Lee Johnson v. Monsanto

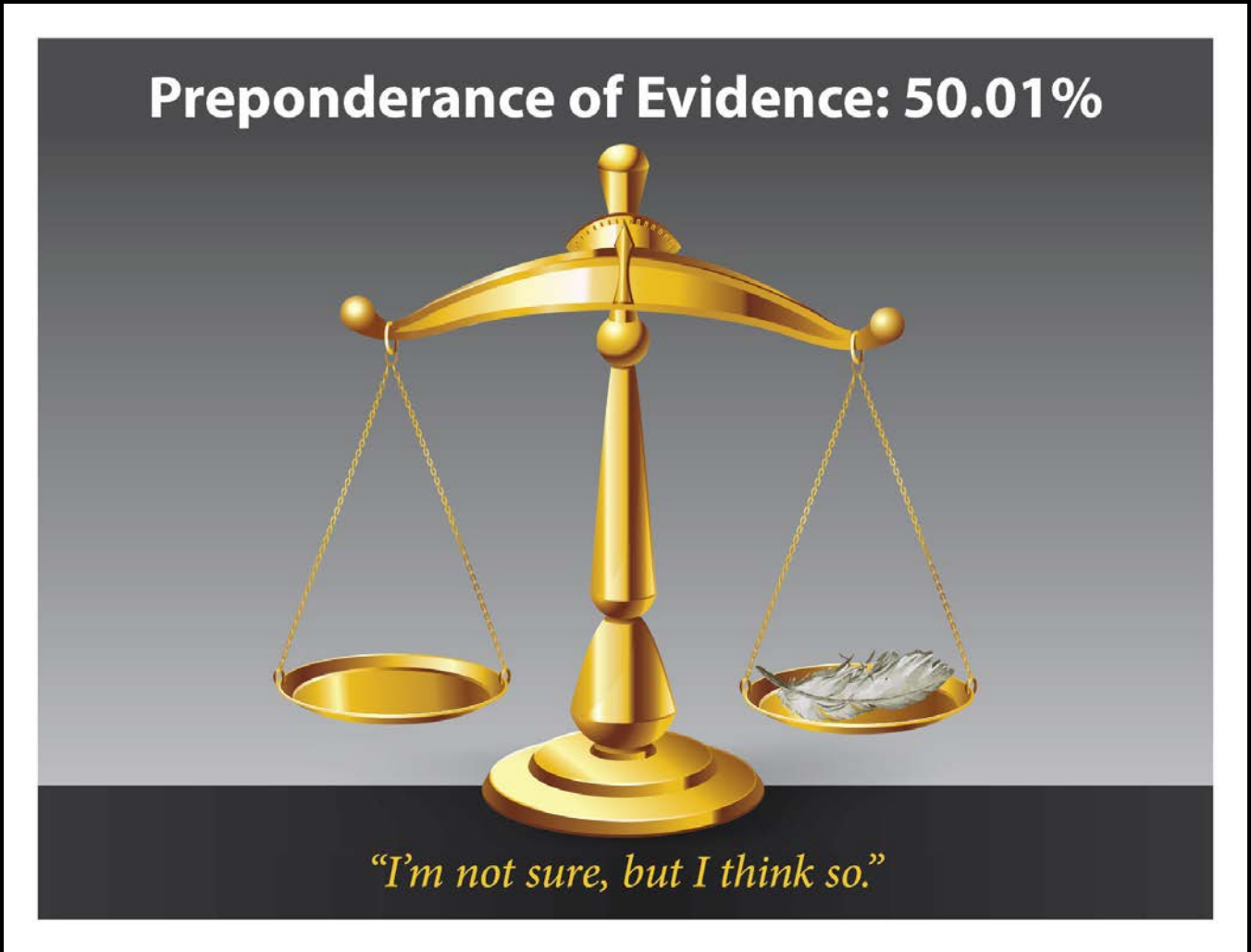
3 Core Questions

1. Can Roundup be a substantial contributing factor in causing cancer?

2. Was Roundup a substantial contributing factor in causing Mr. Johnson's cancer?

3. Did Monsanto act with conscious disregard of human health?

1. Can Roundup be a substantial contributing factor in causing cancer?



1. Can Roundup be a substantial contributing factor in causing cancer?

Jury Instruction

A substantial factor in causing harm is a factor that a reasonable person would consider to have contributed to the harm. It must be more than a remote or trivial factor. It does not have to be the only cause of the harm. Conduct is not a substantial factor in causing harm if the same harm would have occurred without that conduct.

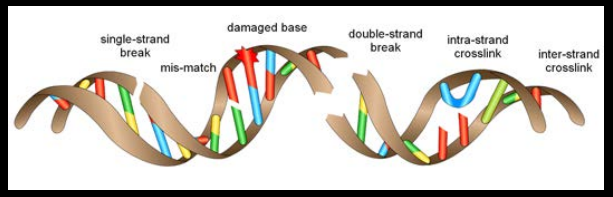
1. Can Roundup be a substantial contributing factor in causing cancer?

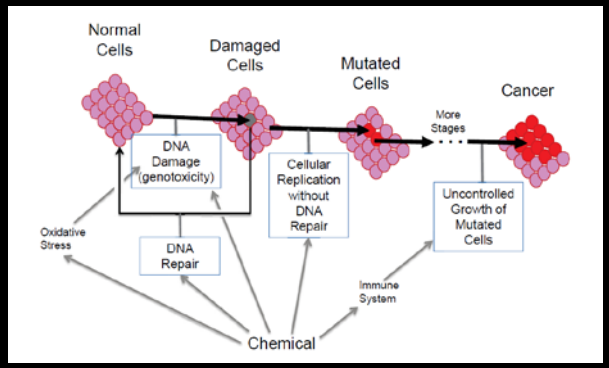
Three Pillars of Cancer Science

Epidemiology

Toxicology

Mechanism





Three Pillars of Cancer Science

Plaintiff's Experts Each Examined All the Data





Monsanto's Experts Did Not


(Epi only)


(Rodent only)


(AHS only)

Three Pillars of Cancer Science

Jury Instruction

The following exhibits may be admitted for the limited purpose of evaluating Monsanto's state of mind regarding the state of the science and for no other purpose:

1. EPA, Office of Prevention, Pesticides and Toxic Substances, Reregistration Eligibility Decision (RED) Glyphosate (Sept. 1993) [Exhibit DX2489]; and

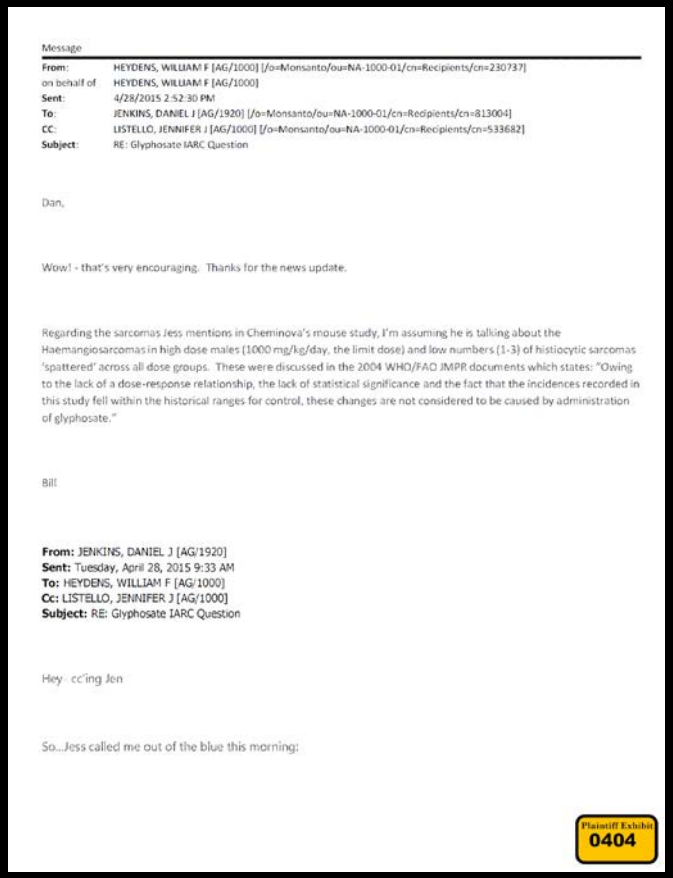
2. EPA, Office of Pesticide Programs, Glyphosate Issue Paper: Evaluation of Carcinogenic Potential dated September 12, 2016 [Exhibit DX2481].

Three Pillars of Cancer Science

- Scientific Advisory Panel
- EPA ignored guidelines
- “Dog in the fight”
- Made mistakes before
- Glyphosate only
- Jess Rowland

Three Pillars of Cancer Science

EPA & Jess Rowland



Message

From: HEYDENS, WILLIAM F [AG/1000] [/o=Monsanto/ou=NA-1000-01/cn=Recipients/cn=230737]
on behalf of HEYDENS, WILLIAM F [AG/1000]
Sent: 4/28/2015 2:52:30 PM
To: JENKINS, DANIEL J [AG/1920] [/o=Monsanto/ou=NA-1000-01/cn=Recipients/cn=813004]
CC: LISTELLO, JENNIFER J [AG/1000] [/o=Monsanto/ou=NA-1000-01/cn=Recipients/cn=533682]
Subject: RE: Glyphosate IARC Question

Dan,

Wow! - that's very encouraging. Thanks for the news update.

Regarding the sarcomas Jess mentions in Cheminova's mouse study, I'm assuming he is talking about the Haemangiosarcomas in high dose males (1000 mg/kg/day, the limit dose) and low numbers (1-3) of histiocytic sarcomas 'spattered' across all dose groups. These were discussed in the 2004 WHO/FAO JMPR documents which states: "Owing to the lack of a dose-response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for control, these changes are not considered to be caused by administration of glyphosate."

Bill

From: JENKINS, DANIEL J [AG/1920]
Sent: Tuesday, April 28, 2015 9:33 AM
To: HEYDENS, WILLIAM F [AG/1000]
Cc: LISTELLO, JENNIFER J [AG/1000]
Subject: RE: Glyphosate IARC Question

Hey cc'ing Jen

So...Jess called me out of the blue this morning:

Plaintiff Exhibit
0404

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Dan Jenkins
U.S. Agency Lead

Regulatory Affairs
Monsanto Company
1300 I St., NW
Suite 450 East
Washington, DC 20005

C[redacted]

[redacted]

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MONGLY00957758

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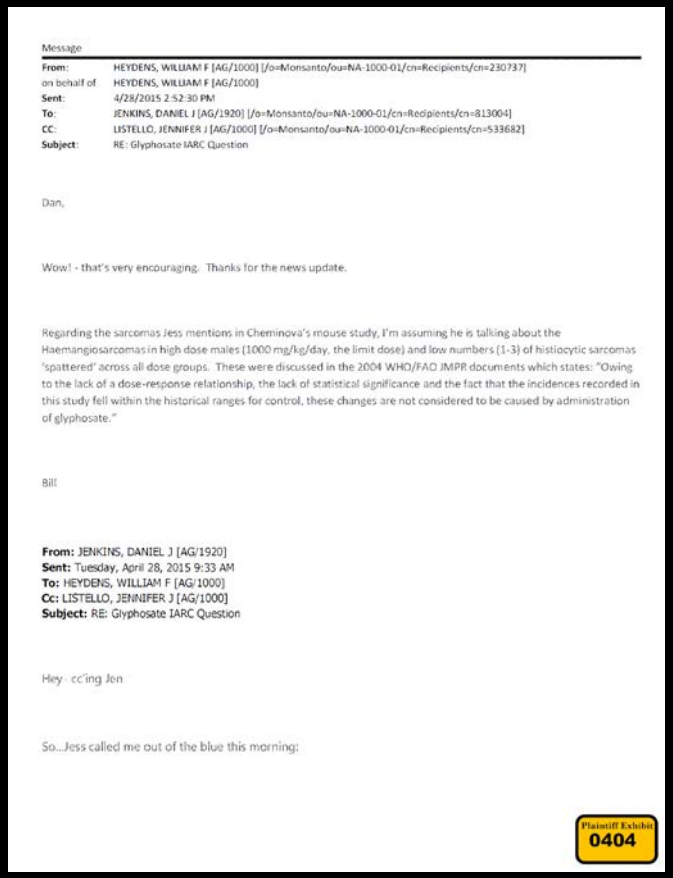
Three Pillars of Cancer Science

EPA & Jess Rowland



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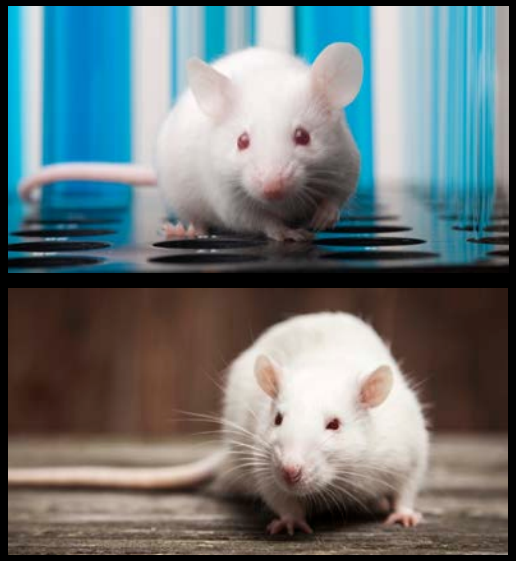
MONGLY00957758

Three Pillars of Cancer Science

Epidemiology

IARC Classification
1. Sufficient
2. Limited
3. Inadequate
4. Lack of Carcinogenicity

Toxicology



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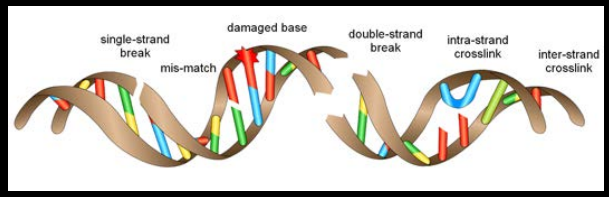
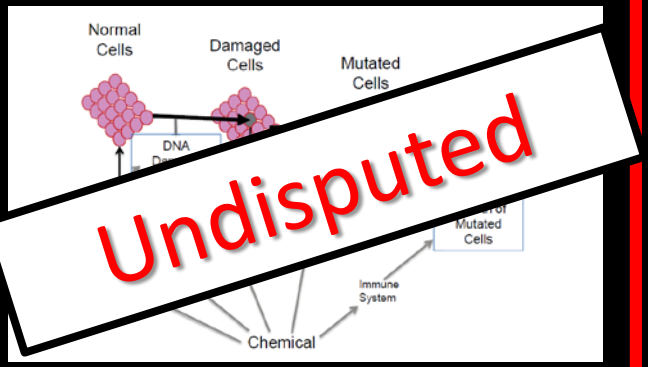
Mechanism



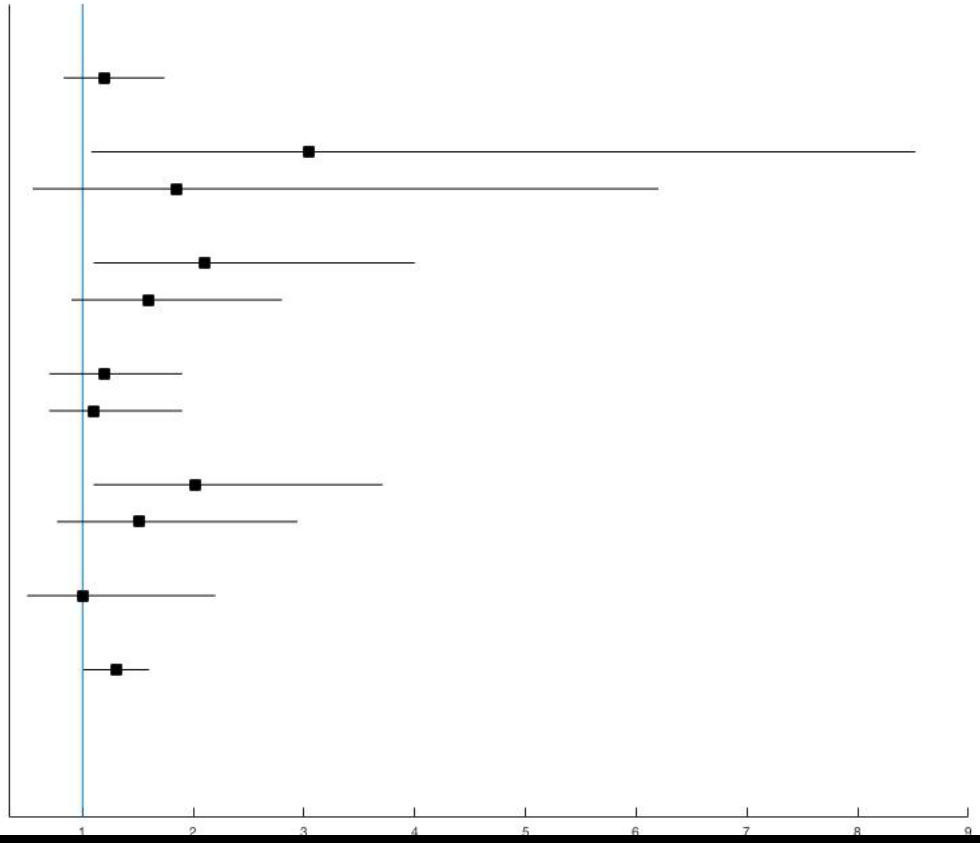


IARC Classification
1. Strong
2. Moderate
3. Weak

Three Pillars of Cancer Science

Epidemiology – Ever/Never

Study	RR	Lower	Upper
McDuffie et al. (2001)			
no pesticide adjustment	1.20	0.83	1.74
Hardell et al. (2002)			
no pesticide adjustment	3.04	1.08	8.52
adjusted for pesticides	1.85	0.55	6.20
De Roos et al. (2003)			
adjusted for pesticides	2.10	1.10	4.00
Bayesian modeling	1.60	0.90	2.80
De Roos et al. (2005)			
no pesticide adjustment	1.20	0.70	1.90
adjusted for pesticides	1.10	0.70	1.90
Eriksson et al., (2008)			
no pesticide adjustment	2.02	1.10	3.71
adjusted for pesticides	1.51	0.77	2.94
Orsi et al. (2009)			
no pesticide adjustment	1.00	0.50	2.20
Meta-Analysis: Model 1			
most adjusted analysis	1.30	1.01	1.60
Andreotti et al. (2018)			
not provided			



Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

- Confounding
- Proxy "bias"
- North American Pooled Project
- Agricultural Health Study

Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

Confounding Is Not A Major Concern

Dr. Mucci

Q. Doctor, you said that one of the great accomplishments of epidemiology was that it helped expose that tobacco was associated with lung cancer; right?
A. Yes.
Q. And isn't it true that when that fight was happening in the epidemiology world, the tobacco companies kept saying, it's confounders?
A. Maybe. I'm sure they did, yes.
Dr. Mucci Cross, pg. 4372:3-10

Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

Confounding Is Not A Major Concern

Dr. Goldstein

Message

From: GOLDSTEIN, DANIEL A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=527246]
Sent: 2/26/2015 8:08:31 PM
To: VICINI, JOHN L [AG/1000] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=56908]; REYNOLDS, TRACEY L [AG/1000] [/O=MONSANTO/OU=Na-1000-01/cn=recipients/cn=133378]
CC: SACHS, ERIC S [AG/1000] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=171736]
Subject: ACSH

While I would love to have more friends and more choices, we don't have a lot of supporters and can't afford to lose the few we have....

I am well aware of the challenges with ACSH and know Eric has valid concerns- so I can assure you I am not all starry-eyed about ACSH- they have PLENTY of warts- but:

You WILL NOT GET A BETTER VALUE FOR YOUR DOLLAR than ACSH:

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Plaintiff Exhibit
0321

Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

Confounding Is Not A Major Concern

Dr. Goldstein

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Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

Confounding Is Not A Major Concern

Dr. Benbrook

Q. The ACSH, what position did it take with regards to tobacco?
A. They were one of the scientific organizations that held out to the end and argued that the science really wasn't clear about tobacco causing cancer.
Q. Talked about how too many confounding factors; right?
A. That's certainly one of the arguments that's brought up.
Dr. Benbrook Direct, pg. 3902:19-3903:10

Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

- Confounding
- Proxy "bias"
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Three Pillars of Cancer Science

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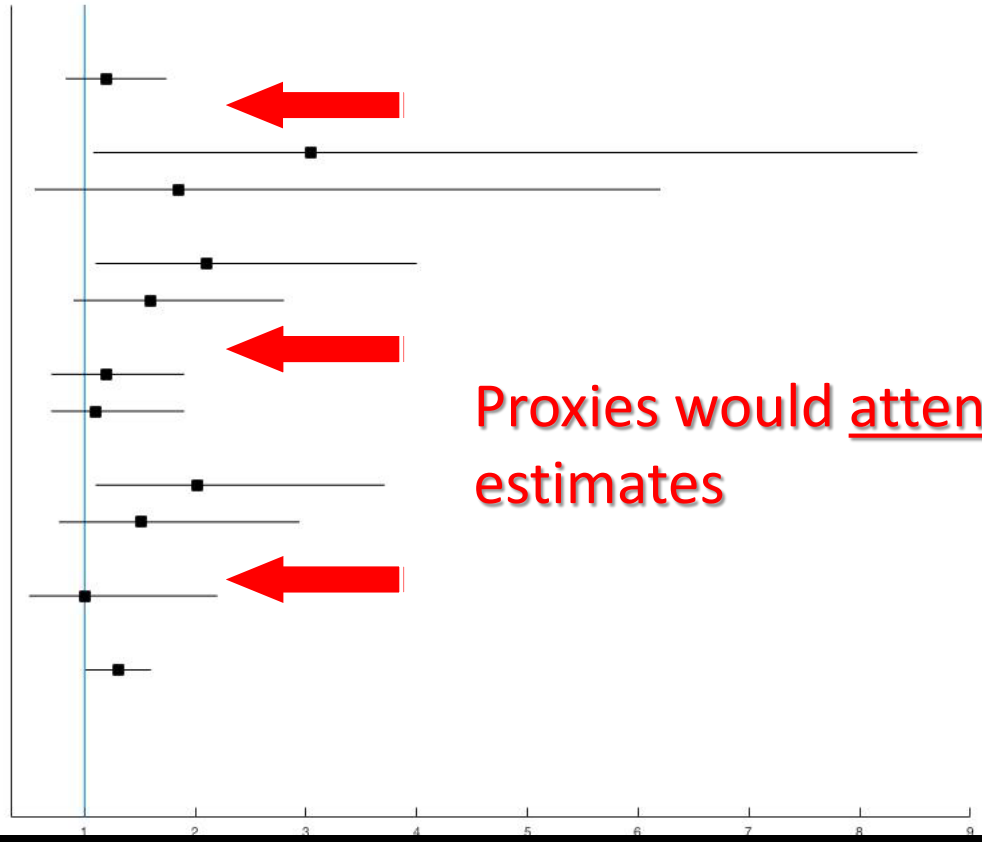


Proxies would attenuate estimates

Three Pillars of Cancer Science

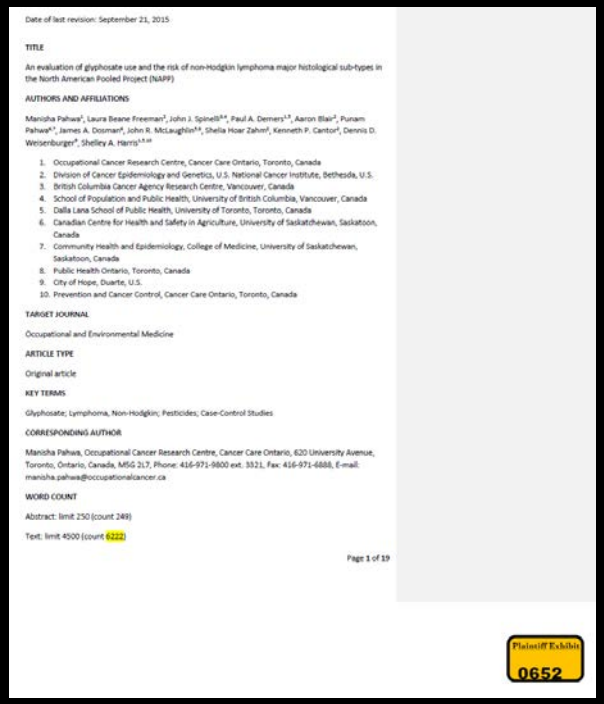
Epidemiology – Monsanto's Arguments

- Confounding
- Proxy "bias"
- North American Pooled Project (NAPP)
- Agricultural Health Study

Three Pillars of Cancer Science

Epidemiology – Monsanto's Arguments

NAPP Supports IARC and NHL Association



Date of last revision: September 21, 2015

TITLE

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

AUTHORS AND AFFILIATIONS

Manisha Pahwa[1], Laura Beane Freeman[2], John J. Spinelli[3,4], Paul A. Demers[1,5], Aaron Blair[2], Punam Pahwa[6], James A. Dosman[6], John R. McLaughlin[7,8], Shelia Hoar Zahm[2], Kenneth P. Cantor[9], Dennis D. Weisenburger[10], Shelley A. Harris[1,5,10]

1. Occupational Cancer Research Centre, Cancer Care Ontario, Toronto, Canada
2. Division of Cancer Epidemiology and Genetics, U.S. National Cancer Institute, Bethesda, U.S.
3. British Columbia Cancer Agency Research Centre, Vancouver, Canada
4. School of Population and Public Health, University of British Columbia, Vancouver, Canada
5. Dalla Lana School of Public Health, University of Toronto, Toronto, Canada
6. Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Canada
7. Community Health and Epidemiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada
8. Public Health Ontario, Toronto, Canada
9. City of Hope, Duarte, U.S.
10. Prevention and Cancer Control, Cancer Care Ontario, Toronto, Canada

TARGET JOURNAL

Occupational and Environmental Medicine

ARTICLE TYPE

Original article

KEY TERMS

Glyphosate; Lymphoma, Non-Hodgkin; Pesticides; Case-Control Studies

CORRESPONDING AUTHOR

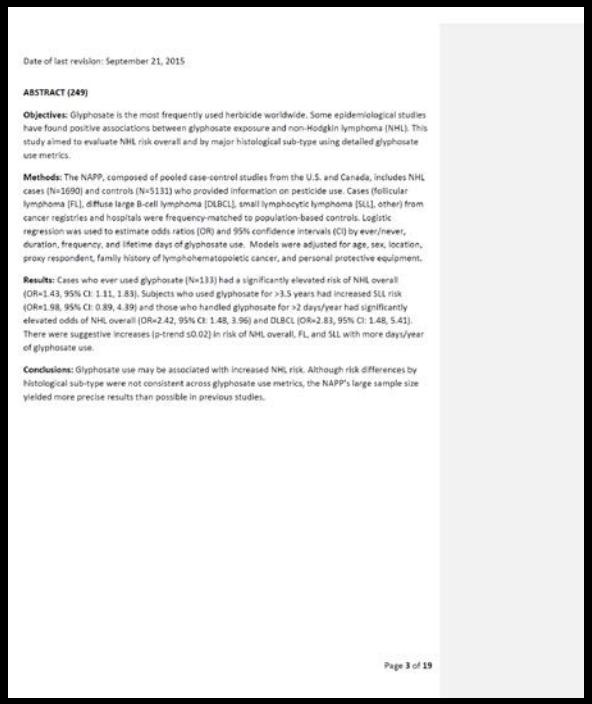
Manisha Pahwa, Occupational Cancer Research Centre, Cancer Care Ontario, 620 University Avenue, Toronto, Ontario, Canada, M5G 2L7, Phone: 416-971-9800 ext. 3321, Fax: 416-971-6888, E-mail: manisha.pahwa@occupationalcancer.ca

WORD COUNT

Abstract: limit 250 (count 249)

Text: limit 4500 (count 6222)

Plaintiff Exhibit 0652



Date of last revision: September 21, 2015

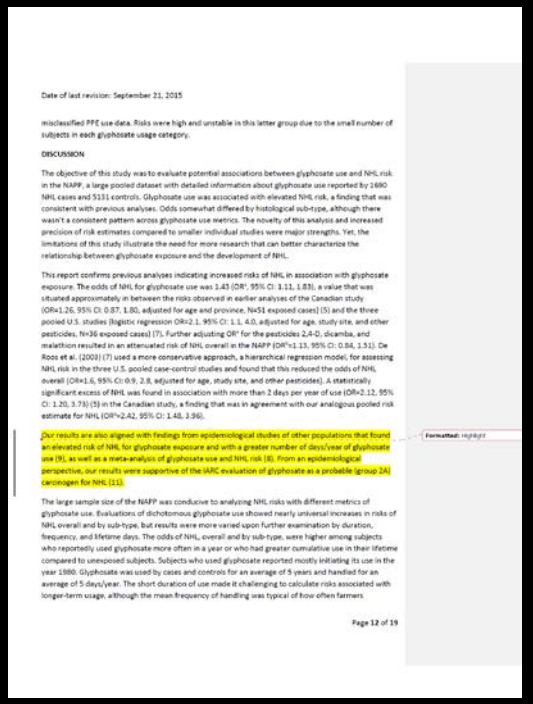
ABSTRACT (249)

Objectives: Glyphosate is the most frequently used herbicide worldwide. Some epidemiological studies have found positive associations between glyphosate exposure and non-Hodgkin lymphoma (NHL). This study aimed to evaluate NHL risk overall and by major histological sub-type using detailed glyphosate use metrics.

Methods: The NAPP, composed of pooled case-control studies from the U.S. 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 (N=133) had a significantly elevated risk of NHL overall (OR=1.43, 95% CI: 1.11, 1.83). Subjects who used glyphosate for >3.5 years had increased SLL risk (OR=1.98, 95% CI: 0.89, 4.39) and those who handled glyphosate for >2 days/year had significantly elevated odds of NHL overall (OR=2.42, 95% CI: 1.48, 3.96) and DLBCL (OR=2.83, 95% CI: 1.48, 5.41). There were suggestive increases (p-trend ≤0.02) in risk of NHL overall, FL, and SLL with more days/year of glyphosate use.

Conclusions: Glyphosate use may be associated with increased NHL risk. Although risk differences by histological sub-type were not consistent across glyphosate use metrics, the NAPP's large sample size yielded more precise results than possible in previous studies.



misclassified PPE use data. Risks were high and unstable in this latter group due to the small number of subjects in each glyphosate usage category.

DISCUSSION

The objective of this study was to evaluate potential associations between glyphosate use and NHL risk in the NAPP, a large pooled dataset with detailed information about glyphosate use reported by 1690 NHL cases and 5131 controls. Glyphosate use was associated with elevated NHL risk, a finding that was consistent with previous analyses. Odds somewhat differed by histological sub-type, although there wasn't a consistent pattern across glyphosate use metrics. The novelty of this analysis and increased precision of risk estimates compared to smaller individual studies were major strengths. Yet, the limitations of this study illustrate the need for more research that can better characterize the relationship between glyphosate exposure and the development of NHL.

This report confirms previous analyses indicating increased risks of NHL in association with glyphosate exposure. The odds of NHL for glyphosate use was 1.43 (OR¹; 95% CI: 1.11, 1.83), a value that was situated approximately in between the risks observed in earlier analyses of the Canadian study (OR=1.26, 95% CI: 0.87, 1.80, adjusted for age and province, N=51 exposed cases) (5) and the three pooled U.S. studies (logistic regression OR=2.1, 95% CI: 1.1, 4.0, adjusted for age, study site, and other pesticides, N=36 exposed cases) (7). Further adjusting OR¹ for the pesticides 2,4-D, dicamba, and malathion resulted in an attenuated risk of NHL overall in the NAPP (OR¹=1.13, 95% CI: 0.84, 1.51). De Roos et al. (2003) (7) used a more conservative approach, a hierarchical regression model, for assessing NHL risk in the three U.S. pooled case-control studies and found that this reduced the odds of NHL overall (OR=1.6, 95% CI: 0.9, 2.8, adjusted for age, study site, and other pesticides). A statistically significant excess of NHL was found in association with more than 2 days per year of use (OR=2.12, 95% CI: 1.20, 3.73) (5) in the Canadian study, a finding that was in agreement with our analogous pooled risk estimate for NHL (OR¹=2.42, 95% CI: 1.48, 3.96).

Our results are also aligned with findings from epidemiological studies of other populations that found an elevated risk of NHL for glyphosate exposure and with a greater number of days/year of glyphosate use (9), as well as a meta-analysis of glyphosate use and NHL risk (8). From an epidemiological perspective, our results were supportive of the IARC evaluation of glyphosate as a probable (group 2A) carcinogen for NHL (11).

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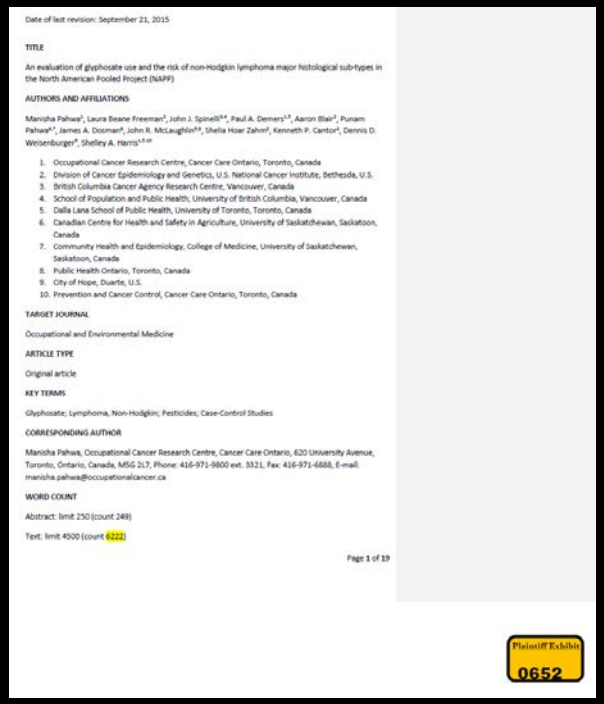

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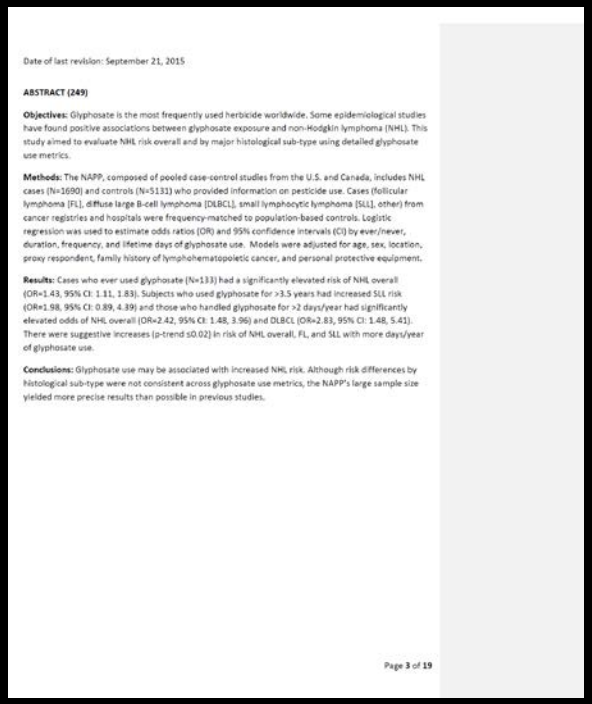

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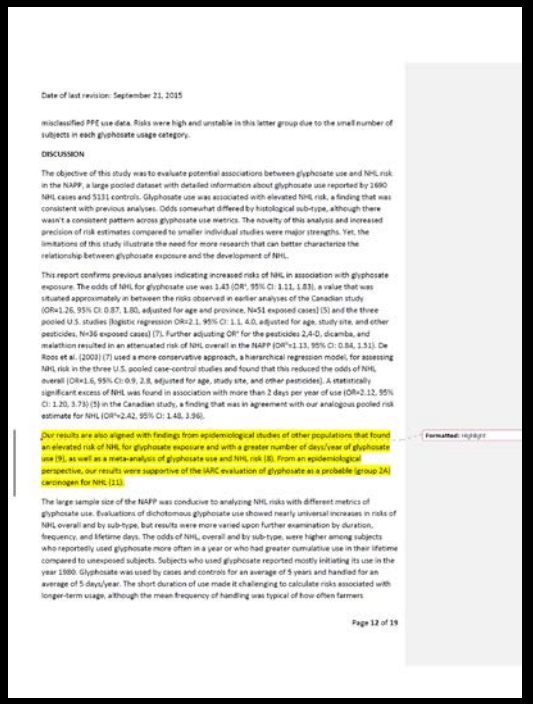

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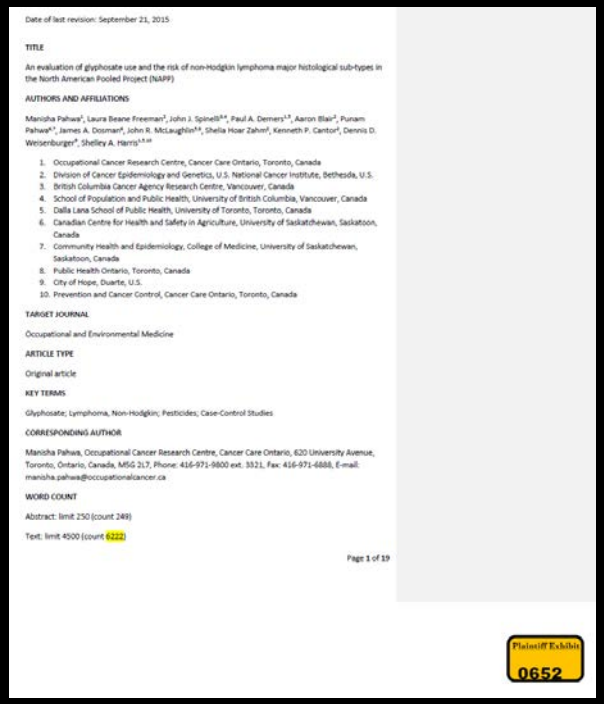

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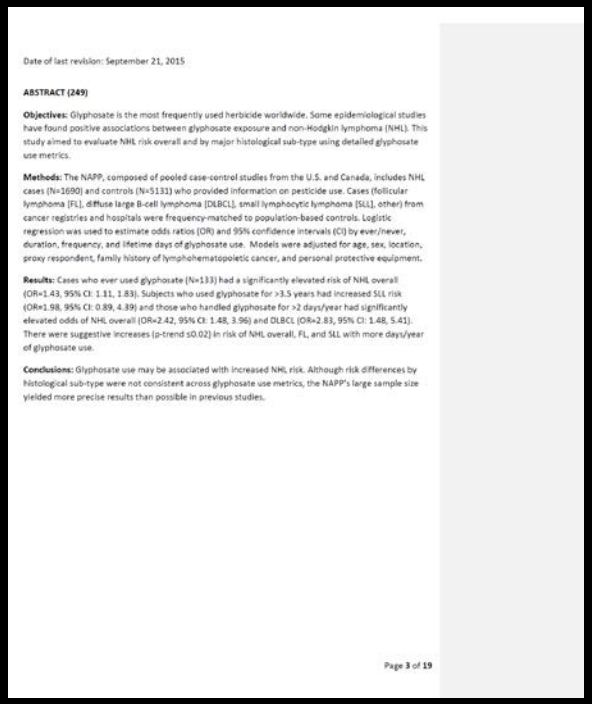

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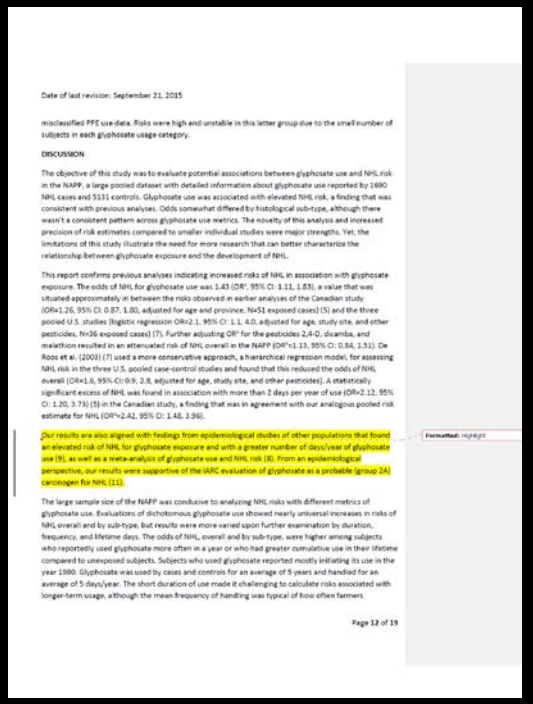

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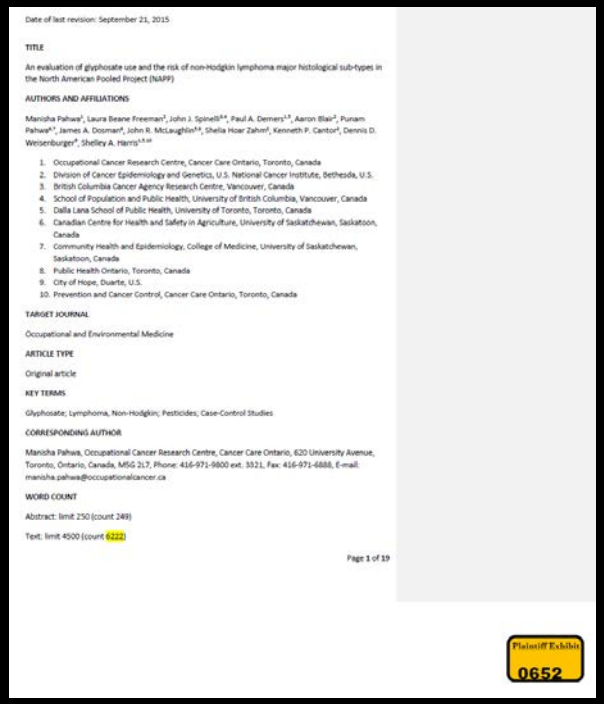

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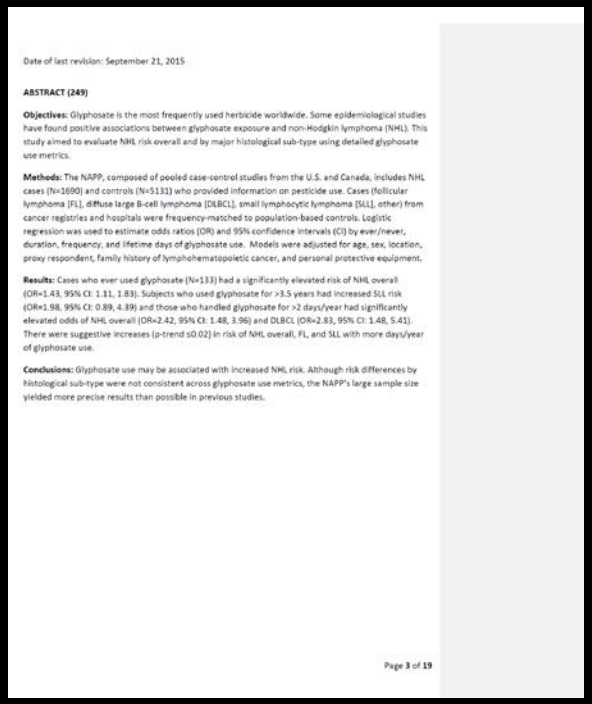

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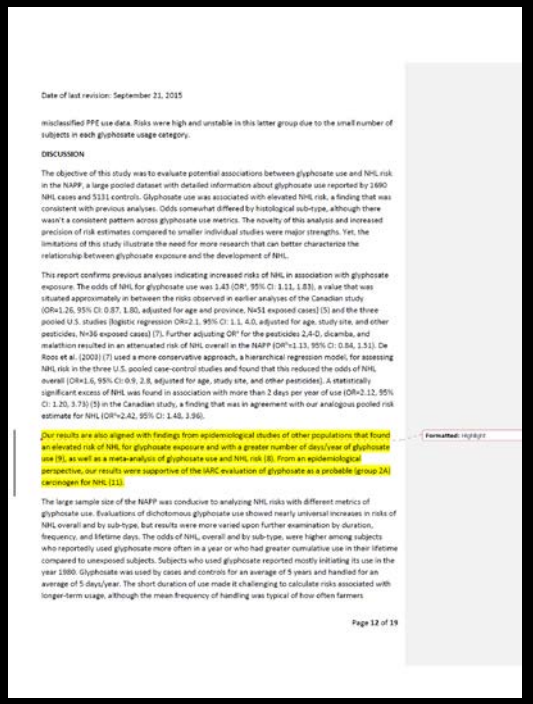

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The large sample size of the NAPP was conducive to analyzing NHL risks with different metrics of glyphosate use. Evaluations of dichotomous glyphosate use showed nearly universal increases in risks of NHL overall and by sub-type, but results were more varied upon further examination by duration, frequency, and lifetime days. The odds of NHL, overall and by sub-type, were higher among subjects who reportedly used glyphosate more often in a year or who had greater cumulative use in their lifetime compared to unexposed subjects. Subjects who used glyphosate reported mostly initiating its use in the year 1980. Glyphosate was used by cases and controls for an average of 5 years and handled for an average of 5 days/year. The short duration of use made it challenging to calculate risks associated with longer-term usage, although the mean frequency of handling was typical of how often farmers

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NAPP Supports IARC and NHL Association

Because NAPP was not published, IARC could not consider this information, which would have strengthened the epidemiology data.

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- Confounding
- Proxy "bias"
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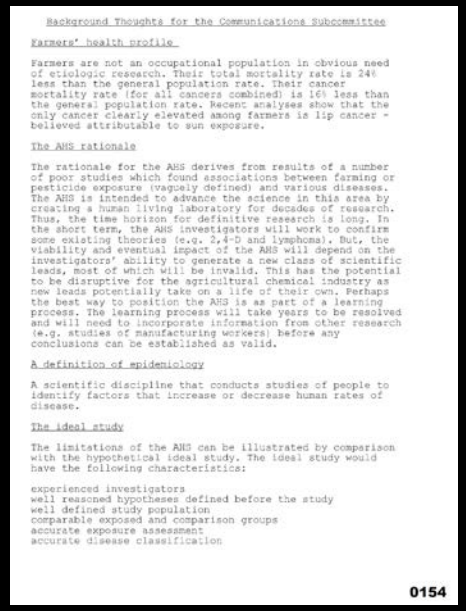

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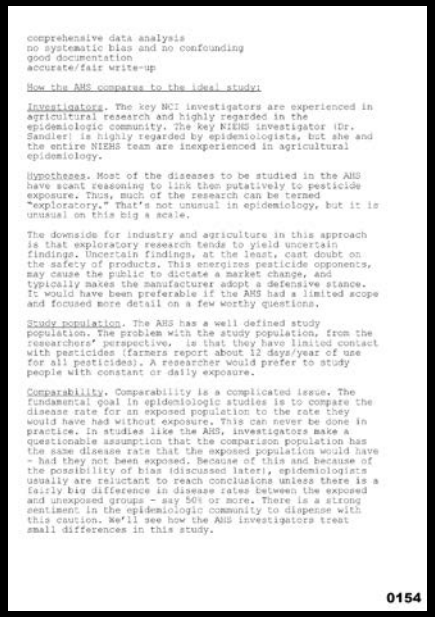

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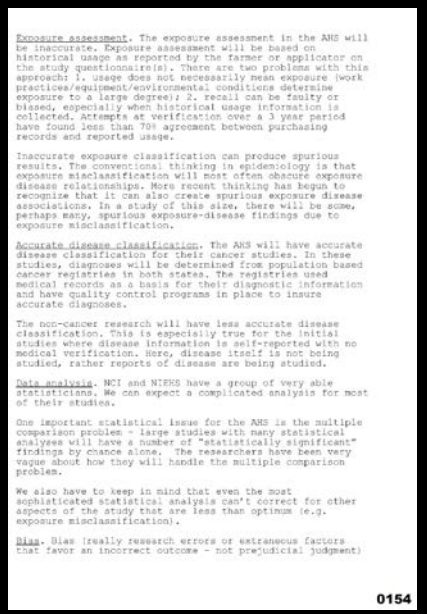

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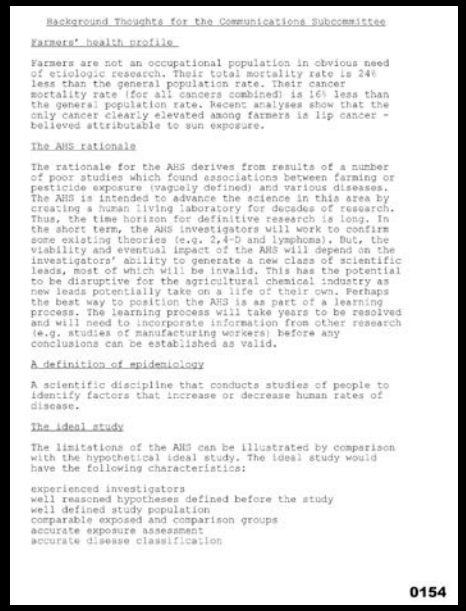

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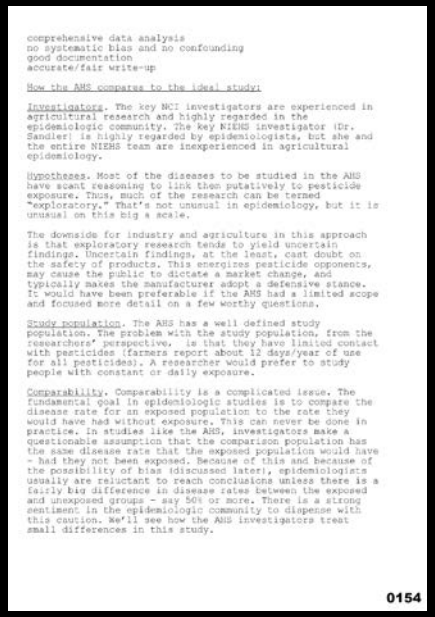

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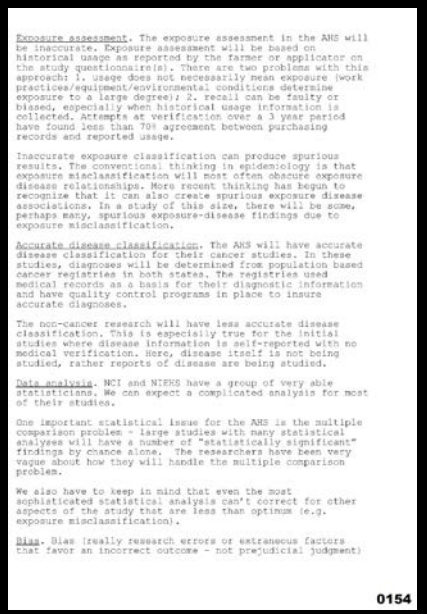

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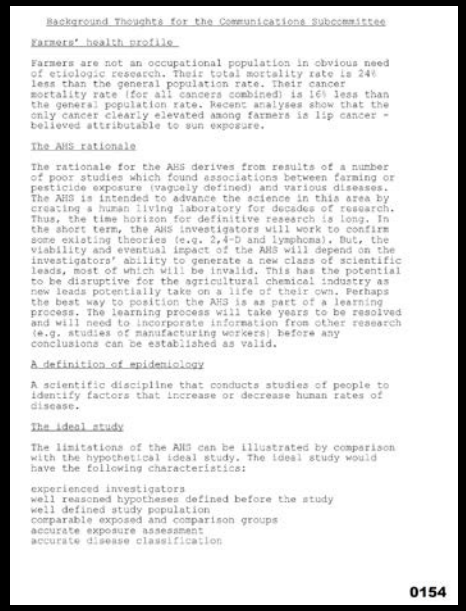

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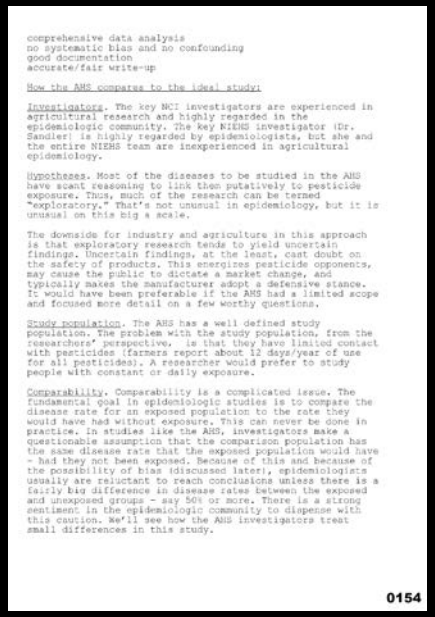

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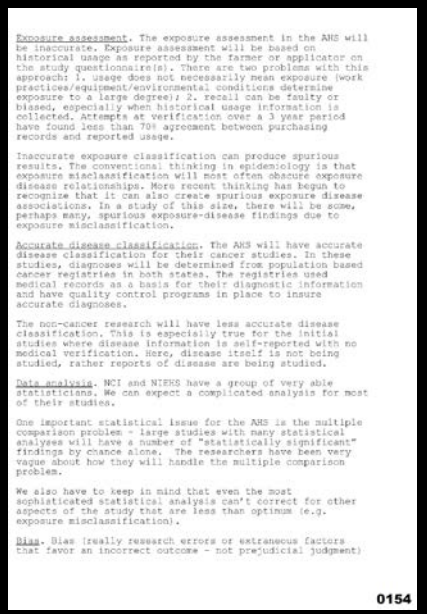

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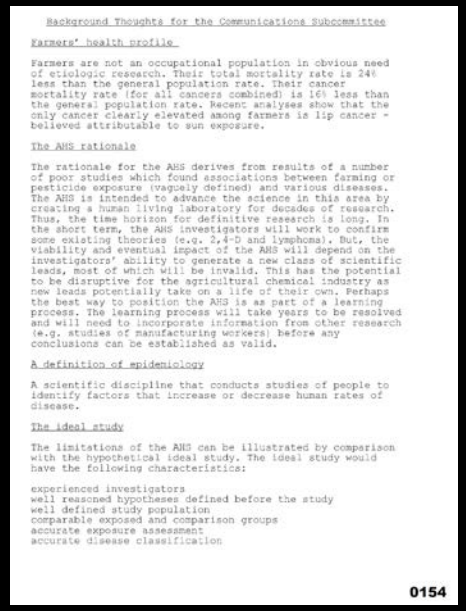

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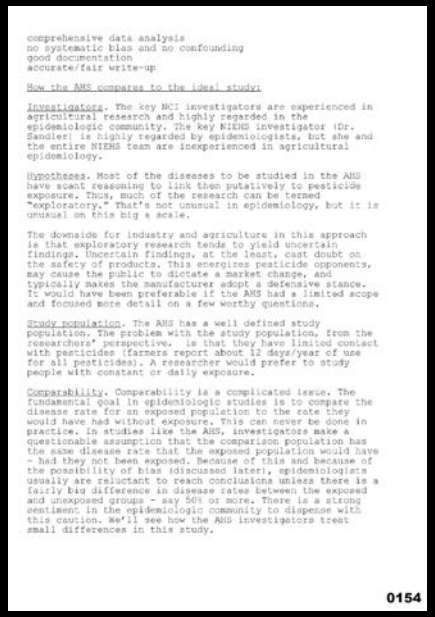

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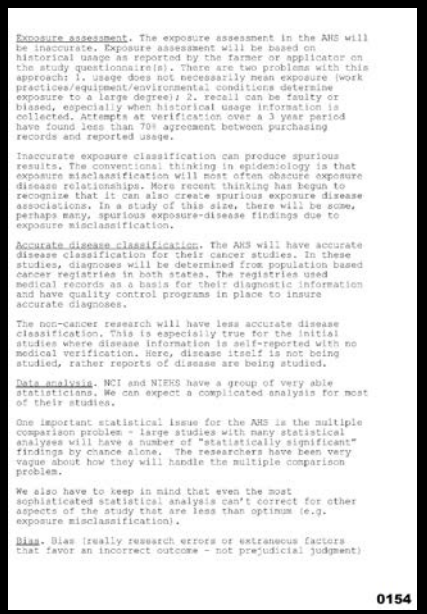

Exposure assessment. The exposure assessment in the AHS will be inaccurate. Exposure assessment will be based on historical usage as reported by the farmer or applicator on the study questionnaire(s). There are two problems with this approach: 1. usage does not necessarily mean exposure (work practices/equipment/environmental conditions determine exposure to a large degree); 2. recall can be faulty or biased, especially when historical usage information is collected. Attempts at verification over a 3 year period have found less than 70% agreement between purchasing records and reported usage.

Inaccurate exposure classification can produce spurious results. The conventional thinking in epidemiology is that exposure misclassification will most often obscure exposure disease relationships. More recent thinking has begun to recognize that it can also create spurious exposure disease associations. In a study of this size, there will be some, perhaps many, spurious exposure-disease findings due to exposure misclassification.

Accurate disease classification. The AHS will have accurate disease classification for their cancer studies. In these studies, diagnoses will be determined from population based cancer registries in both states. The registries used medical records as a basis for their diagnostic information and have quality control programs in place to insure accurate diagnoses.

The non-cancer research will have less accurate disease classification. This is especially true for the initial studies where disease information is self-reported with no medical verification. Here, disease itself is not being studied, rather reports of disease are being studied.

Data analysis. NCI and NIEHS have a group of very able statisticians. We can expect a complicated analysis for most of their studies.

One important statistical issue for the AHS is the multiple comparison problem - large studies with many statistical analyses will have a number of "statistically significant" findings by chance alone. The researchers have been very vague about how they will handle the multiple comparison problem.

We also have to keep in mind that even the most sophisticated statistical analysis can't correct for other aspects of the study that are less than optimum (e.g. exposure misclassification).

Bias. Bias (really research errors or extraneous factors that favor an incorrect outcome - not prejudicial judgment)

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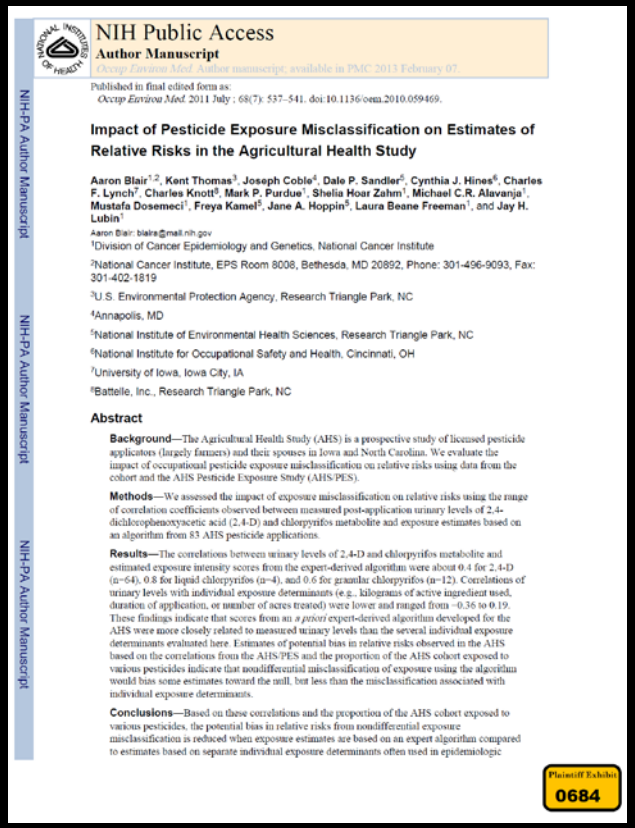

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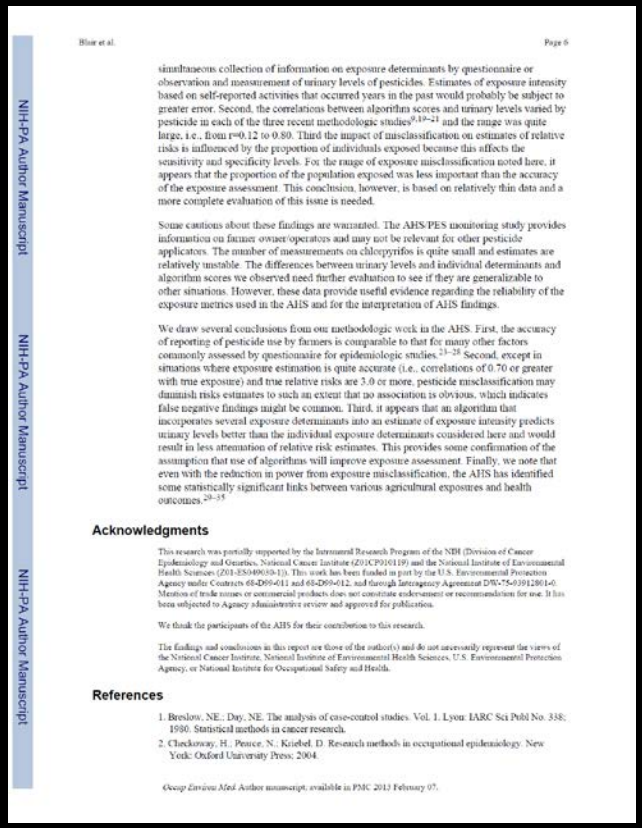

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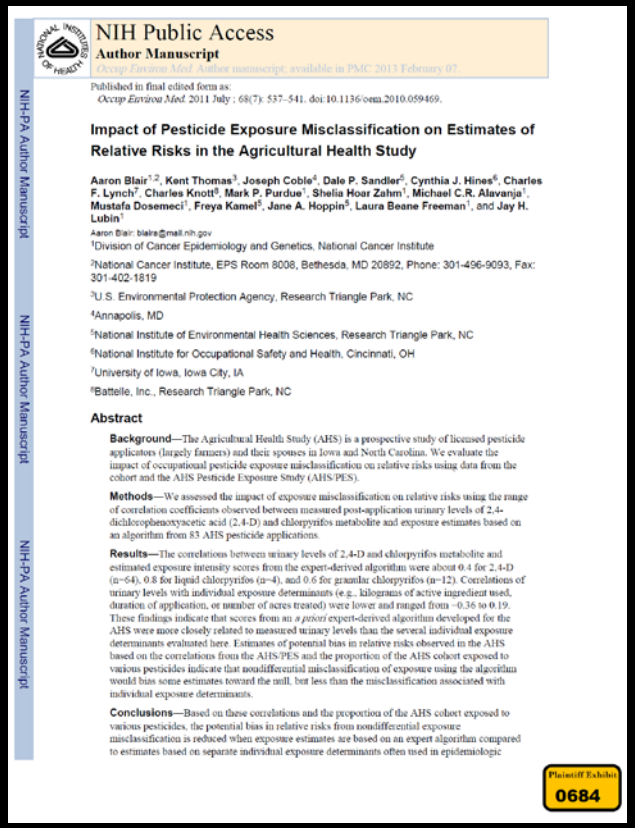

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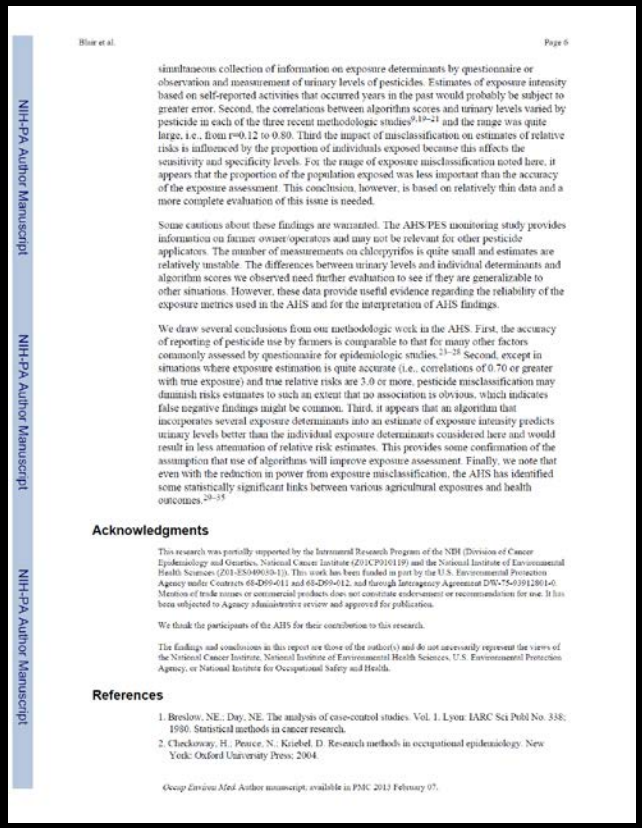

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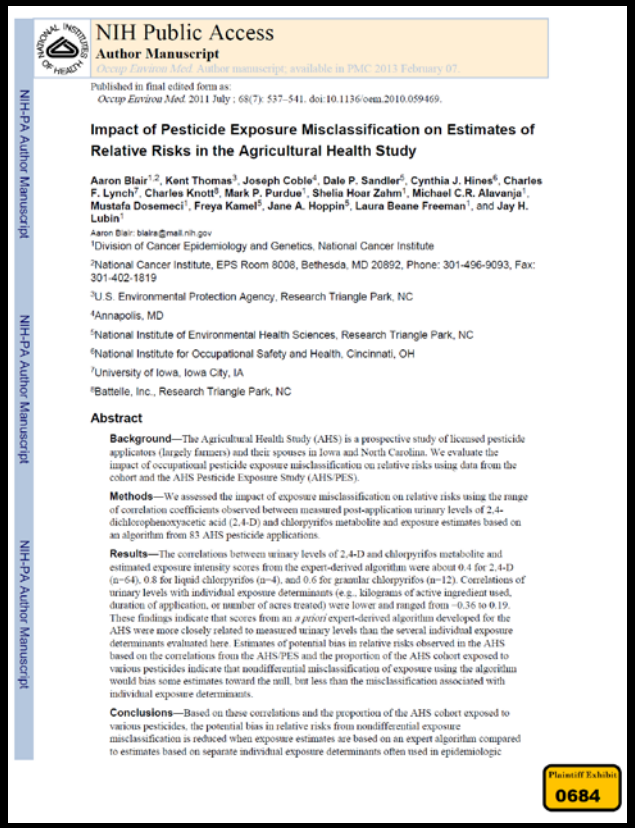

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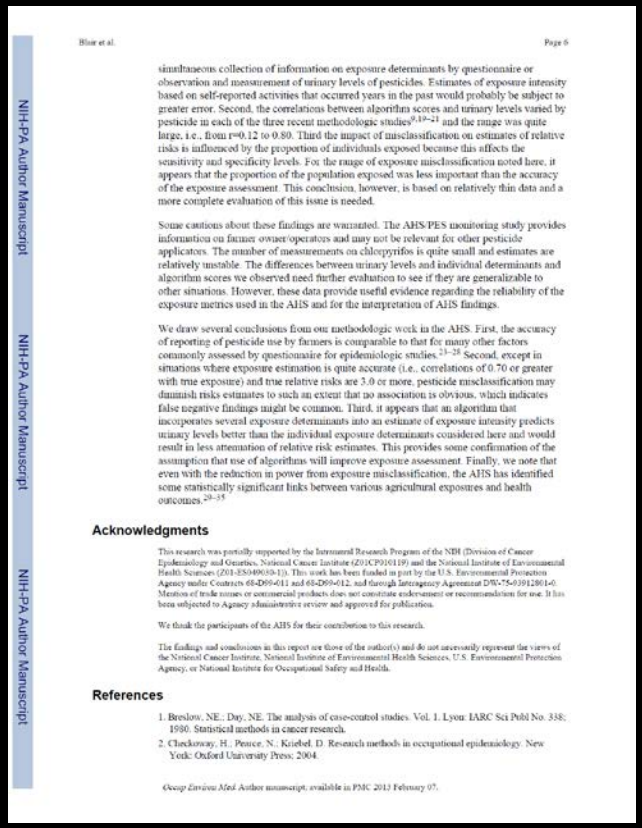

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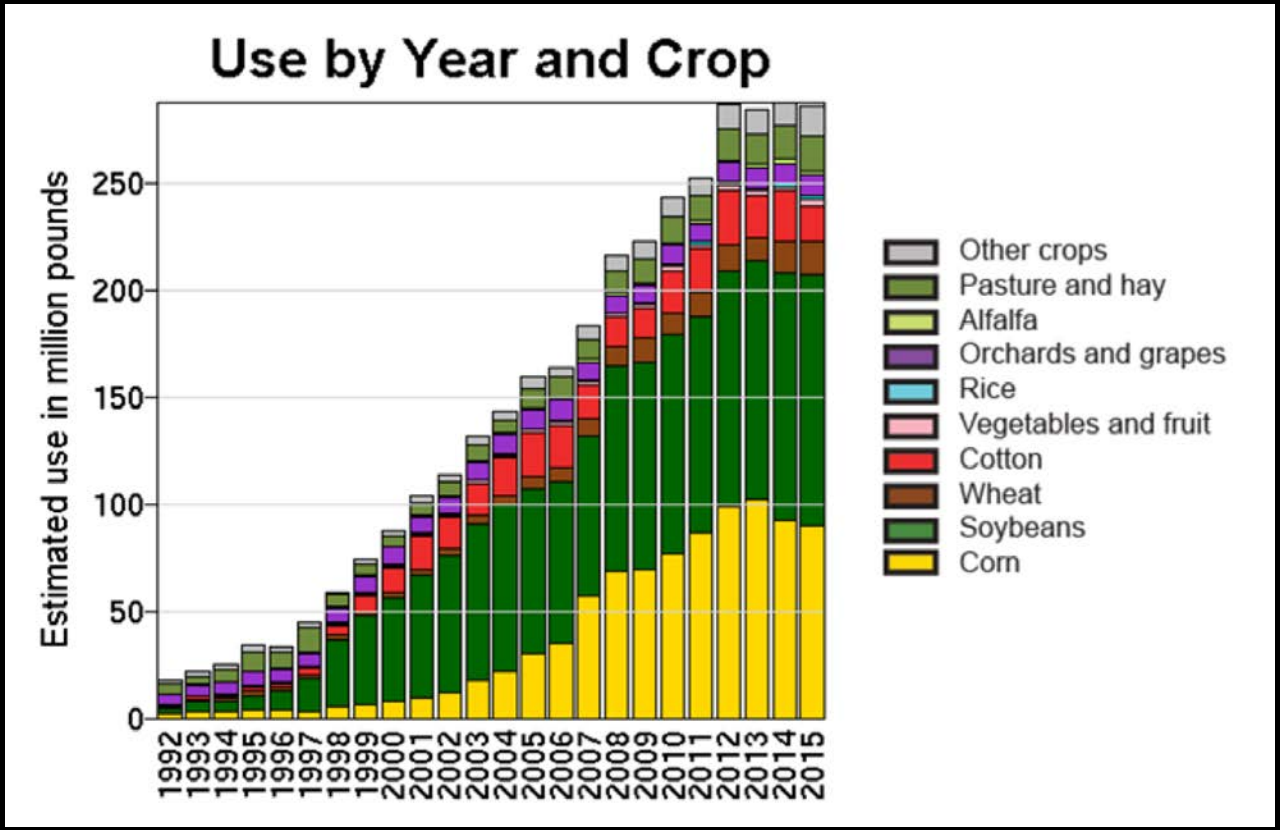

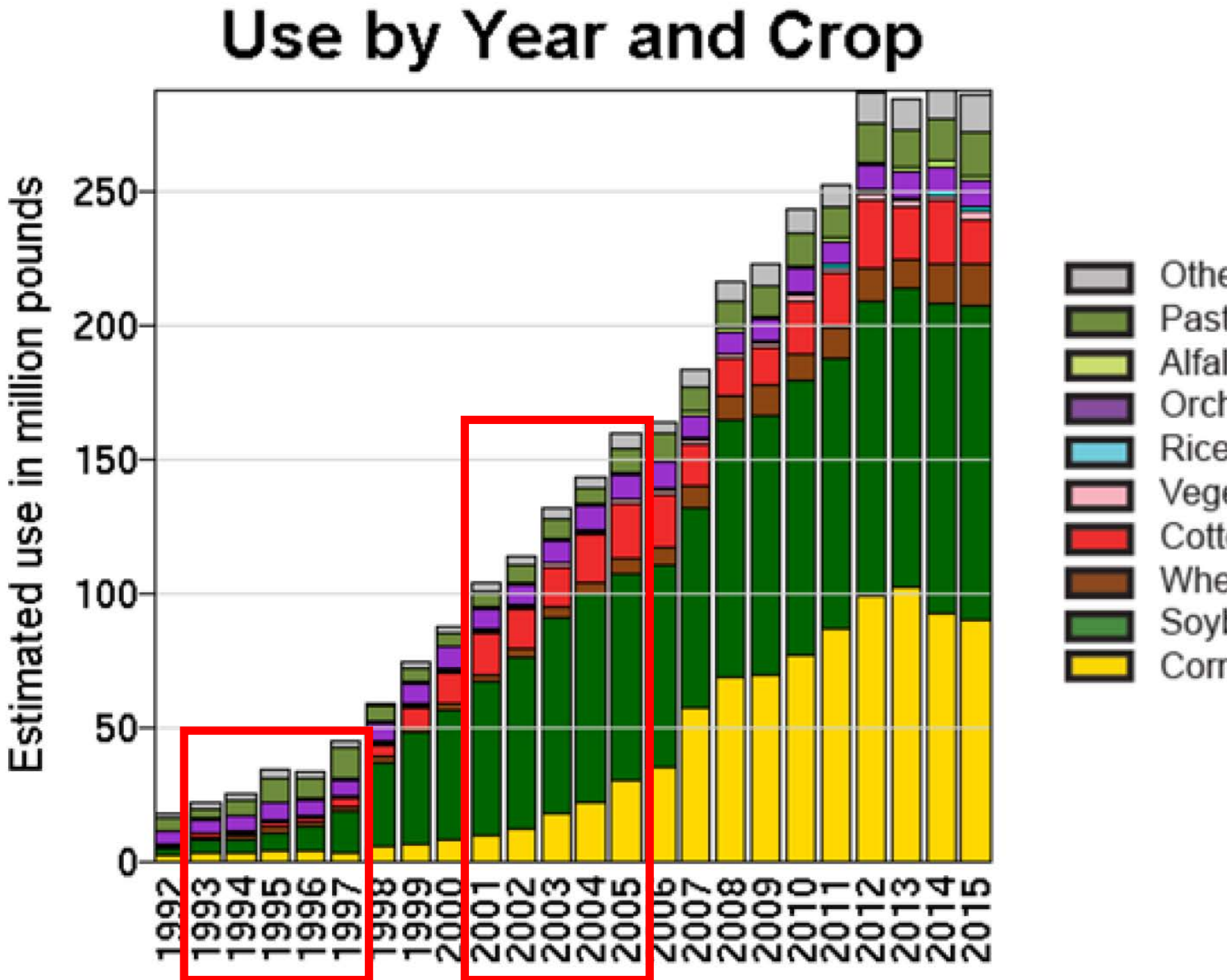
Use by Year and Crop
Estimated use in million pounds
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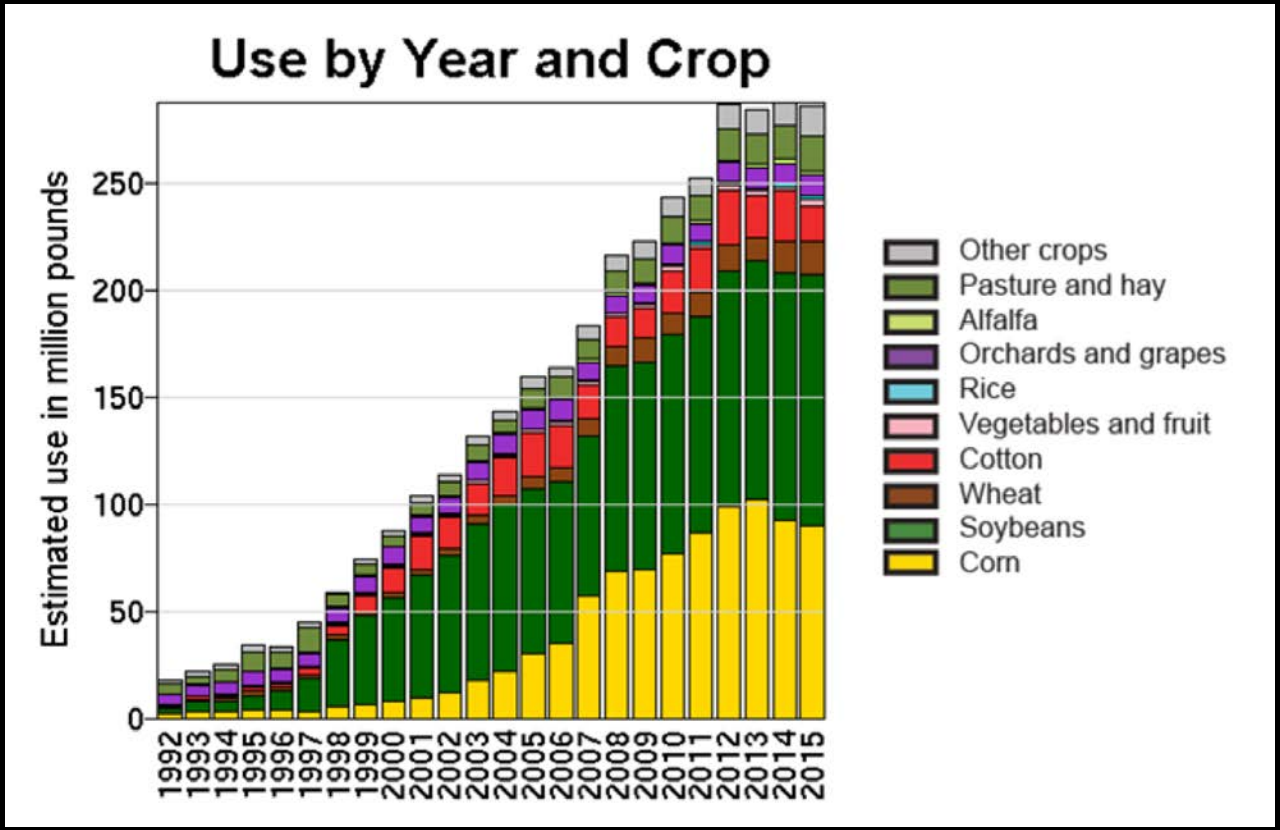

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- Confounding
- Proxy "bias"
- North American Pooled Project (NAPP)
- Agricultural Health Study (AHS)

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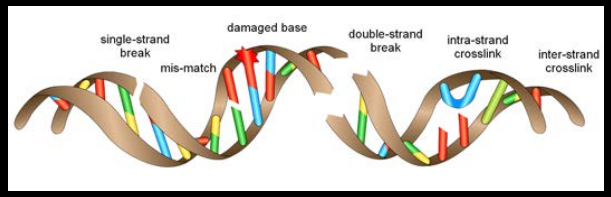
Epidemiology

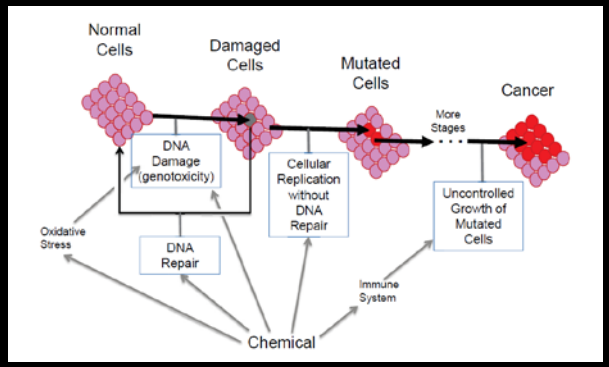
IARC Classification
1. Sufficient
2. Limited
3. Inadequate
4. Lack of Carcinogenicity

Toxicology

IARC Classification
1. Sufficient
2. Limited
3. Inadequate
4. Lack of Carcinogenicity

Mechanism





IARC Classification
1. Strong
2. Moderate
3. Weak

Three Pillars of Cancer Science

Toxicology

Mice Studies – Tumor Chart

Knezevich & Hogan (1983)	Atkinson (1993) *Limited*	Sugimoto (1997)	Wood (2009)	Kumar (2001)
Kidney carcinomas or adenomas Trend Dose M / F	Malignant lymphoma Trend Dose M / F	Kidney carcinomas or adenomas Trend Dose M / F	Malignant lymphoma Trend Dose M / F	Kidney carcinomas or adenomas Trend Dose M / F
Spleen composite lymphosarcoma Trend Dose M / F	Hemangiosarcoma Trend Dose M / F	Malignant lymphoma Trend Dose M / F	Mul. malignant tumors or neoplasms Trend Dose M / F	Malignant lymphoma Trend Dose M / F
		Hemangiosarcoma Trend Dose M / F	Lung adenocarcinoma Trend Dose M / F	Hemangioma Trend Dose M / F
		Hemangioma Trend Dose M / F		
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		Harderian gland adenoma Trend Dose M / F		

Plaintiff Exhibit 1020

Rat Studies – Tumor Chart

Lankas (1981)	Stout & Ruecker (1990)	Atkinson (1993) *Limited*	Enemoto (1997)	Suresh (1996) *Limited*	Brammer (2001)	Wood (2009)
Testicular interstitial cell tumors Trend Dose M / F	Thyroid C-Cell carcinomas or adenomas Trend Dose M / F	Thyroid follicular carcinomas or adenomas Trend Dose M / F	Kidney carcinomas or adenomas Trend Dose M / F		Hepatocellular carcinomas or adenomas Trend Dose M / F	Skin kera-toacanthoma Trend Dose M / F
Thyroid C-Cell carcinomas or adenomas Trend Dose M / F	Pancreatic islet cell tumors Trend Dose M / F	Skin kera-toacanthoma Trend Dose M / F	Skin kera-toacanthoma Trend Dose M / F			Mammary gland carcinomas or adenomas Trend Dose M / F
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	Adrenal cortical carcinomas Trend Dose M / F					
	Skin kera-toacanthoma Trend Dose M / F					

Plaintiff Exhibit 1021

Rat Studies – Tumor Chart

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Thyroid C-Cell carcinomas or adenomas Trend Dose M / F	**Pancreatic islet cell tumors** Trend Dose M / F	**Skin kera-toacanthoma** Trend Dose M / F	**Skin kera-toacanthoma** Trend Dose M / F			**Mammary gland carcinomas or adenomas** Trend Dose M / F
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Plaintiff Exhibit
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Three Pillars of Cancer Science

Toxicology

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		Hemangiosarcoma Trend Dose M / F	Lung adenocarci	

Plaintiff Exhibit 1020

Sufficient

tudies – Tumor Chart

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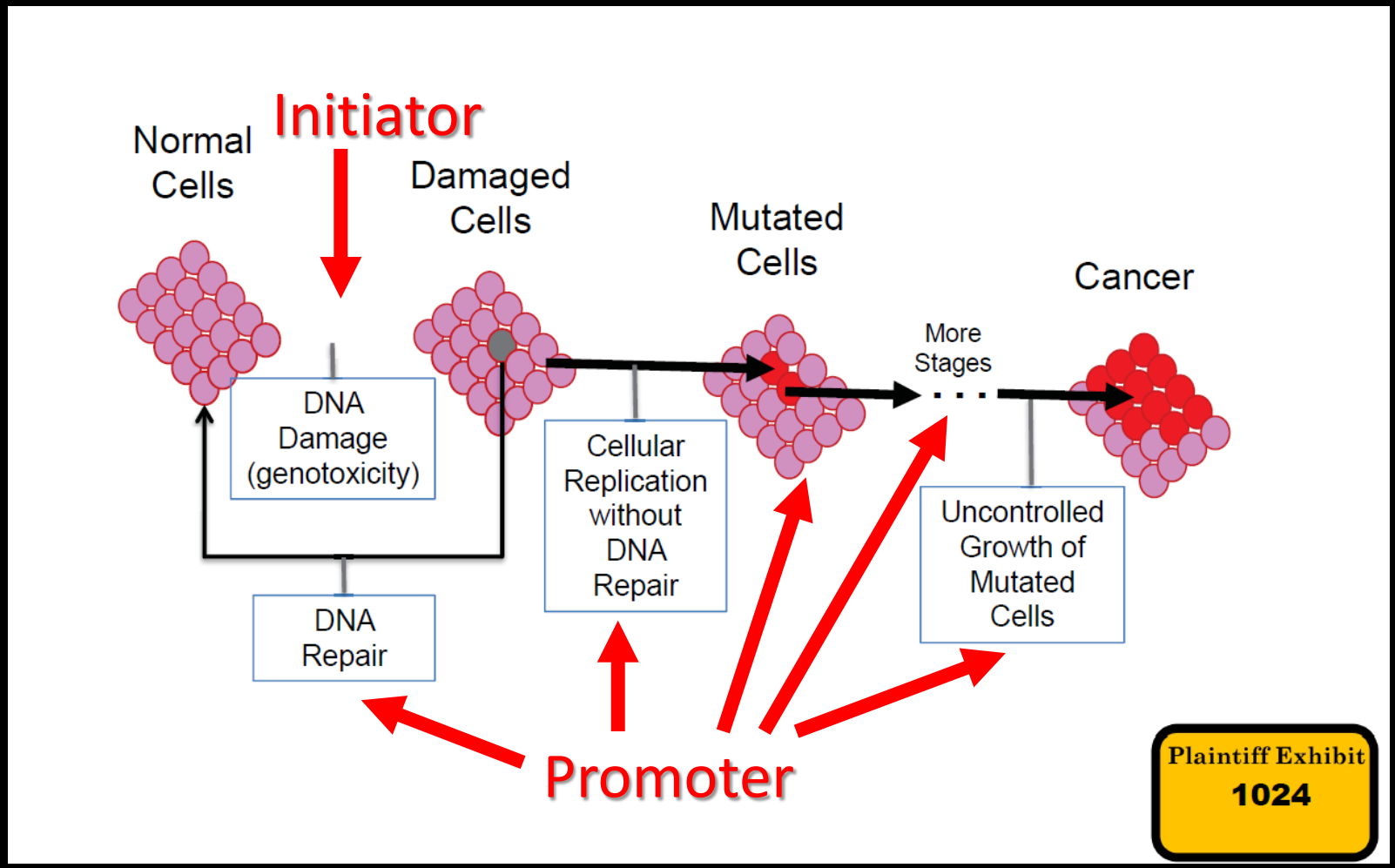
Three Pillars of Cancer Science

Toxicology



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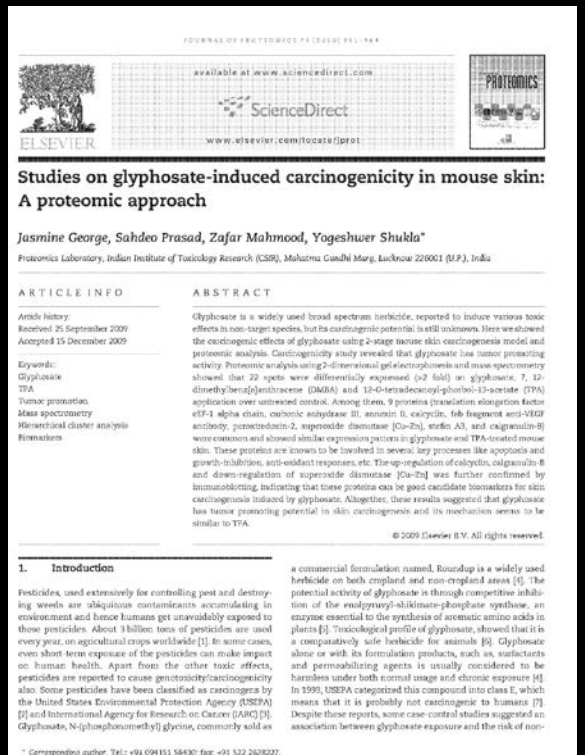

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PROTEOMICS

Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach

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ABSTRACT

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

1. Introduction

Pesticides, used extensively for controlling pest and destroying weeds are ubiquitous contaminants accumulating in environment and hence humans get unavoidably exposed to these pesticides. About 3 billion tons of pesticides are used every year, on agricultural crops worldwide [1]. In some cases, even short-term exposure of the pesticides can make impact on human health. Apart from the other toxic effects, pesticides are reported to cause genotoxicity/carcinogenicity also. Some pesticides have been classified as carcinogens by the United States Environmental Protection Agency (USEPA) [2] and International Agency for Research on Cancer (IARC) [3]. Glyphosate, N-(phosphonomethyl) glycine, commonly sold as a commercial formulation named, Roundup is a widely used herbicide on both cropland and non-cropland areas [4]. The potential activity of glyphosate is through competitive inhibition of the enolpyruvyl-shikimate-phosphate synthase, an enzyme essential to the synthesis of aromatic amino acids in plants [5]. Toxicological profile of glyphosate, showed that it is a comparatively safe herbicide for animals [6]. Glyphosate alone or with its formulation products, such as, surfactants and permeabilizing agents is usually considered to be harmless under both normal usage and chronic exposure [4]. In 1993, USEPA categorized this compound into class E, which means that it is probably not carcinogenic to humans [7]. Despite these reports, some case-control studies suggested an association between glyphosate exposure and the risk of non-

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Hodgkin's lymphoma [8,9]. In another study, both technical grade glyphosate and Roundup were shown to cause a rapid increase in cell division in human breast cancer cells [10]. Glyphosate has also been shown as a skin irritant [11]. Regarding the genotoxic potential, glyphosate exposure to human lymphocytes *in vitro* resulted in increased sister chromatid exchanges [12], chromosomal aberrations [13], and indicators of oxidative stress [14]. A recent study from our laboratory also showed the clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice [15]. These reports prompted us to investigate its carcinogenic effect in long-term animal bioassay.

To evaluate toxicity/carcinogenicity induced by physical and chemical agents including pesticides, various test systems have been employed in bacteria, rodents and mammalian cells [16–18]. Each of these environmental challenges causes changes in DNA conformation, alterations in the levels of mRNA and protein expression, and post-translational modifications such as phosphorylation and glycosylation of proteins specific to each stressor [19]. In recent years, there has been considerable interest in linking carcinogenic/toxic responses to gene and protein expression. Toxicoproteomics has received a lot of attention as a valuable tool to search reliable early predictive toxicity markers in response to environmental stimuli [20]. Two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS); a high throughput technique allows proteins of interest to be identified by their expression and/or modification pattern rather than using the traditional approach of translating gene expression data. Biomarkers can be used to identify causal associations and to make better quantitative estimates of those associations at relevant levels of exposure [21]. Yamamoto et al. [22] have utilized proteomic approach to identify potential biomarker candidates of hepatotoxicant exposure in rat liver.

Skin is the largest organ in the body and dermal contact is one of the most probable routes of human exposure to pesticides, thus, mouse skin model represents a logical experimental choice [23]. As the long-term bioassay for carcinogenicity is expansive, time consuming and involves a large number of animals and ethical issues, development of biomarkers after short-term exposure are needed. The present investigation was carried out to study the carcinogenic potential of glyphosate and to identify differentially expressed proteins, using 2-DE and MS analysis after treatment with glyphosate, a known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and tumor initiator, 7, 12-dimethylbenz[a]anthracene (DMBA) in mouse skin. Altered proteins identified through proteomic approach in our study may be potentially useful as early biomarkers, to detect the adverse effects of glyphosate.

2. Materials and methods

2.1. Materials

The commercial formulation of the herbicide glyphosate (N-phosphonomethyl glycine) Roundup Original® (glyphosate 41%, POEA=15%—Monsanto Company, St. Louis, MO, USA) was used, which contains 360 g/l glyphosate acid equivalent as the isopropylamine salt and was procured from local market. Immobilized pH gradient (IPG) strips and 0.5% pH 3–10 IPG buffer were purchased from Bio-Rad Laboratories (Hercules, CA, USA). DMBA, TPA, CHAPS, DTT, and beta-actin (clone AC-74) antibody were from Sigma-Aldrich (Missouri, USA). DNase/RNase was from Bangalore Genei (Bangalore, India). The rest of the chemicals used in the study were of analytical grade of purity and procured locally.

2.2. Animals and treatments

2.2.1. Carcinogenicity study

Male, Swiss albino mice (12–15 g body weight [b.wt]) were taken from Indian Institute of Toxicology Research (IITR) animal breeding colony and acclimatized for 1 week. The ethical approval for the experiment was obtained from institutional ethical committee. The animals were kept under standard laboratory conditions (temperature 23 ± 2 °C, relative humidity 55 ± 5%) and were fed with synthetic pellet basal diet (Ashirwad, Chandigarh, India) and tap water *ad libitum*. Animals were randomly divided into 8 groups of 20 animals each. Hair were clipped in the dorsal region with proper care in an area of 2 cm^2 using electrical clippers, not lubricated with oil or grease. The long-term treatment was given as described earlier [24]. Briefly,

Group I Untreated control (No treatment).
Group II Glyphosate alone (25 mg/kg b.wt, topically 3 times per week).
Group III DMBA + TPA (Single topical application of DMBA, 52 μg/mouse followed 1 week later by thrice a week application of TPA, 5 μg/mouse).
Group IV Glyphosate (a) + TPA (Single topical application of glyphosate, 25 mg/kg b.wt followed 1 week later by TPA application as in group III).
Group V Glyphosate (m) + TPA (Thrice a week topical application of glyphosate, 25 mg/kg b.wt for 3 weeks [total of 9 applications], followed 1 week later by TPA application as in group III).
Group VI DMBA (Single topical application of DMBA, 52 μg/mouse).
Group VII TPA (Thrice a week topical application of TPA, 5 μg/mouse).
Group VIII DMBA + glyphosate (Single topical application of DMBA [as in group III], followed 1 week later by topical treatment of glyphosate, 25 mg/kg b.wt thrice per week).

Vehicle for glyphosate, DMBA and TPA were 50% ethanol and acetone respectively.

Animals from all the groups were examined every week for gross morphological changes including body weight changes, development and volume of squamous cell papillomas (tumors) locally on the skin during the entire study period and tumors larger than 1 mm diameter, were included in the total number of tumors. Tumor volume per tumor bearing mouse was calculated in each group using formula $V = D \times d^2 \times \pi/6$ (where D = bigger dimension and d = smaller dimension). All the surviving animals were sacrificed at the end of the study period, i.e. 32 weeks for complete carcinogenic, tumor initiating and promoting studies.

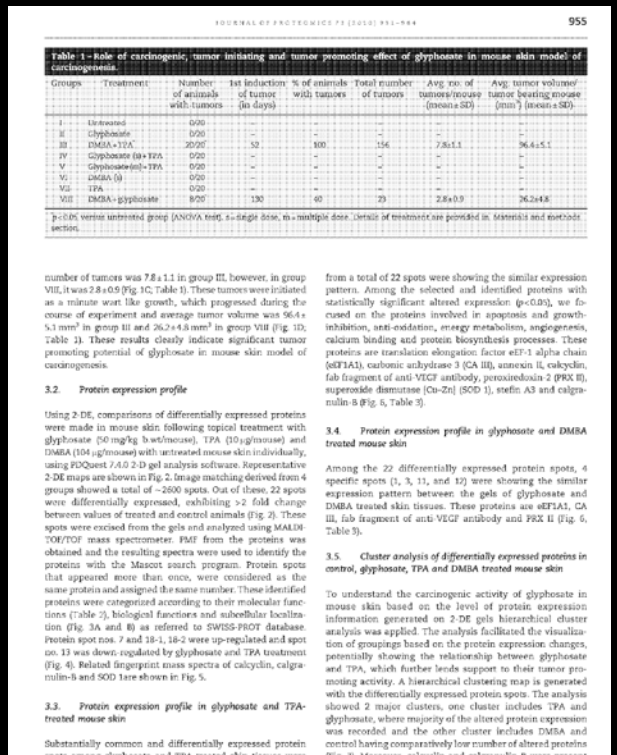

Table 1 – Role of carcinogenic, tumor initiating and tumor promoting effect of glyphosate in mouse skin model of carcinogenesis.

Groups	Treatment	Number of animals with tumors	1st induction (in days)	% of animals with tumors	Total number of tumors	Avg no. of tumors/mouse (mean ± SD)	Avg tumor volume/ tumor bearing mouse (mm^3) (mean ± SD)
I	Untreated	0/20	–	–	–	–	–
II	Glyphosate	0/20	–	–	–	–	–
III	DMBA + TPA	20/20	52	100	156	7.8 ± 1.1	96.4 ± 5.1
IV	Glyphosate (a) + TPA	0/20	–	–	–	–	–
V	Glyphosate (m) + TPA	0/20	–	–	–	–	–
VI	DMBA (s)	0/20	–	–	–	–	–
VII	TPA	0/20	–	–	–	–	–
VIII	DMBA + glyphosate	8/20	130	40	23	2.8 ± 0.9	26.2 ± 4.8

$p < 0.05$ versus untreated group (ANOVA test), s = single dose, m = multiple dose. Details of treatment are provided in Materials and methods section.

number of tumors was 7.8 ± 1.1 in group III, however, in group VIII, it was 2.8 ± 0.9 (Fig. 1C; Table 1). These tumors were initiated as a minute wart like growth, which progressed during the course of experiment and average tumor volume was 96.4 ± 5.1 mm^3 in group III and 26.2 ± 4.8 mm^3 in group VIII (Fig. 1D; Table 1). These results clearly indicate significant tumor promoting potential of glyphosate in mouse skin model of carcinogenesis.

3.2. Protein expression profile

Using 2-DE, comparisons of differentially expressed proteins were made in mouse skin following topical treatment with glyphosate (50 mg/kg b.wt/mouse), TPA (10 μg/mouse) and DMBA (104 μg/mouse) with untreated mouse skin individually, using PDQuest 7.4.0 2-D gel analysis software. Representative 2-DE maps are shown in Fig. 2. Image matching derived from 4 groups showed a total of ~2600 spots. Out of these, 22 spots were differentially expressed, exhibiting >2 fold change between values of treated and control animals (Fig. 2). These spots were excised from the gels and analyzed using MALDI-TOF/TOF mass spectrometer. PMF from the proteins was obtained and the resulting spectra were used to identify the proteins with the Mascot search program. Protein spots that appeared more than once, were considered as the same protein and assigned the same number. These identified proteins were categorized according to their molecular functions (Table 2), biological functions and subcellular localization (Fig. 3A and B) as referred to SWISS-PROT database. Protein spot nos. 7 and 18-1, 18-2 were up-regulated and spot no. 13 was down-regulated by glyphosate and TPA treatment (Fig. 4). Related fingerprint mass spectra of calcyclin, calgranulin-B and SOD 1are shown in Fig. 5.

3.3. Protein expression profile in glyphosate and TPA-treated mouse skin

Substantially common and differentially expressed protein spots among glyphosate and TPA-treated skin tissues were quantitatively analyzed individually. Comparison between the gels of glyphosate and TPA revealed that 13 specific proteins spots (1, 2, 3, 6-1, 6-2, 7, 8, 11, 12, 13, 15, 18-1, and 18-2) from a total of 22 spots were showing the similar expression pattern. Among the selected and identified proteins with statistically significant altered expression ($p < 0.05$), we focused on the proteins involved in apoptosis and growth-inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes. These proteins are translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu–Zn] (SOD 1), stefin A3 and calgranulin-B (Fig. 6, Table 3).

3.4. Protein expression profile in glyphosate and DMBA treated mouse skin

Among the 22 differentially expressed protein spots, 4 specific spots (1, 3, 11, and 12) were showing the similar expression pattern between the gels of glyphosate and DMBA treated skin tissues. These proteins are eEF1A1, CA III, fab fragment of anti-VEGF antibody and PRX II (Fig. 6, Table 3).

3.5. Cluster analysis of differentially expressed proteins in control, glyphosate, TPA and DMBA treated mouse skin

To understand the carcinogenic activity of glyphosate in mouse skin based on the level of protein expression information generated on 2-DE gels hierarchical cluster analysis was applied. The analysis facilitated the visualization of groupings based on the protein expression changes, potentially showing the relationship between glyphosate and TPA, which further lends support to their tumor promoting activity. A hierarchical clustering map is generated with the differentially expressed protein spots. The analysis showed 2 major clusters, one cluster includes TPA and glyphosate, where majority of the altered protein expression was recorded and the other cluster includes DMBA and control having comparatively low number of altered proteins (Fig. 7). Moreover, calcyclin and calgranulin-B were present only in the cluster of glyphosate and TPA whereas SOD 1 is higher in DMBA and control cluster in comparison to other cluster.

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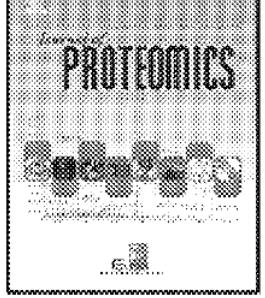

Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach

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Three Pillars of Cancer Science

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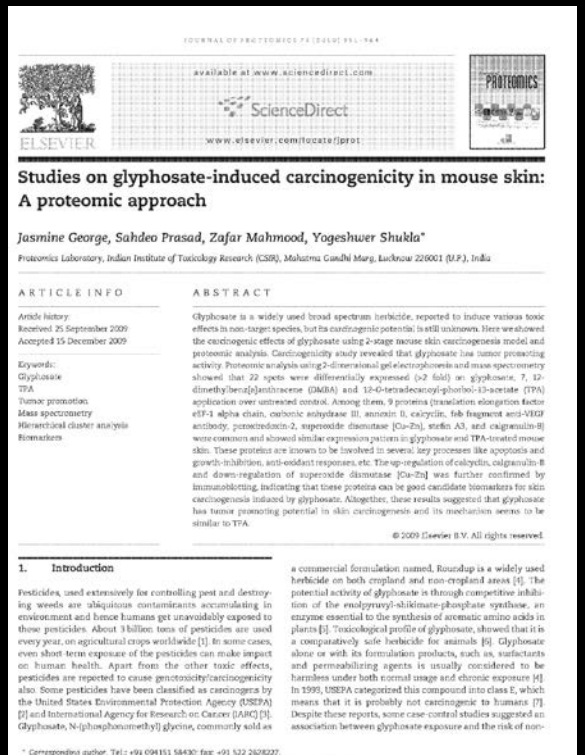

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PROTEOMICS

Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach

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ABSTRACT

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

1. Introduction

Pesticides, used extensively for controlling pest and destroying weeds are ubiquitous contaminants accumulating in environment and hence humans get unavoidably exposed to these pesticides. About 3 billion tons of pesticides are used every year, on agricultural crops worldwide [1]. In some cases, even short-term exposure of the pesticides can make impact on human health. Apart from the other toxic effects, pesticides are reported to cause genotoxicity/carcinogenicity also. Some pesticides have been classified as carcinogens by the United States Environmental Protection Agency (USEPA) [2] and International Agency for Research on Cancer (IARC) [3]. Glyphosate, N-(phosphonomethyl) glycine, commonly sold as a commercial formulation named, Roundup is a widely used herbicide on both cropland and non-cropland areas [4]. The potential activity of glyphosate is through competitive inhibition of the enolpyruvyl-shikimate-phosphate synthase, an enzyme essential to the synthesis of aromatic amino acids in plants [5]. Toxicological profile of glyphosate, showed that it is a comparatively safe herbicide for animals [6]. Glyphosate alone or with its formulation products, such as, surfactants and permeabilizing agents is usually considered to be harmless under both normal usage and chronic exposure [4]. In 1993, USEPA categorized this compound into class E, which means that it is probably not carcinogenic to humans [7]. Despite these reports, some case-control studies suggested an association between glyphosate exposure and the risk of non-

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To evaluate toxicity/carcinogenicity induced by physical and chemical agents including pesticides, various test systems have been employed in bacteria, rodents and mammalian cells [16–18]. Each of these environmental challenges causes changes in DNA conformation, alterations in the levels of mRNA and protein expression, and post-translational modifications such as phosphorylation and glycosylation of proteins specific to each stressor [19]. In recent years, there has been considerable interest in linking carcinogenic/toxic responses to gene and protein expression. Toxicoproteomics has received a lot of attention as a valuable tool to search reliable early predictive toxicity markers in response to environmental stimuli [20]. Two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS); a high throughput technique allows proteins of interest to be identified by their expression and/or modification pattern rather than using the traditional approach of translating gene expression data. Biomarkers can be used to identify causal associations and to make better quantitative estimates of those associations at relevant levels of exposure [21]. Yamamoto et al. [22] have utilized proteomic approach to identify potential biomarker candidates of hepatotoxicant exposure in rat liver.

Skin is the largest organ in the body and dermal contact is one of the most probable routes of human exposure to pesticides, thus, mouse skin model represents a logical experimental choice [23]. As the long-term bioassay for carcinogenicity is expansive, time consuming and involves a large number of animals and ethical issues, development of biomarkers after short-term exposure are needed. The present investigation was carried out to study the carcinogenic potential of glyphosate and to identify differentially expressed proteins, using 2-DE and MS analysis after treatment with glyphosate, a known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and tumor initiator, 7, 12-dimethylbenz[a]anthracene (DMBA) in mouse skin. Altered proteins identified through proteomic approach in our study may be potentially useful as early biomarkers, to detect the adverse effects of glyphosate.

2. Materials and methods

2.1. Materials

The commercial formulation of the herbicide glyphosate (N-phosphonomethyl glycine) Roundup Original® (glyphosate 41%, POEA=15%—Monsanto Company, St. Louis, MO, USA) was used, which contains 360 g/l glyphosate acid equivalent as the isopropylamine salt and was procured from local market. Immobilized pH gradient (IPG) strips and 0.5% pH 3–10 IPG buffer were purchased from Bio-Rad Laboratories (Hercules, CA, USA). DMBA, TPA, CHAPS, DTT, and beta-actin (clone AC-74) antibody were from Sigma-Aldrich (Missouri, USA). DNase/RNase was from Bangalore Genei (Bangalore, India). The rest of the chemicals used in the study were of analytical grade of purity and procured locally.

2.2. Animals and treatments

2.2.1. Carcinogenicity study

Male, Swiss albino mice (12–15 g body weight [b.wt]) were taken from Indian Institute of Toxicology Research (IITR) animal breeding colony and acclimatized for 1 week. The ethical approval for the experiment was obtained from institutional ethical committee. The animals were kept under standard laboratory conditions (temperature 23 ± 2 °C, relative humidity 55 ± 5%) and were fed with synthetic pellet basal diet (Ashirwad, Chandigarh, India) and tap water *ad libitum*. Animals were randomly divided into 8 groups of 20 animals each. Hair were clipped in the dorsal region with proper care in an area of 2 cm^2 using electrical clippers, not lubricated with oil or grease. The long-term treatment was given as described earlier [24]. Briefly,

Group I Untreated control (No treatment).
Group II Glyphosate alone (25 mg/kg b.wt, topically 3 times per week).
Group III DMBA + TPA (Single topical application of DMBA, 52 μg/mouse followed 1 week later by thrice a week application of TPA, 5 μg/mouse).
Group IV Glyphosate (a) + TPA (Single topical application of glyphosate, 25 mg/kg b.wt followed 1 week later by TPA application as in group III).
Group V Glyphosate (m) + TPA (Thrice a week topical application of glyphosate, 25 mg/kg b.wt for 3 weeks [total of 9 applications], followed 1 week later by TPA application as in group III).
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Group VII TPA (Thrice a week topical application of TPA, 5 μg/mouse).
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Vehicle for glyphosate, DMBA and TPA were 50% ethanol and acetone respectively.

Animals from all the groups were examined every week for gross morphological changes including body weight changes, development and volume of squamous cell papillomas (tumors) locally on the skin during the entire study period and tumors larger than 1 mm diameter, were included in the total number of tumors. Tumor volume per tumor bearing mouse was calculated in each group using formula $V = D \times d^2 \times \pi/6$ (where D = bigger dimension and d = smaller dimension). All the surviving animals were sacrificed at the end of the study period, i.e. 32 weeks for complete carcinogenic, tumor initiating and promoting studies.

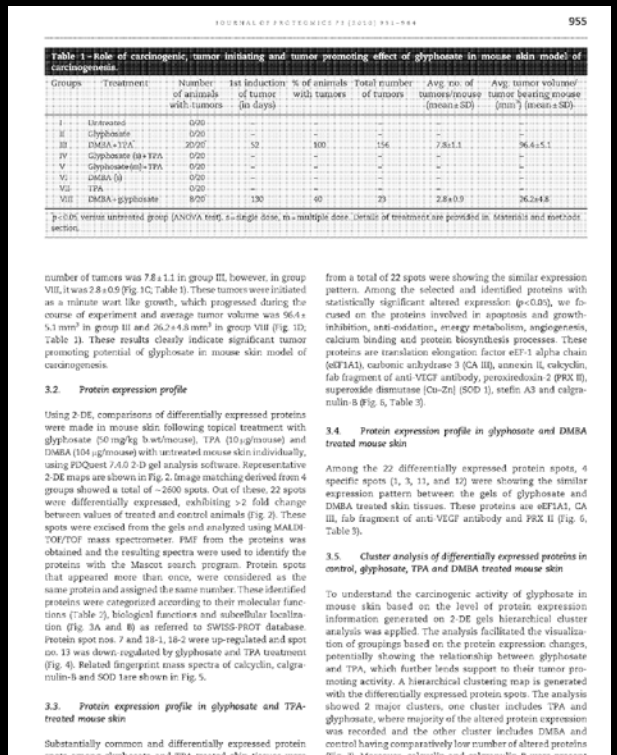

Table 1 – Role of carcinogenic, tumor initiating and tumor promoting effect of glyphosate in mouse skin model of carcinogenesis.

Groups	Treatment	Number of animals with tumors	1st induction (in days)	% of animals with tumors	Total number of tumors	Avg no. of tumors/mouse (mean ± SD)	Avg tumor volume/ tumor bearing mouse (mm^3) (mean ± SD)
I	Untreated	0/20	–	–	–	–	–
II	Glyphosate	0/20	–	–	–	–	–
III	DMBA + TPA	20/20	52	100	156	7.8 ± 1.1	96.4 ± 5.1
IV	Glyphosate (a) + TPA	0/20	–	–	–	–	–
V	Glyphosate (m) + TPA	0/20	–	–	–	–	–
VI	DMBA (s)	0/20	–	–	–	–	–
VII	TPA	0/20	–	–	–	–	–
VIII	DMBA + glyphosate	8/20	130	40	23	2.8 ± 0.9	26.2 ± 4.8

$p < 0.05$ versus untreated group (ANOVA test), s = single dose, m = multiple dose. Details of treatment are provided in Materials and methods section.

number of tumors was 7.8 ± 1.1 in group III, however, in group VIII, it was 2.8 ± 0.9 (Fig. 1C; Table 1). These tumors were initiated as a minute wart like growth, which progressed during the course of experiment and average tumor volume was 96.4 ± 5.1 mm^3 in group III and 26.2 ± 4.8 mm^3 in group VIII (Fig. 1D; Table 1). These results clearly indicate significant tumor promoting potential of glyphosate in mouse skin model of carcinogenesis.

3.2. Protein expression profile

Using 2-DE, comparisons of differentially expressed proteins were made in mouse skin following topical treatment with glyphosate (50 mg/kg b.wt/mouse), TPA (10 μg/mouse) and DMBA (104 μg/mouse) with untreated mouse skin individually, using PDQuest 7.4.0 2-D gel analysis software. Representative 2-DE maps are shown in Fig. 2. Image matching derived from 4 groups showed a total of ~2600 spots. Out of these, 22 spots were differentially expressed, exhibiting >2 fold change between values of treated and control animals (Fig. 2). These spots were excised from the gels and analyzed using MALDI-TOF/TOF mass spectrometer. PMF from the proteins was obtained and the resulting spectra were used to identify the proteins with the Mascot search program. Protein spots that appeared more than once, were considered as the same protein and assigned the same number. These identified proteins were categorized according to their molecular functions (Table 2), biological functions and subcellular localization (Fig. 3A and B) as referred to SWISS-PROT database. Protein spot nos. 7 and 18-1, 18-2 were up-regulated and spot no. 13 was down-regulated by glyphosate and TPA treatment (Fig. 4). Related fingerprint mass spectra of calcyclin, calgranulin-B and SOD 1are shown in Fig. 5.

3.3. Protein expression profile in glyphosate and TPA-treated mouse skin

Substantially common and differentially expressed protein spots among glyphosate and TPA-treated skin tissues were quantitatively analyzed individually. Comparison between the gels of glyphosate and TPA revealed that 13 specific proteins spots (1, 2, 3, 6-1, 6-2, 7, 8, 11, 12, 13, 15, 18-1, and 18-2) from a total of 22 spots were showing the similar expression pattern. Among the selected and identified proteins with statistically significant altered expression ($p < 0.05$), we focused on the proteins involved in apoptosis and growth-inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes. These proteins are translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu–Zn] (SOD 1), stefin A3 and calgranulin-B (Fig. 6, Table 3).

3.4. Protein expression profile in glyphosate and DMBA treated mouse skin

Among the 22 differentially expressed protein spots, 4 specific spots (1, 3, 11, and 12) were showing the similar expression pattern between the gels of glyphosate and DMBA treated skin tissues. These proteins are eEF1A1, CA III, fab fragment of anti-VEGF antibody and PRX II (Fig. 6, Table 3).

3.5. Cluster analysis of differentially expressed proteins in control, glyphosate, TPA and DMBA treated mouse skin

To understand the carcinogenic activity of glyphosate in mouse skin based on the level of protein expression information generated on 2-DE gels hierarchical cluster analysis was applied. The analysis facilitated the visualization of groupings based on the protein expression changes, potentially showing the relationship between glyphosate and TPA, which further lends support to their tumor promoting activity. A hierarchical clustering map is generated with the differentially expressed protein spots. The analysis showed 2 major clusters, one cluster includes TPA and glyphosate, where majority of the altered protein expression was recorded and the other cluster includes DMBA and control having comparatively low number of altered proteins (Fig. 7). Moreover, calcyclin and calgranulin-B were present only in the cluster of glyphosate and TPA whereas SOD 1 is higher in DMBA and control cluster in comparison to other cluster.

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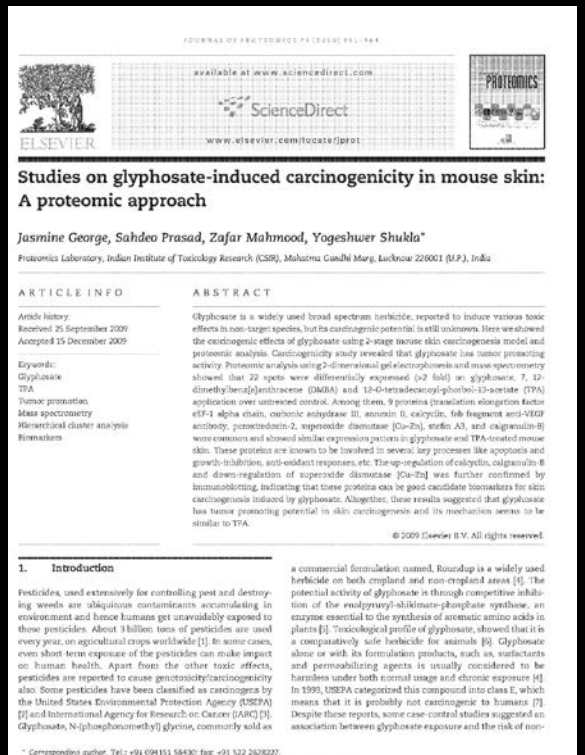

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2.2. Animals and treatments

2.2.1. Carcinogenicity study

Male, Swiss albino mice (12–15 g body weight [b.wt]) were taken from Indian Institute of Toxicology Research (IITR) animal breeding colony and acclimatized for 1 week. The ethical approval for the experiment was obtained from institutional ethical committee. The animals were kept under standard laboratory conditions (temperature 23 ± 2 °C, relative humidity 55 ± 5%) and were fed with synthetic pellet basal diet (Ashirwad, Chandigarh, India) and tap water *ad libitum*. Animals were randomly divided into 8 groups of 20 animals each. Hair were clipped in the dorsal region with proper care in an area of 2 cm^2 using electrical clippers, not lubricated with oil or grease. The long-term treatment was given as described earlier [24]. Briefly,

Group I Untreated control (No treatment).
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Group IV Glyphosate (a) + TPA (Single topical application of glyphosate, 25 mg/kg b.wt followed 1 week later by TPA application as in group III).
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Vehicle for glyphosate, DMBA and TPA were 50% ethanol and acetone respectively.

Animals from all the groups were examined every week for gross morphological changes including body weight changes, development and volume of squamous cell papillomas (tumors) locally on the skin during the entire study period and tumors larger than 1 mm diameter, were included in the total number of tumors. Tumor volume per tumor bearing mouse was calculated in each group using formula $V = D \times d^2 \times \pi/6$ (where D = bigger dimension and d = smaller dimension). All the surviving animals were sacrificed at the end of the study period, i.e. 32 weeks for complete carcinogenic, tumor initiating and promoting studies.

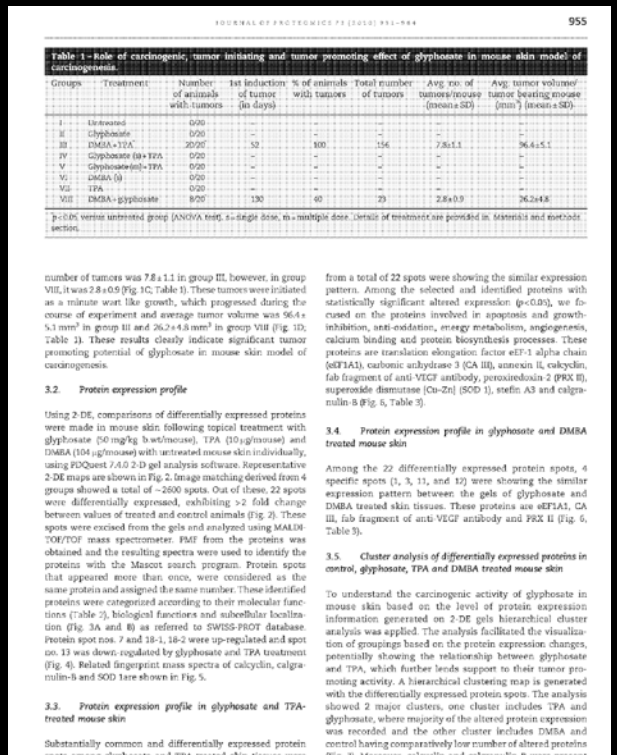

Table 1 – Role of carcinogenic, tumor initiating and tumor promoting effect of glyphosate in mouse skin model of carcinogenesis.

Groups	Treatment	Number of animals with tumors	1st induction (in days)	% of animals with tumors	Total number of tumors	Avg no. of tumors/mouse (mean ± SD)	Avg tumor volume/ tumor bearing mouse (mm^3) (mean ± SD)
I	Untreated	0/20	–	–	–	–	–
II	Glyphosate	0/20	–	–	–	–	–
III	DMBA + TPA	20/20	52	100	156	7.8 ± 1.1	96.4 ± 5.1
IV	Glyphosate (a) + TPA	0/20	–	–	–	–	–
V	Glyphosate (m) + TPA	0/20	–	–	–	–	–
VI	DMBA (s)	0/20	–	–	–	–	–
VII	TPA	0/20	–	–	–	–	–
VIII	DMBA + glyphosate	8/20	130	40	23	2.8 ± 0.9	26.2 ± 4.8

$p < 0.05$ versus untreated group (ANOVA test), s = single dose, m = multiple dose. Details of treatment are provided in Materials and methods section.

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Using 2-DE, comparisons of differentially expressed proteins were made in mouse skin following topical treatment with glyphosate (50 mg/kg b.wt/mouse), TPA (10 μg/mouse) and DMBA (104 μg/mouse) with untreated mouse skin individually, using PDQuest 7.4.0 2-D gel analysis software. Representative 2-DE maps are shown in Fig. 2. Image matching derived from 4 groups showed a total of ~2600 spots. Out of these, 22 spots were differentially expressed, exhibiting >2 fold change between values of treated and control animals (Fig. 2). These spots were excised from the gels and analyzed using MALDI-TOF/TOF mass spectrometer. PMF from the proteins was obtained and the resulting spectra were used to identify the proteins with the Mascot search program. Protein spots that appeared more than once, were considered as the same protein and assigned the same number. These identified proteins were categorized according to their molecular functions (Table 2), biological functions and subcellular localization (Fig. 3A and B) as referred to SWISS-PROT database. Protein spot nos. 7 and 18-1, 18-2 were up-regulated and spot no. 13 was down-regulated by glyphosate and TPA treatment (Fig. 4). Related fingerprint mass spectra of calcyclin, calgranulin-B and SOD 1are shown in Fig. 5.

3.3. Protein expression profile in glyphosate and TPA-treated mouse skin

Substantially common and differentially expressed protein spots among glyphosate and TPA-treated skin tissues were quantitatively analyzed individually. Comparison between the gels of glyphosate and TPA revealed that 13 specific proteins spots (1, 2, 3, 6-1, 6-2, 7, 8, 11, 12, 13, 15, 18-1, and 18-2) from a total of 22 spots were showing the similar expression pattern. Among the selected and identified proteins with statistically significant altered expression ($p < 0.05$), we focused on the proteins involved in apoptosis and growth-inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes. These proteins are translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu–Zn] (SOD 1), stefin A3 and calgranulin-B (Fig. 6, Table 3).

3.4. Protein expression profile in glyphosate and DMBA treated mouse skin

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3.5. Cluster analysis of differentially expressed proteins in control, glyphosate, TPA and DMBA treated mouse skin

To understand the carcinogenic activity of glyphosate in mouse skin based on the level of protein expression information generated on 2-DE gels hierarchical cluster analysis was applied. The analysis facilitated the visualization of groupings based on the protein expression changes, potentially showing the relationship between glyphosate and TPA, which further lends support to their tumor promoting activity. A hierarchical clustering map is generated with the differentially expressed protein spots. The analysis showed 2 major clusters, one cluster includes TPA and glyphosate, where majority of the altered protein expression was recorded and the other cluster includes DMBA and control having comparatively low number of altered proteins (Fig. 7). Moreover, calcyclin and calgranulin-B were present only in the cluster of glyphosate and TPA whereas SOD 1 is higher in DMBA and control cluster in comparison to other cluster.

Table 1 – Role of carcinogenic, tumor initiating and tumor promoting effect of glyphosate in mouse skin model of carcinogenesis.

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III	DMBA + TPA*	20/20*	52	100	156	7.8±1.1	96.4±5.1
IV	Glyphosate (s) + TPA	0/20	–	–	–	–	–
V	Glyphosate (m) + TPA	0/20	–	–	–	–	–
VI	DMBA (s)	0/20	–	–	–	–	–
VII	TPA	0/20	–	–	–	–	–
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3.2. *Protein expression profile*

Using 2-DE, comparisons of differentially expressed proteins from a total of 22 spots were showing the similar expression pattern. Among the selected and identified proteins with statistically significant altered expression ($p<0.05$), we focused on the proteins involved in apoptosis and growth-inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes. These proteins are translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu–Zn] (SOD 1), stefin A3 and calgranulin-B (Fig. 6, Table 3).

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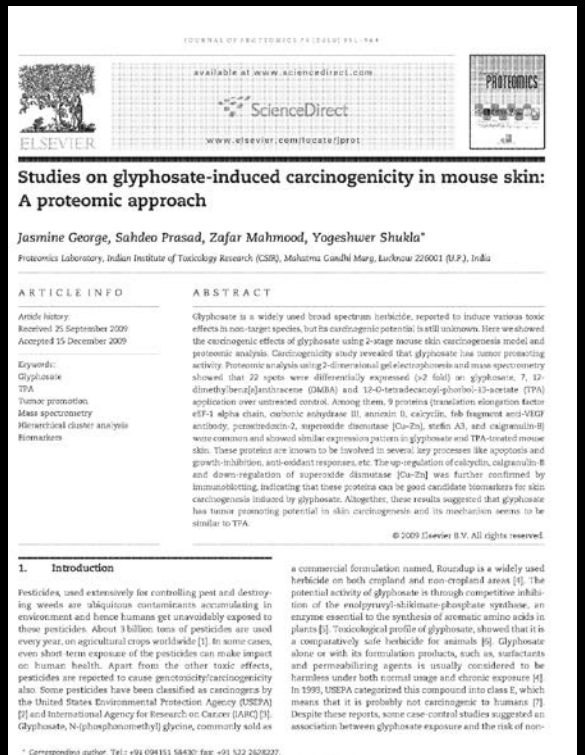
Toxicology



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PROTEOMICS

Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach

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ABSTRACT

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

1. Introduction

Pesticides, used extensively for controlling pest and destroying weeds are ubiquitous contaminants accumulating in environment and hence humans get unavoidably exposed to these pesticides. About 3 billion tons of pesticides are used every year, on agricultural crops worldwide [1]. In some cases, even short-term exposure of the pesticides can make impact on human health. Apart from the other toxic effects, pesticides are reported to cause genotoxicity/carcinogenicity also. Some pesticides have been classified as carcinogens by the United States Environmental Protection Agency (USEPA) [2] and International Agency for Research on Cancer (IARC) [3]. Glyphosate, N-(phosphonomethyl) glycine, commonly sold as a commercial formulation named, Roundup is a widely used herbicide on both cropland and non-cropland areas [4]. The potential activity of glyphosate is through competitive inhibition of the enolpyruvyl-shikimate-phosphate synthase, an enzyme essential to the synthesis of aromatic amino acids in plants [5]. Toxicological profile of glyphosate, showed that it is a comparatively safe herbicide for animals [6]. Glyphosate alone or with its formulation products, such as, surfactants and permeabilizing agents is usually considered to be harmless under both normal usage and chronic exposure [4]. In 1993, USEPA categorized this compound into class E, which means that it is probably not carcinogenic to humans [7]. Despite these reports, some case-control studies suggested an association between glyphosate exposure and the risk of non-

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Hodgkin's lymphoma [8,9]. In another study, both technical grade glyphosate and Roundup were shown to cause a rapid increase in cell division in human breast cancer cells [10]. Glyphosate has also been shown as a skin irritant [11]. Regarding the genotoxic potential, glyphosate exposure to human lymphocytes *in vitro* resulted in increased sister chromatid exchanges [12], chromosomal aberrations [13], and indicators of oxidative stress [14]. A recent study from our laboratory also showed the clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice [15]. These reports prompted us to investigate its carcinogenic effect in long-term animal bioassay.

To evaluate toxicity/carcinogenicity induced by physical and chemical agents including pesticides, various test systems have been employed in bacteria, rodents and mammalian cells [16–18]. Each of these environmental challenges causes changes in DNA conformation, alterations in the levels of mRNA and protein expression, and post-translational modifications such as phosphorylation and glycosylation of proteins specific to each stressor [19]. In recent years, there has been considerable interest in linking carcinogenic/toxic responses to gene and protein expression. Toxicoproteomics has received a lot of attention as a valuable tool to search reliable early predictive toxicity markers in response to environmental stimuli [20]. Two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS); a high throughput technique allows proteins of interest to be identified by their expression and/or modification pattern rather than using the traditional approach of translating gene expression data. Biomarkers can be used to identify causal associations and to make better quantitative estimates of those associations at relevant levels of exposure [21]. Yamamoto et al. [22] have utilized proteomic approach to identify potential biomarker candidates of hepatotoxicant exposure in rat liver.

Skin is the largest organ in the body and dermal contact is one of the most probable routes of human exposure to pesticides, thus, mouse skin model represents a logical experimental choice [23]. As the long-term bioassay for carcinogenicity is expansive, time consuming and involves a large number of animals and ethical issues, development of biomarkers after short-term exposure are needed. The present investigation was carried out to study the carcinogenic potential of glyphosate and to identify differentially expressed proteins, using 2-DE and MS analysis after treatment with glyphosate, a known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and tumor initiator, 7, 12-dimethylbenz[a]anthracene (DMBA) in mouse skin. Altered proteins identified through proteomic approach in our study may be potentially useful as early biomarkers, to detect the adverse effects of glyphosate.

2. Materials and methods

2.1. Materials

The commercial formulation of the herbicide glyphosate (N-phosphonomethyl glycine) Roundup Original® (glyphosate 41%, POEA=15%—Monsanto Company, St. Louis, MO, USA) was used, which contains 360 g/l glyphosate acid equivalent as the isopropylamine salt and was procured from local market. Immobilized pH gradient (IPG) strips and 0.5% pH 3–10 IPG buffer were purchased from Bio-Rad Laboratories (Hercules, CA, USA). DMBA, TPA, CHAPS, DTT, and beta-actin (clone AC-74) antibody were from Sigma-Aldrich (Missouri, USA). DNase/RNase was from Bangalore Genei (Bangalore, India). The rest of the chemicals used in the study were of analytical grade of purity and procured locally.

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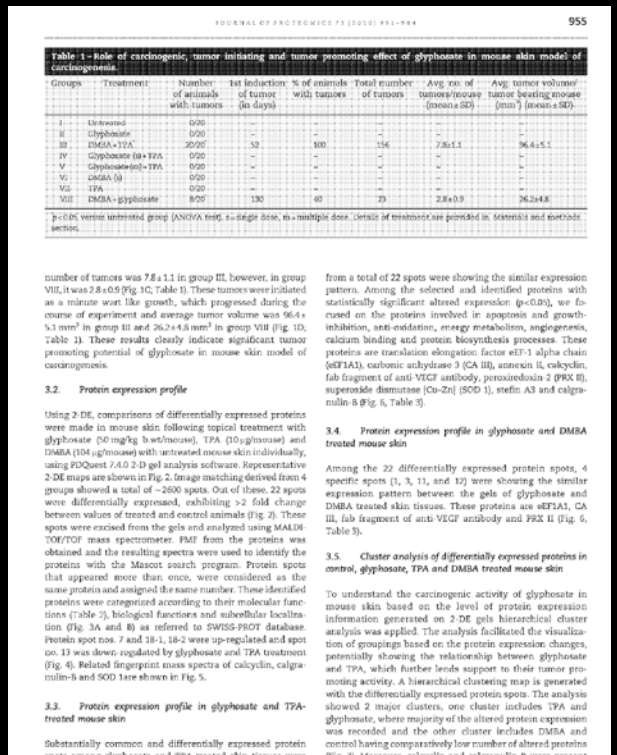

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3.2. Protein expression profile

Using 2-DE, comparisons of differentially expressed proteins were made in mouse skin following topical treatment with glyphosate (50 mg/kg b.wt/mouse), TPA (10 μg/mouse) and DMBA (104 μg/mouse) with untreated mouse skin individually, using PDQuest 7.4.0 2-D gel analysis software. Representative 2-DE maps are shown in Fig. 2. Image matching derived from 4 groups showed a total of ~2600 spots. Out of these, 22 spots were differentially expressed, exhibiting >2 fold change between values of treated and control animals (Fig. 2). These spots were excised from the gels and analyzed using MALDI-TOF/TOF mass spectrometer. PMF from the proteins was obtained and the resulting spectra were used to identify the proteins with the Mascot search program. Protein spots that appeared more than once, were considered as the same protein and assigned the same number. These identified proteins were categorized according to their molecular functions (Table 2), biological functions and subcellular localization (Fig. 3A and B) as referred to SWISS-PROT database. Protein spot nos. 7 and 18-1, 18-2 were up-regulated and spot no. 13 was down-regulated by glyphosate and TPA treatment (Fig. 4). Related fingerprint mass spectra of calcyclin, calgranulin-B and SOD 1are shown in Fig. 5.

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Substantially common and differentially expressed protein spots among glyphosate and TPA-treated skin tissues were quantitatively analyzed individually. Comparison between the gels of glyphosate and TPA revealed that 13 specific proteins spots (1, 2, 3, 6-1, 6-2, 7, 8, 11, 12, 13, 15, 18-1, and 18-2) from a total of 22 spots were showing the similar expression pattern. Among the selected and identified proteins with statistically significant altered expression ($p < 0.05$), we focused on the proteins involved in apoptosis and growth-inhibition, anti-oxidation, energy metabolism, angiogenesis, calcium binding and protein biosynthesis processes. These proteins are translation elongation factor eEF-1 alpha chain (eEF1A1), carbonic anhydrase 3 (CA III), annexin II, calcyclin, fab fragment of anti-VEGF antibody, peroxiredoxin-2 (PRX II), superoxide dismutase [Cu–Zn] (SOD 1), stefin A3 and calgranulin-B (Fig. 6, Table 3).

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Among the 22 differentially expressed protein spots, 4 specific spots (1, 3, 11, and 12) were showing the similar expression pattern between the gels of glyphosate and DMBA treated skin tissues. These proteins are eEF1A1, CA III, fab fragment of anti-VEGF antibody and PRX II (Fig. 6, Table 3).

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Three Pillars of Cancer Science

Toxicology

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Sent: Thursday, August 06, 2015 9:55 AM
To: 'Ashley Roberts Intertek'; FARMER, DONNA R [AG/1000]
Subject: RE: Keith

Ashley,
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That said, the surfactant in the formulation will come up in the tumor promotion skin study because we think it played a role there.

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He has asked if we need to give any consideration to exposures of formulants in the commercial product, at least in applicators? I was under the impression these were inert but reading a response this morning in the Ecologist makes it sound like it is the combination that is toxic!!!

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Three Pillars of Cancer Science

Epidemiology

IARC Classification
1. Sufficient
2. Limited
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4. Lack of Carcinogenicity

Toxicology

IARC Classification
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2. Limited
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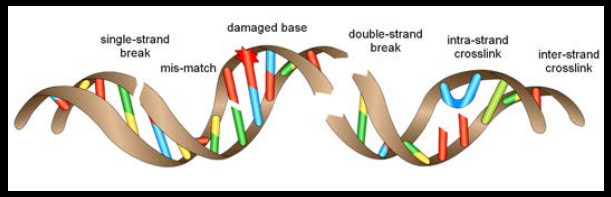
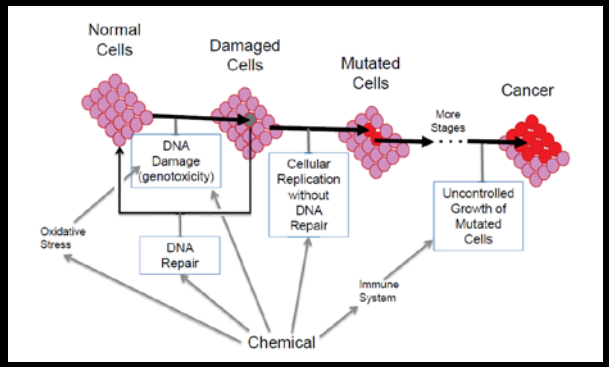
Mechanism





IARC Classification
1. Strong
2. Moderate
3. Weak

3 Core Questions

YES

1. Can Roundup be a substantial contributing factor in causing cancer?

2. Was Roundup a substantial contributing factor in causing Mr. Johnson's cancer?

3. Did Monsanto act with knowing disregard of human health?

2. Was Roundup a substantial contributing factor in causing Mr. Johnson's cancer?

Jury Instruction

A substantial factor in causing harm is a factor that a reasonable person would consider to have contributed to the harm. It must be more than a remote or trivial factor. It does not have to be the only cause of the harm. Conduct is not a substantial factor in causing harm if the same harm would have occurred without that conduct.

Mr. Johnson's Cancer

Jun. 2012

Mr. Johnson is promoted to integrated pest manager for Benicia School District

Mr. Johnson's Cancer

Full spraying season
50 gallons / hour

Jun. 2012

Mr. Johnson is promoted to integrated pest manager for Benicia School District

Aug. 2012

Mr. Johnson completes first spraying season using truck sprayer

Mr. Johnson's Cancer

Full spraying season
50 gallons / hour

Intermittent spraying

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Mr. Johnson's Cancer

May. 2013

Mr. Johnson begins second intensive spraying season

Mr. Johnson's Cancer

Summer 2013

Mr. Johnson gets massive exposure during Mary Farmar incident

May. 2013

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Mr. Johnson's Cancer

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Sep. 2013

Mr. Johnson presents to doctor with several wasp stings. Medical record at the time states "Negative for rash" Tr. 3131:8.

Mr. Johnson's Cancer

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Mr. Johnson presents for lumbar injury, medical record states "no deformity, no shift, no scarring, no swelling." Tr. 3232:16-17

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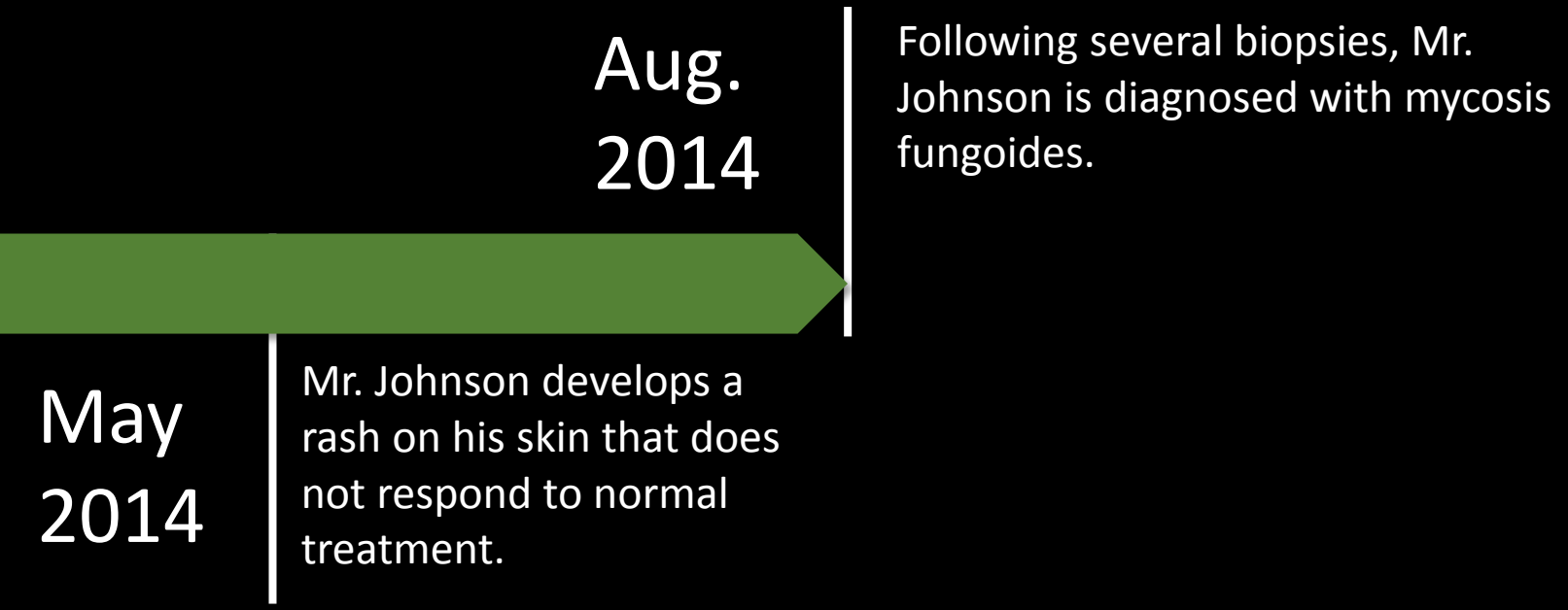
Mr. Johnson's Cancer



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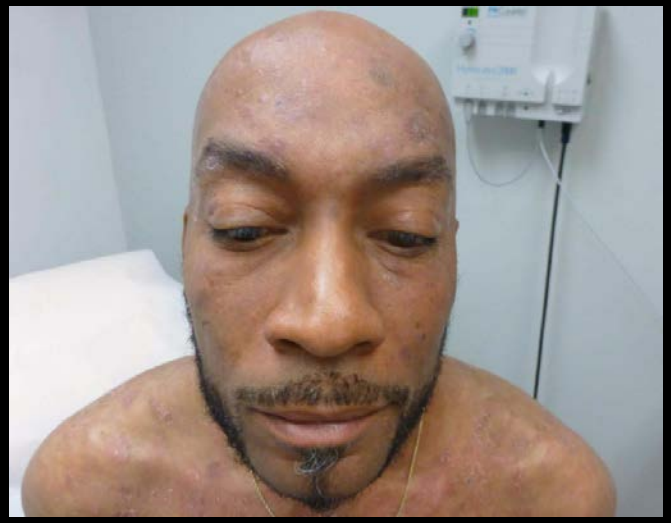
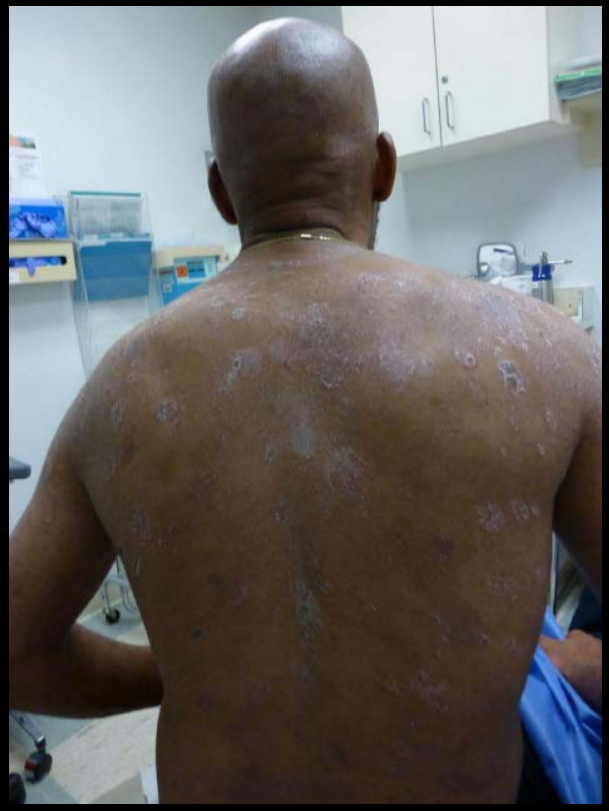
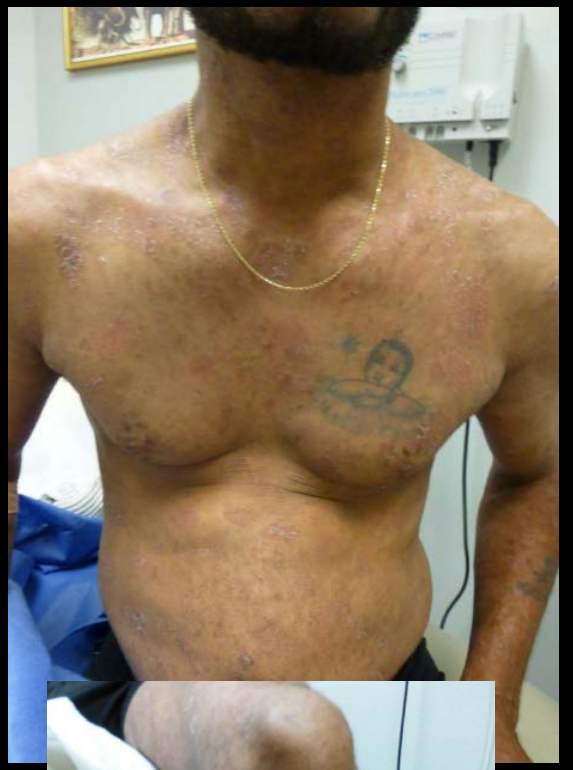
Mr. Johnson begins treatment with Dr. Ofodile at Kaiser.

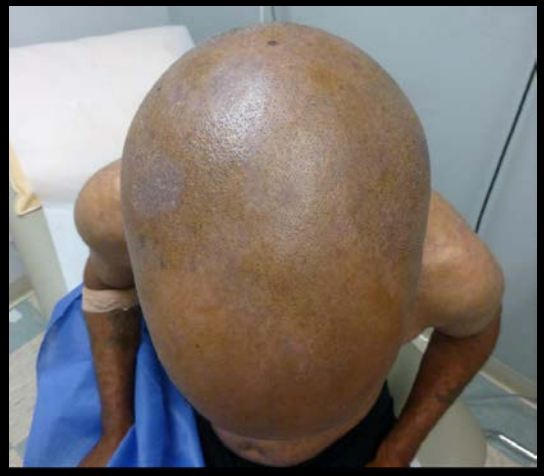
Mr. Johnson's Cancer

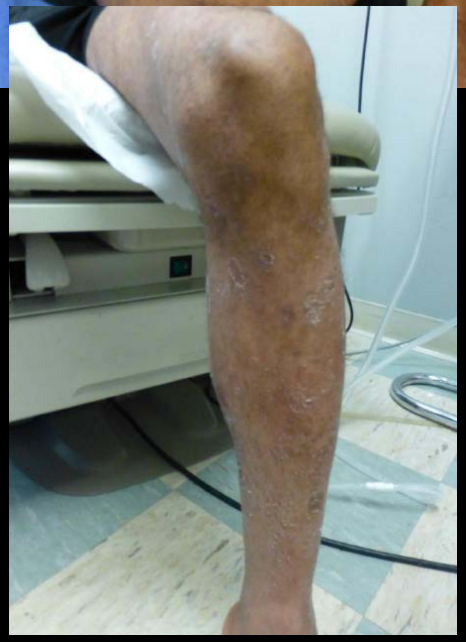
Oct. 2014











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Oct. 2014

IARC announces investigation into glyphosate

Message

From: HEYDENS, WILLIAM F [AG/1000] [redacted@monsanto.com]
Sent: 10/15/2014 9:08:37 PM
To: [redacted]@monsanto.com]
CC: [redacted]@monsanto.com; FARMER, DONNA R [AG/1000] [redacted]@monsanto.com]; SALTMIRAS, DAVID A [AG/1000] [redacted@monsanto.com]; KOCH, MICHAEL S [AG/1000] [redacted@monsanto.com]
Subject: IARC Evaluation of Glyphosate

[redacted]

It is my recollection that you notified the EU-GTF of this IARC evaluation, but I am not aware that there has been any talk of approaching the GTF about providing funding to fight this because it is not considered in the remit of achieving Annex I renewal. If so, is this really the case? I thought the EU evaluation could go well into the summer of 2015, and wouldn't an adverse IARC evaluation have the real potential to impact the results of the Annex I renewal?

I really started thinking about this after our phone call yesterday with the outside epidemiology experts that Donna lined up. The bottom line of the call was that there really is no meaningful publication that we can complete prior to the February submission to positively impact the epidemiology discussion outcome in March. One has to consider that this situational timing did not happen by chance and that more than just pure bad luck is working against glyphosate.

And while we have vulnerability in the area of epidemiology, we also have potential vulnerabilities in the other areas that IARC will consider, namely, exposure, genetox, and mode of action (David has the animal onco studies under

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And while we have vulnerability in the area of epidemiology, we also have potential vulnerabilities in the other areas that IARC will consider, namely, exposure, genetox, and mode of action (David has the animal onco studies under control). If there is a force working against glyphosate, there is ample fodder to string together to help the cause even though it is not scientifically justified in its purest form. Putting all this in the proper perspective will be quite resource intensive, so can't we consider approaching the GTF? Recall that the PAG already agreed to fund the onco publication 2+ years ago for this exact reason.

Thanks.

Bill

Mr. Johnson's Cancer

Oct. 2014

IARC announces investigation into glyphosate

Mr. Johnson's Cancer

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Nov. 2014

Mr. Johnson calls Monsanto looking for answers.

Message

From: GOLDSTEIN, DANIEL A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=527246]
Sent: 11/11/2014 8:19:51 PM
To: BIEHL, PATRICIA M [AG-Contractor/1045] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=208718]
Subject: RE: Ranger Pro Exposure

I will call him. The story is not making any sense to me at all.

Dan

From: BIEHL, PATRICIA M [AG-Contractor/1045]
Sent: Tuesday, November 11, 2014 2:12 PM
To: GOLDSTEIN, DANIEL A [AG/1000]
Subject: Ranger Pro Exposure

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314-[redacted]
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[redacted]@monsanto.com

Plaintiff Exhibit
0332

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Plaintiff Exhibit 0292

Mr. Johnson Continues to Spray

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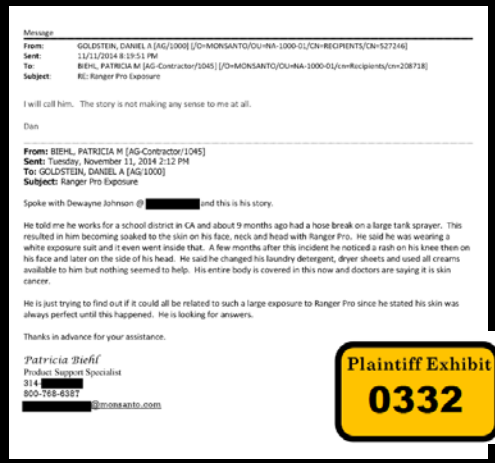

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Plaintiff Exhibit 0332

From: HEYDENS, WILLIAM F [AG/1000]
Sent: Thursday, February 19, 2015 7:53 AM
To: FARMER, DONNA R [AG/1000]
Cc: KOCH, MICHAEL S [AG/1000]; SALTMIRAS, DAVID A [AG/1000]; HODGE-BELL, KIMBERLY C [AG/1000]
Subject: RE: IARC Planning

Donna,

Per our phone call with John the other day, the next two most important things that we need to do are the Meta-analysis publication and the Ag Health Study Follow-up publication, assuming we can get our hands on the data in a reasonable timeframe. I feel confident that we will have organizational support for doing these projects, so I think we need to start setting them up now.

For the meta-analysis, please contact Elizabeth, let her know we would like her/Ellen to do this, and get a cost estimate from her.

For the AHS data, I heard 2 action items during our call: first - get with the lawyers to initiate the FOI process; second - contact [redacted] and get him lined up to do the analysis when we get the data; also, get a cost estimate from him.

For the overall plausibility paper that we discussed with John (where he gave the butadiene example), I'm still having a little trouble wrapping my mind around that. If we went full-bore, involving experts from all the major areas (Epi, Tox, Genetox, MOA, Exposure - not sure who we'd get), we could be pushing $250K or maybe even more. A less expensive/more palatable approach might be to involve

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One thing we could do now on this is to contact Roger McClellan at CRC and see if they would be amenable to putting this publication in *Crit. Rev. Toxicol.* John said he knew that Roger had done such a publication in the past. David, since you have worked with Roger on the other papers, would you be willing to contact him to judge his willingness to publish such a paper?

Any other thoughts welcomed.

Bill

Political Science

- Unfortunately, we are facing regulatory reviews with increased focus on
  - Claims in the peer reviewed literature, irrespective of the quality of the science
  - Stakeholder input including activist researchers
  - Political pressure on outcomes – e.g. POEAs in Germany
  - Reduced pesticide use in general
- Williams et al. (2000) has served us well in toxicology over the last decade
- We need a stronger arsenal of robust scientific papers to support the safe use of our products as we face the next set of chemistry registration reviews across the globe
- With increasing business interests in South America, a local network credible expert scientists is crucial to facilitate scientifically robust and objective regulatory evaluations of our products *We have not determined exactly what we should & could do here. I would modify bullet to reflect that we need to determine an appropriate & do-able (i.e., we can get someone to pay for it course of action here*

[redacted]@monsanto.com

0332

Plaintiff Exhibit
0373

Mr. Johnson's Cancer

Oct. 2014 — IARC announces investigation into glyphosate

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Plaintiff Exhibit 0292

Mr. Johnson Continues to Spray

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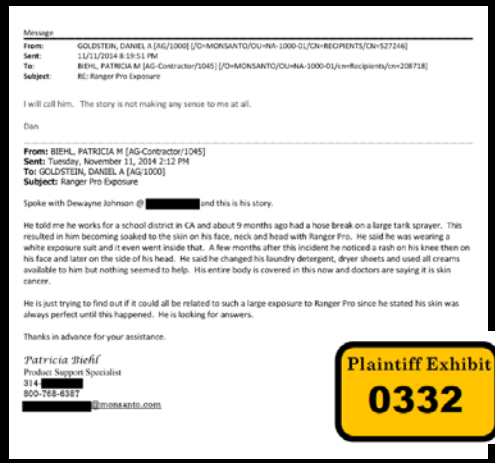

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Plaintiff Exhibit 0332

Mr. Johnson's Cancer

Plaintiff Exhibit
0292

Draft Feb 23, 2015

ATTACHMENT A: PREPAREDNESS AND ENGAGEMENT PLAN FOR IARC CARCINOGEN RATING OF GLYPHOSATE

Last Updated: February 17, 2015

OBJECTIVES FOR PREPAREDNESS & ENGAGEMENT

- Protect the reputation and FTO of Roundup by communicating the safety of glyphosate
- Amplify science-based information to prevent unfounded claims from becoming popular opinion
- Provide cover for regulatory agencies to continue making re-registration decisions based on science

AUDIENCES

- IARC Panelists and Observers
- Regulators
- Stakeholders
- Farmer Customers

STRATEGIES/TACTICS

PRE-IARC

1. **Amplification of Scientific Studies**
   - Support the development of three new papers on glyphosate focused on epidemiology and toxicology
   - Work with RPSA and Strategic Communications to amplify existing studies and new papers
     - Authors work directly with scientific journals to issue alerts and news releases on new bodies of work
     - RPSA posts blog from first-person viewpoint of Monsanto's David Saltmiras, co-author of one of the

Mr. Johnson's Cancer

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0292

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- Develop a "toolkit" containing key information and resources
  - Identify any message shortcomings and address through updates to monsanto.com/glyphosate and through US and EU blog posts
- Work with RPSA, Stakeholder Outreach Team, Industry Affairs, Government Affairs, US Business, Global CE and Regulatory teams, etc. to engage industry partners
  - Tier 1: Crop Life International / European Crop Protection Association / GMO Answ identify committees that are best to engage
  - Tier 2: Academics (AgBioChatter), Biofortified, Sense About Science, Genetic Litera Academics Review
  - Tier 3: Alert food companies via Stakeholder Engagement team (IFIC, GMA, CFI) fo strategy" to provide early education on glyphosate residue levels, describe science-based studies versus agenda-driven hypotheses
  - Tier 4: Inoculate key grower associations

Plaintiff Exhibit 0292

3. Address New Allegations

- Respond quickly and publically to new pseudoscience cancer studies
- Identify / request third-party experts to blog, op/ed, tweet and/or link, repost, retweet, etc.

POST-IARC

4. Orchestrate Outcry with IARC Decision ~ March 10, 2015

- Industry conducts robust media / social media outreach on process and outcome
  - [Sense About Science?] leads industry response and provides platform for IARC observers and industry spokesperson
  - CLI and other associations issue press releases

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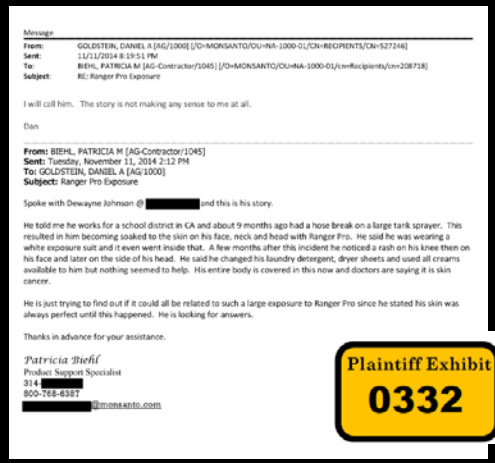

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He is just trying to find out if it could all be related to such a large exposure to Ranger Pro since he stated his skin was always perfect until this happened. He is looking for answers.

Thanks in advance for your assistance.

Patricia Biehl
Product Support Specialist
314-[redacted]
800-768-6387
[redacted]@monsanto.com

Plaintiff Exhibit 0332

Mr. Johnson's Cancer

Oct. 2014 — IARC announces investigation into glyphosate

Feb. 2015 — Monsanto plans to attack IARC ruling.

Mr. Johnson Continues to Spray

Nov. 2014 — Mr. Johnson calls Monsanto looking for answers.

Message
From: GOLDSTEIN, DANIEL A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=527246]
Sent: 11/11/2014 8:19:51 PM
To: BIEHL, PATRICIA M [AG-Contractor/1045] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=208718]
Subject: RE: Ranger Pro Exposure

I will call him. The story is not making any sense to me at all.

Dan

From: BIEHL, PATRICIA M [AG-Contractor/1045]
Sent: Tuesday, November 11, 2014 2:12 PM
To: GOLDSTEIN, DANIEL A [AG/1000]
Subject: Ranger Pro Exposure

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Plaintiff Exhibit
0332

Mar. 2015 — IARC classifies glyphosate as Class 2A carcinogen

International Agency Research on Cancer
World Health Organization

Mr. Johnson's Cancer

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Plaintiff Exhibit 0292

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Mar. 2015 — IARC classifies glyphosate as Class 2A carcinogen

International Agency Research on Cancer
World Health Organization

Mr. Johnson’s Cancer

Mar. 2015

Mr. Johnson reaches out to Missouri Regional Poison Control, seeking answers.

Human Exposure / Adverse Effect Incidents
Involving Monsanto Agricultural Products

Reporting Categories: H-A, H-B, H-C
Reporting Period: March 1, 2015 – March 31, 2015

Substance:	Ranger Pro Herbicide from Monsanto
Serial Number:	32283189
Date:	03/27/2015
Medical Outcome:	Major Effect H-B
EPA Reg. No.	524-517
Active Ingredients:	Glyphosate 41%
State:	California
History and Notes:	Caller states he has been using Ranger Pro as part of his job for 2 to 3 years. He has recently been diagnosed with cutaneous T cell lymphoma. He has concerns about continuing to use Roundup as part of his job and questions if Roundup could be a source of his cancer. As the call progressed, caller said that doctors are unsure as to how to treat his condition and they are not even sure if it is cancer. Caller states that he works with Ranger Pro using a 50 gallon tank and also using a backpack sprayer. He dilutes 10 ounces

Active Ingredients:	Glyphosate 41%
State:	California
History and Notes:	Caller states he has been using Ranger Pro as part of his job for 2 to 3 years. He has recently been diagnosed with cutaneous T cell lymphoma. He has concerns about continuing to use Roundup as part of his job and questions if Roundup could be a source of his cancer. As the call progressed, caller said that doctors are unsure as to how to treat his condition and they are not even sure if it is cancer. Caller states that he works with Ranger Pro using a 50 gallon tank and also using a backpack sprayer. He dilutes 10 ounces of the Roundup per gallon (3.0%) for the 50 gallon tank and 4 ounces of Roundup per gallon (1.25%) when using the backpack sprayer. He recalls having been exposed to Roundup twice in the past 2 to 3 years, both from the backpack leaking/malfunctioning. In one case, he was wearing personal protective equipment (PPE) but it soaked through the PPE and his clothing. Recently, he has had a swollen foot and the MD's cannot figure out what is going on. The caller's level of fear is rising over his continued use of Ranger Pro. He states he continues to get unexplained rashes and nodules over his body. MRPC discussed the product toxicity. The symptoms are not an expected response from the product. Advised MRPC is available, if the treating MD has any questions.

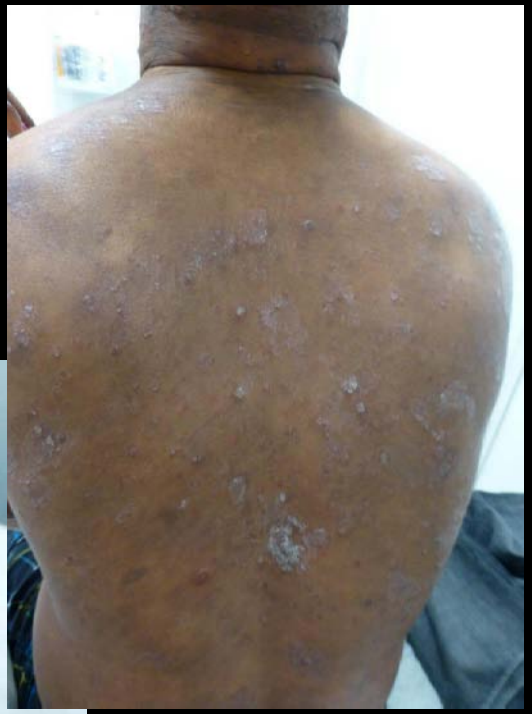
Mr. Johnson’s Cancer

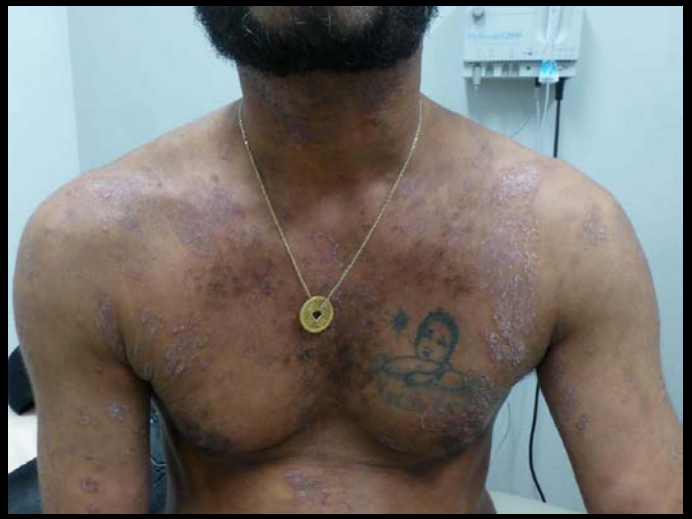
Mar. 2015

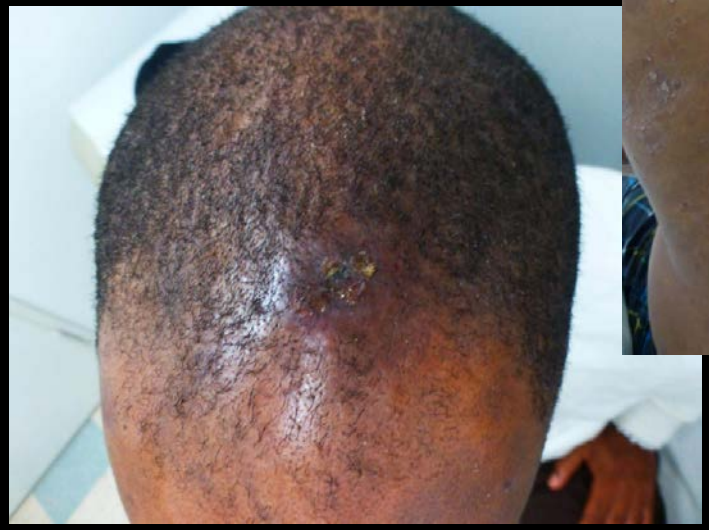
Mr. Johnson reaches out to Missouri Regional Poison Control, seeking answers.

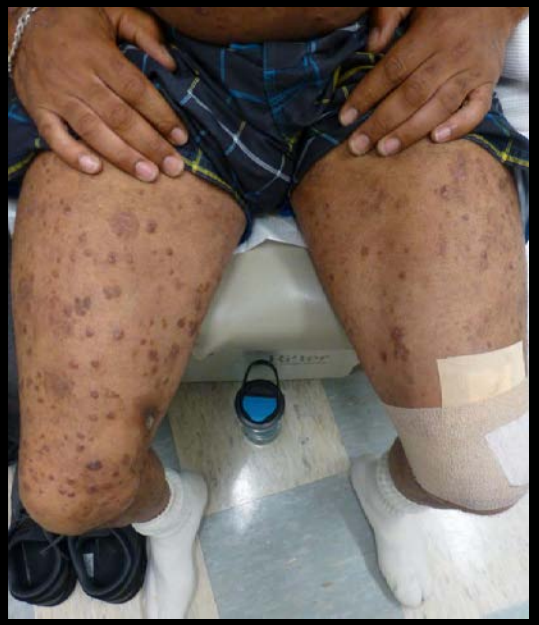
Mr. Johnson's Cancer

Mar. 2015









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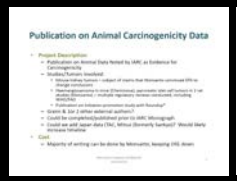

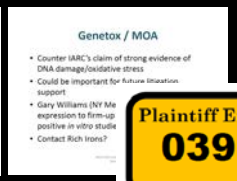

May 2015

Monsanto raises litigation concerns.

Proposal for Post-IARC Meeting Scientific Projects

DRAFT

May 11, 2015

Mr. Johnson's Cancer

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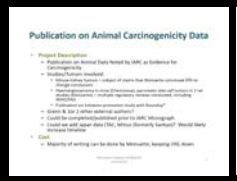

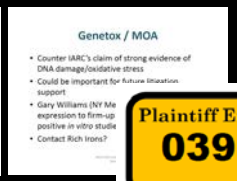

May 2015

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Why do more?

- Severe stigma attached to Group 2A Classification
- Aaron Blair continues to defend work & exaggerate number of studies w/ association while ignoring AHS
- In response to our critique, can expect IARC to beef-up monograph as much as possible
- IARC plans to pool data globally in the future
  - Blair announced at meeting that he has already put together an unofficial work group to begin the process
  - North American Pooled Project (NAPP) already underway and early results reported in 2014
  - Believe this will be used to move pesticides to Group 1
- Provide additional support ('air cover') for future regulatory reviews
  - Broad EU review recently recommended by BfR
  - Other regulatory agencies stated they will review after Monograph publishes
- ASTDR evaluation
- [redacted]
- Litigation support

Mr. Johnson's Cancer

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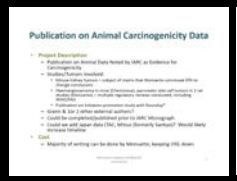

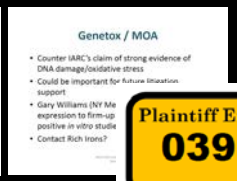

May 2015

Monsanto raises litigation concerns.

Genetox / MOA

- Counter IARC's claim of strong evidence of DNA damage/oxidative stress
- Could be important for future litigation support
- Gary Williams (NY Medical College) - Use gene expression to firm-up non-genotoxic MOA in positive *in vitro* studies with formulations
- Contact Rich Irons?

Mr. Johnson's Cancer

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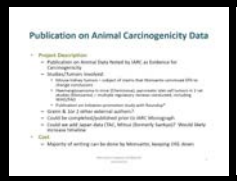

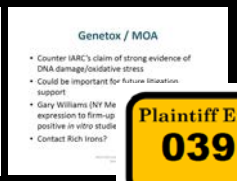

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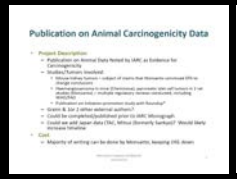

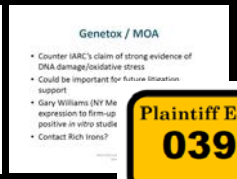

Professional Markets

Wilbur-Ellis Company
1427 Abbott Street
Salinas, CA 93901
Cell: (408) [redacted]
Fax: (650) [redacted]
[redacted]@wilburellis.com

-----Original Message-----
From: GOULD, STEVEN D [AG/1000] [mailto[redacted]@monsanto.com]
Sent: Thursday, September 10, 2015 1:08 PM
To: Greg Fernald
Subject: RE: [redacted]Moves to Label Monsanto's Roundup 'Carcinogenic' | [redacted]

He sent to me too. It's hard to understand how against all science and law the can do this.

Steve Gould

Steven D. Gould
Monsanto IT&O
Account Manager
951-894-[redacted]office
951-704-[redacted]mobile
monsantoITO.com

-----Original Message-----
From: Greg Fernald [mailt[redacted]@wilburellis.com]

Plaintiff Exhibit
0290

To: HARDY, JOHN O [AG/1000] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=53364]
Subject: Fwd: [redacted]Moves to Label Monsanto's Roundup 'Carcinogenic' | [redacted]

I liked this analogy from Greg

Steve Gould

Begin forwarded message:

From: Greg Fernald <[redacted]@wilburellis.com>
Date: September 10, 2015 at 3:19:21 PM PDT
To: "GOULD, STEVEN D [AG/1000]" <steven.d.gould@monsanto.com>
Subject: RE:[redacted] **Moves to Label Monsanto's Roundup 'Carcinogenic' |** [redacted]

We are being overrun by liberals and morons...sort of like a zombie movie, so we just have to start taking them out one at a time, starting with the elections next year.

Greg Fernald
Professional Markets

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Mr. Johnson's Cancer

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Sent: 9/10/2015 10:41:00 PM
To: HARDY, JOHN O [AG/1000] [/O=MONSANTO/OU=NA-1000-01/cn=Recipients/cn=53364]
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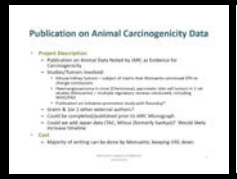

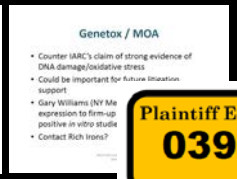

cost on CA Municipalities Markets

By Steve Gould

Volumes:

I would estimate a gradual 2-3 year loss of up to 200 K REGS of all tiers of Roundup IT&O Glyphosate.

High Tier 65K

Mid Tier 75 K

Low Tier 60K

The volume will come from customers like CALTRANS. Caltrans Volume is a mix of high and mid tier and we stand to loss 5-10K depending on internal decisions

School districts are another big risk with the healthy schools act and increased attention. They frequently use PROMAX and PRO Concentrate today.

Airports, arenas, stadiums, municipal buildings any public facility especially if it goes out to bid could be affected

Cities, Counties, reservoirs, wildlife management areas and other similar locations could also be affected.

We will not really know the costs until we see how the agencies react and how many of them must

Low Tier 60K

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We will not really know the costs until we see how the agencies react and how many of them must address this.

This volume is based on discussing with distributor representatives, Monsanto sales volume estimates and knowledge of the market.

Customers that I am aware have already stopped using Glyphosate since the IARC ruling:

Irvine Unified School District and several bay area cities and school districts.

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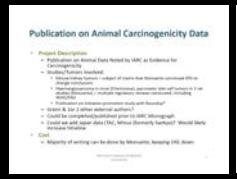

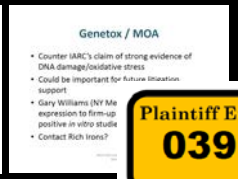

Plaintiff Exhibit 0391

May 2015 — Monsanto raises litigation concerns.

Sept. 2015 — Monsanto raises concern that IARC finding will reduce sales in California school districts.

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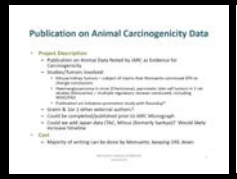

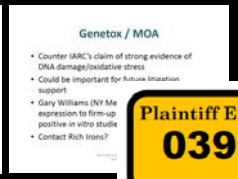

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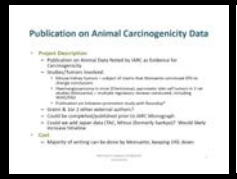
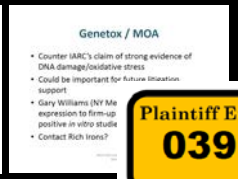


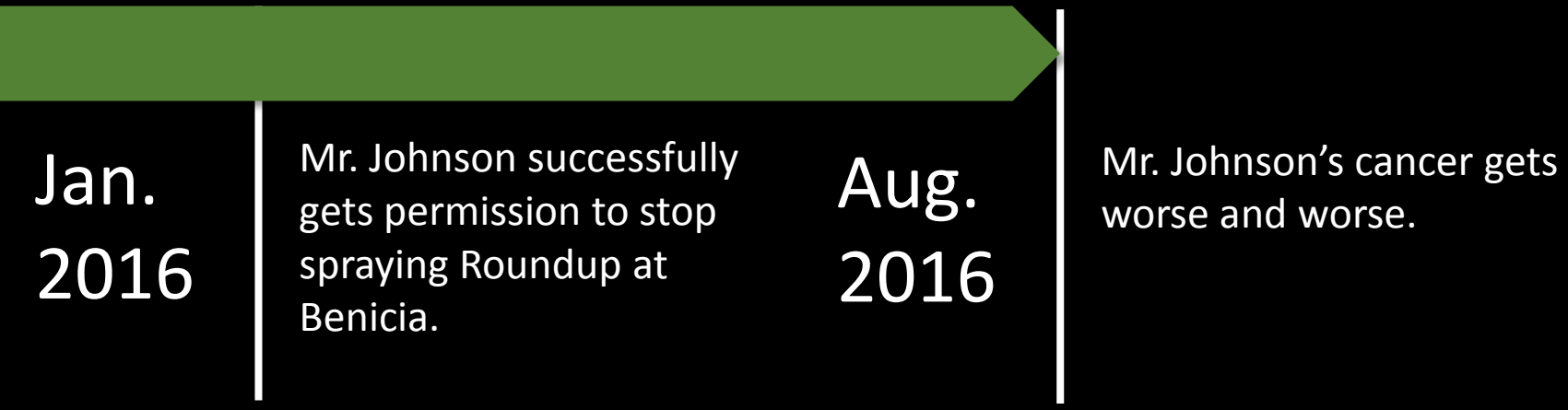


Mr. Johnson's Cancer

Jan. 2016

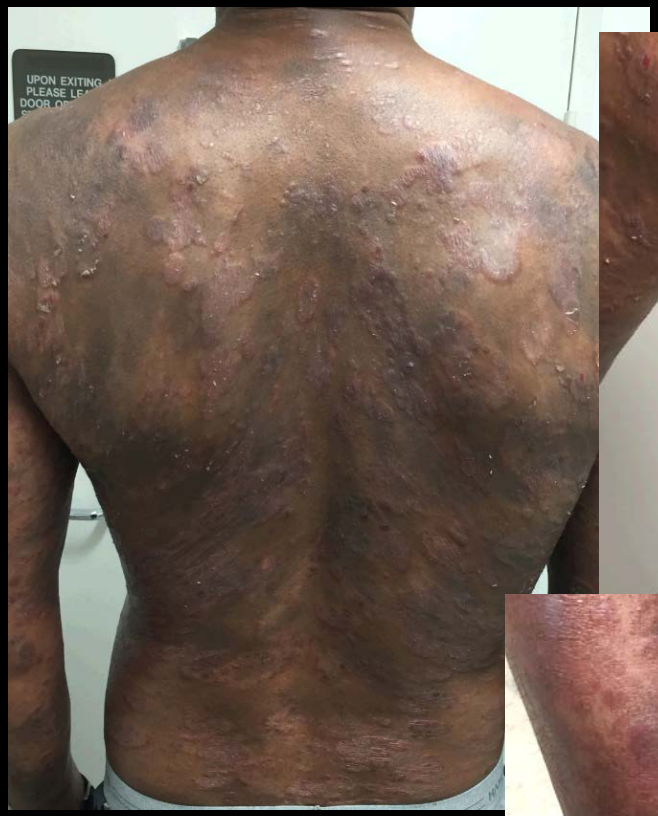
Mr. Johnson successfully gets permission to stop spraying Roundup at Benicia.

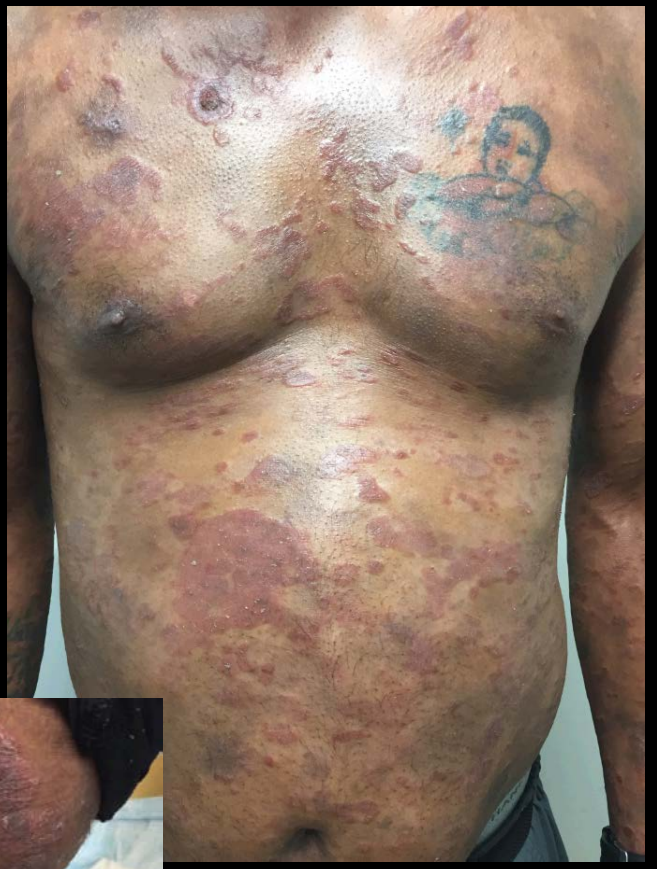
Mr. Johnson’s Cancer

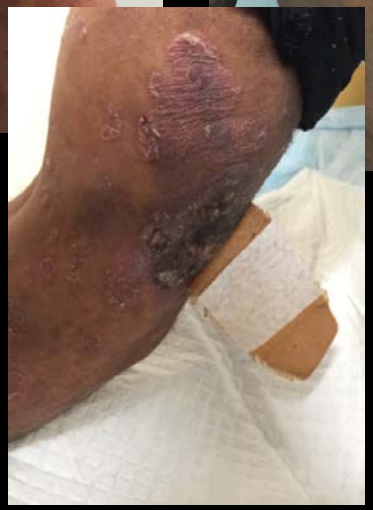


Mr. Johnson's Cancer

Aug. 2016

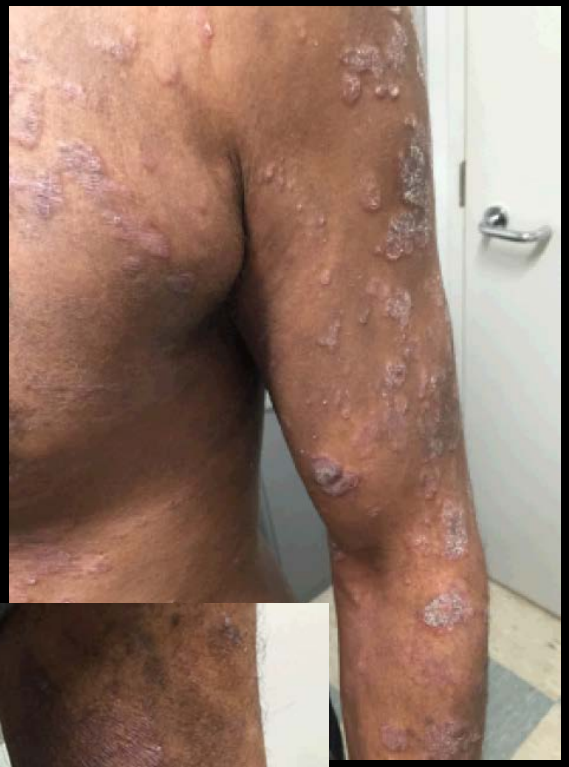


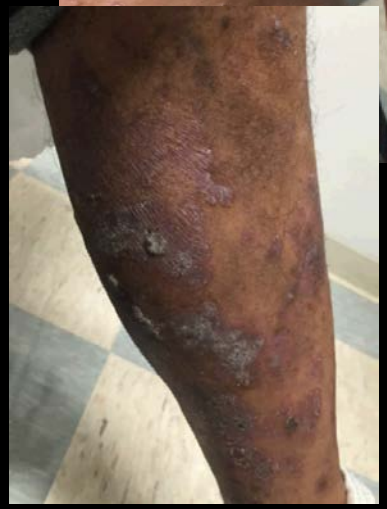


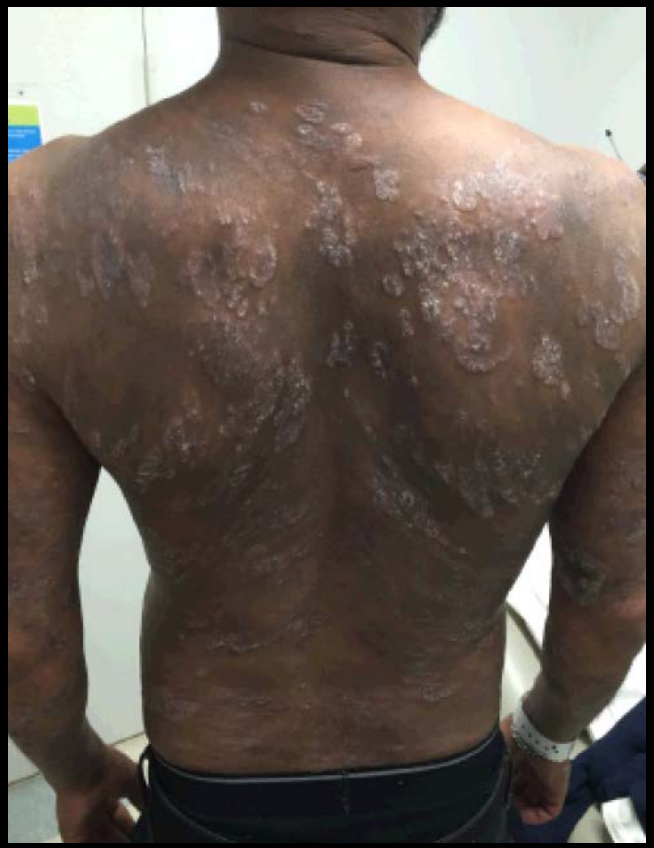


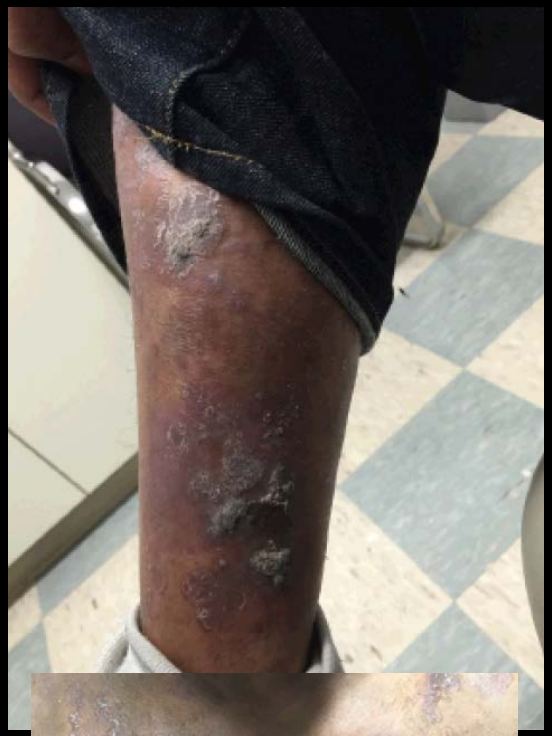
Mr. Johnson's Cancer

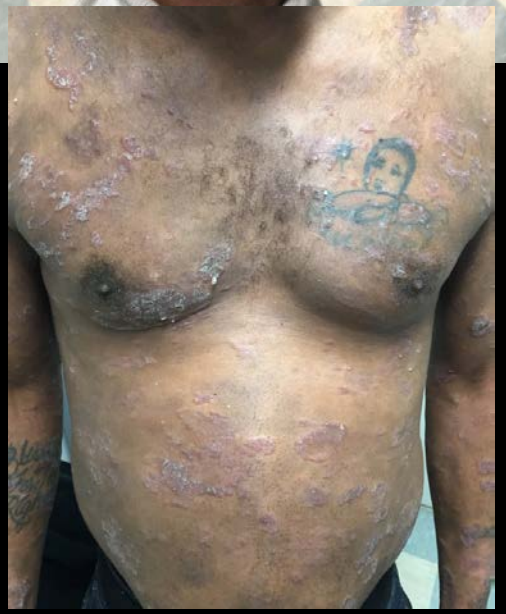
Dec. 2016





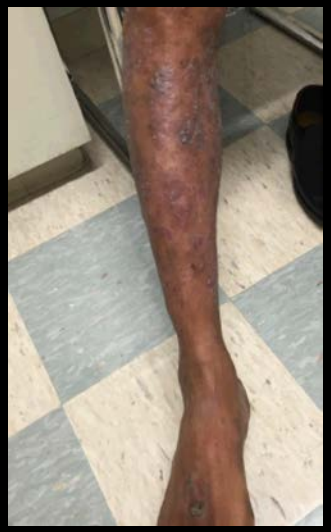


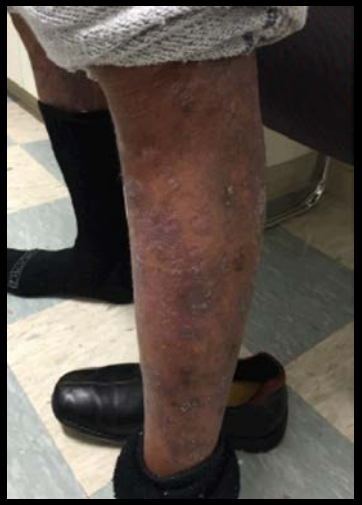


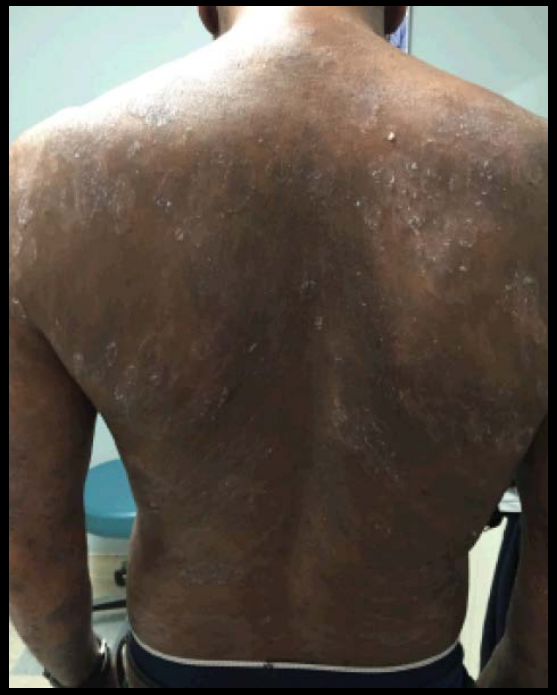


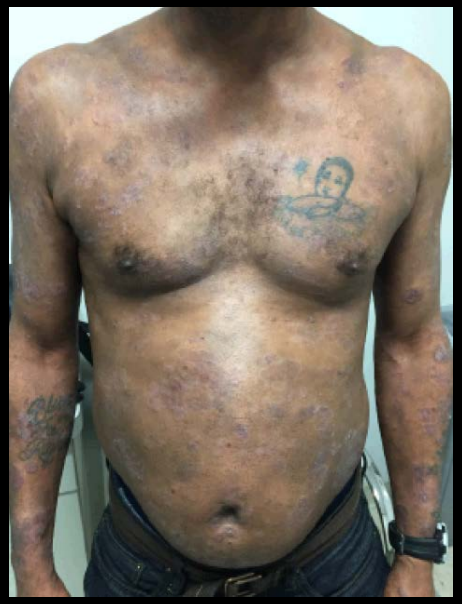
Mr. Johnson's Cancer

Jan. 2017



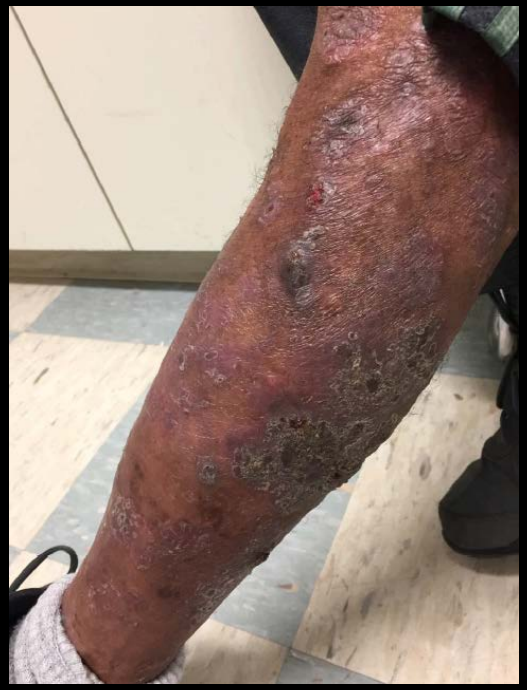


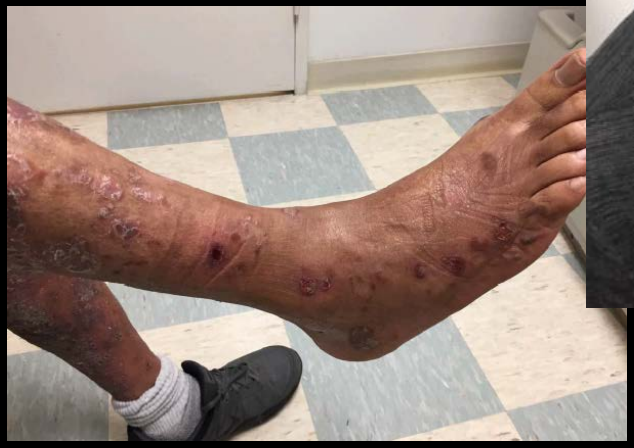


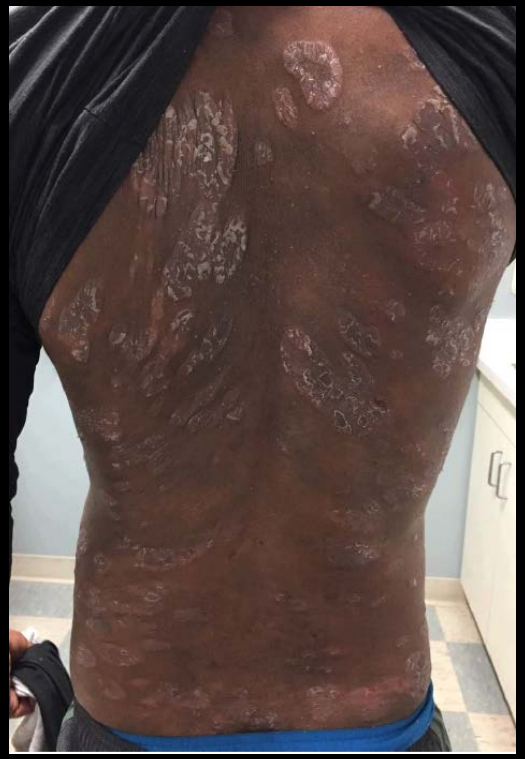


Mr. Johnson's Cancer

June 2017

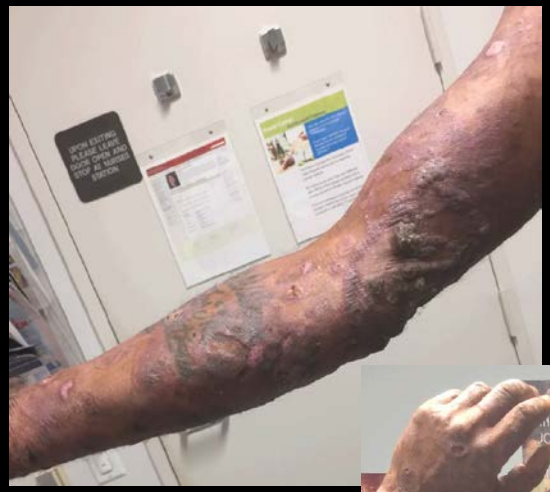


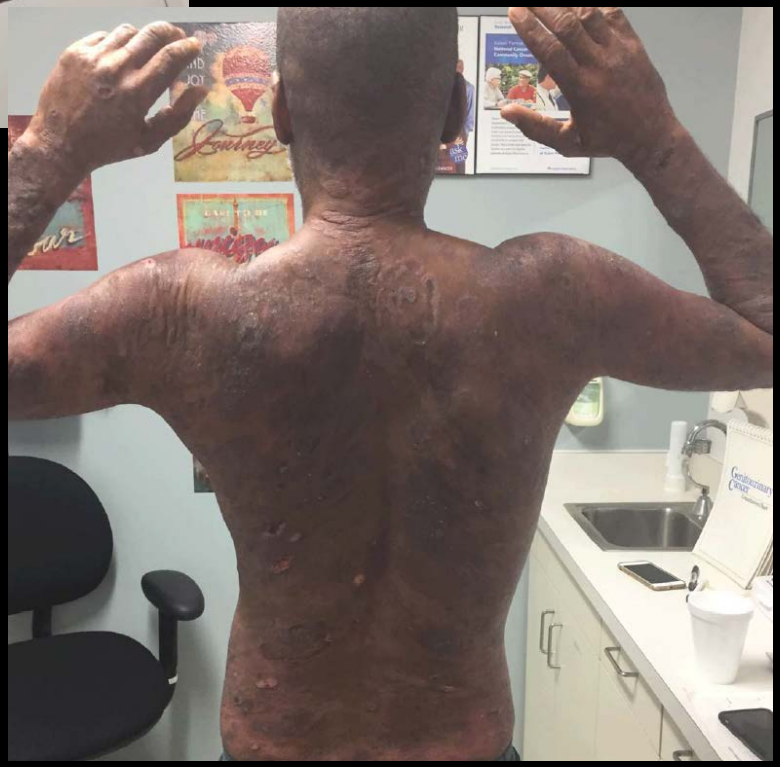


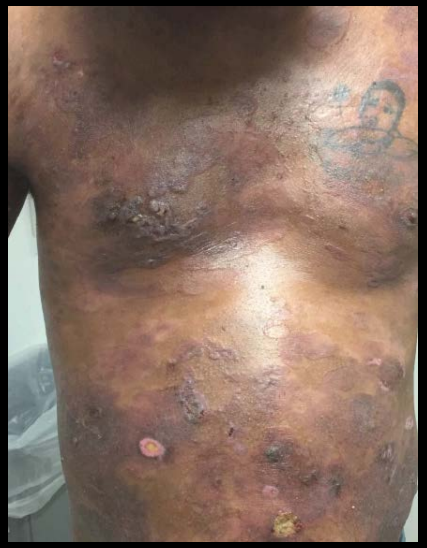


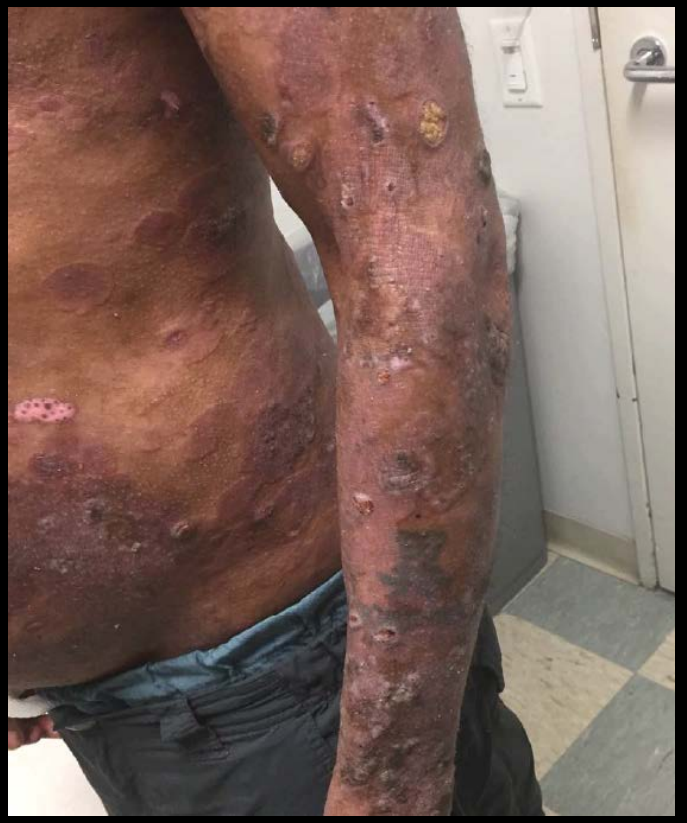
Mr. Johnson's Cancer

Nov. 2017



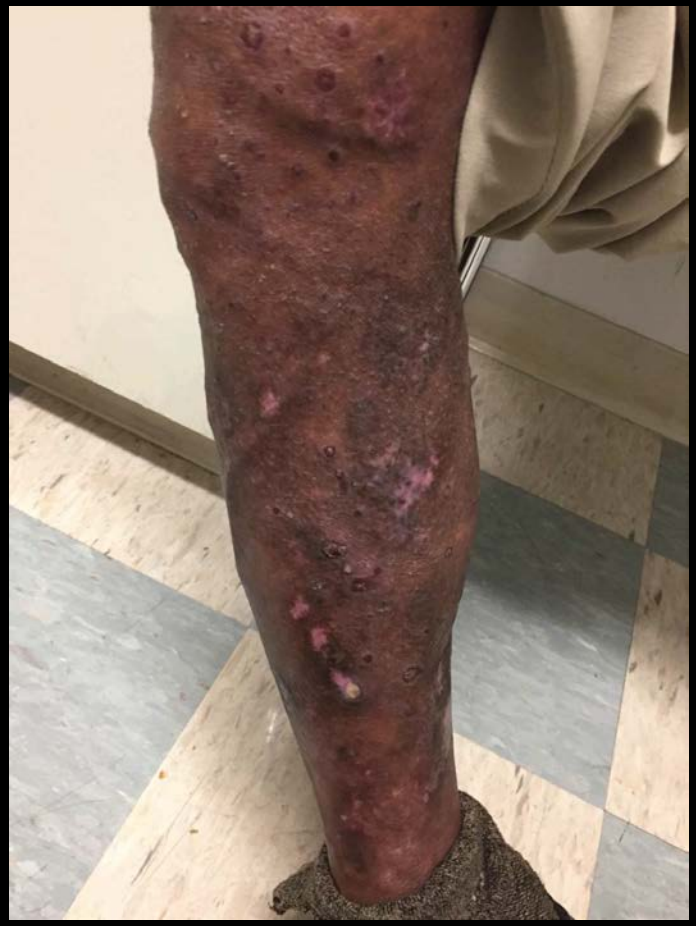


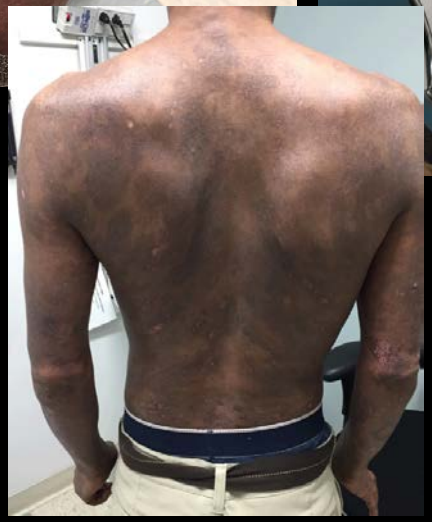




Mr. Johnson's Cancer

Jan. 2018





Mr. Johnson's Cancer

Absent a miracle, Mr. Johnson will not live to see 2020.

Mr. Johnson's Cancer

Dr. Nahban

Differential:

- ~~Age~~
- Race
- ~~Immunosuppressive drugs~~
- ~~Autoimmune disease~~
- Roundup exposures
- ~~Other chemical exposures~~
- ~~UV / Sun exposure~~
- ~~Viruses (HIV, HHV8, etc.)~~

Compensatory Damages

- Economic damages: $2,253,209.32
- Non-economic damages
  - physical pain
  - mental suffering
  - loss of enjoyment of life
  - disfigurement
  - physical impairment
  - grief
  - anxiety
  - humiliation
  - emotional distress

Compensatory Damages

- Economic damages: $2,253,209.32
- Non-economic damages

Past:

4 years: $4,000,000

Future:

2-33 years: $33,000,000

$37,000,000

Compensatory Damages

- Economic damages: $2,253,209.32
- Non-economic damages: $37,000,000

TOTAL

$39,253,209.23

3 Core Questions

YES 1. Can Roundup be a substantial contributing factor in causing cancer?

YES 2. Was Roundup a substantial contributing factor in causing Mr. Johnson's cancer?

3. Did Monsanto act with knowing disregard of human health?

3. Did Monsanto act with knowing disregard of human health?

Jury Instruction

"Malice" means that Monsanto acted with intent to cause injury or that Monsanto's conduct was despicable and was done with a willful and knowing disregard of the rights or safety of another. A person acts with knowing disregard when he or she is aware of the probable dangerous consequences of his or her conduct and deliberately fails to avoid those consequences.

3. Did Monsanto act with knowing disregard of human health?

Jury Instruction

Certain facts must be proved by clear and convincing evidence, which is a higher burden of proof. This means the party must persuade you that it is highly probable that the fact is true.

3. Did Monsanto act with knowing disregard of human health?

    1. Dr. Parry's reports
    2. Ghostwriting
    3. Freedom to operate
    4. Attacking IARC
    5. Refusing to test
    6. Targeting schools after IARC
    7. MSDS v. consumer label
    8. Never calling Mr. Johnson back

Jury Instruction

In view of Monsanto's financial condition, what amount is necessary to punish it and discourage future wrongful conduct?

Net Worth: $6.6 Billion

Cash on hand: $3.1 Billion

Interest (2%): $62 Million

$373,000,000

Verdict Form