

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

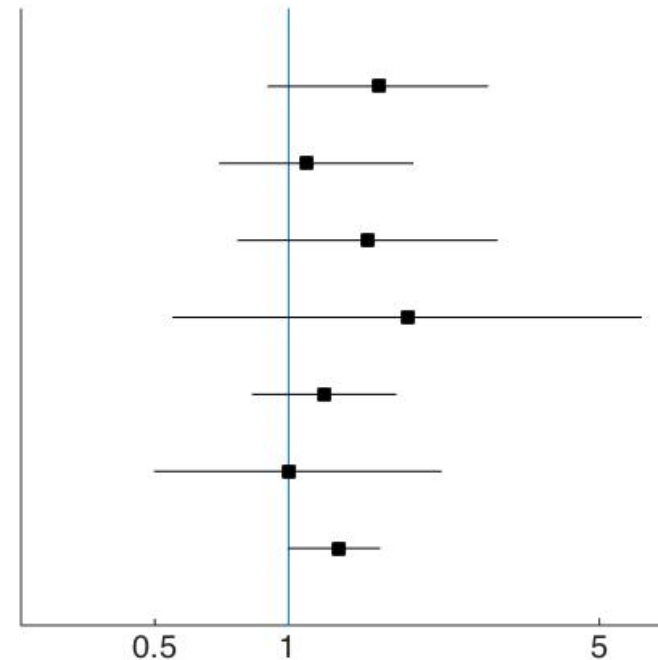
# Meta Analyses

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



# 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 <sup>1</sup>	Top Dose <sup>2</sup>	Renal Tumors	Hemangio-sarcomas	Malignant Lymphoma
1983 <sup>5</sup>	CrI:CD-1	24	4,841	+ <sup>3</sup>		
1993 <sup>5</sup>	?:CD-1	24	1,000		+	+/- <sup>4</sup>
1997	CrJ:CD-1	18	4,843	+	+	+
2001	SW	18	1,460	+		+/- <sup>6</sup>
2009	CrI: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<sup>1</sup> From the Individual Studies

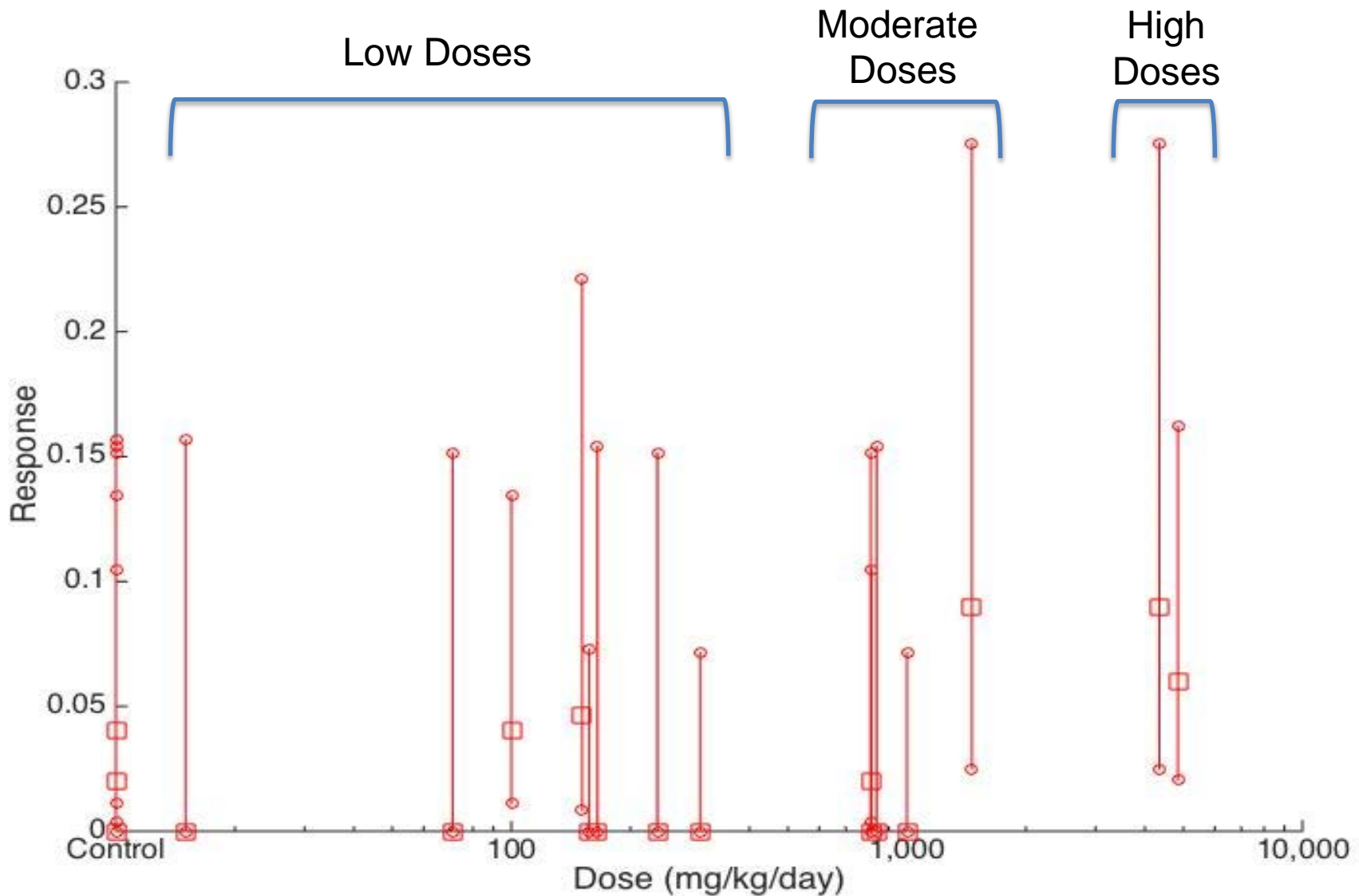
Year	Strain	Length	Doses (mg/kg/d)	Response	p-Trend (p-poly3) <sup>2</sup>
1983	Crl:CD-1	24	157, 814, 4841	1/50, 0/49, 1/50, 3/50	<b>0.03 (0.03)</b>
1993	?:CD-1	24	100, 300, 1000	2/50, 2/50, 0/50, 0/50	0.94 (0.94)
1997	CrJ:CD-1	18	165, 838, 4348	0/50, 0/50, 0/50, 2/50	<b>0.008 (0.009)</b>
2001	SW	18	15, 151, 1460	0/49, 0/49, 1/50, 2/50	<b>0.04 (0.04)</b>
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

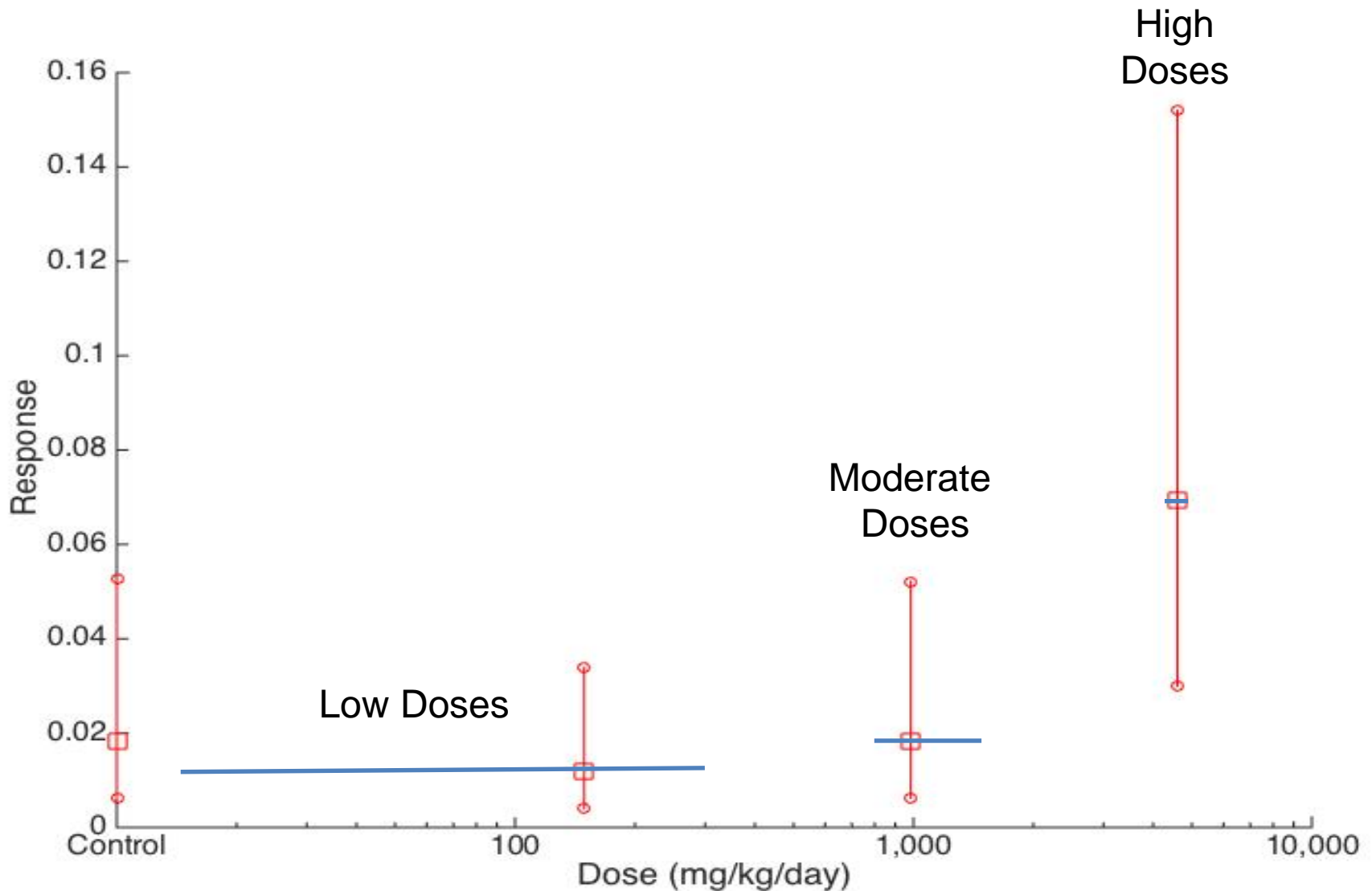
2 – Poly-3 adjustment used to predict response at 24 months from response at 18 months; see Bailer and Portier (1988)



# 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 <sup>1</sup>	Historical Trend <sup>2</sup>
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)

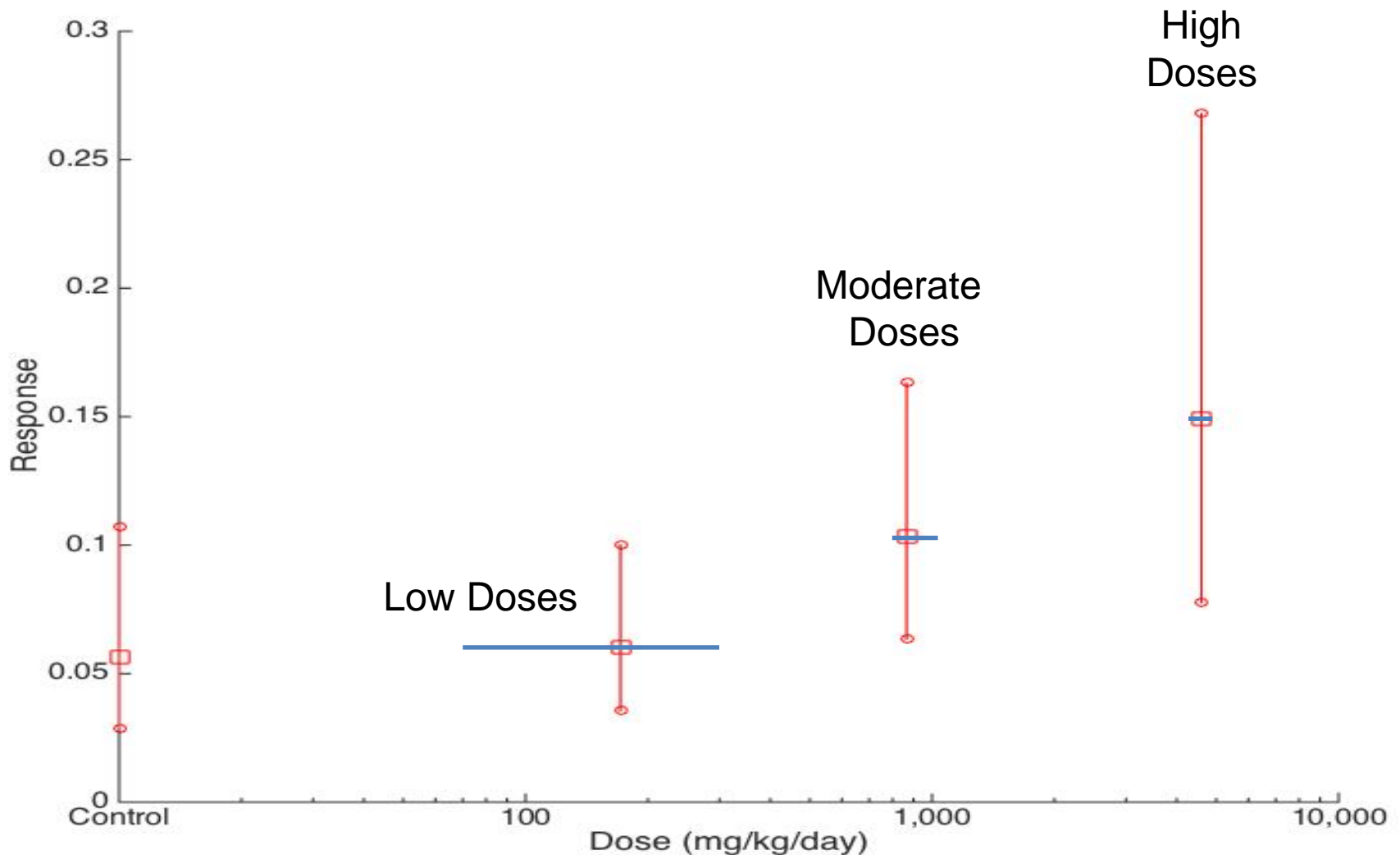
# Analysis of Male Mouse Malignant Lymphoma From the Individual Studies

Year	Strain	Length	Doses (mg/kg/d)	Response	p-Trend (p-poly3) <sup>2</sup>
1983	CrI: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	<b>0.008 (0.012)</b>
2001	SW	18	15, 151, 1460	10/49, 15/49, 16/49, 19/49	<b>0.05 (0.09)</b>
2009	CrI:CD-1	18	71, 234, 810	0/51, 1/51, 2/51, 5/51	<b>0.004 (0.005)</b>

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-1 mice poly-3 adjusted and clustered by similar doses



# Malignant Lymphomas in Male Mice

Study	Approx. Trend	Exact Trend <sup>1</sup>	Historical Trend <sup>2</sup>
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<sup>1</sup> From the Individual Studies

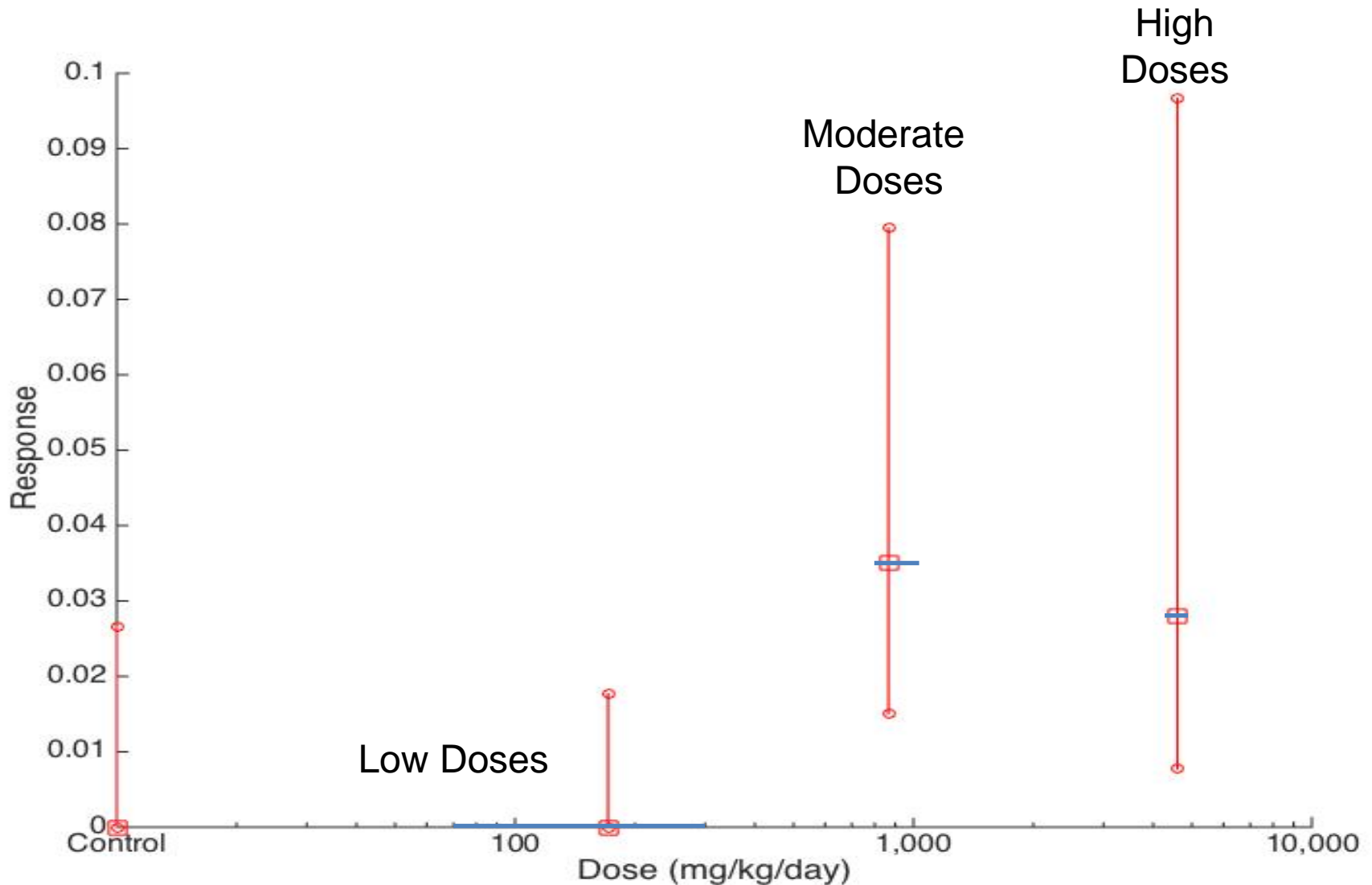
Year	Strain	Length	Doses (mg/kg/d)	Response	p-Trend (p-poly3) <sup>2</sup>
1983	CrI:CD-1	24	157, 814, 4841	0/50, 0/49, 1/50, 0/50	0.63 (0.63)
1993	?:CD-1	24	100, 300, 1000	0/50, 0/50, 0/50, 4/50	<b>0.0004 (0.0004)</b>
1997	CrJ:CD-1	18	165, 838, 4348	0/50, 0/50, 0/50, 2/50	<b>0.008 (0.009)</b>
2001	SW	18	15, 151, 1460	0/50, 0/50, 2/50, 0/50	0.724 (0.724)
2009	CrI:CD-1	18	71, 234, 810	0/51, 0/51, 0/51, 0/51 <sup>3</sup>	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 <sup>1</sup>	Historical Trend <sup>2</sup>
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 <sup>1</sup>	Top Dose <sup>2</sup>	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 <sup>1</sup>	Top Dose <sup>2</sup>	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)
+Chruscielska 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	<ol style="list-style-type: none"> <li>1. <b>Largely positive</b> studies: <ul style="list-style-type: none"> <li>• in human cells <i>in vitro</i>,</li> <li>• in mammalian model systems <i>in vivo</i> and <i>in vitro</i>,</li> <li>• studies in other non-mammalian organisms</li> </ul> </li> <li>2. <b>Generally positive</b> studies in liver <i>in vivo</i> in mammals</li> <li>3. <b>Mixed</b> results for kidney and bone marrow <i>in vivo</i> in mammals</li> <li>4. <b>Consistently negative</b> results from tests in bacterial assays</li> </ol>	<ul style="list-style-type: none"> <li>• Biomarkers of DNA adducts</li> <li>• Biomarkers of various types of chromosomal damage</li> </ul>
Glyphosate formulations	Strong	<ol style="list-style-type: none"> <li>1. <b>Evidence in exposed humans:</b> <ul style="list-style-type: none"> <li>• three studies of genotoxicity endpoints in community residents exposed to glyphosate formulations, two of which reported positive associations</li> <li>• 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</li> </ul> </li> <li>2. <b>Largely positive</b> studies: <ul style="list-style-type: none"> <li>• in human cells <i>in vitro</i>,</li> <li>• in mammalian model systems <i>in vivo</i> and <i>in vitro</i>,</li> <li>• studies in other non-mammalian organisms</li> </ul> </li> <li>3. <b>Generally negative</b> results from tests in bacterial assays</li> <li>4. The <b>pattern</b> of tissue specificity of genotoxicity endpoints observed with glyphosate formulations is similar to that observed with glyphosate alone</li> </ol>	<ul style="list-style-type: none"> <li>• Chromosomal damage (micronuclei) in circulating blood cells from humans</li> <li>• Biomarkers of DNA adducts</li> <li>• Biomarkers of various types of chromosomal damage</li> </ul>
AMPA	Moderate	<ol style="list-style-type: none"> <li>1. Two human <i>in vitro</i> studies</li> <li>2. One mammalian <i>in vivo</i> study</li> <li>3. One mammalian <i>in vitro</i> study</li> <li>4. One study in eel</li> </ol>	While the number of studies is not large, all of the studies were <b>positive</b>

# 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	<ol style="list-style-type: none"> <li>1. Rodent studies <i>in vivo</i> (including similar effects observed in many tissues)</li> <li>2. Rodent cells <i>in vitro</i></li> <li>3. Human cells <i>in vitro</i></li> </ol>	<ul style="list-style-type: none"> <li>• Lipid peroxidation markers</li> <li>• Oxidative DNA adducts</li> <li>• Dysregulation of antioxidant enzymes</li> <li>• Some studies challenged this mechanism experimentally (e.g., by co-administering antioxidants)</li> </ul>
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)<sup>1</sup>
  - 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**<sup>1</sup>
  - 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)