Dr. James Bus

IARC USE OF “OXIDANT STRESS”

IARC Use of "Oxidant Stress" Mode of Action in Glyphosate Cancer Classification Evaluation

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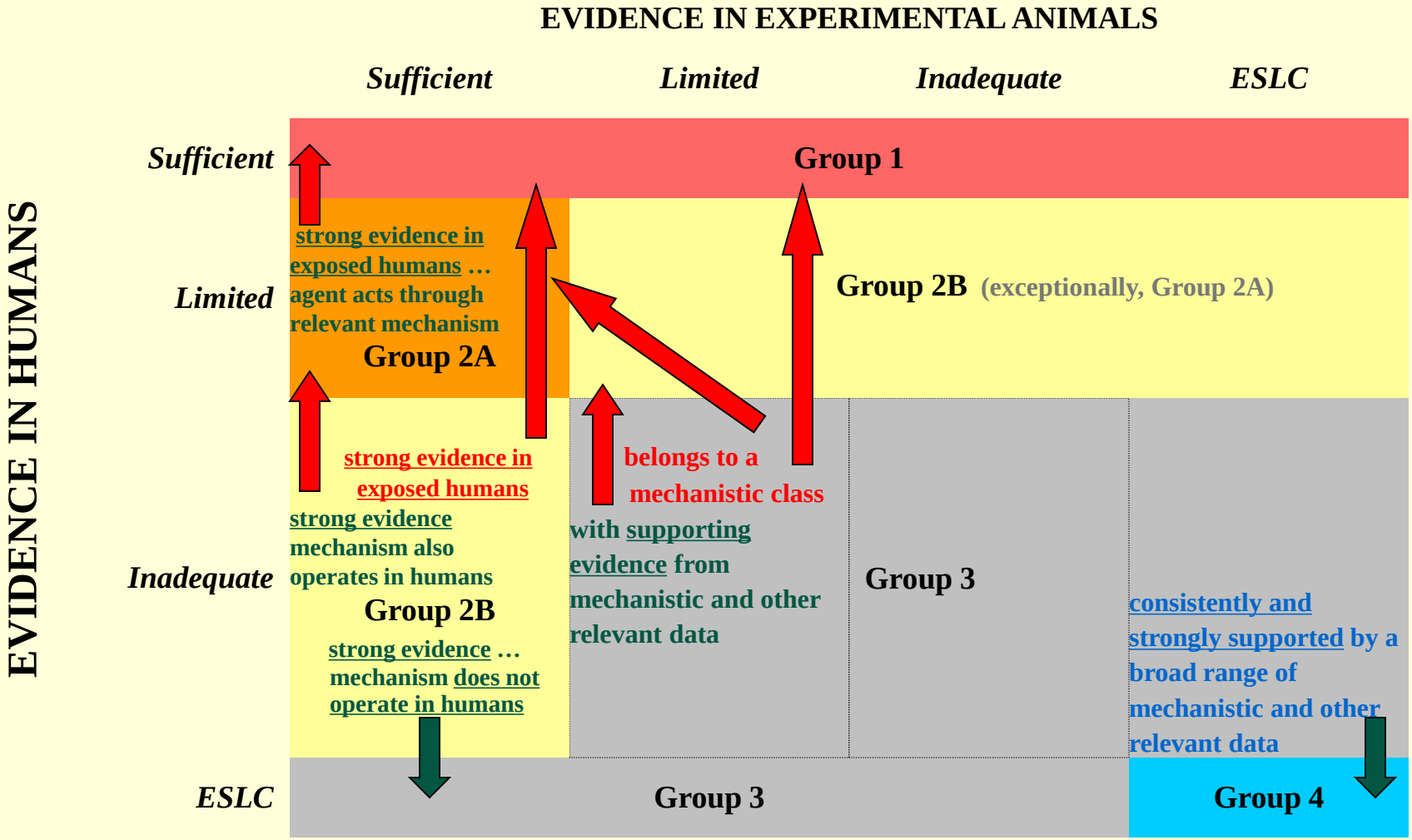
Mechanistic data can be pivotal when the human data are not conclusive - IARC



Portier, 2015; Modified from Vincent Cogliano, IARC

IARC Organizing Principles: MoA Data

10 Key Characteristics of Human Carcinogens

- Electrophilic/Metabolically activated
- Genotoxic
- Alters DNA repair or genomic instability
- Epigenetic alterations
- Oxidative Stress
- Chronic inflammation
- Immunosuppressive
- Modulates receptor mediated effects
- Causes immortalization
- Alters cell proliferation, cell death, or nutrient supply

(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
    - oxidative stress commonly reported in Group III chemicals;
  - ***Did not*** consider key counterfactuals
    - paraquat and diquat: prototypical oxidant stressors – **not animal carcinogens**

IARC Strategy for Use of 10 Key Characteristics

- "…no broadly accepted systematic method for identifying, organizing, and summarizing mechanistic data **for the purpose of decision making in cancer hazard identification"**
- "…approach may be ***difficult to translate to agents with controversial or limited mechanistic evidence*** [emphasis added] "
- "…collected information **can be organized to form hypotheses and evaluate the evidentiary support for mechanistic events as a function of relevant aspects (e.g., dose, species, temporality**)"

(Smith et al., EHP 124: 713-721, 2016)

- **Does IARC's mechanistic evaluation of glyphosate "oxidative stress" follow established mode of action analysis practices?**

Literature Cited by IARC as Evidence of Glyphosate "Oxidative Stress" is Limited

IARC Citations (type)	Citations (number)	Tested as formulation or mixture (number)	Oxidant stress limited to formulation or mixture	Single dose	Single time point	Non-relevant tissue or species[a]	Single and/or limited method(s) for detection of oxidant stress
Human (in vitro)	7	4	4	4	7	7	7
Non-human (mammalian)	7	7	5	6	6	5	7
Non-mammalian	19	19	13[b]	7	10	NA	8

[a] Assumes IARC controversial conclusion of "sufficient evidence" of kidney tumors and hemangiosarcomas in male mice

[b] No or equivocal evidence of oxidative stress reported in 6 of cited studies

Dose Relevance: IARC Human (*in vitro*) Evaluation

- **Whole animal dose context** (Anadon et al., ToxLett, 2009):
  - Rat: glyphosate 400 mg/kg gavage plasma C_{max} = 4.6 µg/ml
- ***In vitro* test concentrations**
  - 4/7 studies tested at LC_{50}: 40(F,L), 376(F,L), 8450(G,S), and 3718(G,S) µg/ml
  - Glyphosate (L) negative at 900 µg/ml; positive (F, same study) at 40 µg/ml, 24hr
  - Glyphosate (PL) positive at 580 µg/ml, 4hr
  - Glyphosate (RBC) positive at 42 µg/ml, 1hr (single ROS biomarker)

F = Formulation; G = Glyphosate; S = HaCaT skin cells; L= HepG2 liver cells; PL = primary lymphocytes; RBC = erythrocytes

» Test concentrations 9-820X higher than C_{max} plasma concentrations after 400 mg/kg oral glyphosate

Dose Relevance: IARC Non-human Mammalian

- **Glyphosate human systemic daily doses (biomonitoring):**
  - Max dose: Farmer = 4 µg/kg/day; Spouse = 0.04 µg/kg/day; Children = 0.8 µg/kg/day (Aquavella et al., 2004)
  - Other studies: 0.1-5 µg/kg/day max (reviewed in Bus 2015)
- **Animal doses:**
  - 2/7 studies: glyphosate at 10 (15 doses) or 300 mg/kg (1 dose) ***ip***
  - 2/7 studies: formulation at 50 or 200 mg/kg (1 dose) ***ip***
  - 1/7 studies: formulation at 50 mg/kg (1 dose) **dermal**
  - 2/7 studies: formulation at 50, 500 mg/kg **gavage** or 0.38% **drinking water**
  - 1/7 studies: mixture of glyphosate (10 mg/kg), zineb (15 mg/kg) and dimethoate (15 mg/kg) ***ip***

Test doses 2,500–75,000X higher than maximally exposed farmer

IARC Non-Mammalian Evaluation

- **All studies tested as formulations, rendering interpretative value to mammalian toxicity uncertain**
- **6/19 studies from same laboratory resulted in negative or equivocal findings using enzyme-modified COMET assay only in native-captured European eel species**
- **2/19 studies included glyphosate only:**
  - 1 study: glyphosate negative using enzyme-modified COMET assay in European eel
  - 1 study: glyphosate down-regulated SOD and up-regulated CAT transcript expression zebrafish testes at single dose
- **1 study was a mixture only study of 8 pesticides in oysters.**

IARC "Oxidant Stress": Application in Practice

- No evidence of **integrated data analyses** using mode of action (or other) framework evaluation
  - Oxidant stress literature simply "binned"
  - Relationships to other key characteristics not considered
  - **Dose, temporality, coherence, consistency, target organ relevance, etc. *not addressed***
- Glyphosate determined as having "strong" evidence of oxidant stress
  - No criteria identified for differentiating "weak", "moderate" or "strong" evidence
- Despite deficiencies of analysis, "oxidant stress" used as basis for support of IARC 2A glyphosate decision