# Carcinogenicity of Glyphosate
## A Systematic Review of the Available Evidence

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# Recent Cancer Assessments of Glyphosate

- IARC – March, 2015
  - Probable human carcinogen
- EFSA – November, 2015
  - Unlikely to pose a carcinogenic hazard to humans
- Portier et al. – January, 2016
  - Probable human carcinogen
- FAO/WHO Joint Meeting on Pesticides Residue (JMPR) – March, 2016
  - Unlikely to pose a carcinogenic risk to humans from exposure through the diet
- CLP Proposal (Germany, BAuA, Federal Institute for Occupational Safety and Health) – May, 2016 (draft)
  - no hazard classification for carcinogenicity is warranted
- USEPA – September, 2016 (draft)
  - Not likely to be carcinogenic to humans at doses relevant to human health risk assessment

# Table 1: Human Epidemiology Studies

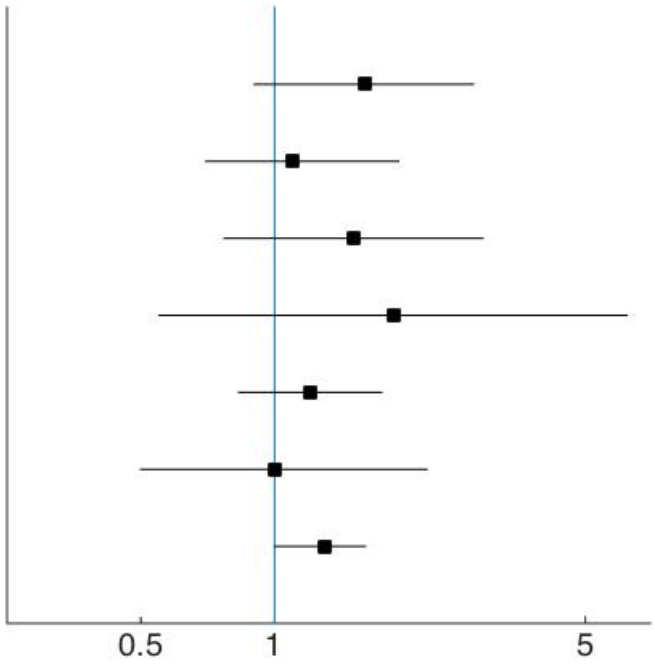
| Study | Type | Size | Findings | Exposed Cases |
|---|---|---|---|---|
| Agricultural Health Study *(De Rooset al., 2005)* | Cohort – licensed pesticide applicators | 52 395 (+32 347 spouses), 92 cases, 4-8 years follow-up | 1.1 (0.7-1.9) C<br>0.7 (0.4-1.4) 21-56% tertile compared to <20% tertile<br>0.9 (0.5-1.6) 21-56% tertile compared to >57% tertile<br>(31 cases no quantification of exposure) | 73 |
| US Midwest *(De Roos et al., 2003)* | Pooled analysis 3 case-control studies | NHL: 650 cases, 1933 controls | 2.1 (1.1-4) U<br>1.6 (0.9-2.8) C | 36<br>36 |
| Cross-Canada *(McDuffie et al., 2001)* | Population-based case-control study | 517 cases, 1506 controls | 1.2 (0.83-1.74) U<br>1.0 (0.63-1.57) ≤2 d/Y<br>2.12 (1.2-3.73) >2 d/Y | 51<br>28<br>23 |
| Swedish Case-Control Study *(Eriksson et al., 2008)* | Population-based case-control study | 910 cases, 1016 control | 2.02 (1.1-3.71) U<br>1.51 (0.77-2.94) C<br>1.69 (0.7-4.07) ≤10 d/Y<br>2.36 (1.04-5.37) >10 d/Y<br>1.11 (0.24-5.08) ≤10 Y<br>2.26 (1.16-4.4) >10 Y | 29<br>29<br>12<br>17<br>NR<br>NR |
| Swedish Case-Control Study *(Hardell et al., 1999)* | Population-based case-control study | 404 cases, 741 control (limited power) | 2.3 (0.4-13) U<br>5.8 (0.6-5.4) C (not specified) | 4<br>NR |
| France Case-Control *(Orsi et al, 2009)* | Hospital-based case-control study | 244 cases, 456 controls | 1.0 (0.5-2.2) U | 12 |
| Swedish Case-Control Study *(Hardell et al., 2002)* | Population-based case-control study | 515 cases, 1141 controls | 3.04 (1.08-8.5) U<br>1.85 (0.55-6.2) C (not specified) | 8<br>8 |
| US Case-Control Study *(Lee et al., 2004)* | Population-based case-control study | 872 cases, 2381controls | 1.4 (0.98-2.1) U – no asthma<br>1.2 (0.4-3.3) U - asthma | 53<br>6 |

# Meta Analyses

| Study | Included Studies | Findings |
|---|---|---|
| Schinasi and Leon, 2014 | McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003 and 2005; Eriksson et al., 2008; Orsi et al., 2009) | 1.5 (1.1-2.0) |
| IARC Monograph Working Group | McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003 and 2005; Eriksson et al., 2008; Orsi et al., 2009) | 1.3 (1.103-1.65) – used adjusted risk estimates from Hardell et al., 2003 and Eriksson et al., 2008 |
| Chang and Delzell, 2016 | McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003 and 2005; Eriksson et al., 2008; Orsi et al., 2009) | 1.3 (1.0-1.6) |

# Tree Plot of Epidemiology Studies
(using analyses corrected for potential confounders)

| Study | RR | Lower | Upper | Weight |
|---|---|---|---|---|
| De Roos et al. (2003) | 1.600 | 0.900 | 2.800 | 16.2 |
| De Roos et al. (2005) | 1.100 | 0.700 | 1.900 | 21.0 |
| Eriksson et al., (2008) | 1.510 | 0.770 | 2.940 | 11.6 |
| Hardell et al. (2002) | 1.850 | 0.550 | 6.200 | 3.6 |
| McDuffie et al. (2001) | 1.200 | 0.830 | 1.740 | 38.1 |
| Oris et al. (2009) | 1.000 | 0.500 | 2.200 | 9.5 |
| Meta-Analysis | 1.300 | 1.000 | 1.600 | 100.0 |




Chang and Delzell (2016)

# Summary of Human Evidence

- Limited Evidence in Humans
  - IARC, Portier et al.
- Insufficient evidence in humans
  - EFSA, CLP Proposal, EPA (draft)
- Definition of Limited Evidence (CLP Guidance, 2015; IARC 2006)
  - limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. (3.6.2.2.3.a)

# Carcinogenicity Studies in Male Mice

| Year | Strain | Length[1] | Top Dose[2] | Renal Tumors | Hemangio-sarcomas | Malignant Lymphoma |
|---|---|---|---|---|---|---|
| 1983[5] | Crl:CD-1 | 24 | 4,841 | +[3] | | |
| 1993[5] | ?:CD-1 | 24 | 1,000 | | + | +/-[4] |
| 1997 | CrJ:CD-1 | 18 | 4,843 | + | + | + |
| 2001 | SW | 18 | 1,460 | + | | +/-[6] |
| 2009 | Crl:CD-1 | 18 | 810 | | | + |

1 – months; 2 – mg/kg bw/day; 3 - + indicates a p-value of <0.05 as calculated by BfR using the Armitage linear trend test in proportions; 4 – p=0.08; 5 – studies evaluated in IARC review; 6 – p=0.053

+ indicates studies evaluated by IARC

Table based on Table 5.3-1 in the EFSA Renewal Assessment Report, Addendum I (8/31/2015)

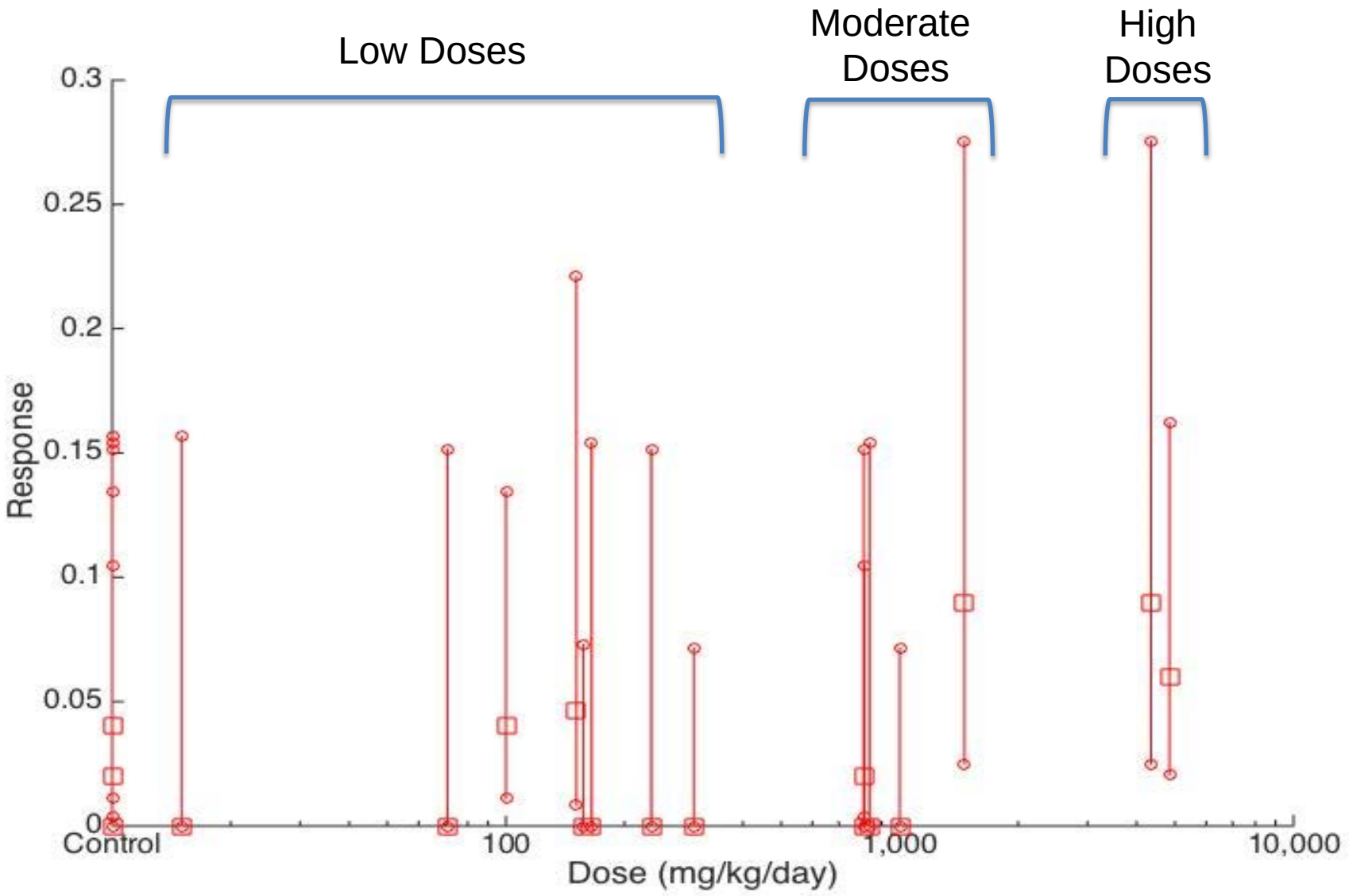
# Analysis of Male Mouse Renal Tumors[1] From the Individual Studies

| Year | Strain | Length | Doses (mg/kg/d) | Response | p-Trend (p-poly3)[2] |
|---|---|---|---|---|---|
| 1983 | Crl:CD-1 | 24 | 157, 814, 4841 | 1/50, 0/49, 1/50, 3/50 | **0.03 (0.03)** |
| 1993 | ?:CD-1 | 24 | 100, 300, 1000 | 2/50, 2/50, 0/50, 0/50 | 0.94 (0.94) |
| 1997 | CrJ:CD-1 | 18 | 165, 838, 4348 | 0/50, 0/50, 0/50, 2/50 | **0.008 (0.009)** |
| 2001 | SW | 18 | 15, 151, 1460 | 0/49, 0/49, 1/50, 2/50 | **0.04 (0.04)** |
| 2009 | Crl:CD-1 | 18 | 71, 234, 810 | 0/51, 0/51, 0/51, 0/51 | - |

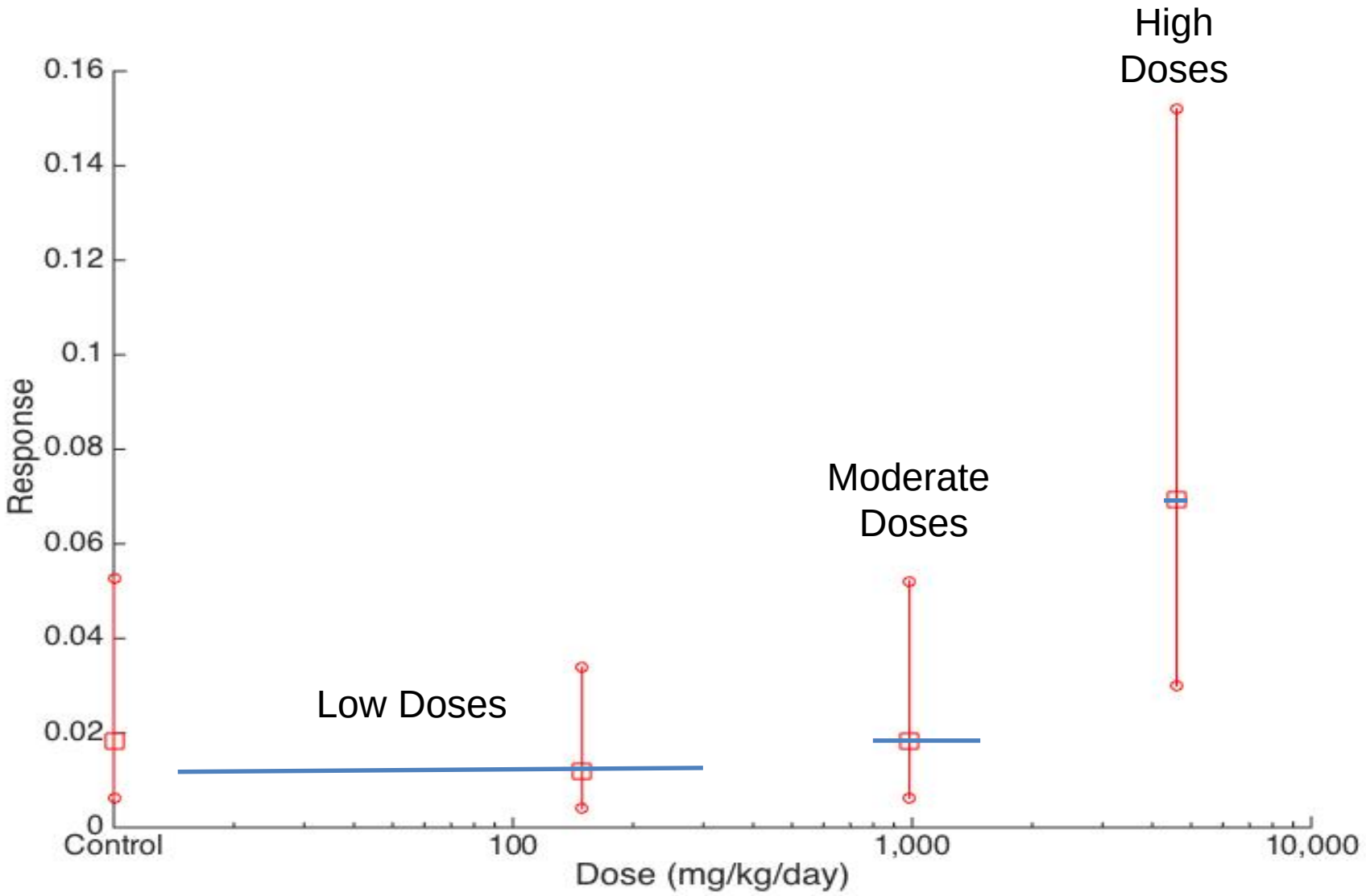
1 – Giknis and Clifford, 2005 historical control rate=0.0038, 43 of 52 studies had no tumors, 7 had 1 tumor and 2 had 2 tumors

2 – Poly-3 adjustment used to predict response at 24 months from response at 18 months; see Bailer and Portier (1988)

# Renal tumors in male mice poly-3 adjusted showing individual dose groups



# Renal tumors in male mice poly-3 adjusted and clustered by similar doses



# Renal Tumors in Male Mice

| Study | Approx. Trend | Exact Trend[1] | Historical Trend[2] |
|---|---|---|---|
| Knezevich and Hogan, 1983 | 0.033 | 0.063 | 0.009 |
| Atkinson, 1993b | 0.94 | 0.982 | 1 |
| Sugimoto, 1997 | 0.008 | 0.061 | 0.009 |
| Kumar, 2001 | 0.04 | 0.059 | 0.011 |
| Wood et al., 2009b | 0.5 | 1 | 0.629 |
| All experiments combined | <0.001 | 0.003 | 0.004 |
| All CD-1 Studies Combined | <0.001 | 0.005 | 0.008 |
| All experiments combined, doses<1500 | 0.212 | 0.209 | 0.206 |
| All CD-1 experiments combined, doses<1000 | 0.851 | 0.856 | 0.867 |

1 – Exact test is based upon a permutation test with fixed marginals.
2 - Historical trend test is based upon historical control data from Giknis and Clifford (2005)

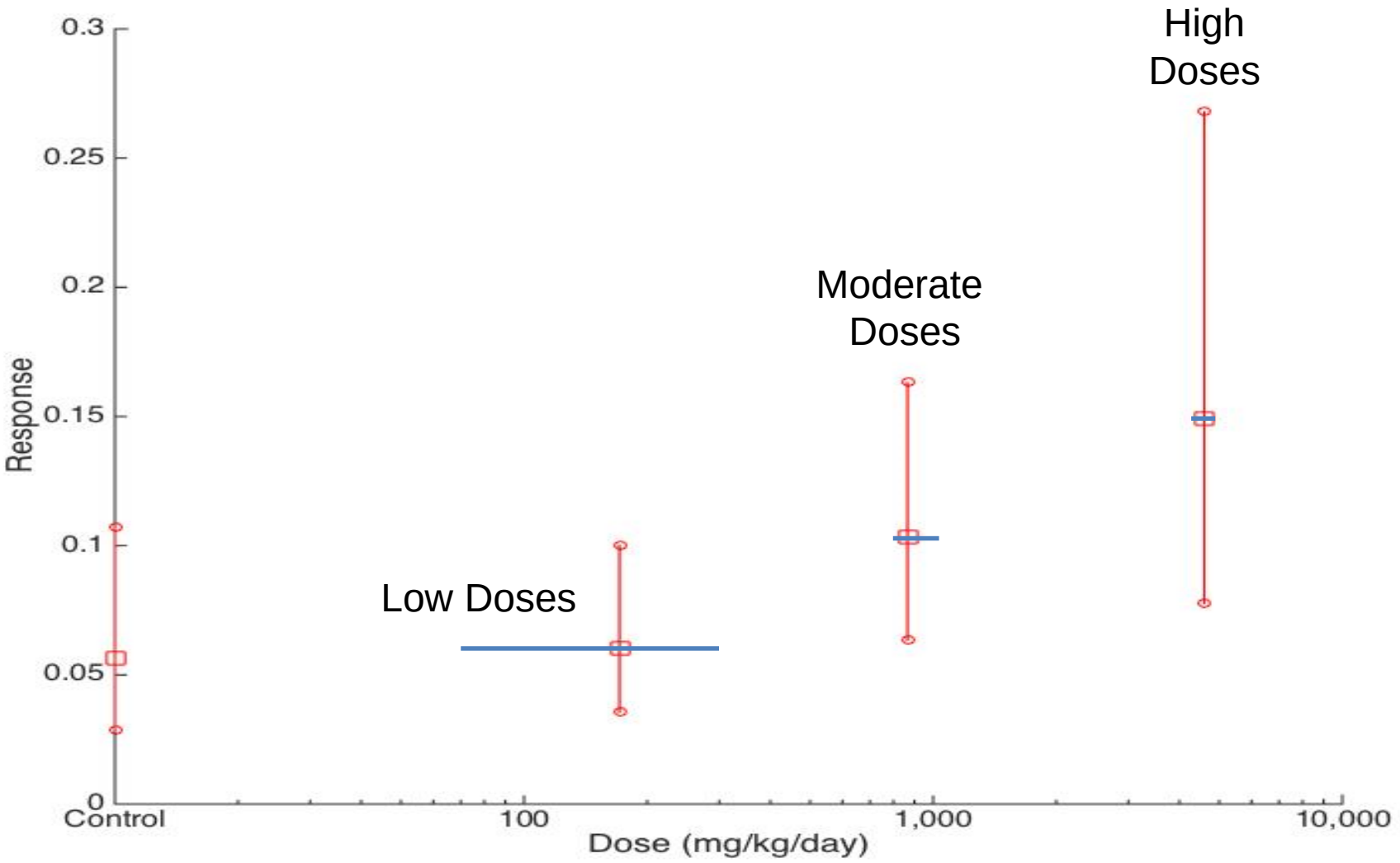
# Analysis of Male Mouse Malignant Lymphoma From the Individual Studies

| Year | Strain | Length | Doses (mg/kg/d) | Response | p-Trend (p-poly3)[2] |
|---|---|---|---|---|---|
| 1983 | Crl:CD-1 | 24 | 157, 814, 4841 | 2/50, 5/49, 4/50, 2/50 | 0.51 (0.51) |
| 1993 | ?:CD-1 | 24 | 100, 300, 1000 | 4/50, 2/50, 1/50, 6/50 | 0.08 (0.08) |
| 1997 | CrJ:CD-1 | 18 | 165, 838, 4348 | 2/50, 2/50, 0/50, 6/50 | **0.008 (0.012)** |
| 2001 | SW | 18 | 15, 151, 1460 | 10/49, 15/49, 16/49, 19/49 | **0.05 (0.09)** |
| 2009 | Crl:CD-1 | 18 | 71, 234, 810 | 0/51, 1/51, 2/51, 5/51 | **0.004 (0.005)** |

1 – Giknis and Clifford, 2005 historical control rate=0.045 (0.027 in 18 month and 0.06 in 24 month), 8 of 26 18-month studies had no tumors, 3 of 26 24-month studies had no tumors

2 – Poly-3 adjustment used to predict response at 24 months from response at 18 months; see Bailer and Portier (1988)

# Malignant lymphomas in male CD-1mice poly-3 adjusted and clustered by similar doses



# Malignant Lymphomas in Male Mice

| Study | Approx. Trend | Exact Trend[1] | Historical Trend[2] |
|---|---|---|---|
| Knezevich and Hogan, 1983 | 0.515 | 0.736 | 0.484 |
| Atkinson, 1993b | 0.076 | 0.095 | 0.087 |
| Sugimoto, 1997 | 0.008 | 0.02 | 0.013 |
| Kumar, 2001 | 0.053 | 0.105 | 0.072 |
| Wood et al., 2009b | 0.004 | 0.008 | 0.007 |
| All experiments combined | 0.173 | 0.426 | 0.172 |
| All CD-1 Studies Combined | 0.015 | 0.084 | 0.021 |
| All experiments combined, doses<1500 | <0.001 | 0.002 | 0.001 |
| All CD-1 experiments combined, doses<1000 | 0.031 | 0.036 | 0.039 |

1 – Exact test is based upon a permutation test with fixed marginals.
2 - Historical trend test is based upon historical control data from Giknis and Clifford (2005)

# Analysis of Male Mouse Hemangiosarcomas[1] From the Individual Studies

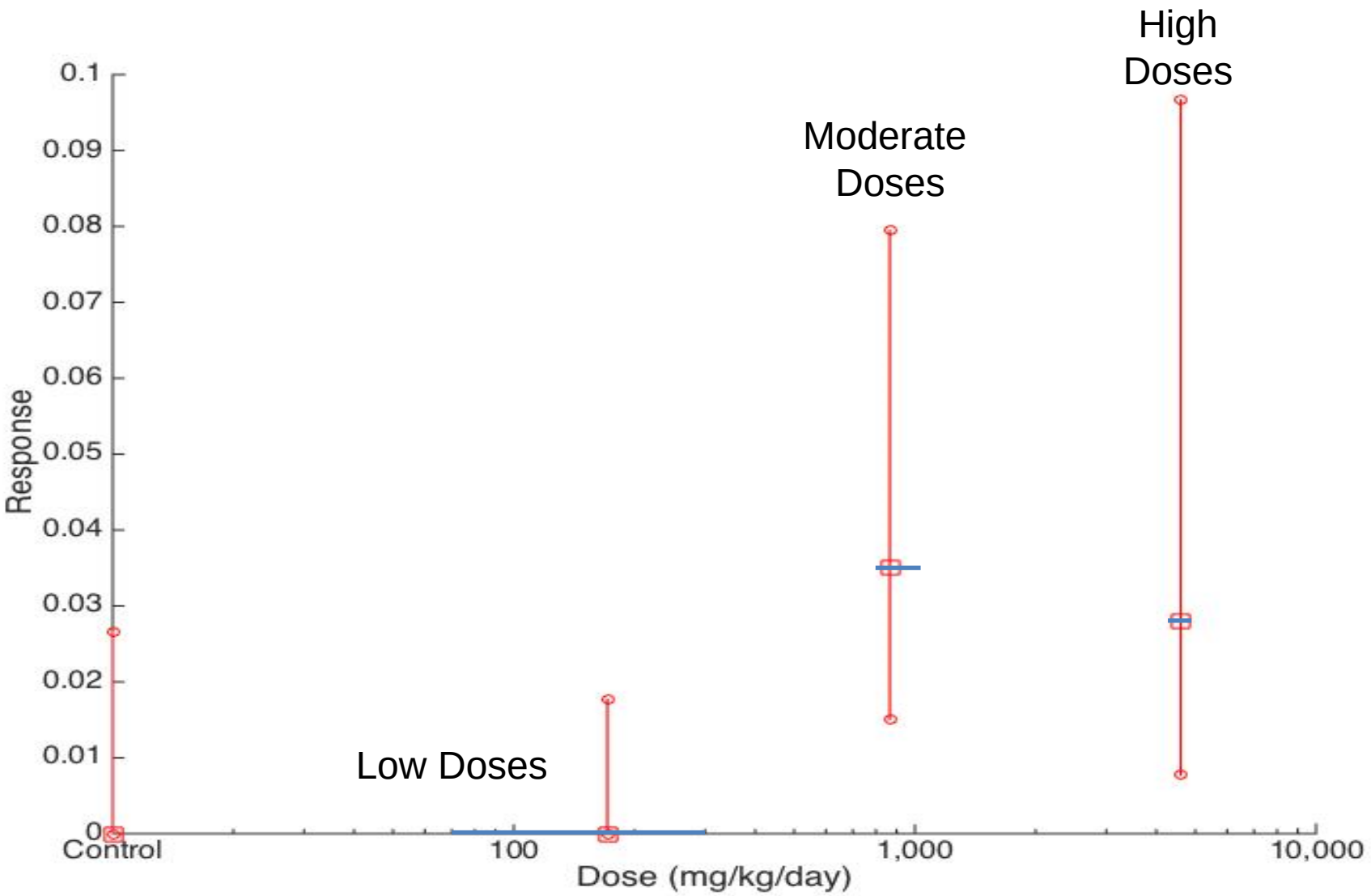
| Year | Strain | Length | Doses (mg/kg/d) | Response | p-Trend (p-poly3)[2] |
|---|---|---|---|---|---|
| 1983 | Crl:CD-1 | 24 | 157, 814, 4841 | 0/50, 0/49, 1/50, 0/50 | 0.63 (0.63) |
| 1993 | ?:CD-1 | 24 | 100, 300, 1000 | 0/50, 0/50, 0/50, 4/50 | **0.0004 (0.0004)** |
| 1997 | CrJ:CD-1 | 18 | 165, 838, 4348 | 0/50, 0/50, 0/50, 2/50 | **0.008 (0.009)** |
| 2001 | SW | 18 | 15, 151, 1460 | 0/50, 0/50, 2/50, 0/50 | 0.724 (0.724) |
| 2009 | Crl:CD-1 | 18 | 71, 234, 810 | 0/51, 0/51, 0/51, 0/51[3] | 0.5 (0.50 |

1 – Giknis and Clifford, 2005 historical control rate=0.01 (0 in 18 month and 0.018 in 24 month), all of 26 18-month studies had no tumors, 18 of 26 24-month studies had no tumors

2 – Poly-3 adjustment used to predict response at 24 months from response at 18 months; see Bailer and Portier (1988)

3 – CLP Proposal Table 42 lists tumor counts for this study of 2/51, 1/51, 2/51 and 1/51. However, these rates include hemangiomas from liver and kidney, making them different from the other studies and not applicable for the comparisons that follow

# Hemangiosarcomas in male CD-1 mice poly-3 adjusted and clustered by similar



# Hemangiosarcomas in Male Mice

| Study | Approx. Trend | Exact Trend[1] | Historical Trend[2] |
|---|---|---|---|
| Knezevich and Hogan, 1983 | 0.628 | 0.5 | 0.592 |
| Atkinson, 1993b | <0.001 | 0.004 | <0.001 |
| Sugimoto, 1997 | 0.008 | 0.061 | 0.021 |
| Kumar, 2001 | 0.5 | 0.494 | 0.621 |
| Wood et al., 2009b | 0.5 | 1 | 0.49 |
| All experiments combined | 0.041 | 0.056 | 0.060 |
| All CD-1 Studies Combined | 0.024 | 0.044 | 0.041 |
| All experiments combined, doses<1500 | 0.007 | 0.016 | 0.014 |
| All CD-1 experiments combined, doses<1000 | <0.001 | <0.001 | <0.001 |

1 – Exact test is based upon a permutation test with fixed marginals.
2 - Historical trend test is based upon historical control data from Giknis and Clifford (2005)

# Carcinogenicity Studies in Rats

| Year | Strain | Length[1] | Top Dose[2] | Finding |
|---|---|---|---|---|
| +Atkinson et al., 1993 | SD | 24 | 1000 | none |
| +Lankas, 1981 | SD | 26 | ~32 | inadequate dose, testicular tumors (M), pancreas islet cell aden. (M, weak) |
| +Stout & Ruecker, 1990 | SD | 24 | 1183 | liver aden. (M), pancreas islet cell aden. (M), thyroid aden. (F) |
| Enemoto, 1997 | SD | 24 | 1127 | none |
| Pavkov & Wyand, 1987 | SD | 24 | 41.8 | inadequate dose and purity |

1 – months; 2 – mg/kg bw/day;
+ indicates studies evaluated by IARC

# Carcinogenicity Studies in Rats

| Year | Strain | Length[1] | Top Dose[2] | Finding |
|---|---|---|---|---|
| +Seralini et al., 1993 | SD | 24 | 2250 mg/L in water | inadequate, mammary tumors |
| +Suresh, 1996 | Wistar | 24 | 886 | none |
| Wood et al., 2004 | Wistar | 24 | 1229.7 | mammary gland tumors (F) |
| Brammer, 2001 | Wistar | 24 | 1,498 | Liver aden. (M) |
| +Chru-scielska et al., 2000 | Wistar | 24 | 2250 mg/L in water | inadequate documentation |
| +Syngenta, 1996 | Wistar | 12 | 1409 | Inadequate length of study |

1 – months; 2 – mg/kg bw/day;
+ indicates studies evaluated by IARC

# Summary of Animal Cancer Data

- Sufficient Evidence
  - IARC, Portier et al.
- Insufficient Evidence
  - EFSA, CLP Proposal, USEPA, WHO/JMPR
- Definition of Sufficient Evidence (CLP Guidance, 2015; IARC, 2006)
  - sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.
  - A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites
-

# Glyphosate Monograph – Mechanistic and Other Considerations:

## Key Characteristic of Carcinogens #2 (Genotoxic)

| Agent | Strength of the evidence | Evidence base includes | Endpoints considered in the evaluation |
|---|---|---|---|
| Glyphosate | Strong | 1. ***Largely positive*** studies:<br>• in human cells *in vitro*,<br>• in mammalian model systems *in vivo* and *in vitro*,<br>• studies in other non-mammalian organisms<br>2. ***Generally positive*** studies in liver *in vivo* in mammals<br>3. ***Mixed*** results for kidney and bone marrow *in vivo* in mammals<br>4. ***Consistently negative*** results from tests in bacterial assays | • Biomarkers of DNA adducts<br>• Biomarkers of various types of chromosomal damage |
| Glyphosate formulations | Strong | 1. ***Evidence in exposed humans***:<br>• three studies of genotoxicicity endpoints in community residents exposed to glyphosate formulations, two of which reported positive associations<br>• one of these studies examined subjects before and after aerial spraying and found a significant increase in micronuclei after exposure in 3 of 4 different geographical areas<br>2. ***Largely positive*** studies:<br>• in human cells *in vitro*,<br>• in mammalian model systems *in vivo* and *in vitro*,<br>• studies in other non-mammalian organisms<br>3. ***Generally negative*** results from tests in bacterial assays<br>4. The ***pattern*** of tissue specificity of genotoxicity endpoints observed with glyphosate formulations is similar to that observed with glyphosate alone | • Chromosomal damage (micronuclei) in circulating blood cells from humans<br>• Biomarkers of DNA adducts<br>• Biomarkers of various types of chromosomal damage |
| AMPA | Moderate | 1. Two human *in vitro* studies<br>2. One mammalian *in vivo* study<br>3. One mammalian *in vitro* study<br>4. One study in eel | While the number of studies is not large, all of the studies were ***positive*** |

# Glyphosate Monograph – Mechanistic and Other Considerations:

## Key Characteristic of Carcinogens #5 (Oxidative Stressor)

| Agent | Strength of the evidence | Evidence base includes | Endpoints considered in the evaluation |
|---|---|---|---|
| Glyphosate | Strong | 1. Rodent studies *in vivo* (including similar effects observed in many tissues)<br>2. Rodent cells *in vitro*<br>3. Human cells *in vitro* | • Lipid peroxidation markers<br>• Oxidative DNA adducts<br>• Dysregulation of antioxidant enzymes<br>• Some studies challenged this mechanism experimentally (*e.g.*, by co-administering antioxidants) |
| Glyphosate formulations | Strong | | |
| AMPA | Strong | | |

# Conclusions

- Glyphosate should be listed as a Category 1B Carcinogen
  - animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)[1]
  - In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals[1]
  - In this case limited evidence in humans and sufficient in animals

1. Guidance on the Application of the CLP Criteria, Table 3.6.1 (2015)