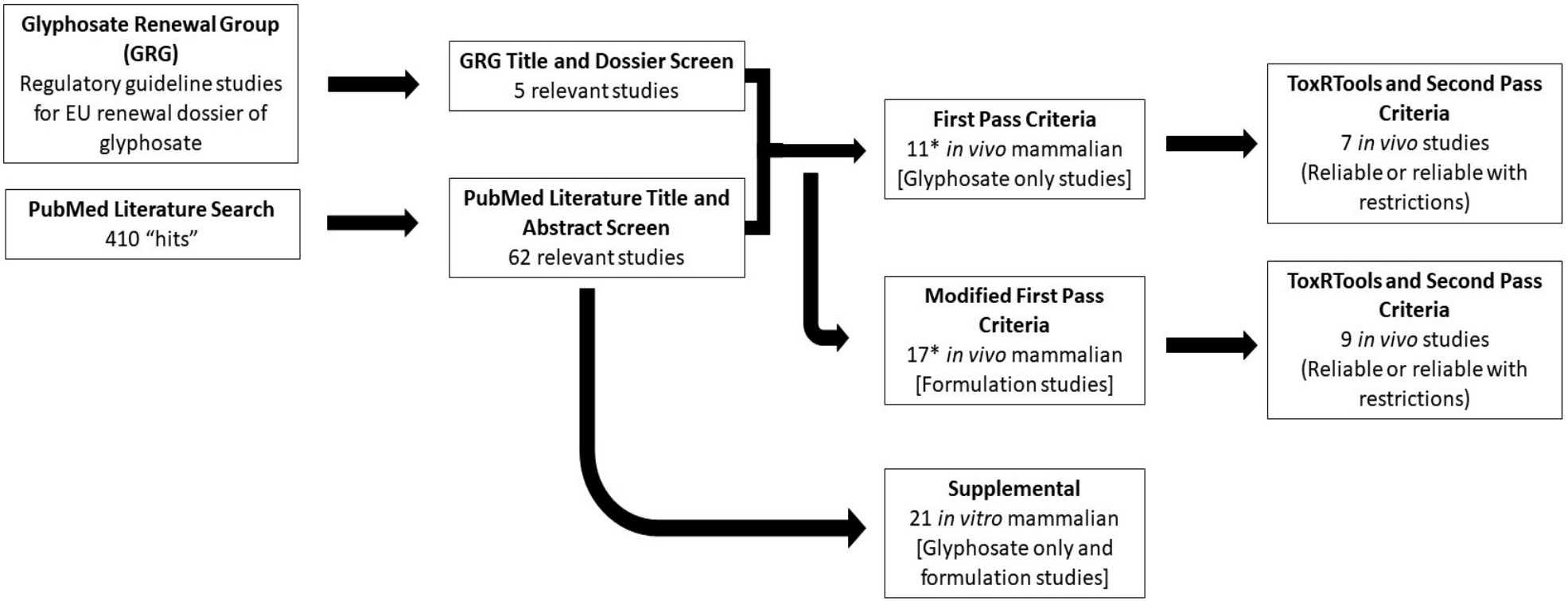
Literature Search

- Broad search on PubMed and ProQuest (which includes databases such as Medline, Toxline, others)
- First-pass inclusion criteria, including
  - Full experimental reports, publicly available
- Second-tier assessment
  - ToxRTools ranking with emphasis on experimental design and conduct, data analysis and reporting
  - Loosened criteria regarding formulation vehicle controls to allow inclusion of formulation studies
  - Overall ranking of 1 (reliable) to 3 (unreliable, excluded from review)

Methodology Deficiencies

- Critical deficiencies that led to some papers or specific endpoints being considered unreliable and therefore excluded
  - Litter was not the experimental unit in developmental studies
    - Litter of origin must be accounted for in experimental design and statistical procedures
    - 7 of 9 developmental studies did not do this
  - Inadequate methods were used for neuronal cell counting evaluations, not unbiased stereology
  - Subjective assessments were made without the observer being unaware of treatment

Search Results



*One study tested glyphosate alone as well as a formulation and is counted in both sections

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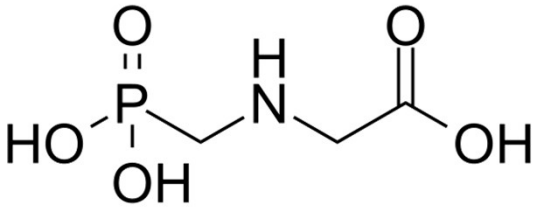
Glyphosate alone

- Available literature
  - 5 regulatory studies
  - Guideline studies: neurotoxicity (acute, 90-day), chronic cancer bioassay (1, 2 yr)
    - FOB, MA, neuropathology; p.o. (gavage or feed)
  - 2 published studies
    - MA, neurochemistry, neuropharmacology; i.p.
    - Neurochemistry; p.o.



BUT concerns with i.p. dosing:

- Absorption differences: p.o. << i.p.
- Time to peak effect differences: p.o. slower than i.p.
- Glyphosate is acidic, may → peritoneal irritation
- i.p. data less useful for regulatory decisions

Glyphosate alone Behavior/Neuropathology

- Regulatory studies
  - Acute: 2000 mg/kg p.o. (highest dose)
  - Repeated: ≥1077 mg/kg/d across studies (2 subchronic, 2 chronic)
    - Acute time of peak effect – clinical signs (↓ activity, gait and posture changes)
    - Sporadic behavioral effects* in most studies
    - No neuropathological changes in any study

Example of inconsistent effects: No clear dose-response, no greater effect with repeated dosing

Subchronic motor activity[1] % change from control where significant				
Dose intake (mg/kg/d)	2 wk	1 mo	2 mo	3 mo
M 77	↑58%	↑38%	--	--
395	--	--	--	↓47%
1499	--	--	--	--

*Inconsistent effects, considered random and not related to treatment

[1]SafePharm, 2006

Glyphosate alone
Behavior/ Neurochemistry

- One published study[1]
  - 3 doses (50-150 mg/kg/d) i.p., 6 injections over 2 weeks
    - ↓ MA after each dose (inconsistent across doses and each subsequent dose)
    - ↓ DA receptor binding (all doses) at 2 days (not 16) after last dose in 1 of 2 regions
    - No changes in monoamine neurotransmitter levels 2 or 16 days after last dose
    - Transiently ↓ localized release of DA (high dose) (microdialysis measurement)
    - No changes in TH^+-positive cell count 2 or 16 days after last dose (2 brain regions)
  - Kinetic aspects of i.p. dosing probably influence results and confound interpretations

[1]Hernández-Plata et al., 2015

Glyphosate alone Neurochemistry

- Second published study[1]
  - 4 doses (35-800 mg/kg/d) p.o., 3 hr after 6 days dosing
  - Alterations in each monoamine neurotransmitter and/or metabolites in at least 1 brain region (out of 5) at ≥75 mg/kg/d
  - Inconsistencies in dose-response over regions, for example:
    - Striatum (highest DA innervation) had ↓ DA (22%) only at highest dose
    - Midbrain (intermediate control DA levels) showed no dose-response at ≥ 75 mg/kg/d (13, 14, 17%)
    - Prefrontal cortex (lowest DA levels) had ↓ DA (48, 53, 83%)
  - Likely transient effect (compared to i.p. study)
  - Multiple statistical analyses without correction
  - Functional significance unknown

[1]Martínez et al., 2018

Glyphosate alone
Summary

- Regulatory studies
  - Only highest dose produced acute behavioral but no neuropathological effects
  - Studies were standardized and acceptable for risk assessment
- Two published studies
  - Effects on MA and neurochemistry at lower doses
  - But data limited in terms of dosing regimens, interpretability of endpoints, and route of exposure
  - Results less useful for regulatory purposes

Glyphosate Formulations

- Studies that have compared glyphosate alone to formulations show that most or all of the measured effects were due to formulations
- Quantitative comparisons cannot be made to glyphosate alone or between formulations
- Formulation components differ, change over time, confidential information

Glyphosate Formulations

- 9 published papers
  - 5 laboratories, each used different commercial products
  - Different routes of administration – oral gavage, intranasal, drinking water
  - Different exposure scenarios – acute, intermittent or daily dosing, 8 to 90 days
  - Different endpoints, different methodologies for same endpoints
  - Most used only one dose and/or a single time point
  - Few reported body weight or general toxicity

Glyphosate Formulations Behavior/Motor Activity

- MA most common endpoint across studies
- Some but not complete consistency in effects
  - Acute:
    - No effect (1 study) or both ↓ and ↑ depending on time and test measure (1 study)
  - Repeated:
    - No effect (1 study) or ↓ (4 studies*)

*One study used toxic doses that caused weight loss, decreased weight gain, and decreased relative brain weight

Glyphosate Formulations Behavior/Other Behaviors

- Anxiety behaviors
  - No acute effects (2 studies)
  - Repeated dosing: no effect (1 study) or ↑ anxiety (3 studies*)
- Depressive behaviors
  - Acute: No effect (1 study) or ↑ depression (1 study)
  - Repeated dosing: ↑ (1 study*), both no effect and ↑ depending on test (1 study), or both ↑ or ↓ depending on test (1 study)
- Cognitive behaviors
  - Acute: ↓ aversive memory (1 study)
  - Repeated dosing: ↓ recognition memory (2 studies*), ↓ working and ↓ aversive memory (1 study*)

*One study used toxic doses that caused weight loss, decreased weight gain, and decreased relative brain weight

Glyphosate Formulations
Limitations of Behavioral Data

- Activity differences can confound interpretation
- Different tests used across studies, little comparability
- At least one study used toxic doses, confounds specificity of neurological effects
- In some cases,
  - Inconsistencies in patterns of change
  - No clear impact of dose regimen
  - Data not clearly presented, raw data not provided
  - Pre-test differences that can confound subsequent test data

Glyphosate Formulations Neurochemistry/Neuropathology

- Neuronal cell count
  - One study: ↓ cells with DA and 5HT markers, but at toxic doses*
- Neurotransmitter enzymes
  - AChE: varied outcomes. No effect (1 study), ↓in 1 of 6 brain regions (1 study), or ↓ in 3 regions (1 study*)
  - Glutamate transaminases: 1 study, inconsistent ↓ and ↑ in different brain regions
- Neuropathology
  - One study: no histopathological changes

*Study used toxic doses that caused weight loss, decreased weight gain, and decreased relative brain weight

Glyphosate Formulations Developmental

- Two papers described appropriate litter allocations
  - Same laboratory (same study?)
- Slightly accelerated development of one behavior and one physical landmark (many measured)
  - ≤ One day faster, no dose-response
- Behavioral effects in adult offspring at PND45 and 90
  - ↓ MA, ↓ anxiety, ↓ recognition memory (pre-test differences)
- Neurochemical changes
  - ↓ glutamate transaminases in some regions; no consistent dose-response
  - ↓ AChE

Glyphosate Formulations Summary

- Behavior
  - Most studies showed various behavioral effects
  - Some inconsistencies and differences across studies weaken weight-of-evidence conclusions
- Neuropathology/neurochemistry
  - Fewer studies, varied measures with little overlap
  - Mostly negative or inconsistent effects
- Developmental
  - Behavioral and neurochemical effects, requires replication
- Being formulations, data not useful for risk assessment or quantitative comparisons across studies

Glyphosate
In vitro

- 21 identified studies
  - 2 excluded since they used formulations directly on cells
  - 12 came from same laboratory, validating tests for neurotoxicity screening
    - All showed no effects (glyphosate was negative control in chemical training set)
- Various effects in cell systems but using concentrations that were cytotoxic and/or unattainable in real life (e.g., ≥1 mM)
- One study reported altered cell migration and differentiation in neural stem cells at lower concentrations (≥ 4 µM)
  - But this was not seen in other papers that used similar cell systems and measures, requires replication

Summary of Literature

- Limited literature
  - Variable methods and results limit weight-of-evidence conclusions
- Published studies not sufficient to inform risk assessment, but may generate testable hypotheses for additional research
- More rigorous and broader studies of glyphosate alone are needed
  - Improve usefulness of published literature for risk assessment
  - Augment existing regulatory studies

Conclusions of Glyphosate Neurotoxicity

- Glyphosate alone: regulatory studies mostly negative, and two published papers showed potential effects but data inadequate for risk assessment
- Formulations: variety of effects but results were complicated and problematic, inadequate for quantitative risk assessment
- Almost all *in vitro* data were negative at relevant exposures

- This review did not result in conclusive evidence of glyphosate neurotoxicity that would inform risk assessment decisions

Thank you for your time!