Weight-of-evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations (GBFs) and aminomethylphosphonic acid (AMPA)

David Brusick, Marilyn Aardema, Larry Kier, David Kirkland, Gary Williams
In 2015, IARC published the Glyphosate Monograph of Volume 112, and concluded that there was strong evidence supporting that “glyphosate can operate through two key characteristics of known human carcinogens” - genotoxicity and induction of oxidative stress.

Genotoxicity was used to support IARC classification of glyphosate as probably carcinogenic to humans, Group 2A. Many previous published and regulatory reviews agreed that proper use of glyphosate and GBFs does not pose a genotoxic or carcinogenic hazard/risk.

The IARC conclusion was therefore inconsistent with these other reviews.

Importantly, the IARC Monograph did not consider a large number of regulatory genetic toxicology studies.
Our Review

- Intertek Scientific & Regulatory Consulting convened several panels
  - Exposure, epidemiology, carcinogenicity, genotoxicity
- Our panel conducted an independent review of all of the genotoxicity data (including data not reviewed by IARC)
- A weight of evidence approach (WoE) was used to evaluate the results present in the glyphosate (and related materials) dataset.
- Our review found some areas of agreement with IARC, but also identified some major differences between the conclusions of the two assessments
- Our report has been accepted for publication in a special issue of Critical Reviews in Toxicology, vol. 46, sup1, 56–74.
- I will not discuss oxidative stress in this presentation since it will be addressed by Dr James Bus.
Interpretation of complex data sets

- Chemicals in widespread use 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 an approach that is both systematic and critical.
- On the next slides we describe our approach to the evaluation of the genotoxic potential of glyphosate.
Key considerations (1)

- Rigorous human population monitoring studies (sufficient sample sizes, knowledge of exposure, adjusted for confounding factors) can offer highly relevant information.

- However, confounding factors beyond age, gender, smoking, alcohol, tobacco, and medicines used need to be considered
  - Diet, disease status (e.g. presence of inflammatory diseases), seasonal variation and physical stress are also important

- We assigned a high weight to measurement of micronuclei (MN) in exposed humans.
Data from **different cell types** need to be carefully considered. Many scientists now recommend using human rather than rodent derived cell lines.

However, cell lines with e.g. p53 mutations are typically more susceptible to genetic damage. Therefore p53-deficient human and rodent cell lines expected to generate similar results.

OECD guidelines state “*At the present time, the available data do not allow firm recommendations to be made but suggest it is important, when evaluating chemical hazards to consider the p53 status, genetic (karyotype) stability, DNA repair capacity and origin (rodent versus human) of the cells chosen for testing.*”

Thus, *in vitro* mammalian cell results should be interpreted with caution, and the weight they contribute to an overall assessment should take account of the potential limitations.
“Misleading” positive results (in terms of predicting cancer) are to be expected in a large dataset such as exists for glyphosate, and understanding their impact on evaluation and interpretation of genotoxicity results is important.

“Misleading” responses may be:

- Non-predictive – positive responses with non-carcinogens due to low specificity of mammalian cell tests.
- Secondary responses - indirect consequences of high cytotoxicity, high osmolality, low pH etc (not due to direct DNA-reactivity).
- Due to technical deficiencies - poor study design, mistakes during conduct of a test, or inappropriate evaluation of data.

A weight of evidence (WoE) approach should be used

Individual test methods should be assigned a weight that is consistent with their contribution to the overall evidence.

We applied 4 considerations:
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 (i.e. determine an aggregate of the weights within a single test category)

- Tests with greater ability to extrapolate results 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).
We therefore assigned weights according to the following 4 categories:

- **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.
- **Low weight** – the end point is indicative of primary DNA damage, is not unequivocally linked to mechanisms of tumorigenicity, and the test system has low specificity.
- **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.
- **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>
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<tbody>
<tr>
<td>DNA binding (adduct formation) <em>in vitro</em></td>
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<tr>
<td>DNA binding (adduct formation) <em>in vivo</em></td>
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<td>SSB/DSB <em>in vitro</em> (including comet)</td>
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<td>SSB/DSB <em>in vivo</em> (including comet)</td>
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<td>SCEs <em>in vitro</em></td>
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<td>SCEs <em>in vivo</em></td>
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<td>Oxidative DNA Damage <em>in vitro</em></td>
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<td>Oxidative DNA Damage <em>in vivo</em> (detection of 8-OHdG adducts)</td>
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<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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<td>Micronuclei <em>in vitro</em></td>
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<td>Micronuclei <em>in vivo</em> (including human studies)</td>
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<td>Chromosomal aberrations <em>in vitro</em></td>
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<tr>
<td>Chromosomal aberrations <em>in vivo</em> (including human studies)</td>
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<tr>
<td>Gene mutation in bacteria (Ames Test)</td>
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<tr>
<td>Gene mutation mammalian <em>in vitro</em></td>
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<tr>
<td>Gene mutation <em>in vivo</em></td>
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Different approaches?

- We used the considerations and WoE approaches discussed above to assess the various genotoxicity results with glyphosate.
- Whilst we agree with some of the IARC assessments, we disagree on others.
- Some of the differences may be due to different approaches.
  - Section 4.2.1 of the IARC Monograph does not provide sufficient information regarding the strategy employed in assessing the WoE
  - We do not know if studies were assigned variable weights in accordance with the criteria discussed above.
  - It appears that IARC considered *in vitro* studies in human cells as carrying more weight than rodent *in vivo* studies
  - IARC did not appear to assign additional weight to relevance of the genotoxicity endpoint to tumour initiation, quality of study performance, *in vitro* vs. *in vivo* data or reproducibility of responses.
We agree with IARC there is sufficient evidence to conclude that glyphosate and GBFs appear to induce DNA strand breaks and possibly MN in vitro in mammalian and non-mammalian systems, and SCEs in mammalian systems.

However, it is not possible to accurately characterize or classify genotoxic hazard/risk or carcinogenesis mechanisms based on these results alone.

As stated in the OECD overview (2016) regarding test weights, “When evaluating the mutagenic potential of a chemical, more weight should be given to the measurement of permanent DNA changes (i.e. mutations) than to DNA damage events that are reversible.”

Consequently, positive responses in “indicator tests” (i.e. DNA strand breaks, SCEs) are evidence of exposure but not sufficient to determine effect.

- For compound effect, data from tests measuring the induction of gene mutations or stable chromosomal alterations, particularly in vivo in mammalian systems, must be considered
IARC did not consider the chemical structure of glyphosate in its mechanistic section; however, IARC Monograph Section 5.3 states that glyphosate is not electrophilic.
  ◦ While structural alerts are not as definitive as experimental data, they serve as part of a WoE (Dearfield et al. 2011).

Analysis of the glyphosate structure by DEREK software identified no structural alerts for chromosomal damage, genotoxicity, mutagenicity, or carcinogenicity.

The lack of structural alerts for glyphosate suggests lack of genotoxicity or that genotoxic effects might well be secondary to toxicity or resulting from mechanisms other than DNA-reactivity.

Although formal analysis is not available it does not appear likely that AMPA (glyphosate without a carboxymethyl group) has structural alerts.
A Note on GBFs

- Another aspect of chemistry that should be recognized is the fact that GBFs, while containing glyphosate (often present as a sodium or potassium salt) also contain other components which frequently include surfactants.
- Specific formulations differ in composition and differences may exist between GBFs identified with a common brand name.
- Frequently, GBFs are observed to have greater toxicities than glyphosate.
- Evaluation of genotoxicity results for glyphosate and GBFs should always consider the possibility that effects observed with GBFs may be due to GBF components other than glyphosate and that there may be chemical differences between various GBFs.
Whilst IARC policies and Working Group decisions include published papers, they exclude consideration of additional data from unpublished studies or publicly unavailable governmental reports.

- Although primary GLP study reports were not available, Kier & Kirkland, (2013) was published, and the information provided in the supplementary tables was detailed.
  - However, it was considered by IARC as insufficient regarding details of statistical methods, choice of highest dose tested, and verification of the target tissue exposure.

We believe that **90 genetic toxicology studies** published in Kier and Kirkland (2013), *not reviewed by IARC, should have been considered by IARC* in evaluating the genetic toxicology of glyphosate and GBFs.

Though the primary study reports were not available to IARC, detailed supplementary data extracted from the study reports were provided in Kier and Kirkland (2013) and exceed the weight of data in most of the published papers that were considered by IARC.

Regulatory studies of GBFs and AMPA summarized in Williams et al. (2000) should also have been considered.
We believe the supplementary tables presented in Kier and Kirkland (2013) do contain sufficient detail concerning the robustness of the studies.

- Each study examined was stated to have been conducted to GLP
- Almost all of the studies were reported to have been conducted in accordance with the relevant OECD test guidelines applicable at the time of the study.
- If statistical analysis was performed (not commonly performed or required for Ames tests) this is given as a footnote to the supplementary tables in Kier & Kirkland (2013), together with the statistical method used, and whether the results were significant.

In addition, the information in the following slides was available within Kier & Kirkland (2013):
For Ames tests, the concentrations tested, critical aspects of the methods (e.g. plate incorporation or pre-incubation for the Ames tests, inducing agent for the S9 and its final concentration, and number of replicate cultures) were detailed in every table. *Thus, it is clear what top concentrations were used, whether they complied with the maximum concentration/dose as recommended in OECD guidelines, or whether they were defined by toxicity.*

Almost all of the many Ames tests on glyphosate used a top concentration of 5000 µg/plate (maximum required) unless limited by toxicity. All of the required strains, including either TA102 or *E. coli*, were used in the regulatory studies included in Kier and Kirkland (2013).

The Ames tests on GBFs used quite variable top concentrations. Some went as high as 5000 µg/plate, but others only reached <100 µg/plate, seemingly limited by toxicity.

- Since we know glyphosate *per se* is not very toxic in the bacterial tests, the GBF toxicity is presumably caused by the other components of the formulations, which were more toxic in some GBFs than in others.
Mammalian cell assays on glyphosate generally reached top concentrations of 500-5000 µg/mL, even when prolonged (48 hour) treatments were performed in the CA studies.

- Thus, many of these studies exceeded 10 mM (1690 µg/mL for glyphosate).
- There were no regulatory mammalian cell tests on GBFs.

All except one of the regulatory *in vivo* MN tests on glyphosate achieved a top oral dose of at least 2000 mg/kg.

- In 1 oral study the top dose was only 30 mg/kg, seemingly because of lethality at higher doses, yet much higher doses were tolerated in other studies using the same acute dosing regimen.
- Several studies using i.p. injection had lower top doses because of greater toxicity.

Thus, all of the regulatory *in vivo* MN studies on glyphosate met or exceeded the required top dose.
The regulatory *in vivo* bone marrow MN and CA studies in Kier and Kirkland (2013) generally did not report evidence of target organ toxicity (e.g. %PCE) or include analysis of plasma.

- Was the bone marrow exposed?

IARC states that about 1/3 of glyphosate administered orally to rodents is absorbed and excreted, largely unchanged, in urine.

- This indicated that the bone marrow, a well-perfused tissue, is highly likely to have been exposed to glyphosate in rodents treated orally.

Definitive evidence of absorption and systemic distribution of glyphosate in rodents (including distribution in bone marrow in rats dosed i.p. or orally) is also contained in a summary of regulatory toxicokinetic studies (JMPR 2006).

- Published data also indicate absorption and systemic distribution of glyphosate administered by i.v. or oral routes in rats, (Brewster et al. 1991; Anadon et al. 2009) and by the dietary route in mice (Chan & Mahler 1992).

Thus, *in the regulatory rodent in vivo MN and CA tests*, target organ exposure would have been achieved.
Based on the above, we believe the exclusion by IARC of the 90 additional (mainly GLP) studies was not justified
  ◦ Because this was a published paper
  ◦ Supplementary data was much more extensive than in many papers that were included by IARC

We believe this failure resulted in an inaccurate assessment of glyphosate, GBFs and AMPA’s genotoxic hazard/risk potential.

When the additional studies are combined with the published data evaluated by IARC, the patterns of positive and negative results change
  ◦ Particularly for glyphosate and GBFs, where there were many GLP studies
  ◦ Particularly for those studies identified as high weight

There were very few GLP studies on AMPA, so these are not shown in the following charts
Profile of Results for glyphosate from IARC

No. of studies

- High Weight (IARC only)
  - Negative: 5
  - Positive: 1

- Moderate Weight (IARC only)
  - Negative: 4
  - Positive: 3

- Low Weight (IARC only)
  - Negative: 2
  - Positive: 5

Legend:
- Green: Negative
- Red: Positive
Profile of Results for glyphosate with Added GLP Studies

No. of Studies

- High Weight (Total)
  - Negative: 40
  - Positive: 0

- Moderate Weight (Total)
  - Negative: 10
  - Positive: 5

- Low Weight (Total)
  - Negative: 5
  - Positive: 5
Profile of Results for GBFs from IARC

No. of studies

- High Weight (IARC only)
- Moderate Weight (IARC only)
- Low Weight (IARC only)

- Negative
- Positive

For High Weight (IARC only), there are 8 studies. There are 2 studies for Moderate Weight (IARC only) and 2 studies for Low Weight (IARC only).
Profile of Results for GBFs with Added GLP Studies

No. of Studies

<table>
<thead>
<tr>
<th>Weight Level</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Weight (Total)</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Moderate Weight (Total)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Low Weight (Total)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Test methods identified as Low Weight produced the highest frequency of positive responses, whether evaluated by IARC alone (8 of 9) or from all studies combined (8 of 11).

The highest frequencies of positive responses were from endpoints and systems most likely to yield “misleading” positive results.

- This was the case whether taken from those evaluated by IARC alone or from all studies combined.

A minority of High Weight studies gave positive results for glyphosate, GBFs or AMPA in the both IARC and our evaluations.

- 6 out of 15 High Weight studies were positive in the IARC evaluation
- However, only 8 out of 92 High Weight studies were positive in our evaluation, with all studies combined
Summary of *in vitro* and *in vivo* findings (1)

- 40 Ames tests on glyphosate, GBFs and AMPA, not reviewed by IARC, were all negative.
- Glyphosate does not induce gene mutations in mammalian cells *in vitro*.
  - There are no *in vitro* mammalian cell gene mutation data for GBFs or AMPA, and no gene mutation data *in vivo*.
- Glyphosate, GBFs and AMPA are not clastogenic *in vitro*.
- Glyphosate is also not clastogenic *in vivo*.
  - Some positive *in vivo* CA studies with GBFs are all subject to concerns regarding their reliability or biological relevance.
- There is limited evidence that glyphosate induces MN *in vitro*. Although this could be a reflection of increased statistical power in the *in vitro* MN studies, the absence of induction of CA suggests possible threshold-mediated aneugenic effects.
- There is strong evidence that glyphosate does not induce MN *in vivo*. 
Summary of *in vitro* and *in vivo* findings (2)

- There is no convincing evidence that GBFs or AMPA induce MN *in vitro*.
- The overwhelming majority of *in vivo* MN studies on GBFs gave negative results.
  - Conflicting and limited data do not allow a conclusion on *in vivo* induction of MN by AMPA.
- There is evidence that glyphosate and GBFs can induce DNA strand breaks *in vitro*, but these might be secondary to toxicity since they did not lead to chromosome breaks.
  - There is limited evidence of transient DNA strand breakage for glyphosate and GBFs *in vivo*, but for glyphosate at least these are not associated with DNA adducts. These results were assigned a low weight.
- There is evidence that glyphosate and AMPA do not induce UDS in cultured hepatocytes.
- Some reports of induction of SCE *in vitro* by glyphosate and GBFs, and one report of SCE induction *in vivo* by a GBF, do not contribute to the overall evaluation of genotoxic potential since the mechanism of induction and biological relevance of SCE are unclear (negligible weight).
Glyphosate or GBFs have been tested for genotoxicity in a variety of non-mammalian species (other than the Ames test), and these publications appear to be included in the IARC review.

- There are 2 positive and 1 negative CA studies with glyphosate in plants
- CA studies have mainly been published for GBFs and showed predominantly positive results for MN in fish and amphibians.

A larger number of comet assays in fish and other non-mammalian species *in vitro* reported as giving mainly positive results for glyphosate.

- Larger numbers of positive comet results are available for GBFs in fish and amphibian/reptile studies. One positive fish comet study is reported for AMPA.

We are not aware of any internationally accepted guidelines for such non-mammalian test systems, or any databases of acceptable negative control data or positive control responses, and no results from validation studies suggesting concordance with carcinogenicity.

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.
Both major endpoints measured in the majority of non-mammalian tests (i.e. MN and comet) might well produce positive responses that are secondary to toxic effects.

Many of these tests involve exposure by immersion in, or surface contact with, the test material in water.

- This is not a standard or relevant route of exposure for *in vivo* mammalian systems and may introduce route-specific unique toxic and genotoxic effects.
- This is particularly a concern for GBFs which commonly contain surfactants.

Therefore, we did not consider data from a majority of the non-mammalian systems and non-standard tests with glyphosate, GBF, and AMPA to have significant weight in the overall genotoxicity evaluation, especially given the large number of standard core studies for gene mutations and chromosomal damage available in mammalian systems.
Human Biomonitoring Studies (1)

- IARC reviewed 3 biomonitoring studies
- DNA breakage (comet assay) was reported in human individuals exposed to GBF spraying (Paz-y-Miñó et al., 2007), however:
  - The comet assay is an ‘indicator’ test and primary DNA damage does not accumulate, so the consequences of the observed DNA breaks remain unknown
  - It is not clear which blood cells were scored for comets, or if it was all cells in the blood
  - Median comet tail length was exactly 25.0 μM for 20/21 unexposed control individuals!
  - Signs of clinical toxicity were reported, and the GBF application rate was some 20x higher than recommended. The clinical signs were consistent with acute intoxication associated with severe exposures, suggesting the DNA damage might have been secondary to toxicity.
- We regard this study as “inconclusive” in terms of evidence for *in vivo* human genotoxic effects relevant to induction of mutations or cancer.
Paz-y-Miño et al. (2011) reported negative results for induction of chromosomal changes in individuals from the same areas where GBF spraying had occurred 2 years previously.

The absence of chromosomal aberrations supports the presumption that the DNA strand breaks identified in the earlier (Paz-y-Miño et al. 2007) study were either repaired or lethal and did not persist as lesions which could be expressed as chromosomal aberrations in cultured lymphocytes in the follow-up study.
Bolognesi et al. (2009) reported a significant but small, transient and inconsistent induction of MN in individuals living in 3 areas where aerial spray application of glyphosate occurred, but concluded that any risk was “low”.

However, no statistically significant increase in the frequency of MN was observed in individuals that actually reported direct exposure to the spray compared to individuals who lived in the spray area but were not present during spraying (see graph on next slide)
Mean Frequency of MN in Self-Reported Exposures to Glyphosate Spray in Areas Where Aerial Application Occurred, From Bologensi et al 2009; Table 4. Data from Valle del Cauca not shown in graph since only 1 individual reported exposure. Graph provided by K. Solomon.
There was no statistically significant difference in post-spray MN frequency between different categories of self-reported spray exposure, nor between “no exposure” and any self-reported spray exposure for any of the three regions.

The lack of significant correlation between increased post-spraying MN frequencies and self-reported spray exposure, and inconsistency with application rates, indicate that the MN effects observed in this study cannot be associated with GBF exposure.

We agree with the authors that, based on the Bradford Hill criteria (Hill 1965), it is not possible to assign causality to the MN increases observed.

Although IARC indicated qualifications in their initial discussion of this study, these qualifications are not indicated in their Summary, and the unqualified study is given very high emphasis in the IARC Evaluation section as strong evidence for a genotoxic mechanism operating in humans.
Based on the above, we 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.
The table below compares the characteristics found with confirmed genotoxic carcinogens (Bolt et al. 2004; Petkov et al. 2015) and the genotoxic activity profiles for glyphosate, AMPA and GBFs. 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, GBFs, AMPA study data</th>
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</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 or AMPA 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 or AMPA</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>
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</table>
Overall Conclusions

- There is substantial evidence, particularly in Ames tests (not reviewed by IARC), that glyphosate, GBFs, and AMPA do not induce gene mutation from either direct or oxidative induced mechanisms.
- The evidence indicating that glyphosate can produce (unstable) CAs that could indicate a potential to induce stable chromosome mutations in mammalian systems is very limited, conflicting and potentially due to secondary mechanisms.
- The failure of glyphosate and GBFs to induce responses in well-validated test systems with robust experimental protocols that are characteristic of genotoxic carcinogens, does not support the conclusion that either glyphosate or GBFs might act via a genotoxic mode of action.
- The evidence for oxidative stress/damage as a mechanism or predictor of carcinogenesis is unconvincing. Repeated exposure to ROS most likely leads to adaptive responses, mitigating the mutagenicity of oxidative DNA lesions.
  - Studies directed toward a better understanding this relationship for glyphosate or GBF related exposures have not been reported.
- There is little or no reliable evidence that GBFs, at levels experienced across a broad range of end-user exposures, pose any human genotoxic hazard/risk.
We concluded that the IARC assessment of classifications regarding strong evidence of genotoxicity and oxidative stress capabilities of glyphosate, GBFs, and AMPA is not supported by the available data.

A critical review of the complete dataset supports a conclusion that glyphosate (including GBFs and AMPA) does not pose a genotoxic hazard and, therefore, should not be considered support for the classification of glyphosate as a genotoxic carcinogen.
Acknowledgements

- Intertek Scientific & Regulatory Consulting (Mississauga, Ontario, Canada) for convening the expert panel and providing organisational and documentary support
Back-up slides
## Studies not included by IARC

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Endpoint</th>
<th>Glyphosate (Pos/Neg)</th>
<th>GBFs (Pos/Neg)</th>
<th>AMPA (Pos/Neg)</th>
<th>Total (Pos/Neg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Reverse Mutation</td>
<td>Gene Mutation</td>
<td>0/19</td>
<td>0/20</td>
<td>0/1</td>
<td>0/40</td>
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<tr>
<td>Mammalian <em>In Vitro</em></td>
<td>Gene Mutation</td>
<td>0/2</td>
<td>ND</td>
<td>ND</td>
<td>0/2</td>
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<tr>
<td></td>
<td>Chromosome Aberrations</td>
<td>1/5</td>
<td>1/0</td>
<td>ND</td>
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<tr>
<td></td>
<td>Micronucleus</td>
<td>2/0*</td>
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<td>SCE</td>
<td>ND</td>
<td>1/0</td>
<td>ND</td>
<td>1/0</td>
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<tr>
<td>Mammalian <em>In Vivo</em></td>
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<tr>
<td></td>
<td>Micronucleus</td>
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<td>0/17</td>
<td>0/1</td>
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<tr>
<td></td>
<td>SCE</td>
<td>ND</td>
<td>1/0</td>
<td>ND</td>
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<td>Total</td>
<td></td>
<td>3/41</td>
<td>6/37</td>
<td>0/3</td>
<td>9/81</td>
</tr>
</tbody>
</table>

*= inconclusive studies not included in count
Dr. James Bus

IARC USE OF “OXIDANT STRESS”
IARC Use of “Oxidant Stress” Mode of Action in Glyphosate Cancer Classification Evaluation

James S. Bus PhD, DABT, ATS, Exponent, Inc.
Glyphosate Task Force Webinar
November 10, 2016
Mechanistic data can be pivotal when the human data are not conclusive - IARC

**EVIDENCE IN EXPERIMENTAL ANIMALS**

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<tr>
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**EVIDENCE IN HUMANS**

- **Sufficient**: strong evidence in exposed humans … agent acts through relevant mechanism
- **Limited**: strong evidence in exposed humans … mechanism also operates in humans
- **Inadequate**: strong evidence … mechanism does not operate in humans
- **ESLC**: consistently and strongly supported by a broad range of mechanistic and other relevant data

Portier, 2015; Modified from Vincent Cogliano, IARC
IARC Organizing Principles: MoA Data

10 Key Characteristics of Human Carcinogens

- Electrophilic/Metabolically activated
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- Chronic inflammation
- Immunosuppressive
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(Smith et al., EHP 124: 713-721, 2016)
Identification of 10 Key Characteristics

- “…[a review of] agents documented and listed as human carcinogens showed a number of characteristics that are shared among many carcinogenic agents”
  - Analysis based only on IARC Group I chemicals
  - Individual “characteristics” superficially supported by literature
    - oxidative stress rationale based on two reviews
  - Analysis did not examine if any of 10 key characteristics also are found in Group III chemicals
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  - Did not consider key counterfactuals
    - paraquat and diquat: prototypical oxidant stressors – not animal carcinogens
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- “…no broadly accepted systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision making in cancer hazard identification”
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<tr>
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\(^a\) Assumes IARC controversial conclusion of “sufficient evidence” of kidney tumors and hemangiosarcomas in male mice

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