Association Between Increasing Agricultural Use of 2,4-D and Population Biomarkers of Exposure: Findings from the National Health and Nutrition Examination Survey, 2001-2012

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Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is one of the most widely used herbicides in the United States. It is listed as an active ingredient in more than 600 agricultural and residential use products in the US.[1]

Since the 1990s **2,4-D use** on corn, soybeans, and cotton has been **rapidly increasing** in agriculture due to:

- Spread of glyphosate-resistant weeds requiring alternative and combination herbicides.
- Approval of crops resistant to both **glyphosate and 2,4-D** (DuoEnlist®, Enlist E3®).
- Regulatory restrictions on competing dicamba-glyphosate GMO crops.[3]

In 2018, **IARC** classified 2,4-D as a **"possible human carcinogen"** due to its ability to increase **oxidative stress** in the human cell.[8] Research has demonstrated associations between 2,4-D and various acute and chronic health conditions, such as:

- **Cancer** outcomes (Non-Hodgkin's Lymphoma and pediatric Leukemia)[9,11]
- **Birth Defects**[5,10]
- Hypothyroidism[6]
- Allergic/Non-Allergic Wheeze[7]

Objectives

Due to massive changes in agricultural practices over the past several decades, changes in the volume of herbicides applied over time have not been linear. Therefore, the study had a two-pronged objective:

- To determine whether **2,4-D exposure is changing over time**;
- To determine if increased **agricultural use of 2,4-D is associated with increased human exposure to 2,4-D** in biomarker analysis.

Methods

Human Exposure

NHANES includes about 5,000 participants from approximately 15 U.S. counties each year, with urine samples collected from approximately 1/3 of the participants. **NHANES 2,4-D urine concentration levels in five cycles** (2001/2002, 2003/2004, 2007/2008, 2009/2010, 2011/2012) were evaluated and exposure levels were dichotomized at the level of the highest limit of detection across all cycles (0.40 ug/L).

Pesticide Use

Data on agricultural use of pesticides is collected by several different US agencies, including as part of the National Agricultural Statistics Survey by the United States Department of Agricultural and various reports by the United States Geological Service. These data have been compiled into the **Pesticide Use Database System (PUDS),**[4] from which 2,4-D crop application was estimated.

Results

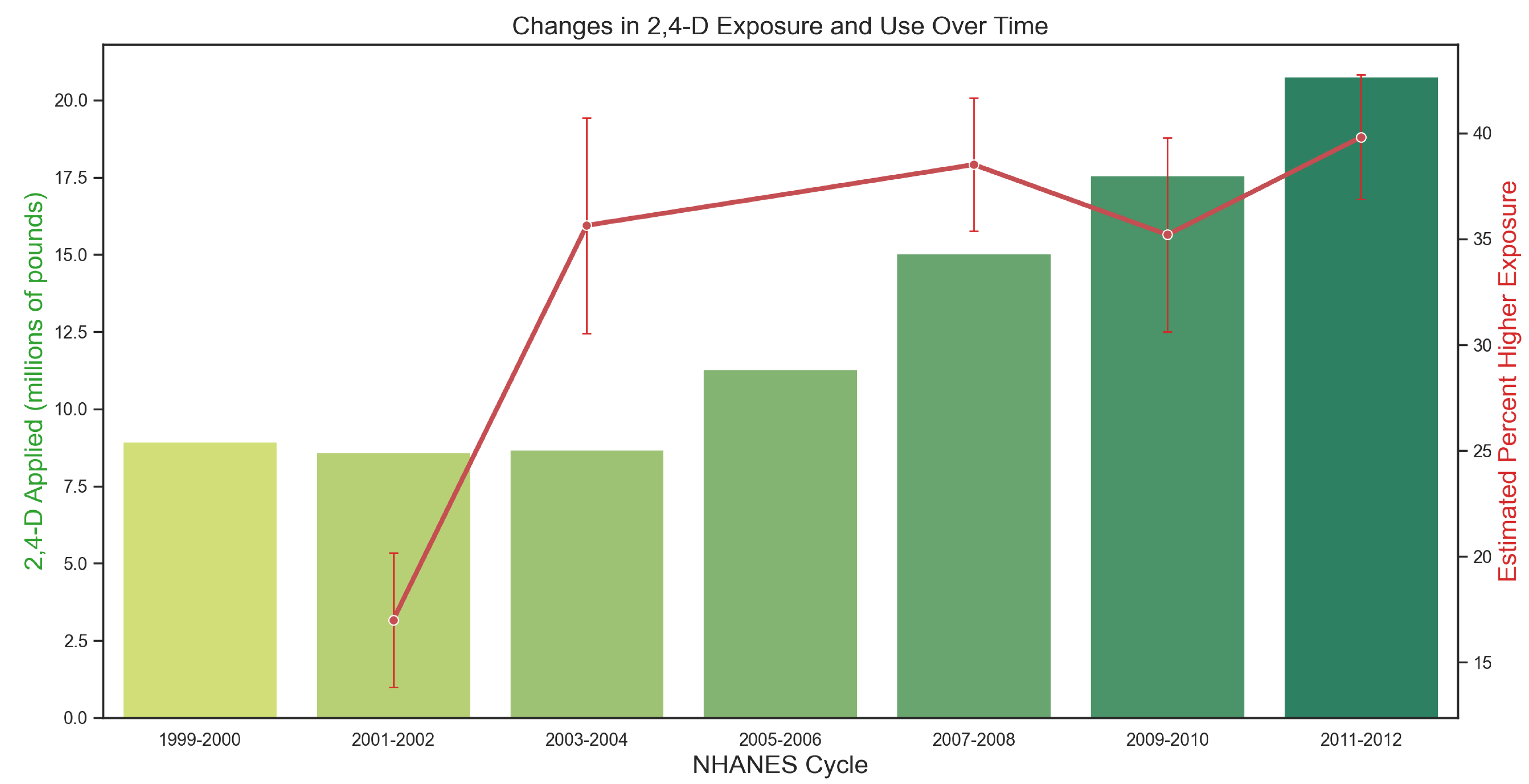


Figure 1. Changes in use of 2,4-D applied (*bar chart - left*) and percent Higher Exposure[1] in urinary concentrations (line chart, with 95% confidence bars – right) over NHANES cycle series.

[1] Defined as measurements above the highest limit of detection across cycles (0.04 ug/L), approximately the top tertile of exposure.

Across 5 cycles between 2001-2012

- 12,059 participants
- 3855 (31.25%) at Higher Exposure Level

There was a statistically significant increase in prevalence from **17.0% in 2001 to 39.8% in 2012** over the series of surveys (p<.0001).

For each **one-million-pound** increase in field application of 2,4-D, **prevalence of Higher Exposure increased by 6.5%.**

Prevalence of Higher Exposure was higher in some vulnerable groups:

- Children **age 6-11**,
- Older Adults **age 60+,** and
- **Women of childbearing age.**

Variable	All Participants	Age 6-11	Age 12-20	Age 20-59	Age 60+	Female, Age 20-44	Male, Age 20-44
	n=12059	n= 1887	n=2491	n=5152	n=2529	n=1796	n=1582
Age	1.009 (1.006-1.011)	0.885 (0.813-0.963)	0.873 (0.787-0.968)	1.021 (1.014-1.028)	1.016 (0.998-1.035)	1.014 (0.990-1.037)	1.014 (0.996-1.032)
Race/Ethnicity							
Non-Hispanic White	*	*	*	*	*	*	*
Mexican American	0.859 (0.680-1.085)	0.810 (0.577-1.136)	0.980 (0.706-1.361)	1.078 (0.803-1.447)	0.703 (0.476-1.039)	1.093 (0.674-1.773)	0.951 (0.660-1.371)
Non-Hispanic Black	0.542 (0.452-0.649)	0.511 (0.357-0.733)	0.674 (0.473-0.959)	0.484 (0.378-0.618)	0.591 (0.439-0.797)	0.463 (0.310-0.691)	0.472 (0.334-0.668)
Other	0.661 (0.553-0.820)	0.426 (0.264-0.687)	0.776 (0.552-1.156)	0.751 (0.564-1.000)	0.603 (0.386-0.943)	0.629 (0.399-0.992)	0.724 (0.483-1.086)
Education							
Grade 8 or Less	1.713 (1.489-1.971)	–	0.632 (0.318-1.257)	0.732 (0.528-1.014)	1.189 (0.825-1.713)	1.202 (0.632-2.284)	0.634 (0.363-1.105)
Grade 9-12	0.907 (0.794-1.037)	–	0.858 (0.478-1.539)	0.862 (0.732-1.015)	0.850 (0.660-1.094)	0.845 (0.630-1.134)	1.045 (0.762-1.433)
Some College	*	*	*	*	*	*	*
Poverty Income Ratio	1.073 (1.029-1.118)	1.164 (1.071-1.265)	1.038 (0.948-1.136)	1.046 (0.989-1.107)	1.070 (0.981-1.167)	1.079 (0.967-1.203)	1.025 (0.944-1.114)
Urine Creatinine	1.009 (1.009-1.010)	1.015 (1.011-1.018)	1.008 (1.007-1.010)	1.010 (1.009-1.012)	1.009 (1.007-1.011)	1.011 (1.009-1.013)	1.008 (1.006-1.010)
Agricultural Worker	2.326 (1.446-3.744)	–	1.647 (0.554-4.895)	2.841 (1.557-5.183)	2.591 (0.559-12.014)	1.805 (0.460-7.085)	3.232 (1.624-6.430)
Pounds of 2,4-D Applied	1.065 (1.043-1.087)	1.095 (1.055-1.137)	1.039 (1.000-1.080)	1.056 (1.034-1.079)	1.095 (1.069-1.121)	1.081 (1.049-1.115)	1.037 (1.002-1.073)

Table 1. Adjusted odds ratios for higher level of 2,4-D exposure. * = reference level for comparison;. – = variables not applicable in age group.

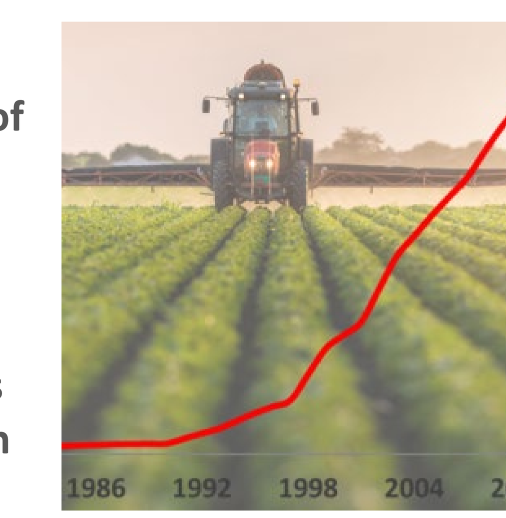
Conclusion

Current situation: The **clear and persistent relationship between exposure status and agricultural use of 2,4-D** in a large nationally representative survey with a sample diverse in age, race/ethnicity, income, and geography, indicates that **agricultural use is indeed an important factor in overall exposure levels in the US.**

Projections: Use of 2,4-D in agricultural applications has increased substantially over the past several decades. Since the most recent year in which 2,4-D was analyzed in an NHANES survey (2011-2012), **2,4-D use in agriculture has been increasing steadily and is expected to continue to do so over at least the next 10 years.** While urinary levels of 2,4-D have historically been substantially below levels considered to be hazardous in human risk assessment,[2] recent research demonstrates that lower levels of 2,4-D exposure may be related to specific health endpoints in humans.

Future Needs:

While the application of 2,4-D is increasing exponentially, human studies to evaluate potential health impacts to populations need to keep pace with these increases.



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