Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

Toxicological re-evaluation of glyphosate

Dept. of Food Safety and Zoonoses (FOS)

http://www.who.int/foodsafety/en/
Context of the JMPR assessment

- In 2015, WHO’s International Agency for Research on Cancer conducted a hazard classification for glyphosate and concluded that it was a probable human carcinogen (Group 2A).

- In May 2016, the WHO’s Joint Meeting on Pesticide Residues had to re-evaluate the toxicity of glyphosate residues present in the diet in particular related to a genotoxic and/or carcinogenic risk for consumers.
Content of the JMPR assessment

- Acute toxicity and Short-term studies of toxicity
- Long-term studies of toxicity and carcinogenicity
- Genotoxicity
- Reproductive and developmental toxicity
- Special studies on Neurotoxicity, Immunotoxicity, Endocrine disruption, Intestinal microflora effects…
Key Acceptability & Weighting Considerations for Genotoxicity Data

- JMPR considers both published studies and data commissioned and owned by industry.

- These data are the same as those submitted by industry to national regulatory authorities when applying for registration and authorization of pesticides.

- These studies are conducted according to strict internationally-accepted requirements.
Key Acceptability & Weighting Considerations for Genotoxicity Data

- More weight given to:
  - higher quality studies in validated or well established models
  - in vivo studies than in vitro studies
  - studies in phylogenetically close species (mammals) than in distant species (*Drosophila*, frogs, fish, caiman, etc.)
  - endpoints considered to be more serious (mutation, chromosome alterations) than to less serious and transient endpoints
  - studies conducted by relevant routes of exposure (e.g. oral) than by non-physiological routes (e.g. intraperitoneal injection).
Genotoxicity studies

- Bacterial studies
  - Non-standard test: 4 positive, no negative
  - Standard Ames test or rec test: 1 positive, 35 negative

  **Glyphosate is non-mutagenic and non-genotoxic in standard bacterial mutagenicity tests**

- In vivo studies in mammals
  - Intraperitoneal administration: 13 positive, 16 negative
  - Oral administration: 4 positive, 29 negative

  **Glyphosate is inactive in vivo in standard mammalian genotoxicity tests when administered by the oral route**
Genotoxicity studies

- **Human Biomonitoring Studies***
  - Measure of frequency of micronuclei in blood lymphocytes shortly after spraying as compared with the pre-spraying samples
  - Control frequencies were unusually low and frequencies among the “exposed” fell at the middle of the normal range
  - Increases did not correlate with application rates
  - Those reporting exposure did not differ from those reporting no exposure
  - Authors stated that damage was “small” and “transient”, “not possible to assign causality to the increases”, “of low biological relevance”, and suggested exposure to other genotoxic agents.

**JMPR considered these results to be equivocal**

* Bolognesi et al. 2009
# Genotoxicity studies - summary

<table>
<thead>
<tr>
<th>Studies evaluated</th>
<th>Quality</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>High to medium</td>
<td>Negative</td>
</tr>
<tr>
<td>In vivo mammals (oral route)</td>
<td>High to medium</td>
<td>Negative</td>
</tr>
<tr>
<td>Human biomonitoring</td>
<td>Medium</td>
<td>Equivocal</td>
</tr>
<tr>
<td>In vitro mammalian cells</td>
<td>Medium to poor</td>
<td>Mixed, mainly positive</td>
</tr>
<tr>
<td>Phylogenetically distant in vivo</td>
<td>Medium to poor</td>
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</table>
Other mechanistic considerations

- Negative for structural alerts for chromosomal damage, genotoxicity, mutagenicity and carcinogenicity using Derek computer program (Kier and Kirkland, 2013).

- Negative in 599 of 620 screening assays for biological activity in PubChem (Tox21) database. Positive responses were primarily related to its mechanism of action in plants and bacteria.

- Little evidence that glyphosate significantly affects the immune system, a key characteristic of agents that cause NHL in humans.
JMPR genotoxicity conclusions

“The overall weight of evidence indicates that administration of glyphosate [...] at doses as high as 2000 mg/kg body weight 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.”
Evaluation process for epidemiological evidence

1. Relevance - For each compound/cancer site combination - did IARC identify positive associations from the body of epidemiological evidence?

   - Yes
   - 6 compound/cancer site combinations

   ACTION - for each relevant compound/cancer site:
   - Identify all papers in IARC Monographs assessing relevant compound/cancer sites (positive and null associations)
   - Identify any papers published since IARC Monograph which address relevant compound/cancer site
   - Search by hand (e.g. check reference lists of identified papers) for any papers potentially missed

   2G papers identified

2. For related papers that examined the same compound/cancer site is this:
   - the most recent publication with longest follow-up for this compound/cancer site? (e.g. cohort studies)
   - the most complete and updated analysis with the greatest number of participants for this compound/cancer site? (e.g. pooled case-control)

   - Yes
   - Exclusion from evaluation for given compound/cancer site
   - Malathion/NHL - 2 papers excluded
   - Diazinon/NHL - 2 papers excluded
   - Diazinon/Lung - 2 papers excluded
   - Glyphosate/NHL - 2 papers excluded

   - No
   - Exclusion from paper for given compound/cancer site

3. Is exposure assessment specific to compound of interest?

   - Yes
   - Paper is relevant and can contribute to quantitative risk assessment (i.e. hazard characterization) for compound/cancer site

   - No
   - Paper is not relevant to risk assessment for compound
   - Diazinon/NHL - 1 paper excluded

4. Quantitative exposure assessment (exposure expressed on a ratio scale)

   - Yes
   - Paper is relevant but cannot contribute information to a quantitative risk assessment

   - No
   - Paper is relevant and can contribute to quantitative risk assessment (i.e. hazard characterization) for compound/cancer site

   ACTIONS - for each relevant paper:
   - Extract information on quantitative exposure units.
   - Describe magnitude of effect/uncertainty
   - Review quality of study based on IARC Monograph and evaluation criteria.
   - Describe exposure assessment and how exposure levels compare to/translate to pesticide residue levels/pathways.

Overall summary

ACTIONS - for each compound/cancer site:
- Characterize hazard for each compound/cancer site from all studies contributing to quantitative risk assessment, e.g. forest plot (or meta-regression, time-permitting).
- Summarize strength of evidence.

Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes
“Overall, there is some evidence of a positive association between glyphosate exposure and risk of Non-Hodgkin Lymphoma from the case–control studies and the overall meta-analysis. However, it is notable that the AHS, which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level.”
Animal Cancer Bioassays

- The original studies for 10 rat and 7 mouse chronic bioassays were reviewed by JMPR.

- Statistical consideration about trend tests
  - In a single sex and species combination of a standard rodent cancer bioassay, histopathology may be performed on 40 individual tissues.
  - Assuming that a control and 3 treatment doses were administered and the statistical analyses consisted of a trend test and pair-wise comparisons between the control and each dose for each tissue, a total of 160 separate statistical tests would be performed.
  - Using a $p \leq 0.05$, one can estimate that 8 of these would be positive by random chance alone.
Animal Cancer Bioassays

- For rat, an increased incidence in tumors was observed in interstitial cell tumors of the testes (1 study over 10), pancreatic islet cell adenoma (1 study over 10), thyroid C-cell tumors (1 study over 10) and skin keratoma (2 studies over 10, males only).

The JMPR concluded that these findings were incidental, in part, because studies that used appreciably higher doses did not find any excess.
Animal Cancer Bioassays

- For mouse suggestive increases in tumor incidences were observed in some of the studies.
  - Lymphomas: positive trend in 3 studies over 7 ($p \leq 0.05$) by the trend test but not in pairwise comparisons.
  - Kidney adenomas: positive trend in 4 studies over 7 significant by the trend test but not in pairwise comparisons.

Note: The doses administered to the mice were up to 50,000 ppm in the diet (approx. 7500 mg/kg in males and 8700 mg/kg in females).
JMPR chronic bioassay conclusions

“The JMPR concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses.”
Interaction with hormone receptor pathways

Glyphosate was tested in a range of validated "in vivo" and "in vitro" assays for its potential to interact with the endocrine system. The studies considered by JMPR as adequate for the evaluation demonstrate no interaction with estrogen or androgen receptor pathways or thyroid pathways.
Microbiological effects of Glyphosate

- **In vitro**
  - Beneficial microbiota are more vulnerable to glyphosate than pathogenic bacteria which can affect the multistep processes in carcinogenesis

- **In vivo**, the (nonsignificant) association seen between glyphosate exposure and non-Hodgkin lymphoma pathogenesis, cannot be explained in the absence of:
  - measurement of glyphosate residues in the (gastro)intestinal tract
  - adverse affects of glyphosate on the normal functioning of the microbiota in the human gastrointestinal tract or mammalian models.
JMPR overall conclusion

“In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet.”
JMPR Expert Group

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http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/