Complementarity of hazard and risk assessments following recent IARC* reviews of pesticide carcinogenicity

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** International Agency for Research on Cancer*

Disclosure of Conflicts of Interest

None

Declaration:

This presentation provides the view of Dr. McLaughlin, and does not reflect the official views of PHO, IARC, U of T or any other organization.

Outline

- Overview of IARC assessment of glyphosate
  - Glyphosate and its uses
  - IARC methodology for carcinogencity assessment
  - Results and conclusions
- Hazard assessment vs. Risk assessment
  - Strengths and limitation
  - Balance and complementarity
- Recent results
- Implications and discussion

What is glyphosate and how is it used?

- Broad-spectrum herbicide
- Used widely in agriculture, forestry and other non-agriculture settings
- "Roundup" ™ introduced in 1974 by Monsanto
- Many companies around the world now produce it
- Glyphosate mixed with water and surfactant to aid absorption by foliage
- Inhibits enzyme essential to plant growth and life that is not found in animals
- Used alone as a herbicide or combination with genetically modified crops that tolerate the herbicide

How does glyphosate work?

- Biological mechanism
  - Inhibits production of EPSPS (5-enolpyruvylshikimate-3-phosphate synthase)
  - Which blocks a pathway (shikimate) that produces aromatic amino acids needed for plant growth and life (e.g., phenylalanine, tyrosine)
  - Shikimate pathway is not present in animals, as the amino acids are derived in diet (leading to high excretion in animals and low toxicity)
  - A version of the enzyme that is resistant to glyphosate (CP4 EPSPS) was engineered into genetically modified crops that can selectively grow after glyphosate use (e.g., soy, corn, canola, cotton)
- Worldwide use of glyphosate and herbicide tolerant crops
  - Aiming to increase crop yield per acre (by reducing competition for moisture, nutrients and sun), lower operating costs (by less spraying, tillage and costs for fuel and labour, offset by higher costs of seed) and soil conservation (by less tillage to remove weeds)

Recent IARC Review of Glyphosate - Summarized in Lancet Oncology, March 2015

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate *(monograph published in July 2015)*

Table: IARC classification of some organophosphate pesticides *(Source: Guyton et al.. Lancet Oncology, online publication March 20, 2015)*

	Activity (current status)	Evidence in humans (cancer sites)	Evidence in animals	Mechanistic evidence	Classification*
Tetrachlorvinphos	Insecticide (restricted in the EU and for most uses in the USA)	Inadequate	Sufficient	..	2B
Parathion	Insecticide (restricted in the USA and EU)	Inadequate	Sufficient	..	2B
Malathion	Insecticide (currently used; high production volume chemical)	Limited (non-Hodgkin lymphoma, prostate)	Sufficient	Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death	2A†
Diazinon	Insecticide (restricted in the USA and EU)	Limited (non-Hodgkin lymphoma, leukaemia, lung)	Limited	Genotoxicity and oxidative stress	2A†
Glyphosate	Herbicide (currently used; highest global production volume herbicide)	Limited (non-Hodgkin lymphoma)	Sufficient	Genotoxicity and oxidative stress	2A†

EU=European Union. *See the International Agency for Research on Cancer (IARC) preamble for explanation of classification system (amended January, 2006). †The 2A classification of diazinon was based on limited evidence of carcinogenicity in humans and experimental animals, and strong mechanistic evidence; for malathion and glyphosate, the mechanistic evidence provided independent support of the 2A classification based on evidence of carcinogenicity in humans and experimental animals.

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IARC Evaluation of Glyphosate

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate *(Guyton et al.. Lancet Oncology, March 2015)*

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be published as volume 112 of the IARC Monographs.[1] The insecticides tetrachlorvinphos to the bioactive metabolite, paraoxon, is similar across species. Although bacterial mutagenesis tests were negative, parathion induced DNA and chromosomal damage in human cells in vitro. Parathion markedly increased rat mammary gland terminal end bud density.[2] Parathion use has been severely restricted since the 1980s. The insecticides malathion and diazinon were classified as "probably aggressive cancers after adjustment for other pesticides.[3] In mice, malathion increased hepatocellular adenoma or carcinoma (combined).[3] In rats, it increased thyroid carcinoma in males, hepatocellular adenoma or carcinoma (combined) in females, and mammary gland adenocarcinoma after subcutaneous injection in females.[4] Malathion is rapidly absorbed and distributed. Metabolism to the

Summary:

- Limited evidence in humans re: non-Hodgkin lymphoma
- Sufficient evidence of cancer in animals
- Mechanistic evidence of genotoxicity and oxidative stress
- Classified as Group 2A (probably carcinogenic)

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International Agency for Research on Cancer (IARC)

- **Mission**: Cancer research for cancer prevention
- Specialized cancer agency of the World Health Organization
- Global scope, aiming to reduce burden of disease and associated suffering, including attention to needs of low-middle income countries
- Promotes international collaboration in cancer research
- Focus on prevention, hence identification of cancer causes so preventive measures are adopted

One IARC Program = *Monographs to Assess Carcinogenicity*

- IARC Monographs are used by governments and health agencies
  - To identify potential carcinogenic hazards
  - To set priorities for conducting risk assessments of chemicals
  - To guide policy development related to prevention of exposures to known or suspected carcinogens

Hazard vs. Risk Assessment

- Hazard Assessment (by IARC)
  - A process to identify any source of potential damage, harm or adverse health effects on something or someone under certain conditions at work. (Canadian Centre for Occupational Health and Safety)
- Risk Assessment (by national regulatory agencies)
  - Human health risk assessment is a process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environments. (US-EPA definition)

Risk (probability of adverse event or effect)	=	Hazard (a recognized threat or potential loss)	x	Probability (probability that the loss will occur)

- Risk may be accepted or tolerated if expected loss is small compared to cost or difficulty of effective countermeasure. Analysis of balance between risks and benefits can also identify acceptable/tolerated risk.

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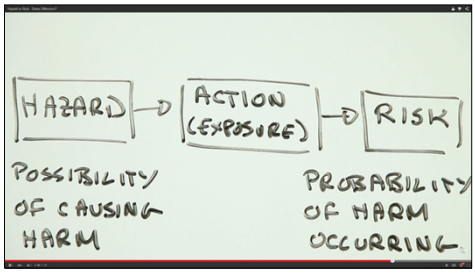
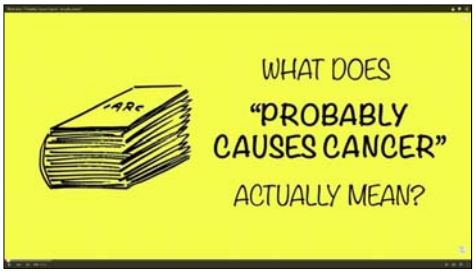
Explaining the link between Hazard and Risk

For ***Hazard*** (the possible)to become ***Risk*** (the probable)

- there must be ***Exposure***
- e.g., communicated by "Risk Bites" (on YouTube) and other sources





Source: Risk Science Centre, University of Michigan School of Public Health

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The IARC Monograph Program

- *IARC Monographs* evaluate:
  - Chemicals
  - Complex substances and mixtures
  - Physical and biological agents
  - Occupational exposures
  - Personal habits
- 112 volumes evaluated 980 agents (as of May 2015)
  - **Group 1 -** 116 classified as ***carcinogenic*** *to humans*
  - **Group 2A** - 73 classified as ***probably carcinogenic*** *to humans*
  - **Group 2B** - 287 classified as ***possibly carcinogenic*** *to humans*
  - **Group 3** - 503 classified as ***not classifiable as to its carcinogenicity*** *to humans*
  - **Group 4** - 1 classified as ***probably not carcinogenic to humans***

What makes IARC Monograph process unique?

- Comprehensive reviews that **integrate all human, experimental, and mechanistic evidence** that is published and peer-reviewed
- Consensus evaluations are carried out by Working Group consisting of world's leading experts on topic
- Rigorous and transparent review and decision making processes
- Strict control of conflicts of interests
  - Before official invitation, all sources of potential conflict must be declared through WHO process (e.g., employment, research, funding, financial)
  - Working Group members are unpaid volunteers
  - Pertinent interests are disclosed and published
- Stakeholders (with conflicts of interest) are permitted as observers to inform and monitor process, but do not vote on decisions
- Process is published in "Preamble", and regularly updated

IARC Monographs: Contents

All pertinent epidemiological (human) studies, animal studies and cancer bioassays

- Study designs and results are reviewed and critiqued

Representative mechanistic data that is relevant/important

- Includes information on (i) toxicokinetics, (ii) representative data on the 10 key characteristics of carcinogens, (iii) data relevant to comparisons across agents and end-points, (iv) cancer susceptibility, and (v) other adverse effects
- Mechanistic and other relevant data for the agent is drawn from representative studies in humans, animals, and *in vitro*

All studies must be *publicly available* (published or accepted)

- *Includes studies published in languages other than English*
- Published and peer-reviewed, including government documents in final form (e.g., does not consider research in progress, articles in preparation, consultant reports, or data that is not publicly available)

Each study summary written or reviewed by someone not associated with the study

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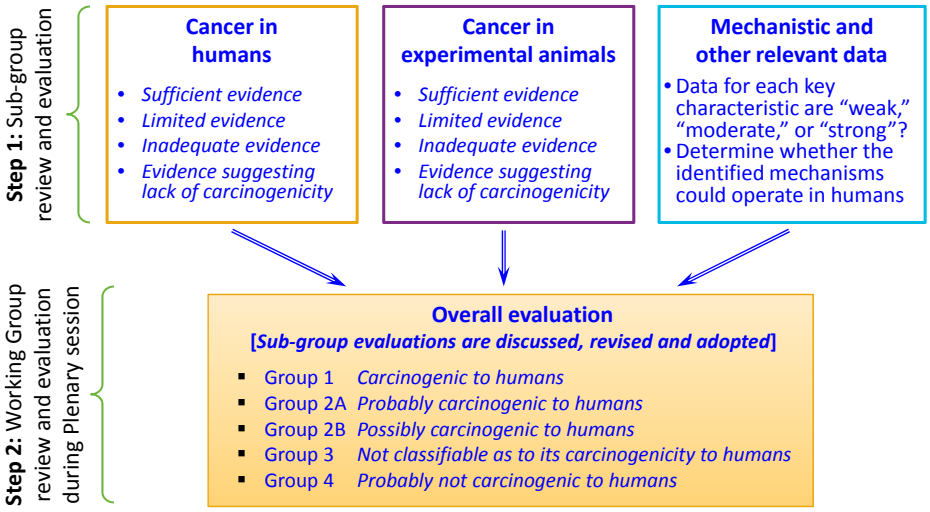
Two-Step Evaluation Process



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Evaluating human data

Cancer in humans — Preamble Part B, Section 6(a)	Cancer in experimental animals	Mechanistic and other relevant data

Sufficient evidence	Causal relationship has been established Chance, bias, and confounding could be ruled out with reasonable confidence
Limited evidence	Causal interpretation is credible Chance, bias, or confounding could not be ruled out
Inadequate evidence	Studies permit no conclusion about a causal association
Evidence suggesting lack of carcinogenicity (ESLC)	Several adequate studies covering the full range of exposure levels are mutually consistent in not showing a positive association at any observed level of exposure Conclusion is limited to cancer sites and conditions studied

Evaluating experimental animal data

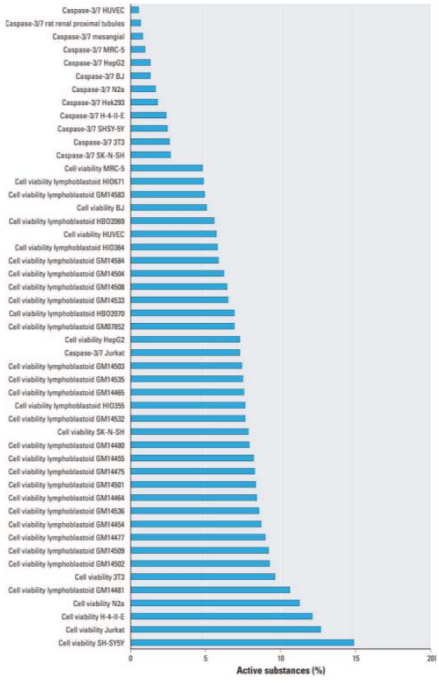
Cancer in humans	Cancer in experimental animals — Preamble Part B, Section 6(b)	Mechanistic and other relevant data

Sufficient evidence	Causal relationship has been established through either: - Multiple positive results (2 species, studies, sexes of GLP) - Single unusual result (incidence, site/type, age, multi-site)
Limited evidence	Data suggest a carcinogenic effect but: (*e.g.*) single study, benign tumours only, promoting activity only
Inadequate evidence	Studies permit no conclusion about a carcinogenic effect
Evidence suggesting lack of carcinogenicity (ESLC)	Adequate studies in at least two species show that the agent is not carcinogenic Conclusion is limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied

Evaluating mechanistic and other data

Cancer in humans	Cancer in experimental animals	Mechanistic and other relevant data — Preamble Part B, Section 6(c)

• Are the mechanistic data "weak," "moderate," or "strong"?	Have the mechanistic events been established? Are there consistent results in different experimental systems? Is the overall database coherent? Has each mechanism been challenged experimentally? Do studies demonstrate that suppression of key mechanistic processes leads to suppression of tumour development?
• Is the mechanism likely to be operative in humans?	Are there alternative explanations? Could different mechanisms operate in different dose ranges, in humans and experimental animals, or in a susceptible group? Note: an uneven level of support for different mechanisms may reflect only the resources focused on each one



New understanding of and methods to assess biological mechanisms involved in cancer etiology

- e.g., "Tox21" at NIEHS

Figure shows high-throughput screen of 50 pathway target assays on 1,408 compounds – up to 15% substances classified as active in each assay

Source: Tice et al. Enviro Health Perspectives 2013; 121: 1289

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Mechanistic and Other Considerations: 10 Key Characteristics of Carcinogens

Key characteristic	Example of relevant evidence
1. Electrophilic or undergo metabolic activation	Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.), formation of DNA and protein adducts
2. Genotoxic	DNA damage (DNA strand breaks, DNA-protein crosslinks, etc.),, gene mutations, cytogenetic changes (e.g. chromosomal, micronucleus)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)
4. Epigenetic Alterations	DNA methylation, histone modification, microRNAs
5. Oxidative Stressor	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Immunosuppressant	Decreased immuno-surveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)
9. Immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or signaling	Increased proliferation, decreased apoptosis, changes in growth factors, signaling related to replication or cell-cycle control, angiogenesis

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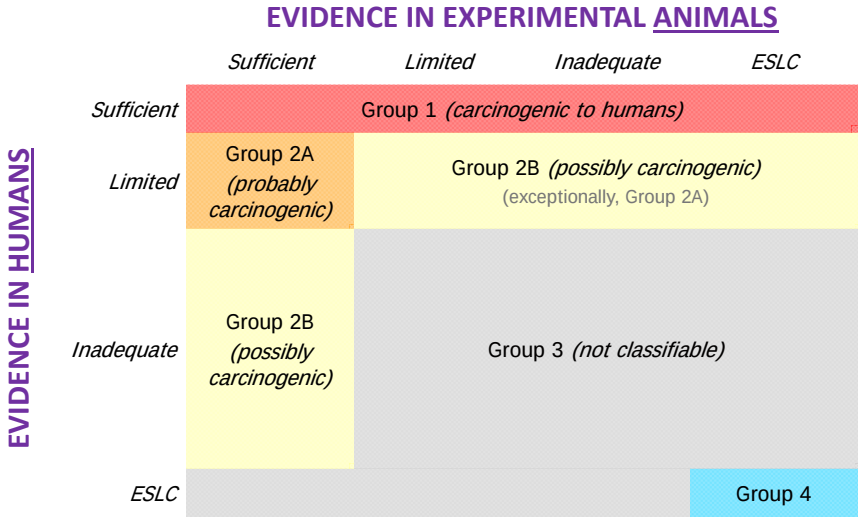
Inference based on integration (matrix) of human and experimental animal evaluations



IARC Glyphosate Review – Human Epidemiological Evidence

Epidemiological Studies that Contribute

Additional features of studies that most inform an evaluation:

- Direct measures of glyphosate use (i.e., rather than inference based on workplace), including to be able to distinguish between multiple agents and mixtures
- Measures to enable gradient of effect to be explored (e.g., frequency, duration, intensity of exposure)
- Data on other risk factors, so confounding can be controlled
- Large sample size to provide statistical power to assess relationships between rare outcomes and exposures

Key Observations:

- Case-Control Studies in 3 populations (US, Canada and Sweden)
  - Positive associations, with higher levels of occupational exposure, that persisted after adjustment (e.g., for confounders and other pesticides)
- Agricultural Health Study (US cohort study)
  - No additional support for association; which diminished consistency

Overall Conclusion: *Limited Evidence (non-Hodgkin lymphoma)*

- Causal interpretation is credible
- Chance, bias and confounding could not be ruled out with confidence

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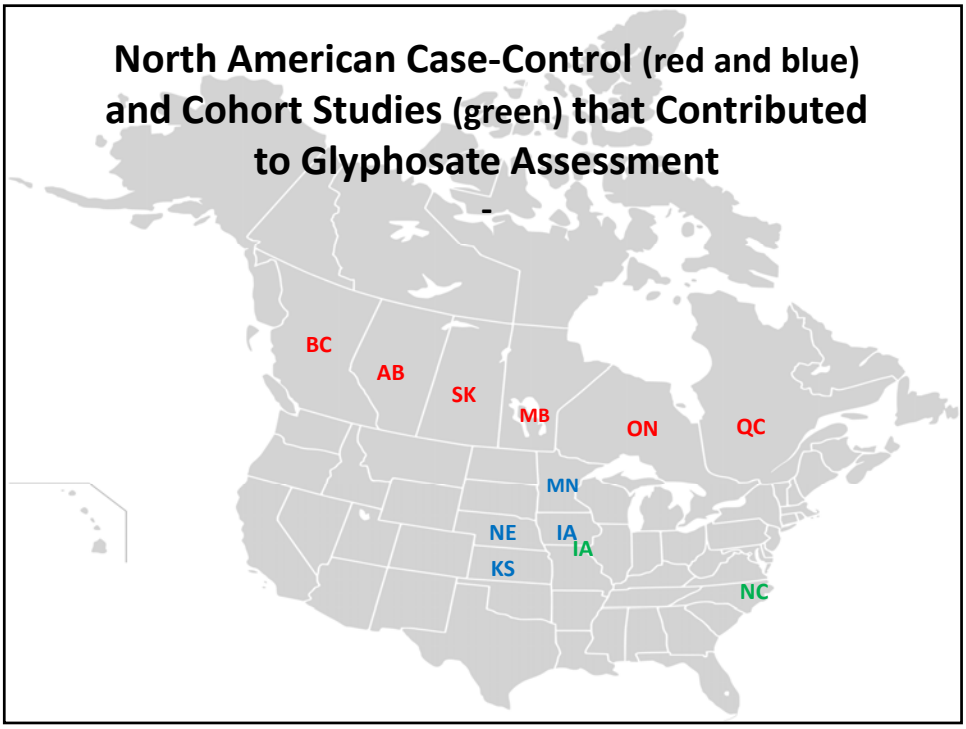
North American Case-Control (red and blue) and Cohort Studies (green) that Contributed to Glyphosate Assessment



Epidemiological Studies that Contributed Most

[published odds ratio (OR), relative risk (RR) and confidence interval (CI)]

McDuffie et al. (2001), Cancer Epidemiol Biomarkers Prev 10:1155

- Cross-Canada Case–Control Study - 517 NHL cases and 1506 controls
- recruited during 1991-94 in six provinces - exposures in early-1990s and before
- **OR = 2.1 (95% CI = 1.2-3.7)** for those with the longest use (adjusted)

De Roos et al. (2003), Occup Environ Med 60:E11

- US Mid-west Pooled Case-Control Studies - in 4 states
- 872 NHL cases and 2569 controls recruited in 1980s - exposures in 1980s and before
- **OR = 2.1 (95%CI = 1.1-4.0)** adjusted for other factors (e.g., pesticides)

De Roos et al. (2005), Environ Health Perspect 113:49

- Agricultural Health Study (cohort of applicators (*n* = 52 394) and their spouses (*n* = 32 347), from Iowa and North Carolina
- Recruited in 1993–97 – exposures in 1990s plus before and after; 92 exposed NHL cases
- Similar exposure questionnaire as case-control studies, but in cohort design
- **RR = 1.1 (95%CI = 0.7-1.9)** adjusted for other pesticides

Eriksson et al. (2008), Int J Cancer 123:1657

- Swedish Case-Control Study - Population-based with men and women
- 910 NHL cases and 1016 controls - recruited in 1999-2002
- **OR = 2.0 (95% CI, 1.1–3.7)** – adjusted - stronger associations with greater use and longer latency; some confounding by other pesticides

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IARC Glyphosate Review – Experimental Animal Evidence

- 1 mouse feeding (glyphosate) study showed significant trend in incidence of ***renal tubule adenoma or carcinoma*** (combined) in male mice; renal tubule carcinoma is a **rare tumour**
- 1 mouse feeding (glyphosate) study showed significant trend in the incidence of ***haemangiosarcoma*** in male mice
- 2 rat feeding (glyphosate) studies showed significant increase in the incidence of *pancreatic islet cell adenoma* (a benign tumour) in male rats
- 1 mouse study (formulation) showed effect on *skin cancer* in an initiation-promotion study
- Several other oral feeding (glyphosate) and drinking water (glyphosate and glyphosate formulation) studies in rats showed no significant effects

Overall conclusion: *Sufficient Evidence*
2 independent studies showing a significant association, particularly for rare tumours

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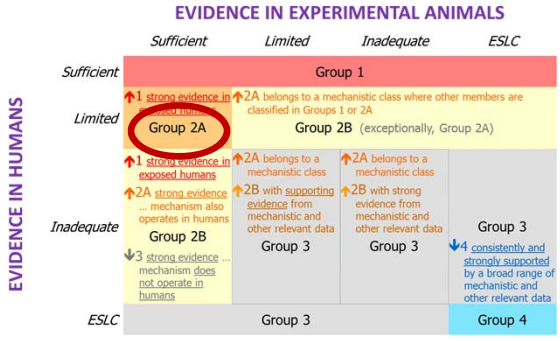
Glyphosate Monograph – Mechanistic Considerations

Key characteristic	Strength of Evidence
1. Electrophilic or ability to undergo metabolic activation	***Not*** *electrophilic*
2. Genotoxic	✓ *(Strong)*
3. Alters DNA repair or causes genomic instability	*No data*
4. Epigenetic Alterations	*No data*
5. Oxidative Stressor	✓ *(Strong)*
6. Induces chronic inflammation	*No data*
7. Immunosuppressant	*Weak*
8. Modulates receptor-mediated effects	*Weak*
9. Immortalization	*No data*
10. Alters cell proliferation, cell death, or nutrient supply	*Weak*

Conclusion: Independent support of the 2A classification

- Mechanistic data provide strong support for genotoxicity, DNA damage and oxidative stress. From *in vitro* human (operate in humans) and experimental animal analyses.
- Certain bioassays negative (eg, bacterial mutagenesis); A potential indirect pathway reported re: a degradation product (aminomethylphosphoric acid (AMPA))

Overall classification of Glyphosate



Lancet Oncology (2015) Summary: Classified as **probably** carcinogenic to humans **(Group 2A)** based on:

- **limited human** evidence (related to non-Hodgkin lymphoma)
- **sufficient** evidence from **animal** studies.
- **mechanistic** evidence provided **independent support** (but no change) for 2A classification (genotoxicity, DNA damage and oxidative stress)

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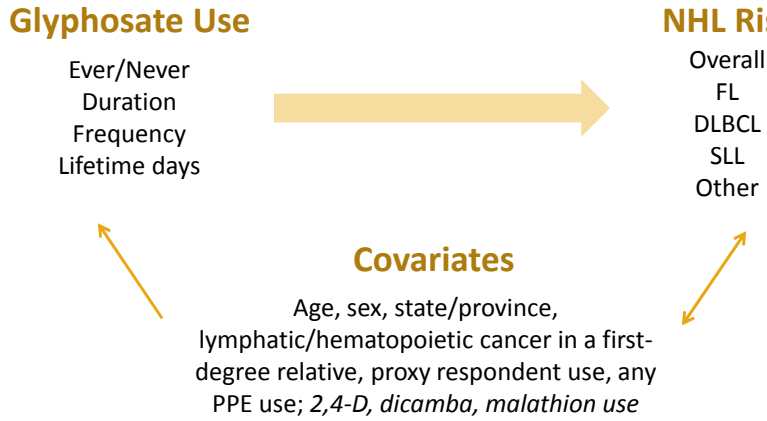
Recent finding from North American Pooled Analysis

(Pahwa et al, presented at International Society for Environmental Epidemiology, 2015)

With 1690 cases and 5131 controls



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Recent finding from North American Pooled Analysis

(Published abstract for presentation at International Society for Environmental Epidemiology, September 2015; Paper is forthcoming.)

Title: An evaluation of glyphosate use and the risks of non-Hodgkin lymphoma major histological sub-types in the North American Pooled Project (NAPP)

Authors: M Pahwa, J Spinelli, L Beane Freeman, P Demers, A Blair, P Pahwa, J Dosman, J McLaughlin, S Hoar Zahm, K Cantor, D Weisenburger, S Harris.

Objectives: Glyphosate is a commonly used herbicide worldwide. Some epidemiological studies have linked exposure with the development of non-Hodgkin lymphoma (NHL), a group of cancers with distinct risk factors and etiologies. This study aimed to evaluate possible associations between glyphosate exposure and NHL risk.
Methods: The NAPP, composed of pooled case-control studies from the US and Canada, includes NHL cases (N=1690) and controls (N=5131) who provided information on pesticide use. Cases (follicular lymphoma [FL], diffuse large B-cell lymphoma [DLBCL], small lymphocytic lymphoma [SLL], other) from cancer registries and hospitals were frequency-matched to population-based controls. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) by ever/never, duration, frequency, and lifetime days of glyphosate use. Models were adjusted for age, sex, location, proxy respondent, family history of lymphohematopoietic cancer, and personal protective equipment.
Results: Cases who ever used glyphosate had elevated NHL risk overall (OR=1.51, 95% CI: 1.18, 1.95). The highest risks were found for "other" sub-types (OR=1.91, 95% CI: 1.20, 3.04). Subjects who used glyphosate for >5 years had increased SLL risk (OR=2.58, 95% CI: 1.03, 6.48). Compared to non-handlers, those who handled glyphosate for >2 days/year had significantly elevated odds of NHL overall (OR=2.66, 95% CI: 1.61, 4.40) and FL (OR=2.36, 95% CI: 1.06, 5.29), DLBCL (OR=3.11, 95% CI: 1.61, 6.00), and other (OR=2.99, 95% CI: 1.10, 8.09) sub-types. There were suggestive increases in NHL risk overall with more lifetime days of use but this trend was not statistically significant (p=0.065).
Conclusion: This study provides some evidence that glyphosate use may be associated with increased NHL risk. Effects may differ by histological sub-type. The large sample size of the NAPP enabled a detailed investigation despite some inconsistent results across different exposure metrics.

From Hazard to Risks and Benefits

IARC Monograph 112 Summary:

	Activity (current status)	Evidence in humans (cancer sites)	Evidence in animals	Mechanistic evidence	Classification*
Tetrachlorvinphos	Insecticide (restricted in the EU and for most uses in the USA)	Inadequate	Sufficient	--	2B
Parathion	Insecticide (restricted in the USA and EU)	Inadequate	Sufficient	--	2B
Malathion	Insecticide (currently used; high production volume chemical)	Limited (non-Hodgkin lymphoma, prostate)	Sufficient	Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death	2A†
Diazinon	Insecticide (restricted in the USA and EU)	Limited (non-Hodgkin lymphoma, leukaemia, lung)	Limited	Genotoxicity and oxidative stress	2A†
Glyphosate	Herbicide (currently used; highest global production volume herbicide)	Limited (non-Hodgkin lymphoma)	Sufficient	Genotoxicity and oxidative stress	2A†

Major Uses

Mosquito control

Crops, Forestry

- Evidence to-date relates to occupational exposures, at high levels, many years ago.
- No evidence related to low levels of use, food consumption, or genetically modified food

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Implications

Must balance **benefits and risks**, and for prevention to be effective, set **exposure/dose** targets based on evidence - e.g.:

- Group 1 = ionizing radiation, air pollution, wood dust, dioxin
- Group 2a = cisplatin

Further considerations:

- Work together as risk and hazard assessments are complementary
- Hazard assessment heightens awareness of importance of attending to exposure, thereby preventing even small risks.
- Special attention on 'who is at risk,' with improved engagement, support and communication
- More high quality and timely data are needed ! (eg, re: exposures)
- Improved environmental exposure assessment is now feasible, so it is time to apply to health and environment issues.
- Build solutions with lessons learned from:
  - Infectious disease surveillance, vaccination and outbreak control
  - Phase IV clinical trials for drugs (post-marketing surveillance)

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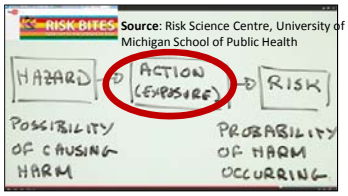
Conclusion

IARC classification of glyphosate as 2A (probable)

- Based on occupational exposures, at higher levels
- Based on exposures in past periods
- Based on NHL alone in humans
- Limitations of evidence for other exposures and outcomes

'Hazard' versus 'Risk'

- For glyphosate there is a probable hazard, due to uncertainty from human health studies, particularly for a rare cancer in the situation of certain types of exposure
- Hazard confers no risk without exposure (at non-trivial dose)
- Hazard and risk assessments are **linked and complementary**
- Central **importance of measuring and understanding exposure**



Source: Risk Science Centre, University of Michigan School of Public Health

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- **Co-investigators in the North American pooled analysis:** Manisha Pahwa, Laura Beane Freeman, Aaron Blair, Kenneth Cantor, Paul Demers, Shelley Harris, Dennis Weisenburger, Shelia Hoar Zahm
- **Funding**: National Health Research and Development Program for the pan-Canadian study; National Institutes for Health (NCI) for the US studies; Canadian Cancer Society Research Institute for the pooled analysis

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Discussion and Questions

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Thank you