A Comprehensive Analysis of the Animal Carcinogenicity Data for Glyphosate from Chronic Exposure Rodent Carcinogenicity Studies

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Collaborative on Health and the Environment

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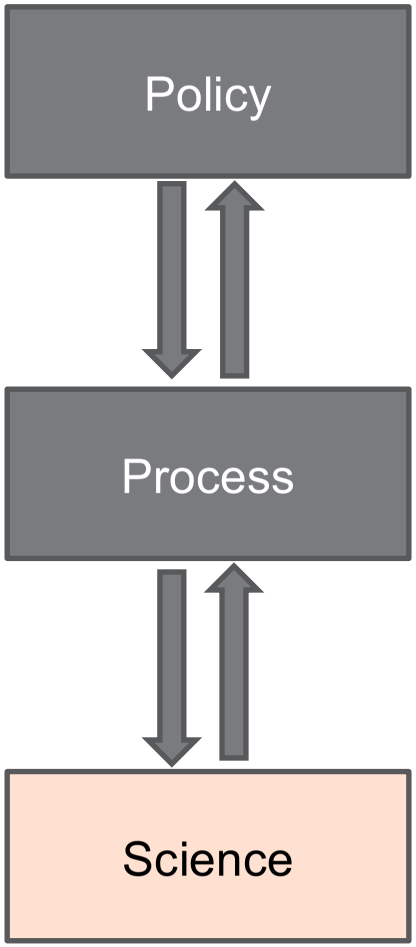
Webinar

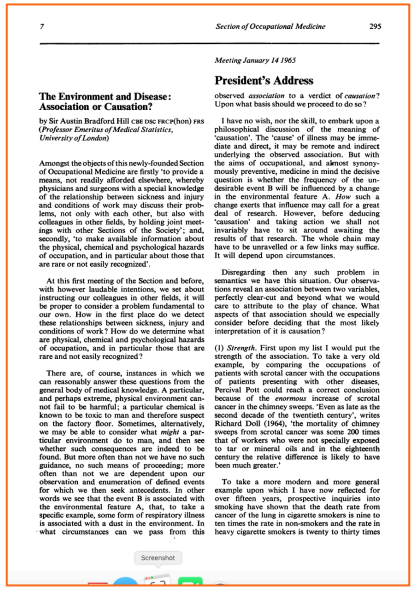
Disclosures

- The opinions expressed here and the analyses done to support those opinions are mine alone.
- I am a consultant for a group of US law firms involved in glyphosate litigation.
- I work part-time as a Senior Contributing Scientist for the Environmental Defense Fund (EDF)
  - On issues related to air pollution, biomonitoring, climate change and public health
  - No work on glyphosate

Policy, Process and Science

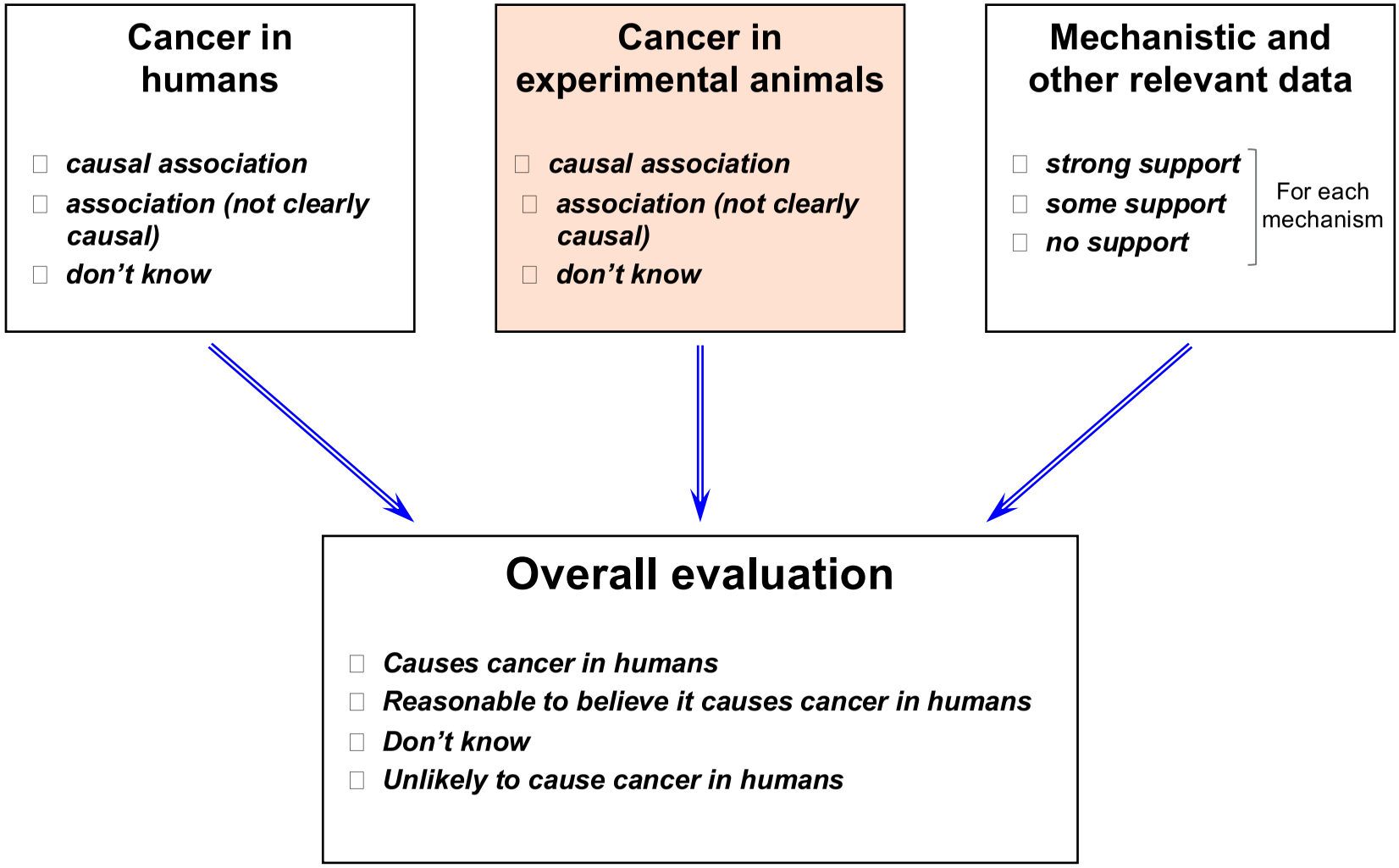


Guidance Documents



Bradford Hill

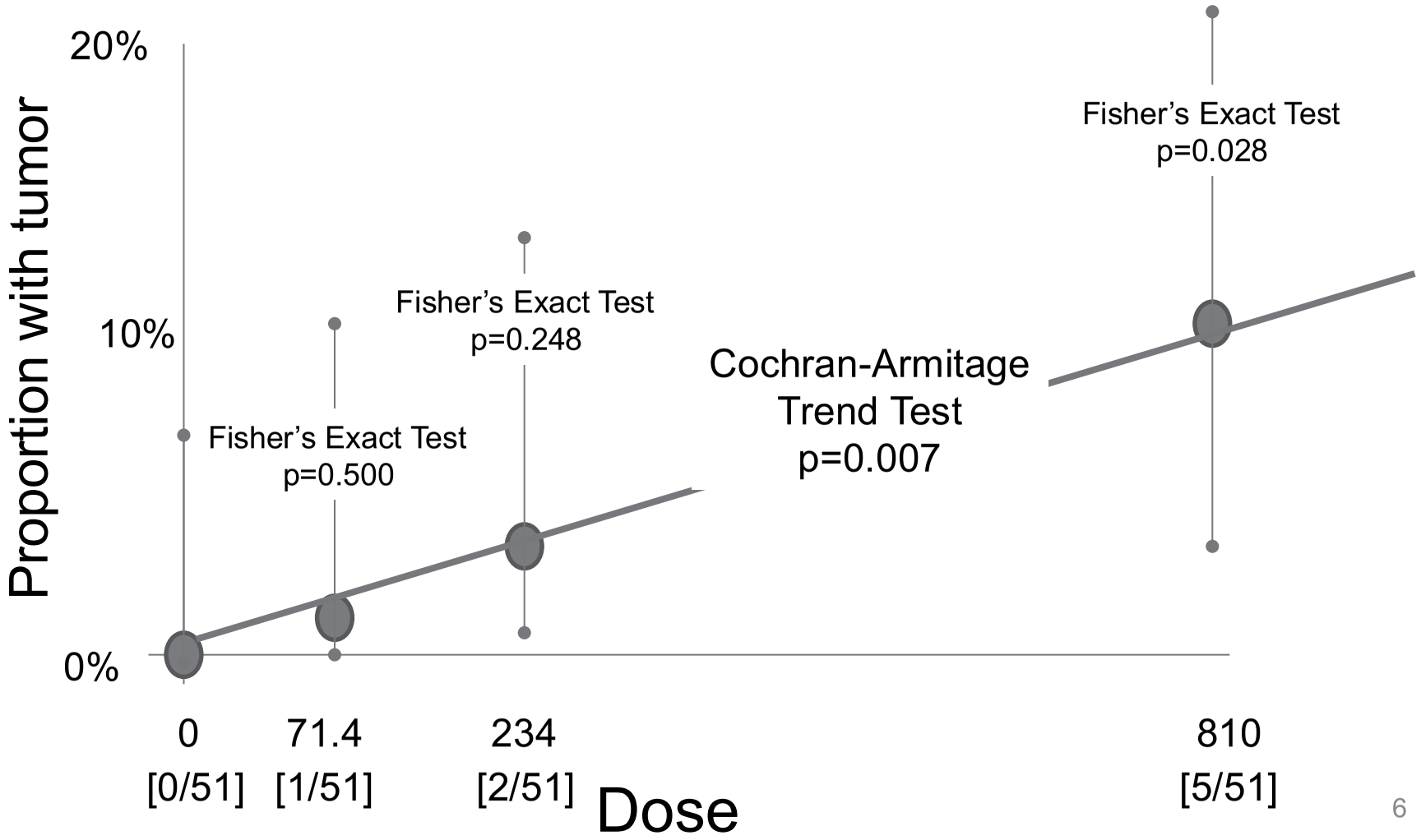
Combining human evidence, animal evidence, and mechanistic evidence



Materials and Methods

- Study Inclusion
  - 21 total animal carcinogenicity studies
  - 13 studies with sufficient detail and quality
- Data Analyzed
  - Individual tumor counts from each study with 3 or more tumors added across all dose groups, and
  - Individual tumor counts from studies not matching 3 tumor minimum but with a significant ($p<0.05$) finding from another study using same sex/strain
  - Tissue pathology in all studies of same sex/strain with at least one significant tumor finding
- Analysis
  - Armitage Linear Trend Test in proportions (one-sided p-value)
    - Fisher Exact Test for pairwise comparisons (non-decisional)
  - Pooled analysis using logistic regression with individual backgrounds and test for homogeneity of slopes (for each sex/species/strain/tumor)
  - Historical controls analysis using Tarone's test (*Biometrics*, 1982)

Malignant Lymphomas
Male CD-1 Mice, Wood et al. (2009)



Long-term chronic dietary exposure toxicity and carcinogenicity studies of glyphosate analyzed in this evaluation.

Study Reference	Duration (months)	Strain		Dietary exposure dose levels (mg/kg/day)	Animals per Group	Purity (%)	Comments on survival and weight
		Mouse	Rat				
A: Knezevich and Hogan (1983) [11]	24	CD-1		M: 0, 157, 814, 4841 F: 0, 190, 955, 5874	50	99.8	No survival differences, slight weight reduction in high dose (M)
B: Atkinson et al. (1993) [12]	24	CD-1		M: 0, 98, 297, 988 F: 0, 102, 298, 1000	50	>97.0	No survival differences, no weight differences
C: Sugimoto (1997) [13]	18	CD-1		M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	50	94.6-95.7	No survival differences, slight weight reduction in mid (F) & high dose (M+F)
D: Wood et al. (2009) [14]	18	CD-1		M: 0, 71.4, 234.2, 810 F: 0, 97.9, 299.5, 1081.2	51	95.7	No survival differences, no weight differences
E: Takahashi (1999a) [15]	18	CD-1		M: 0, 167.6, 685, 7470 F: 0, 93.2, 909, 8690	50	97.5	Reduced survival high dose (M), slight weight reduction in mid (M) & high dose (M+F). This study was only mentioned by JMPR [7] and provides limited tumor data.
F: Kumar (2001) [16]	18	S-A[1]		M: 0, 85.5, 285.2, 1077.4 F: 0, 104.5, 348.6, 1381.9	50	>95.0	No survival differences, no weight differences
G: Lankas (1981) [17]	26		SD[2]	M: 0, 3.05, 10.3, 31.49 F: 0, 3.37, 11.22, 34.02	50	98.7	No survival differences, no weight differences
H: Stout and Ruecker (1990) [18]	24		SD[2]	M: 89, 362, 940 F: 0, 113, 457, 1183	50	98.7	No survival differences, slight weight reduction in high dose (F)
I: Atkinson (1993) [19]	24		SD[2]	M: 0, 11, 112, 320, 1147 F: 0, 12, 109, 347, 1134	50	98.9	No survival differences, slight weight reduction in high dose (M+F)
J: Enemoto (1997) [20]	24		SD[2]	M: 0, 104, 354, 1127 F: 0, 115, 393, 1247	50	95.7	Reduced survival high dose (M), slight weight reduction in high dose (M+F)
K: Suresh (1996) [21]	24		W[3]	M: 0, 6.3, 59.4, 595.2 F: 0, 8.6, 88.5, 886	50	96.8	No survival differences, no weight differences
L: Brammer (2001) [22]	24		W[3]	M: 0, 121, 361, 1214 F: 0, 145, 437, 1498	53	97.6	High-dose survived longer (M), reduced weight highest dose (M+F)
M: Wood et al. (2009) [23]	24		W[3]	M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	51	94.7-97.6	No survival differences, no weight differences

P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test (p≤0.05) or Fisher's exact test (p≤0.05) in male CD-1 mice

Tumor	Individual study p-values for trend[1]					Common Trend
Males	A	B	C	D	E	
Kidney Adenomas	0.442 (0.138)	0.938	0.062 (0.009)[4]	---[2]	0.019	0.006
Kidney Carcinomas	0.063 (<0.001)[4]	0.938	---[2]	---[2]	0.250	0.031
Kidney Adenomas and Carcinomas	0.065 (0.008)[4]	0.981	0.062 (0.009)[4]	---[2]	0.005	<0.001
Malignant Lymphomas	0.754	0.087	0.016	0.007	ND[3]	0.093
Hemangiosarcomas	0.505	0.004	0.062 (0.005)[4]	---[2]	ND[3]	0.033
Alveolar-Bronchiolar Adenomas	0.294	0.231	0.513	0.924	ND[3]	0.384
Alveolar-Bronchiolar Carcinomas	0.918	0.456	0.148	0.028	ND[3]	0.407
Alveolar-Bronchiolar Adenomas and Carcinomas	0.576	0.231	0.294	0.336	ND[3]	0.346

1 – Study A is Knezevich and Hogan (1983), Study B is Atkinson et al. (1993), Study C is Sugimoto (1997), Study D is Wood (2009), Study E is Takahashi (1999); [2] – three dashes "---" indicates all tumor counts were zero; [3] – ND indicates there was no data available for this tumor in this study; [4] – significance against historical controls using Tarone Test (Tarone, 1982)

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Kidney Carcinomas	0.063 (<0.001)[4]	0.938	---[2]	---[2]	0.250	0.031
Kidney Adenomas and Carcinomas	P=0.686		P=0.005		0.005	<0.001
Malignant Lymphomas	0.754	0.087	0.016	0.007	ND[3]	0.093
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P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test (p≤0.05) or Fisher's exact test (p≤0.05) in female CD-1 mice

Tumor	Individual study p-values for trend[1]					Common Trend
Females	A	B	C	D	E	
Hemangiomas	0.631	---[2]	0.002	0.438	ND[3]	0.031
Harderian Gland Adenomas	0.877	---[2]	0.040	0.155	ND[3]	0.155
Harderian Gland Carcinomas	---[2]	---[2]	---[2]	1.000	ND[3]	0.500
Harderian Gland Adenomas and Carcinomas	0.877	---[2]	0.040	0.372	ND[3]	0.184
Alveolar-Bronchiolar Adenomas	0.183	0.136	0.800	0.656	ND[3]	0.996
Alveolar-Bronchiolar Carcinomas	0.985	0.456	0.623	0.601	ND[3]	0.268
Alveolar-Bronchiolar Adenomas and Carcinomas	0.016	0.211	0.842	0.688	ND[3]	0.982
Malignant Lymphomas	0.070[5]	0.484	0.294	0.353	0.050	0.012

[1] – Study A is Knezevich and Hogan (1983), Study B is Atkinson et al. (1993), Study C is Sugimoto (1997), Study D is Wood (2009), Study E is Takahashi (1999); [2] – three dashes "---" indicates all tumor counts were zero; [3] – ND indicates there was no data available for this tumor in this study; [4] – statistically significant against historical controls using Tarone Test (Tarone, 1982); [5] – Spleen composite lymphosarcomas (malignant lymphomas) are also significantly increased in female mice in this study

P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test or Fisher's exact test (p≤0.05) in male Sprague-Dawley rats

Tumor	Individual study p-values for trend[1]				Common Trend
Males	G	H	I	J	
Testicular Interstitial Cell Tumors	0.009	0.296	0.580	0.594	0.461
Pancreas Islet Cell Adenomas	0.512	0.147 (0.007)[3]	0.974	0.859	0.849
Pancreas Islet Cell Carcinomas	0.251	1.000	---	0.500	0.731
Pancreas Islet Cell Adenomas or Carcinomas	0.316	0.206	0.974	0.844	0.875
Thyroid C-cell Adenomas	0.743	0.089	0.278	0.631	0.210
Thyroid C-cell Carcinomas	0.505	0.442	0.495	0.565	0.322
Thyroid C-cell Adenomas and Carcinomas	0.748	0.097	0.197	0.642	0.175
Thyroid Follicular-cell Adenomas	0.122	0.408	0.067	0.966	0.464
Thyroid Follicular-cell Carcinomas	---[2]	0.255	0.443	1.000	0.448
Thyroid Follicular-cell Adenoma and Carcinoma	0.122	0.232	0.099	0.986	0.446
Hepatocellular Adenomas	0.471	0.015	0.325	0.500	0.029
Hepatocellular Carcinomas	0.062	0.637	0.760	0.642	0.803
Hepatocellular Adenomas and Carcinomas	0.173	0.050	0.480	0.690	0.144
Kidney Adenomas	0.938	0.813	1.000	0.004	0.039
Skin Keratoacanthomas	---[2]	0.042	0.047	0.029	<0.001
Skin Basal Cell Tumors	0.251	0.249	1.000	0.004	<0.001

[1] – Study G is Lankas (1981), Study H is Stout and Ruecker (1990), Study I is Atkinson et al. (1993) and Study J is Enemoto (1997); [2] – three dashes "---" indicates all tumor counts were zero; [3] – significance against historical control data using Tarone's test

P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test or Fisher's exact test (p≤0.05) in female Sprague-Dawley rats

Tumor	Individual study p-values for trend[1]				Common Trend
Females	G	H	I	J	
Thyroid C-cell Adenomas	0.679	0.049	0.207	0.912	0.287
Thyroid C-cell Carcinomas	0.003 (<0.001)[3]	0.500	---[2]	---[2]	0.385
Thyroid C-cell Adenomas and Carcinomas	0.072 (0.037)[3]	0.052	0.207	0.912	0.275
Adrenal Cortical Adenoma	0.851	0.603	---[2]	0.626	0.713
Adrenal Cortical Carcinoma	0.386	0.015	0.493	---[2]	0.031
Adrenal Cortical Adenoma and Carcinoma	0.801	0.090	0.493	0.626	0.195

[1] – Study G is Lankas (1981), Study H is Stout and Ruecker (1990), Study I is Atkinson et al. (1993) and Study J is Enemoto (1997); [2] – three dashes "---" indicates all tumor counts were zero; [3] – significance against historical control data using Tarone's test

P-values for the Cochran-Armitage trend test and pooled logistic regression analysis for tumors with at least one significant trend test or Fisher's exact test (p≤0.05) in male and female Wistar rats

Tumor	Individual study p-values for trend[1]			Common Trend
Males	K	L	M	
Hepatocellular Adenomas	0.391	0.008	0.418	0.048
Hepatocellular Carcinomas	0.418	---[2]	1.000	0.492
Hepatocellular Adenomas and Carcinomas	0.286	0.008	0.610	0.029
Pituitary Adenomas	0.376	0.277	0.045	0.057
Pituitary Carcinomas	0.692	---[2]	1.000	0.771
Pituitary Adenomas and Carcinomas	0.454	0.277	0.059	0.073
Skin Keratoacanthomas	---[2]	0.387	0.030	0.032
Adrenal Pheochromocytomas	0.048	0.721	0.306	0.273
Females	K	L	M	
Mammary Gland Adenomas	0.539	0.941	0.062	0.448
Mammary Gland Adenocarcinomas	1.000	0.271	0.042	0.071
Mammary Gland Adenomas and Adenocarcinomas	0.729	0.590	0.007	0.113
Pituitary Adenomas	0.967	0.261	0.014	0.105
Pituitary Carcinomas	1.000	---	0.750	0.748
Pituitary Adenomas and Carcinomas	0.976	0.261	0.017	0.129

[1] – Study E is Suresh (1996), Study F is Brammer (2001), and Study G is Wood et al. (2009); [2] – three dashes "---" indicates all tumor counts were zero

Observed (Obs.) versus expected (Exp.) tumor sites with significant trends in the 13 acceptable rodent carcinogenicity studies using glyphosate

Species	Strain	Sex	Total Sites[1]	Exp. p<0.05	Obs. p<0.05 (prob.)[2]	Exp. p<0.01	Obs. p<0.01
Rat (7 studies)	Sprague-Dawley (4 studies)	M	125	6.3	9 (0.17)	1.3	4 (0.04)
		F	95	4.8	4 (0.52)	1.0	2 (0.25)
	Wistar (3 studies)	M	67	3.4	5 (0.24)	0.7	2 (0.15)
		F	58	2.9	4 (0.33)	0.6	1 (0.44)
Mouse (6 studies)	CD-1 (5 studies)	M	60	3.0	11 (<0.001)	0.6	8 (<0.001)
		F	63	3.2	6 (0.09)	0.6	1 (0.47)
	Albino (1 study)	M	24	0.7	0 (1)	0.1	0 (1)
		F	14	0.7	1 (0.51)	0.1	1 (0.13)
Rats (7 studies)	All (7 studies)	M	192	9.6	14 (0.10)	1.9	6 (0.013)
		F	153	7.7	9 (0.36)	1.5	3 (0.20)
		Both	345	17.3	23 (0.02)	3.5	9 (0.01)
Mice (6 studies)	All (6 studies)	M	74	3.7	11 (0.001)	0.7	8 (<0.001)
		F	77	3.9	7 (0.09)	0.8	2 (0.18)
		Both	151	7.6	18 (0.001)	1.5	10 (<0.001)
All (13 studies)	All (13 studies)	M	266	13.3	25 (0.002)	2.7	14 (<0.001)
		F	230	11.5	16 (0.12)	2.3	5 (0.08)
		Both	496	24.8	41 (0.001)	5.0	19 (<0.001)

1 – number of trend tests actually conducted; 2 – probability of seeing the number of observed significant findings or more

Probability that all findings are false positives

Supporting Evidence
Malignant Lymphomas in Mice

- Significant dose-related increases seen in male and female CD-1 mice
  - marginal increases seen in male and female Swiss albino mice
- Tissue changes
  - Thymus weight ↗
  - Enlarged lymph nodes ↗
  - Enlarged spleens ↗
- Peer-reviewed literature
  - Increase in M-spike in gene-dependent manner homozygous and heterozygous male and female Vk*MYC mice but not in null mice
  - Activation-induced cytidine deaminase (AID) induced in gene-dependent manner in homozygous and heterozygous male and female Vk*MYC mice but not in null mice
  - NHL in epidemiology studies

Summary of level of evidence[1] for tumors observed to have a significant trend in 13 rodent carcinogenicity studies in male and female, mice and rats.[2]

Tumor	Males				Females			
	CD-1 Mouse	Swiss Mouse	SD Rat	Wistar Rat	CD-1 Mouse	Swiss albino mouse	SD Rat	Wistar Rat
Adrenal cortical carcinoma							CE	
Adrenal pheochromocytoma								EE
Alviolar-Bronchiolar tumor	NE				NE			
Harderian gland tumor					NE			
Hemangioma					CE	CE		
Hemangiosarcomas	CE							
Kidney tumor	CE	SE	CE					
Liver adenoma			CE	CE				
Mammary tumor								SE
Malignant lymphoma	CE	SE			CE	SE		
Pancreas Islet Cell tumor			EE					
Pituitary adenomas				SE				SE
Skin basal-cell tumor			CE					
Skin keratoacanthoma			CE	CE				
Thyroid C-cell tumor			EE				EE	
Thyroid follicular-cell tumor			EE					
Testis interstitial-cell Tumor			SE					

1 – CE=clear evidence; SE=some evidence; EE=equivocal evidence; NE=no evidence: 2 – a blank space indicates there is no positive finding in any study for this tumor in this sex/species

Evaluation: Animal Carcinogenicity Data

- Multiple Tumor Types in Different Studies
  - 41 positive (<0.05) trend tests
- Same Tumor in Multiple Studies
  - Kidney Tumors, Skin Keratoacanthoma, Malignant Lymphoma, Hemangiosarcoma, Hepatocellular Adenomas
- Rare tumors Increased
  - Kidney, Hemangiosarcoma (18-month studies), Pacreas Islet Cell tumors, Thyroid C-cell Carcinomas confirmed with formal test against historical controls
- Tumors in Two Strains
  - Skin Keratoacanthoma, Hemangiosarcomas
- Tumors in Two Species
  - Kidney Tumors
- Supporting findings in tissue pathology and peer-reviewed literature
- CONCLUSION
  - **Glyphosate can cause cancer in rodents**

Animal Carcinogenicity Conclusions

USEPA

Based on the weight-of-evidence evaluations, the agency has concluded that none of the tumors evaluated in individual rat and mouse carcinogenicity studies are treatment-related due to lack of pairwise statistical significance, lack of a monotonic dose response, absence of preneoplastic or related non-neoplastic lesions, no evidence of tumor progression, and/or historical control information (when available). Tumors seen in individual rat and mouse studies were also not reproduced in other studies, including those conducted in the same animal species and strain at similar or higher doses.

EFSA

or limited evidence of an association. No evidence of carcinogenicity was confirmed by the large majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pair-wise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/MTD, lack of pre-neoplastic lesions and/or being within historical control range. The statistical significance found in trend analysis (but not in pair-wise comparison) *per se* was balanced against the former considerations. During the teleconference 117, the experts also agreed to the conclusion of the RMS,

IARC Working Group

There is *sufficient evidence* in experimental animals for the carcinogenicity of glyphosate.

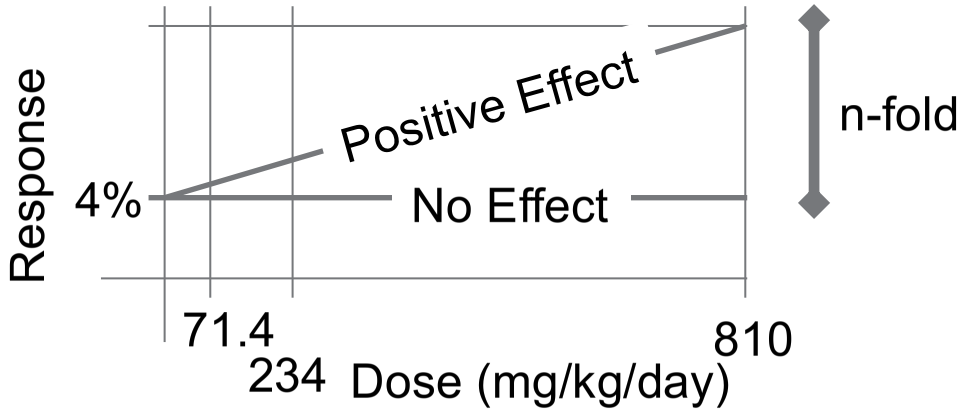
Regulatory Agency Reasons for Excluding all Positive Findings

- Lack of dose-response (statistical power ↘)
  - Monotonic dose-response is generally unlikely
- Trend test positive but not pairwise comparison (statistical power ↘)
- No consistency across studies
  - See pooled analysis
- Differences between sexes
  - Not unusual and many times for no easily explained reason
- Lack of pre-neoplastic lesions
  - Multiple additional tissue changes
  - Multiple peer-reviewed studies supporting findings
- Within range of historical controls (statistical power ↘)
  - Inappropriate use of historical controls (known since 1982)
- Results due to a single high dose potentially at or above the MTD
  - Only one study had survival problems at high dose and it was marginal

False Positive Rates and Power

Cochran-Armitage Linear Trend Test vs Fisher's Exact Test

50 animals per group



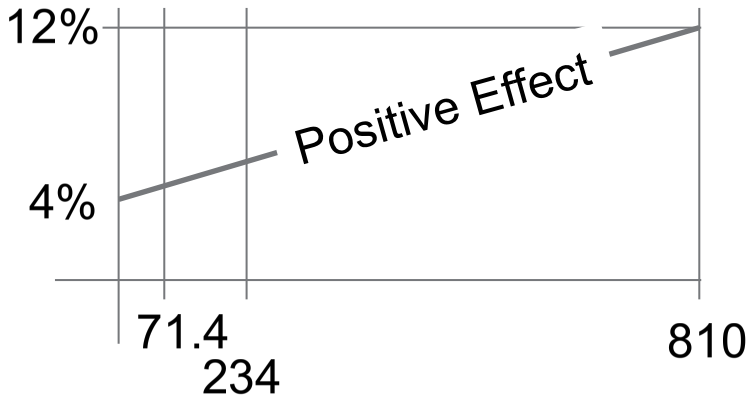
n-fold (response at highest dose)	Percentage of Positive Finding (p=0.05) in 10,000 simulations	
	CA Test	Fisher's Test
0 (4%)	4.4%	2.2%
1 (8%)	23%	11%
2 (12%)	52%	31%
3 (16%)	75%	56%

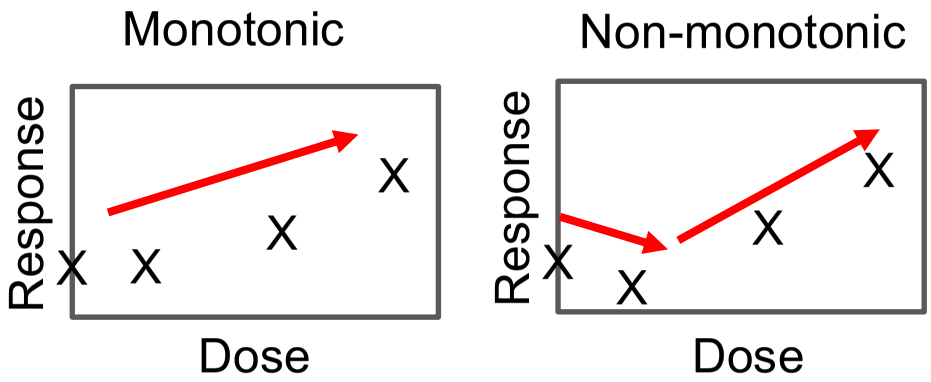
False Positive Rate

Statistical Power

Power of the C-A Trend Test

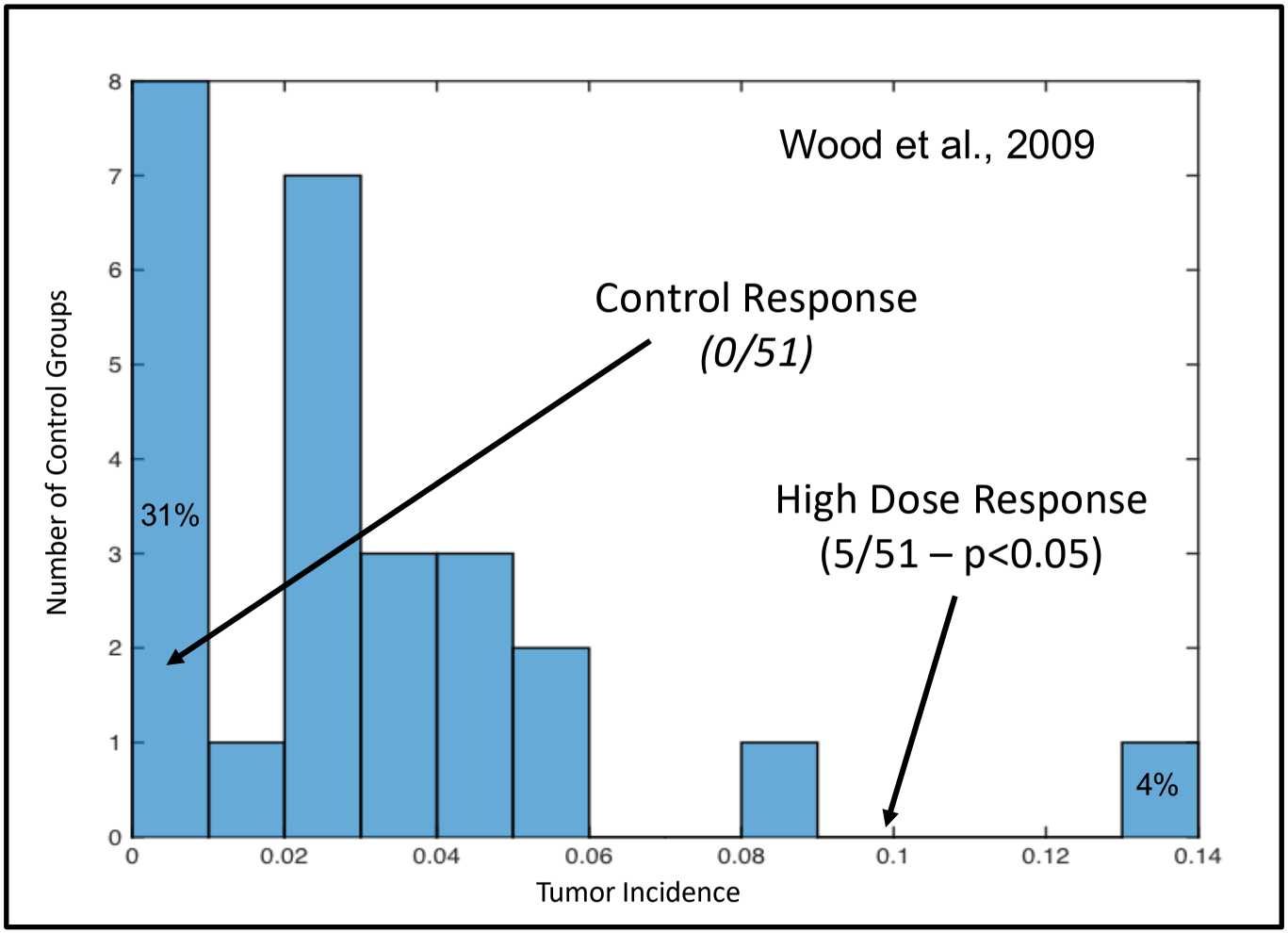
Monotonic Dose-Response and Range of Historical Controls





# Historical Control Datasets	Power		
	CA-Test	Monotonicity	Range of HC
5	52%	24%	49%
10			48%
20			45%
30			42%
50			39%

Historical Controls for Malignant Lymphomas Male CD-1 Mice with Wood et al. (2009)



Tarone (1982) Historical Control P-value 0.003

Historical controls from *Giknis and Clifford, (2005)*

Summary

- Glyphosate causes multiple cancer types to appear in multiple studies in experimental animals
  - This finding is supported by other organ toxicity and peer-reviewed literature
- Using a statistical cut-off of $p \leq 0.05$ loses information; better to present the actual p-value
- Trend tests are the appropriate tool for analyzing these data
  - Requiring other criteria like significant pairwise tests or monotonicity increases the risk of a false negative finding
- A combined analysis is needed to evaluate the overall trend when multiple studies use the same sex/species/strain
  - Just noting the number of studies with positive and negative findings at a particular target is an inadequate evaluation of the data
- Use historical controls properly in evaluating animal carcinogenicity data

Thank You!