Comments on Charge Questions for Consideration by EPA FIFRA SAP Review of Glyphosate

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David Kirkland, PhD
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Consumer Exposure and Glyphosate Residues
Introduction

• Dietary exposure is the principal route that the general population is exposed through glyphosate
  – EPA’s assessment presents a unrefined dietary risk assessment
    – A refined analysis is available from Europe
  – As carcinogenicity studies are generally conducted at high doses, it is useful to understand how these doses compare to doses experienced in the general population through residues on food

• John Acquavella will discuss operators
Risk Assessment

• Identify Hazard
• Determine Exposure
• Calculate Risk which may result from exposures to a substance
  – Risk = f (hazard, exposure)
Calculating theoretical consumer exposure

- Basic paradigm described previously is used
- Start with very conservative assumptions
- Can apply various levels of refinement dependant on
  - Data available
  - How far you need to refine exposure
- Regulatory authorities around the world generally do not refine below a level where safety can be clearly demonstrated
Glyphosate and Consumer Exposure

• Low dietary exposure
• Low GI absorption via oral ingestion (ca. 20%)
• Very low dermal absorption (human in vitro < 1% absorption)
• Virtually all absorbed glyphosate is excreted in urine
• Virtually all studies show no detectable levels in breast milk
  – One study using a non-validated method of analysis reported biologically implausible results
Theoretical Consumer Exposure in The EPA Cancer Assessment

- Carried out using DEEM
  - Very conservative approach
  - Equivalent to the Theoretical Maximum Daily Intake or TMDI in Europe
  - Used default adjustment factors
    - Takes account of potential increases in residues as a result of processing
TMDI Can be Refined with Readily Available and Relevant Information

- A refined assessment has been conducted in Europe
  - TMDI: All foods contain glyphosate residues at the MRL
  - National Estimate Dietary Intakes (NEDI): Replaces MRL with the supervised trials median residue (STMR)
  - Refined NEDI: Includes changes in residue levels during processing and also uses residues found in monitoring
  - National Refinements: Accounts for specific consumption patterns in Ireland and Germany based on cereals and citrus processing
TMDI Can be Refined with Readily Available and Relevant Information

Source: Stephenson, C. and Harris, C. An assessment of dietary exposure to glyphosate using refined deterministic and probabilistic methods. Food and Chemical Toxicology 95 (2016) 28-41
Comparing Chronic Intakes: Expressing Conservatism

<table>
<thead>
<tr>
<th>Source/Study</th>
<th>Intake (mg/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. EPA (DEEM)</td>
<td>0.058–0.223</td>
</tr>
<tr>
<td>Europe (PRiMo)</td>
<td>0.4005</td>
</tr>
<tr>
<td>Europe (PRiMo) median residues</td>
<td>0.018</td>
</tr>
<tr>
<td>Europe (PRiMo) processing</td>
<td>0.0105</td>
</tr>
<tr>
<td>Joint Meeting on Pesticide Residues (JMPR)</td>
<td>0.002–0.013</td>
</tr>
<tr>
<td>Biomonitoring</td>
<td>0.000005-0.0063</td>
</tr>
</tbody>
</table>

Sources:

EFSA. PRIMo – Pesticide Residue Intake Model. PRIMo – Pesticide Residue Intake Model
https://www.efsa.europa.eu/sites/default/files/assets/calculationacutechronic_2.xls

Illustration from Solomon paper

Operator Exposure

Estimated exposure range (passive dosimeter)
0.000001 - 0.054 mg/kg b.m./d

Reference Dose and Toxicology Studies

JMPR/WHO Chronic ADI, 1 mg/kg b.m./d

EPA Chronic RDF, 1.75 mg/kg b.m./d

Relevant doses in toxicology studies
50 - 5400 mg/kg b.m./d

Measured exposure range (biomonitoring)
0.000013 - 0.0046 mg/kg b.m./d

EFSA ADI, 0.5 mg/kg b.m./d

USEPA dietary exposure (DEEM model)
(tolerance-level residues)
0.058 - 0.223 mg/kg b.m./d

USEPA water exposure estimates
-ground and surface water
0.000068 - 0.00027 mg/kg b.m./d
Based on maximum ground water monitoring and est'd surface water conc. from direct application

JMPR dietary exposure (GEMS model)
(median residues)
0.002 - 0.013 mg/kg b.m./d

Food, Water, and Bystander Exposure

Measured general population exposure range
(biomonitoring)
0.000005 - 0.00083 mg/kg b.m./d

RfD or ADI as a consumed dose

RfD or ADI as a systemic dose
What other refinements could be made?

• Adjust for percentage of crop treated
  – Current model assumes
    – All crop eaten will have been treated with glyphosate
    – Will contain residues at the maximum legally permitted limit
    – Not realistic for assessing lifetime exposure
Consumer Exposure Risk Assessment

• If the EPA’s Reference Dose of 1.75 mg/kg bw/day is utilised, the EPA calculated intakes for consumers would be of 3.3–12.7% of this value
• Clearly demonstrates large margins of safety for consumers
Charge Question #2: Epidemiology
EPA Charge Questions

• The Agency’s literature search identified all the relevant studies

• The Agency’s application of quality criteria in assessing the evidence and deemphasizing lower quality studies are appropriate
  – For NHL, there is a clear quality difference between the one cohort study and the 6 case control studies

• The Agency’s conclusions of no association for solid tumors, leukemia, Hodgkin’s lymphoma, and inadequate evidence for multiple myeloma and NHL, are appropriate
Three Themes

• Glyphosate biomonitoring in context
• Exposure differences: NHL cohort versus case control studies
• Analytic considerations:
  – Analytic selection bias in the 2 Swedish NHL case control studies
  – NHL latency – how the cohort and case control studies compare
  – Systematic error and p values for NHL random effects meta-analysis
Urine Biomonitoring Provides Reliable Dose Estimates to Assess Risk

- Glyphosate formulations have very low vapor pressure and dermal penetrability
- Once absorbed, glyphosate is excreted rapidly as the parent compound with no potential for bioaccumulation
- Measuring parent molecule in urine provides a reliable measure of internal dose (Niemann et al. 2015; Solomon 2016)

Sources:
Solomon K. Glyphosate in the general population and in applicators: a critical review of studies on exposures. Crit Rev Toxicol 2016; 46 suppl 1:
Farm Family Exposure Study (FFES)

- Biomonitored farmer-applicators in S. Carolina and Minnesota
  - Glyphosate (n = 48), 2,4-D (n = 32), chlorpyrifos (n = 32)
- Collected 24 hour urine days -1, 0, 1, 2, 3; high compliance (see Baker et al. 2005)
- Glyphosate applications were substantial
  - 1/3rd 10 to 45 acres; 1/3rd 46 to 124 acres; 1/3rd 125 to 439 acres
- Farmer questionnaire about application practices
- Trained field observers recorded actual application practices

Sources:

FFES Glyphosate Urinary levels

Day of Study

<table>
<thead>
<tr>
<th>#</th>
<th>LOD</th>
<th>GeoMean</th>
<th>SD</th>
<th>% &gt; LOD</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>1 ppb</td>
<td>3 ppb</td>
<td>6 ppb</td>
<td>60%</td>
<td>223 ppb</td>
</tr>
</tbody>
</table>

FFES Urinary Levels Varied by Chemical

FFES Internal Doses Varied by Chemical

Cumulative Probability (%)

Systemic Dose in mg/kg BW

Glyphosate Biomonitoring Doses << Regulatory Guidelines and Toxicology Doses

**Human Biomonitoring**

- Measured exposure range (biomonitoring): 0.000013–0.0046 mg/kg b.m./d

**Reference Dose and Toxicology Studies**

- JMPR/WHO Chronic ADI, 1 mg/kg b.m./d
- EPA Chronic RfD, 1.75 mg/kg b.m./d
- EFSA ADI, 0.5 mg/kg b.m./d
- Relevant doses in toxicology studies: 50–5,400 mg/kg b.m./d

Exposure in Epidemiologic Studies
# NHL Studies for Glyphosate by Study Type

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Study Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDuffie 2001</td>
<td>Canada</td>
<td>Case control</td>
</tr>
<tr>
<td>Hardell 2002</td>
<td>Sweden</td>
<td>Case-control</td>
</tr>
<tr>
<td>DeRoos 2003</td>
<td>USA</td>
<td>Case-control</td>
</tr>
<tr>
<td>DeRoos 2005</td>
<td>USA</td>
<td>Cohort</td>
</tr>
<tr>
<td>Eriksson 2008</td>
<td>Sweden</td>
<td>Case-control</td>
</tr>
<tr>
<td>Orsi 2009</td>
<td>France</td>
<td>Case-control</td>
</tr>
<tr>
<td>Cocco 2013</td>
<td>EU (6 countries)</td>
<td>Case-control</td>
</tr>
</tbody>
</table>

### NHL Case Control Studies
#### Glyphosate Exposure Frequency and Results

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of NHL Cases</th>
<th>Number of (%) Exposed Cases</th>
<th>Exposure Metric</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDuffie 2001</td>
<td>517</td>
<td>51 (9.9%)</td>
<td>Any use &gt; 2 days/year</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>Hardell 2002</td>
<td>515</td>
<td>8 (1.6%)</td>
<td>Any use</td>
</tr>
<tr>
<td>DeRoos 2003</td>
<td>650</td>
<td>36 (5.5%)</td>
<td>Any use</td>
</tr>
<tr>
<td>Eriksson 2008</td>
<td>910</td>
<td>29 (3.2%)</td>
<td>Any use &gt; 10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>Orsi 2009</td>
<td>244</td>
<td>12 (4.9%)</td>
<td>Any use</td>
</tr>
<tr>
<td>Cocco 2013</td>
<td>2348</td>
<td>4 (0.2%)</td>
<td>Any use</td>
</tr>
</tbody>
</table>

Adequate Exposure

• Common practice in cohort studies: exclude those with limited exposure potential.
  – “… statistical analysis excluded workers exposed to benzene for < 6 months ... to reduce the potential for including serious health outcomes unlikely to be due to benzene exposure.“

Agricultural Health Study Exposure

- Cohort recruitment focused on frequent pesticide users
- Recruitment of licensed applicators implies knowledgeable self-reporters
- Exposure was collected at enrollment before follow-up for health outcomes; disease cannot affect reporting
- No proxy respondents

Glyphosate Exposure Frequency >> in Ag Health Study than Case Control Studies

- 76% of analysis cohort and 77% of the 92 NHL cases reported glyphosate use
- Cumulative exposure analyses (De Roos et al 2005) suggest a substantial % of cohort members have 21 to 56 or 57+ days of use

<table>
<thead>
<tr>
<th>Number of Exposed NHL Cases</th>
<th>Cumulative Days of Use</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 (48%)</td>
<td>1 to 20 days</td>
<td>1.0</td>
</tr>
<tr>
<td>15 (25%)</td>
<td>21 to 56 days</td>
<td>0.7</td>
</tr>
<tr>
<td>17 (28%)</td>
<td>57 to 2678 days</td>
<td>0.9</td>
</tr>
</tbody>
</table>

- Days of use >> in AHS than in case control studies

Analytic Issues

1. Analytic selection bias in case control studies
2. NHL latency differences across studies
3. Meta-analysis random effects models assumptions
Analytic Selection Bias
Case Control and Cohort Designs are Related

- Can think of a case control study as a cohort study where you identify all the cases and a sample of the underlying population that gave rise to the cases (viz. the controls).
- The controls provide an estimate of the exposure and covariate(s) prevalence in the population that gave rise to the cases.
- The ratio of exposure odds for cases and controls estimates the rate ratio that you would get from a cohort study.

Selection Bias in the Analysis

- The 2 Swedish NHL case control studies (i.e., Hardell 2002; Eriksson 2008) defined “unexposed” as no exposure to glyphosate or other pesticides.
- But, the population that gave rise to the NHL cases included those with exposure to other pesticides.


Analytic Selection Bias Illustrated in Brown et al. 1993

Brown et al. studied 173 MM cases and 650 controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>Unexposed</td>
<td>162</td>
<td>610</td>
</tr>
</tbody>
</table>

% cases exposed = 6%

% controls exposed = 6%

Defining unexposed as non-farmers excludes 100 cases (58% of total) and 338 controls (52% of total) and changes exposure prevalence

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>Unexposed</td>
<td>62</td>
<td>272</td>
</tr>
</tbody>
</table>

% cases exposed = 15%

% controls exposed = 13%

Also, precludes controlling for other farm exposures

Sources:

Latency: NHL Cohort & Case Control Studies

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>Herbicide</td>
<td>1959</td>
<td>Triazine</td>
<td>75–82</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Metolachlor</td>
<td>Herbicide</td>
<td>1976</td>
<td>Acetanilide</td>
<td>63–69</td>
<td>2</td>
<td>3</td>
<td>n/a</td>
<td>++++</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Herbicide</td>
<td>1974</td>
<td>Phosphonoglycine</td>
<td>34–38</td>
<td>5</td>
<td>17</td>
<td>n/a</td>
<td>++++</td>
</tr>
<tr>
<td>Acetochlor</td>
<td>Herbicide</td>
<td>1994</td>
<td>Acetanilide</td>
<td>31–36</td>
<td>7</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2,4-D</td>
<td>Herbicide</td>
<td>1947</td>
<td>Phenoxy acetic acid</td>
<td>29–33</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>Herbicide</td>
<td>1974</td>
<td>Dinitroaniline</td>
<td>24–28</td>
<td>9</td>
<td>10</td>
<td>n/a</td>
<td>++++</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>Herbicide</td>
<td>1963</td>
<td>Dinitroaniline</td>
<td>21–25</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Cyanazine</td>
<td>Herbicide</td>
<td>1971</td>
<td>Triazine</td>
<td>18–22</td>
<td>11</td>
<td>7</td>
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<td>n/a</td>
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<tr>
<td>Alachlor</td>
<td>Herbicide</td>
<td>1969</td>
<td>Acetanilide</td>
<td>13–16</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Chlorpyrifos</td>
<td>Insecticide</td>
<td>1965</td>
<td>Organophosphate</td>
<td>9–13</td>
<td>14</td>
<td>14</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Herbicide</td>
<td>1966</td>
<td>Chloranil</td>
<td>7–10</td>
<td>15</td>
<td>19</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>Dicamba</td>
<td>Herbicide</td>
<td>1956</td>
<td>Benzoic Acid</td>
<td>7–10</td>
<td>16</td>
<td>23</td>
<td>14</td>
<td>25</td>
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<tr>
<td>Mancozeb</td>
<td>Fungicide</td>
<td>1962</td>
<td>Carbamates</td>
<td>7–10</td>
<td>17</td>
<td>21</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>EPTC</td>
<td>Herbicide</td>
<td>1958</td>
<td>Carbamates</td>
<td>9–13</td>
<td>18</td>
<td>8</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Terbufos</td>
<td>Insecticide</td>
<td>1974</td>
<td>Organophosphate</td>
<td>6–9</td>
<td>19</td>
<td>11</td>
<td>n/a</td>
<td>++++</td>
</tr>
<tr>
<td>Dimethenamid</td>
<td>Herbicide</td>
<td>1993</td>
<td>Acetanilide</td>
<td>6–9</td>
<td>20</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Bentazone</td>
<td>Herbicide</td>
<td>1985</td>
<td>Thiadiazine</td>
<td>6–8</td>
<td>21</td>
<td>15</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Propanil</td>
<td>Herbicide</td>
<td>1960</td>
<td>Acetanilide</td>
<td>6–8</td>
<td>22</td>
<td>13</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Simazine</td>
<td>Herbicide</td>
<td>1956</td>
<td>Triazine</td>
<td>5–7</td>
<td>23</td>
<td>28</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>MCPA</td>
<td>Herbicide</td>
<td>1945</td>
<td>Phenoxy acetic acid</td>
<td>5–6</td>
<td>24</td>
<td>25</td>
<td>n/a</td>
<td>14</td>
</tr>
</tbody>
</table>

# Latency: NHL Studies for Glyphosate

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Years Cases Identified</th>
<th>Years since 1974 Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDuffie 2001</td>
<td>Canada</td>
<td>1991–1994</td>
<td>17 to 20</td>
</tr>
<tr>
<td>Hardell 2002</td>
<td>Sweden</td>
<td>1987–1990 (NHL)</td>
<td>13 to 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1987–1992 (HCL)</td>
<td>13 to 18</td>
</tr>
<tr>
<td>Orsi 2009</td>
<td>France</td>
<td>2000–4</td>
<td>26 to 30</td>
</tr>
<tr>
<td>Cocco 2013</td>
<td>EU (6 countries)</td>
<td>1998–2003</td>
<td>24 to 29</td>
</tr>
<tr>
<td>De Roos 2005</td>
<td>USA</td>
<td>1993–2001</td>
<td>19 to 27</td>
</tr>
</tbody>
</table>

Meta-analysis Random Effects Assumptions
Original Articles

"... probabilistic interpretations of conventional statistics are rarely justified and ... may encourage misinterpretations of non-randomized studies."

Randomization, Statistics, and Causal Inference

Sander Greenland

This paper reviews the role of statistics in causal inference. Special attention is given to the need for randomization to justify causal inferences from conventional statistics, and the need for random sampling to justify descriptive inferences. In most epidemiologic studies, randomization and random sampling play little or no role in the assembly of study cohorts. I therefore conclude that probabilistic interpretations of conventional statistics are rarely justified, and that such interpretations may encourage misinterpretation of nonrandomized studies. Possible remedies for this problem include deemphasizing inferential statistics in favor of data descriptors, and adopting statistical techniques based on more realistic probability models than those in common use. (Epidemiology 1990;1:421–429)

Keywords: statistics, causal inference, epidemiologic methods.

In this paper, I wish to review some ideas that, though of long history, seem too often overlooked when epidemiologists use statistical methods. My topic is the role of statistics in causal inference, but I am not here concerned with the issue of confidence intervals versus P values (an issue which, I think, was successfully resolved by Poole (1)). Nor am I here concerned with arguments or philosophies, each of them has attempted to clarify the meaning and limitations of inferential statistics when randomization assumptions fail to hold. Many other writers have put forth either parallel or dissenting views, but my intention is to review some logic rather than the literature (which is vast). I begin with a review of heuristic arguments leading to Fisher's exact test for
Validity Factors in the Glyphosate NHL Studies

<table>
<thead>
<tr>
<th>Potential Bias</th>
<th>Ag Health (cohort) Study</th>
<th>Case Control Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recall bias</td>
<td>no</td>
<td>All 6 studies</td>
</tr>
<tr>
<td>Selection bias</td>
<td>In some analyses</td>
<td>In 4 of 6 studies</td>
</tr>
<tr>
<td>Proxy respondents</td>
<td>no</td>
<td>In 3 of 6 studies</td>
</tr>
<tr>
<td>Confounding control</td>
<td>extensive</td>
<td>Poor 5 of 6 studies</td>
</tr>
</tbody>
</table>
Meta-Analyses and Systematic Error

- When differences between studies are due to systematic factors, the assumptions underlying random effects models for meta-analysis are violated.
- Therefore calculated p values and 95% CIs are not correct.

Sources:
Conclusions: EPA Charge Questions

- The Agency’s literature search identified all the relevant studies.

- The Agency’s application of quality criteria in assessing the evidence and deemphasizing lower quality studies is appropriate.
  - For NHL, the Eriksson 2008 case control study should not be included in the high quality category; the Ag Health Study cohort findings are the most reliable.

- The Agency’s conclusions of no association for solid tumors, leukemia, Hodgkin’s lymphoma, and inadequate evidence for multiple myeloma and NHL, are appropriate.
Charge Question #3: Animal Cancer Bioassays
Charge Question #3:

- EPA’s review and evaluation of relevant laboratory animal carcinogenicity studies
  - Appropriate treatment of 9 rat and 6 mouse cancer bioassays for consideration in the weight-of-evidence analysis
    - However, one rat study (Burnett) was conducted on NNG not glyphosate
  - Appropriate reliance on Guidelines for Carcinogen Risk Assessment (EPA, 2005) and Carcinogenicity Test Guidance (EPA, OECD) for study acceptability
  - Appropriate WoE conclusions that glyphosate is not a carcinogen in any individual rat or mouse study or an animal carcinogen in an integrated WoE analysis of all studies

- Weight-of-Evidence evaluation
- Issues identified for study evaluation:
  - Appropriateness of dose selection to real-world exposure
  - Occurrence of pre-neoplastic/non-neoplastic lesions to support tumor findings
  - Evidence of progression to malignancy
  - Reproducibility of tumor findings
  - Historical control data to inform significance of tumors
  - Statistical evidence of a dose-response
  - Statistical and biological significance of tumor incidence
Animal Carcinogenicity Studies Reviewed by EPA

<table>
<thead>
<tr>
<th>Animal Study</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAT STUDIES</strong></td>
<td></td>
</tr>
<tr>
<td>Burnett et al. (1979)</td>
<td>Albino rats</td>
</tr>
<tr>
<td>Lankas (1981)</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Stout and Ruecker (1990)</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Atkinson et al. (1993a)</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Brammer. (2001)</td>
<td>Wistar rats</td>
</tr>
<tr>
<td>Pavkov and Wyand (1987)</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Suresh (1996)</td>
<td>Wistar rats</td>
</tr>
<tr>
<td>Enemoto (1997)</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Wood et al. (2009a)</td>
<td>Wistar rats</td>
</tr>
<tr>
<td><strong>MOUSE STUDIES</strong></td>
<td></td>
</tr>
<tr>
<td>Reyna and Gordon (1973)</td>
<td>Swiss white mice</td>
</tr>
<tr>
<td>Knezevich and Hogan (1983)</td>
<td>CD-1 mice</td>
</tr>
<tr>
<td>Atkinson et al. (1993b)</td>
<td>CD-1 mice</td>
</tr>
<tr>
<td>Wood et al. (2009b)</td>
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<td>Sugimoto (1997)</td>
<td>CD-1 mice</td>
</tr>
<tr>
<td>Pavkov and Turnier (1987)</td>
<td>CD-1 mice</td>
</tr>
</tbody>
</table>
Dose Selection Considerations in WoE Evaluation of Studies

<table>
<thead>
<tr>
<th>Animal Study</th>
<th>Strain</th>
<th>Adequately High Dosing</th>
<th>Excessively High Dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAT STUDIES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burnett et al. (1979)</td>
<td>Albino rats</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lankas (1981)</td>
<td>Sprague-Dawley rats</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Stout and Ruecker (1990)</td>
<td>Sprague-Dawley rats</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Atkinson et al. (1993a)</td>
<td>Sprague-Dawley rats</td>
<td>Yes</td>
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<tr>
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<td>Sprague-Dawley rats</td>
<td>Yes</td>
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<tr>
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<td>Wistar rats</td>
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<td>Yes</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reyna and Gordon (1973)</td>
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<td>No</td>
<td>No</td>
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<tr>
<td>Knezevich and Hogan (1983)</td>
<td>CD-1 mice</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Atkinson et al. (1993b)</td>
<td>CD-1 mice</td>
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<td>Yes</td>
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<tr>
<td>Wood et al. (2009b)</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Sugimoto (1997)</td>
<td>CD-1 mice</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pavkov and Turnier (1987)</td>
<td>CD-1 mice</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Dose Selection Considerations

- **Adequately high dosing?**
  - “Yes” for 11 of 15 studies
  - No statistical significance by pair-wise comparison for any high dose

- **Met or exceeded Limit Dose of > 1,000 mg/kg-bw/day?**
  - “Yes” for 10 of 15 studies
  - Less weight given to doses ≥ Limit Dose when widely separated from human exposures
Relevance of 1000 mg/kg/day Limit Dose to Human Risk Assessment

- EPA Chronic Toxicity-Carcinogenicity Test Guidance (EPA 870.4300) sets a Limit Dose of 1000 mg/kg/day unless:
  - “...expected human exposure may indicate the need for a higher dose level.”

- OECD Chronic Toxicity-Carcinogenicity Test Guidance (OECD 453) states:
  - “A limit of 1000 mg/kg body weight/day may apply except when human exposure indicates the need for a higher dose level to be used [emphasis added].”

- Human exposure data are available from multiple high-quality human biomonitoring studies (reviewed in Niemann et al., 2014)
  - Average human external doses are less than 0.0005 mg/kg/day
  - Human exposure is 2,000,000-fold lower than 1000 mg/kg/day limit dose in rodent bioassays
Charge Question #3:

- EPA’s conclusion of an absence of preneoplastic or related non-neoplastic lesions and lack of progression to malignancy
  - Terminal sacrifice data do not show progression
  - Interim sacrifice data provide no supporting evidence of preneoplastic lesions
  - No evidence of malignant progression of tumors
- Based on these criteria in part, EPA found no evidence of carcinogenicity in any study
Charge Question #3:

• EPA’s interpretation of conflicting evidence and reproducibility for these studies
  – EPA correctly looked across the studies to evaluate consistency and coherence
  – When the data are evaluated in totality there are no consistent findings
    – Differing or absence of tumors in differing studies
    – No coherence as tumors observed only in one sex or species
    – Lack of statistical significance when adjusted for multiple comparisons and for rare vs common tumor considerations
Charge Question #3:

- EPA’s methodology and interpretation of statistical analyses
  - EPA appropriately calculated all statistics
  - Absence of statistical significance further evidenced by impact of rare vs common tumor considerations on pair-wise and trend analyses
Statistical Analyses to Avoid Excessive False Positives

- Statistical decision rules developed based on whether tumors are rare (background rate \( \leq 1\% \)) or common \( (>1\%) \)
  - NTP (Haseman et al., 1983)
  - FDA (2001)

<table>
<thead>
<tr>
<th>Agency</th>
<th>Rare Tumors (p value)</th>
<th>Common Tumors (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTP – pair-wise comparisons</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>FDA – trend tests</td>
<td>0.025</td>
<td>0.005</td>
</tr>
<tr>
<td>EPA Issue Paper</td>
<td>0.05 for trends and pair-wise</td>
<td></td>
</tr>
</tbody>
</table>
Statistical Adjustment Considerations for Rodent Bioassays

• EPA used Sidak method in pair-wise comparison
  – Only adjusts for multiple dose pair-wise comparisons
  – Does not consider impact of rare vs common tumors

• Haseman (1983) supports pair-wise \( p \)-value adjustment to 0.01 for common tumors

• FDA adjusts \( p \)-values for trend tests of rare and common tumors based on assumption of availability of 2 sex- and 2 species-bioassays
  – 15 studies for glyphosate
Charge Question #3:

- EPA’s use of historical control data to inform significance of tumor findings
  - Historical control incidence was reported for some, but not all, tumor types
    - EPA commented on historical control data viewed as further informing evaluation of 4 tumor types
  - Historical control data indicates all statistically significant identified findings (pair-wise and/or trend) are common spontaneous tumors
Analysis Using FDA and Haseman (1983) Decision Rules

• Only one study with a significant pair-wise comparison with Sidak correction (Lankas, 1981 – testes)
  – Not significant by NTP pair-wise decision rules
  – High dose much lower than other 8 rat bioassays
    – Testes response not observed in any other study

• Nine tumor sites with significant trend tests under EPA criteria of $p = 0.05$
  – Only two tumor trends (male mouse hemangiosarcoma and female mouse hemangioma) remain statistically significant under FDA trend decision rule
    – Both included doses $\geq 1,000$ mg/kg-bw/day (limit dose)
    – No tumor incidence in controls despite high background rates in historical controls
Charge Question #3:

• EPA’s conclusion that tumors observed at high-doses are not relevant for human health risk assessment
  
  – High doses in bioassays are substantially separated from real-world human exposures and EPA RfD of 1.75 mg/kg/day
  
  – No pair-wise tumor responses were statistically significant
  
  – High-dose tumor findings were limited to single sex and/or species, and were not replicated across 15 available rat and mouse bioassays
Charge Question #3: Additional Comments on Statistical Analysis
Focus of Presentation

• Statistical analysis of glyphosate tumor data

• Commentary on other statistical analyses of glyphosate tumor data presented in comments to OPP report (Portier; Tarone)
Glyphosate Rodent Carcinogenicity Data Considered by the EPA

- 9 rat studies
- 6 mouse studies
- 100s of potential tumor trends examined
  - Would be expected to result in some chance findings of significant ($p<0.05$) trends
Key Question 1

• Does the overall frequency of significant trends reported in the 15 studies exceed what would be expected by chance?
Key Question 2

• Is there a consistency of target sites reporting significant trends across the 15 studies?
Key Question 3

• Are there one or more tumor trends reported in the 15 studies that are so strong statistically that they would be extremely unlikely to occur by chance?
### Frequency of Significant Trends

<table>
<thead>
<tr>
<th></th>
<th>Estimated Number</th>
<th>Significant trends Expected (.05)</th>
<th>Significant Trends Observed (.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Trend Tests</td>
<td>568</td>
<td>28.4</td>
<td>11</td>
</tr>
<tr>
<td>Trend Tests for Unique Tumor Sites/Types</td>
<td>368</td>
<td>18.4</td>
<td>7</td>
</tr>
<tr>
<td>Strongest Trend (.002)</td>
<td>1</td>
<td>1.14</td>
<td>1</td>
</tr>
</tbody>
</table>
**Significant Trends Observed: No Consistency or Replication**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sex-Species</th>
<th>Target Site</th>
<th>Replicated?</th>
<th>EPA Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lankas (1981)</td>
<td>Male Rat</td>
<td>Testis</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Wood (2009)</td>
<td>Female Rat</td>
<td>Mammary Gland</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Stout (1990)</td>
<td>Female Rat</td>
<td>Thyroid Gland</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Brammer (2001)</td>
<td>Male Rat</td>
<td>Liver</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Sugimoto (1997)</td>
<td>Female Mouse</td>
<td>Hemangioma</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Wood (2009)</td>
<td>Male Mouse</td>
<td>Malignant Lymphoma</td>
<td>No</td>
<td>Not compound related</td>
</tr>
<tr>
<td>Atkinson (1993)</td>
<td>Male Mouse</td>
<td>Hemangiosarcoma</td>
<td>No</td>
<td>Not compound related</td>
</tr>
</tbody>
</table>
Key Question 1

- Does the overall frequency of significant trends exceed what would be expected by chance?
  - No.
    - Frequency of tumors consistent with chance expectation
Key Question 2

• Is there a consistency of target sites across the studies that show significant trends?

• No.
  – Significant trends observed show no consistency of tumor type and no replication
Key Question 3

• Are there one or more tumor trends that are so strong statistically that they would be extremely unlikely to occur by chance?

• No.
  – Strongest trend observed is consistent with chance expectation
Conclusion

• Glyphosate is not carcinogenic in mice or rats
Conclusions of Other Statistical Evaluations

• **Dr. Robert Tarone:** There is no “convincing evidence that glyphosate induces renal tumors, lymphomas, or hemangiosarcomas in male mice.”

• **Dr. Chris Portier:** “There is very strong evidence in mice for renal tumors, hemangiosarcomas, and malignant lymphomas”. “The thyroid tumors in female rats should be considered a positive finding.”
Dr. Portier’s Evaluation of Glyphosate Rodent Cancer Data

• Used an approximate rather than an exact trend test

• The EPA apparently used exact trend tests, which led Dr. Portier to the mistaken conclusion that the EPA was using two-sided rather than one-sided tests
Dr. Portier’s Evaluation of Glyphosate Rodent Cancer Data

- When questioned by Dr. Tarone regarding the exaggerated statistical significance associated with the approximate trend test, Dr. Portier conceded that “I agree with Dr. Tarone that, in cases where tumors are rare, the approximate p-value can overstate the significance of the findings.” (Portier 11/16 supp. comments)

- Dr. Portier continues to assert that the pooled results for kidney tumors, malignant lymphoma, and hemangiosarcoma are significant
# Comparison of Approximate and Exact Trend Test Results

<table>
<thead>
<tr>
<th>Study</th>
<th>Kidney Tumor Rates</th>
<th>Approximate Test</th>
<th>Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knezevich (1983)</td>
<td>1/49, 0/49, 1/50, 3/50</td>
<td>0.03</td>
<td>0.065</td>
</tr>
<tr>
<td>Sugimoto (1997)</td>
<td>0/50, 0/50, 0/50, 2/50</td>
<td>0.008</td>
<td>0.062</td>
</tr>
<tr>
<td>Kumar (2001)</td>
<td>0/49, 0/49, 1/50, 2/50</td>
<td>0.04</td>
<td>0.063</td>
</tr>
<tr>
<td>Atkinson (1993)</td>
<td>2/50, 2/50, 0/50, 0/50</td>
<td>0.94</td>
<td>0.98 (inverse trend)</td>
</tr>
<tr>
<td>Wood (2009)</td>
<td>0/51, 0/51, 0/51, 0/51</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Comparison of Approximate and Exact Trend Test Results

• Note that none of the three kidney tumor trends that were apparently significant by an approximate test are significant by an exact test.

• Note that the strongest (and only statistically significant) trend is an inverse trend (i.e., a decreasing kidney tumor incidence)
Dr. Portier’s Evaluation of Glyphosate Rodent Cancer Data

- Poly-3 test was inappropriately applied
  - Poly-3 test requires survival data for individual rodents
  - Poly-3 test is designed to correct for survival differences within a study, not to extrapolate 18 month tumor rates to 24 month tumor rates
  - When “adjusting” 18-month study tumor rates, Dr. Portier assumed that all tumor free animals survived until the end of the study
  - No Poly-3 adjustment was conducted for 24-month studies
Dr. Portier’s Evaluation of Glyphosate Rodent Cancer Data

• Considers thyroid c-cell tumors in female rats in Stout study (1990) to be a positive finding
  – Marginally significant trend (2/57, 2/60, 7/59, 6/55) (p = 0.04)
  – Concurrent control rate is abnormally low
  – This significant trend is almost certainly a false positive in light of hundreds of trend tests carried out in rats
Dr. Portier’s Evaluation of Glyphosate Rodent Cancer Data

- Pooling of the mouse data over studies is flawed
  - Cannot combine 18 and 24 month observed tumor rates
  - Poly-3 rates are flawed as noted above
  - Cannot combine disparate doses (differing by as much as 20-fold)
  - Improper to pool data over different strains, labs, and time frames
Proper Use of Historical Control Data

- Study duration should be comparable in historical and concurrent control studies.

- Pathology protocols should be comparable. For example, historical control data for comparison with kidney tumor data in the 1983 Knevich mouse study does not have the same level of review and additional step sectioning that was present in the Knevich study and, therefore, is not a proper comparison.
Conclusion

• Proper statistical analyses of the rodent carcinogenicity data strongly supports OPP’s conclusion that glyphosate does not cause tumors in rodents
Charge Question #4: Genotoxicity
Weight-of-Evidence Evaluation of the Genotoxicity of Glyphosate

Professor David Kirkland, BSc Ph.D.
Kirkland Consulting
Tadcaster, UK
EPA Used Sound Approach to Evaluating Genotoxicity

- Genotoxicity data from many test systems and endpoints.
- Assessment focuses only on test systems that EPA considered relevant for assessing genotoxic risks in humans.
- The totality of the genetic toxicology information evaluated using a weight of evidence approach
  - Involves integration of *in vitro* and *in vivo* results as well as an overall evaluation of the quality, consistency, reproducibility, magnitude of response, dose-response relationship and relevance of the findings.
- Studies evaluating gene mutations and chromosomal aberrations (i.e. permanent DNA damage) given more weight than DNA events that may be transient or reversible such as primary DNA damage (e.g., comet assays).
- *In vivo* studies in mammals were given the greatest weight
  - More weight given to doses and routes of administration considered relevant for evaluating genotoxic risk based on human exposure.
EPA Conclusions

- No convincing evidence that glyphosate induces mutations *in vivo* via the oral route.
- When administered by i.p. injection, the micronucleus (MN) studies were predominantly negative.
  - In the two cases where an increase in MN were reported via this route, the effects occurred above the reported i.p. LD$_{50}$ for mice and were not observed in other i.p. injection studies at similar or higher doses.
- Limited evidence for questionable genotoxic effects in some *in vitro* experiments, but *in vivo* effects were given more weight than *in vitro* effects particularly for same genetic endpoint
  - Consistent with current OECD guidance.
- The only positive findings reported *in vivo* were seen at relatively high doses that are not relevant for human health risk assessment.
Background

• Expert panels were convened to review glyphosate and glyphosate-based formulations (GBFs)
  – Exposure, epidemiology, carcinogenicity, genotoxicity

• The genotoxicity panel conducted an independent review of all of the genotoxicity data (including all regulatory GLP studies)
  – Other panelists: D Brusick, M Aardema, L Kier, G Williams

• The genotoxicity report (Brusick et al. 2016) has been published in a special issue of Critical Reviews in Toxicology, vol. 46, sup1, 56-74.

• Approach and conclusions consistent with EPA
Interpretation of Complex Data Sets

• Chemicals in widespread use, such as glyphosate, are typically subjected to extensive testing for genotoxic activity

• The resultant database will contain studies on different endpoints, varied test systems and exposure methods

• The more common test methods often have multiple data entries in the database

• Proper evaluation of such data sets requires a rigorous weight-of-evidence (WoE) approach that is both systematic and critical
Considerations for WoE

• Data from different cell types need to be carefully considered, and interpreted with caution as recommended in OECD test guidelines
  - p53 status, genetic (karyotype) stability, DNA repair capacity and origin (rodent versus human) of the cells chosen for testing
• “Misleading” positive results are to be expected in a large dataset, and their impact on evaluation of results is important
  - Non-predictive – positive responses with non-carcinogens
  - Secondary responses - indirect consequences of high cytotoxicity, high osmolality, low pH etc
  - Due to technical deficiencies
• In a WoE approach individual test methods, test systems and endpoints should be assigned a weight that is consistent with their contribution to the overall evidence
Categories of Evidence Weighting

• Different assay types have different weights
  – Tests measuring mutations and chromosome damage should have greater weight than “indicator” assays that only measure DNA damage (see recent OECD overview of genotoxicity test guideline revisions, 2016)

• The aggregate strength (robustness of protocols and reproducibility) and quality of evidence in the category also influence the weight
  – Studies conducted to GLP and according to OECD guidelines should have greater weight than studies lacking these attributes
• The number of pieces of evidence within a category influences the weight
  – One or a few divergent responses (+ or -) within a majority of studies with concordant findings should not alter direction and strength of the WoE

• Tests with greater relevance to humans carry greater weight
  – Data from *in vivo* tests (more predictive of potential human hazard) should carry more weight than data from *in vitro* or non-mammalian tests (except the Ames test)
Evidence Weighting

• The Expert Panel assigned weights according to the following 4 categories:

1. **Negligible weight:** The endpoint is not linked to any adverse effect relevant to genetic or carcinogenic hazard/risk and as such is not given weight as evidence of genotoxicity

2. **Low weight:** The endpoint is indicative of primary DNA damage, is not unequivocally linked to mechanisms of tumorigenicity, and the test system has low specificity

3. **Moderate weight:** The endpoint is potentially relevant to tumorigenicity or may be subject to secondary, threshold-dependent mechanisms of induction (e.g. cytotoxic clastogens, aneugens) or the test system exhibits a high rate of misleading positives with respect to carcinogen prediction or mode of action

4. **High weight:** The endpoint is one that has been demonstrated with a high level of confidence to play a critical role in the process of tumorigenicity
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Negligible Weight</th>
<th>Low Weight</th>
<th>Moderate Weight</th>
<th>High Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA binding (adduct formation) <em>in vitro</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA binding (adduct formation) <em>in vivo</em></td>
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<tr>
<td>SSB/DSB <em>in vitro</em> (including comet)</td>
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<tr>
<td>SSB/DSB <em>in vivo</em> (including comet)</td>
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<tr>
<td>SCEs <em>in vitro</em></td>
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<tr>
<td>SCEs <em>in vivo</em></td>
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<tr>
<td>Oxidative DNA damage <em>in vitro</em></td>
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<tr>
<td>Oxidative DNA Damage <em>in vivo</em> (detection of 8-OHdG adducts)</td>
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<tr>
<td>DNA repair effects <em>in vitro</em></td>
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<tr>
<td>DNA repair effects <em>in vivo</em></td>
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<tr>
<td>Micronuclei <em>in vitro</em></td>
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<tr>
<td>Micronuclei <em>in vivo</em></td>
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<tr>
<td>Chromosomal aberrations <em>in vitro</em></td>
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<tr>
<td>Chromosomal aberrations <em>in vivo</em></td>
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<tr>
<td>Gene mutation in bacteria (Ames Test)</td>
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<tr>
<td>Gene mutation in mammalian cells <em>in vitro</em></td>
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<tr>
<td>Gene mutation <em>in vivo</em></td>
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</tr>
</tbody>
</table>

**Principles of WoE consistent with guidance provided by international bodies.**
Which Studies Should Be Included?

• Detailed information on 44 genetic toxicology studies on glyphosate, provided in the supplementary tables of Kier and Kirkland (2013), were not reviewed by IARC but should have been considered

• In the supplementary tables in Kier and Kirkland (2013):
  – Each study examined was stated to have been conducted to GLP.
  – Almost all of the studies were conducted in accordance with the relevant OECD test guidelines applicable at the time of the study
  – Statistical methods (not routine for Ames tests) and level of significance were given as footnotes to the tables
Which Studies Should Be Included?
(continued)

• In addition, methodological details were provided:
  – Bacterial strains tested or cell type used
  – Data on individual replicates
  – Top concentrations and cytotoxic effects
  – Numbers of cells scored
  – Doses and dosing routes for *in vivo* studies
Profile of Results for Glyphosate (all studies)

Number of Studies

- High Weight (Total)
- Moderate Weight (Total)
- Low Weight (Total)

Categories:
- Negative
- Positive
Conclusions from WoE Analysis

• Test methods identified as Low Weight produced the highest frequency of positive responses (5 of 7), and were from endpoints and systems most likely to yield “misleading” positive results.

• The overwhelming majority of High Weight studies gave negative results.
  – Only 2 out of 39 High Weight studies on glyphosate were positive in the Expert Panel evaluation, with all studies combined.
Summary of *In Vitro* Findings

- DEREK SAR analysis of glyphosate identified no structural alerts for chromosomal damage, genotoxicity, mutagenicity, or carcinogenicity
  - Glyphosate is not electrophilic
- 20 Ames tests on glyphosate were negative
- Glyphosate does not induce gene mutations in mammalian cells *in vitro*
Summary of *In Vitro* and *In Vivo* Findings *(continued)*

- Glyphosate does not induce CA *in vitro*
- Glyphosate is not clastogenic *in vivo*
- There is questionable evidence that glyphosate induces MN *in vitro*
  - Unclear whether this is a reflection of increased statistical power in the *in vitro* MN studies, or a reflection of possible threshold-mediated aneugenic effects (supported by absence of induction of CA)
- There is strong evidence that glyphosate does not induce MN *in vivo*
Summary of Other Findings

• There is questionable evidence of transient DNA strand breakage for glyphosate *in vivo*
  – For glyphosate these are not associated with DNA adducts

• Thus, although there is evidence that glyphosate can induce DNA strand breaks *in vitro*, these might be secondary to toxicity since they did not lead to chromosome breaks
Summary of *In Vivo* and Other Findings (continued)

- There is evidence that glyphosate does not induce UDS in cultured hepatocytes
- Some reports of induction of SCE *in vitro* by glyphosate do not contribute to the overall evaluation of genotoxic potential
  - Mechanism of induction and biological relevance of SCE are unclear (therefore given negligible weight)
Non-mammalian Studies

• Glyphosate has been tested for genotoxicity in a variety of non-mammalian species (other than the Ames test)
  – Fish, amphibians, reptiles, plants
  – Many tests involved exposure by immersion in, or surface contact with, the test material in water – non-standard and may produce artifacts

• No internationally accepted guidelines for such non-mammalian test systems
Non-mammalian Studies (continued)

• OECD guidelines specifically state that use of any non-standard test requires justification along with stringent validation including establishing robust historical negative and positive control databases
  – No databases of acceptable negative or positive control responses
  – No results from validation studies indicating concordance with carcinogenicity

• Data from such non-standard tests should not have significant weight in the overall genotoxicity evaluation
Biomonitoring Studies

• Human population monitoring studies of genotoxic endpoints can offer highly relevant information as long as they are rigorous
• 3 biomonitoring studies have been published on GBF exposures
• EPA properly assigned a low quality ranking to the following studies which were not further evaluated in detail
  – Lack of exposure information on glyphosate from all subjects, and/or no quantitative measure of association between glyphosate and a cancer outcome
Biomonitoring Studies – Panel Assessment

• Paz-y-Miño et al. (2007) reported DNA breakage (comet assay) reported in humans exposed to GBF spraying–GBF application rate reported to be around 20x higher than recommended. Signs of clinical toxicity consistent with acute intoxication reported, but at such exposures this does not make sense. DNA damage might have been secondary to toxicity due to unrelated exposures/causes.

• Follow-up study (Paz-y-Miño et al., 2011) reported negative results for chromosomal changes in individuals from the same areas.

• Bolognesi et al. (2009) reported a significant but small, transient and inconsistent induction of MN in individuals living in 3 GBF spray areas, but no statistically significant increase in MN frequency was observed in individuals that actually reported direct exposure to the spray (any risk was “low”)

• Based on the above, the Panel concluded that there was little or no reliable evidence produced in these studies that would support a conclusion that GBFs, at levels experienced across a broad range of end-user exposures, pose any human genotoxic hazard/risk.
Characteristics of Genotoxic Carcinogens

- The table below compares the characteristics found with confirmed genotoxic carcinogens (Bolt et al. 2004; Petkov et al. 2015) and the genotoxic activity profile for glyphosate

*There is virtually no concordance between the two sets of characteristics*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Carcinogens with a Proven Genotoxic Mode of Action</th>
<th>Glyphosate Study Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Profile of Test Responses in Genetic Assays</td>
<td>Positive effects across multiple key predictive endpoints (i.e. gene mutation, CA, aneuploidy) both <em>in vitro</em> and <em>in vivo</em></td>
<td>No clear evidence for induction of gene mutations; no evidence for CA in humans and equivocal findings elsewhere</td>
</tr>
<tr>
<td>Structure Activity Relationships</td>
<td>Positive for structural alerts associated with genetic activity</td>
<td>No structural alerts for glyphosate suggesting genotoxicity</td>
</tr>
<tr>
<td>DNA binding</td>
<td>Agent or breakdown product are typically electrophilic and exhibit direct DNA binding</td>
<td>No unequivocal evidence for electrophilic properties or direct DNA binding by glyphosate</td>
</tr>
<tr>
<td>Consistency</td>
<td>Test results are highly reproducible both <em>in vitro</em> and <em>in vivo</em></td>
<td>Conflicting and/or non-reproducible responses in the same test or test category both <em>in vitro</em> and <em>in vivo</em></td>
</tr>
<tr>
<td>Response Kinetics</td>
<td>Responses are dose-dependent over a wide range of exposure levels</td>
<td>Many positive responses do not show significant dose-related increases</td>
</tr>
<tr>
<td>Susceptibility to Confounding Factors (e.g. Cytotoxicity)</td>
<td>Responses are typically found at non-toxic exposure levels</td>
<td>Positive responses typically associated with evidence of overt toxicity</td>
</tr>
</tbody>
</table>
Overall Conclusions

• The expert panel concluded that a critical weight of evidence review of the complete dataset supports a conclusion that glyphosate does not pose a genotoxic hazard as is consistent with the EPA conclusions.
Charge Question #4:
Genotoxicity
Charge Question #4:

- EPA and our expert panel have followed very similar approaches (in terms of WoE) and reached similar conclusions. Therefore, the Panel agrees with:
  - EPA’s review and evaluation of relevant genotoxicity studies
  - Appropriate identification of over 80 studies
  - Reliance on active/technical glyphosate scientifically justified
    - Exclusion of surfactants is appropriate – EPA concluded that surfactants are not genotoxic
  - Appropriate exclusion of non-mammalian assays
  - Suitable exclusion of 5 studies (faulty design)
  - EPA’s reliance on *in vivo* studies as more relevant to humans, and consideration of all data in WoE evaluation
    - *In vitro* assays are given less weight, except for the Ames test
    - Negative *in vitro* results provide assurance that not likely genotoxic *in vivo*
    - Positive *in vitro* results must be confirmed *in vivo*
Charge Question #4:

- Agree with EPA regarding:
  - Relevance of genotoxicity findings with respect to dose and route of exposure
  - Inconsistent with 5 other studies that were negative for MN induction at equal or higher doses (up to 3,000 mg/kg)
  - No MN induced in mice exposed up to 3,393 mg/kg/day in the diet for 13-weeks (NTP 1992)
  - Strengths and uncertainties associated with WoE and conclusions
    - Some studies report positive genotoxicity but predominantly seen in negligible or low weight categories, or with i.p. route of exposure

- The WoE conclusion is scientifically strong and the data supports glyphosate as not being an in vivo clastogen/genotoxicant
EPA Conclusions Consistent With Other Expert Reviews

• “The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2,000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans.” (JMPR 2016)

• The “Expert Panel concluded that glyphosate, GBFs, and AMPA genotoxicity response profiles are not consistent with characteristics of genotoxic carcinogens.” (Brusick et al. 2016)
Charge Question #5: Characterization of Carcinogenic Potential
Guidelines Emphasize WOE Review

“Users of these cancer guidelines and of the risk assessments that result from the use of these cancer guidelines should consider the entire range of information included in the narrative rather than focusing simply on the descriptor.” (bold in original)
WOE Summary

• Rodent carcinogenicity evaluation across 15 available cancer bioassays
  — No compound-related tumors in individual studies
  — Lack of supporting evidence of carcinogenicity:
    – No monotonic dose-response; no progression from pre-neoplastic to malignant; no reduced latency
    – No consistency across a large number of studies
WOE Summary

• WOE shows glyphosate not a genotoxicant
  – Positive in only 2 of 39 high weight studies
  – Shows none of the characteristics of a genotoxicant

• WOE of epidemiologic data does not support an association for NHL
  – Glyphosate occupational exposures are extremely small
  – Potential bias from many sources limits informativeness of case-control studies
  – Only cohort study (AHS) showed no association
    – More days of glyphosate use in AHS than case-control studies
**Descriptor: Suggestive evidence of carcinogenic potential**

<table>
<thead>
<tr>
<th>Data needs to support classification</th>
<th>WOE supports?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study - not contradicted by other studies of equal quality</td>
<td>NO</td>
</tr>
<tr>
<td>Small increase in a tumor (high background rate in sex/strain) – some but insufficient evidence that observed tumors are due to intrinsic factors and not due to the agent being assessed</td>
<td>NO</td>
</tr>
<tr>
<td>Positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion; supported by other evidence</td>
<td>NO</td>
</tr>
<tr>
<td>Statistically significant increase at one dose only, but no significant response at the other doses and no overall trend</td>
<td>NO</td>
</tr>
</tbody>
</table>
Descriptor: *Not likely to be carcinogenic to humans*

- Used when data are robust and no basis for human hazard

<table>
<thead>
<tr>
<th>Data needs to support classification</th>
<th>WOE supports?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of carcinogenic effect (both sexes) in well-designed, well-conducted studies in at least two appropriate animal species</td>
<td>YES</td>
</tr>
<tr>
<td>Experimental evidence shows that the only carcinogenic effects observed in animals are not relevant to humans</td>
<td>N/A</td>
</tr>
<tr>
<td>Convincing evidence that carcinogenic effects are not likely by a particular exposure route</td>
<td>N/A</td>
</tr>
<tr>
<td>Convincing evidence that carcinogenic effects are not likely below a defined dose range</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Conclusion

• WOE supports classification of *Not likely to be carcinogenic to humans*
  – Consistent with all other global regulatory authorities