

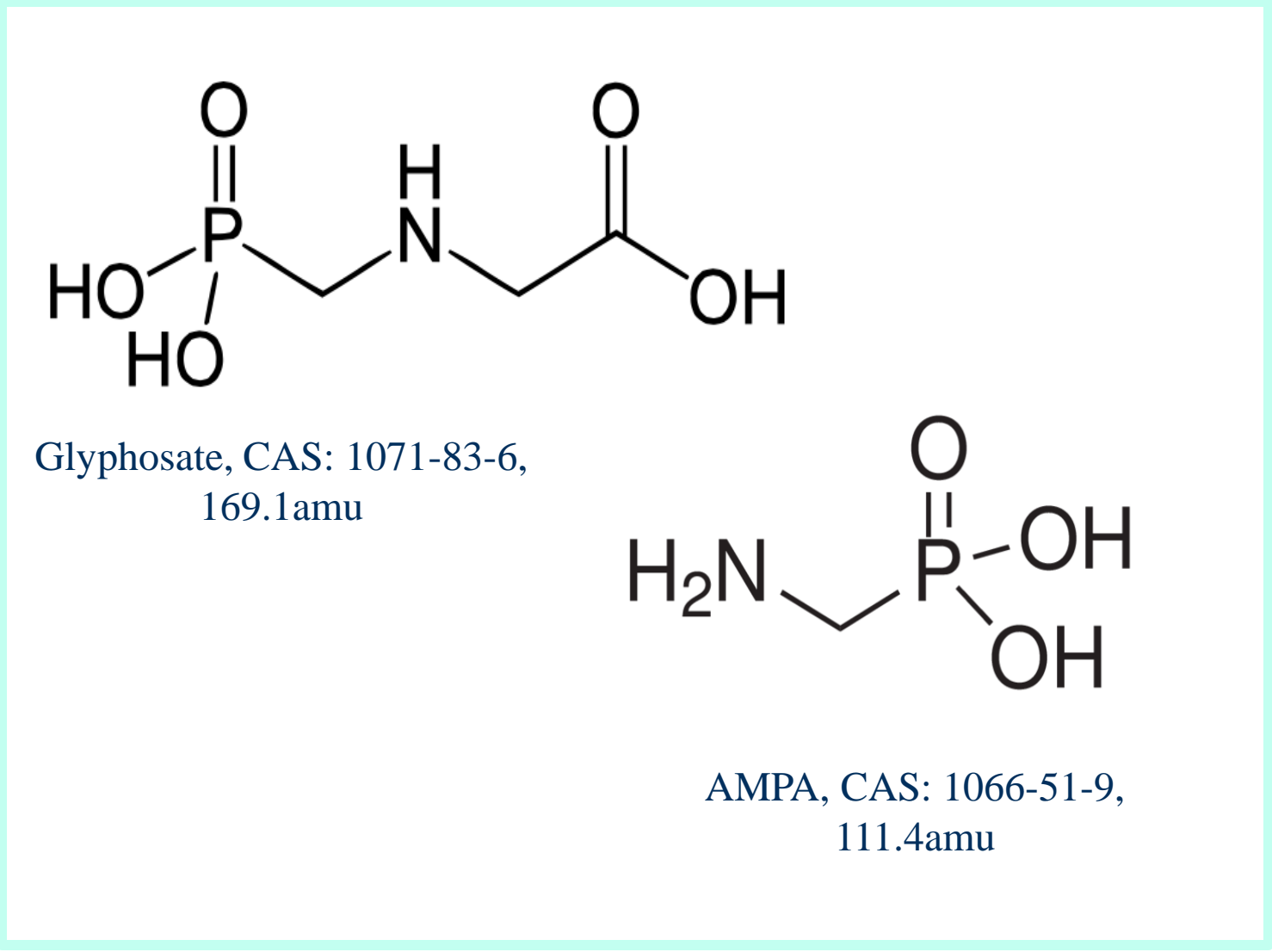


Biomonitoring of glyphosate across the United States in urine and tap water using high-fidelity LC-MS/MS method

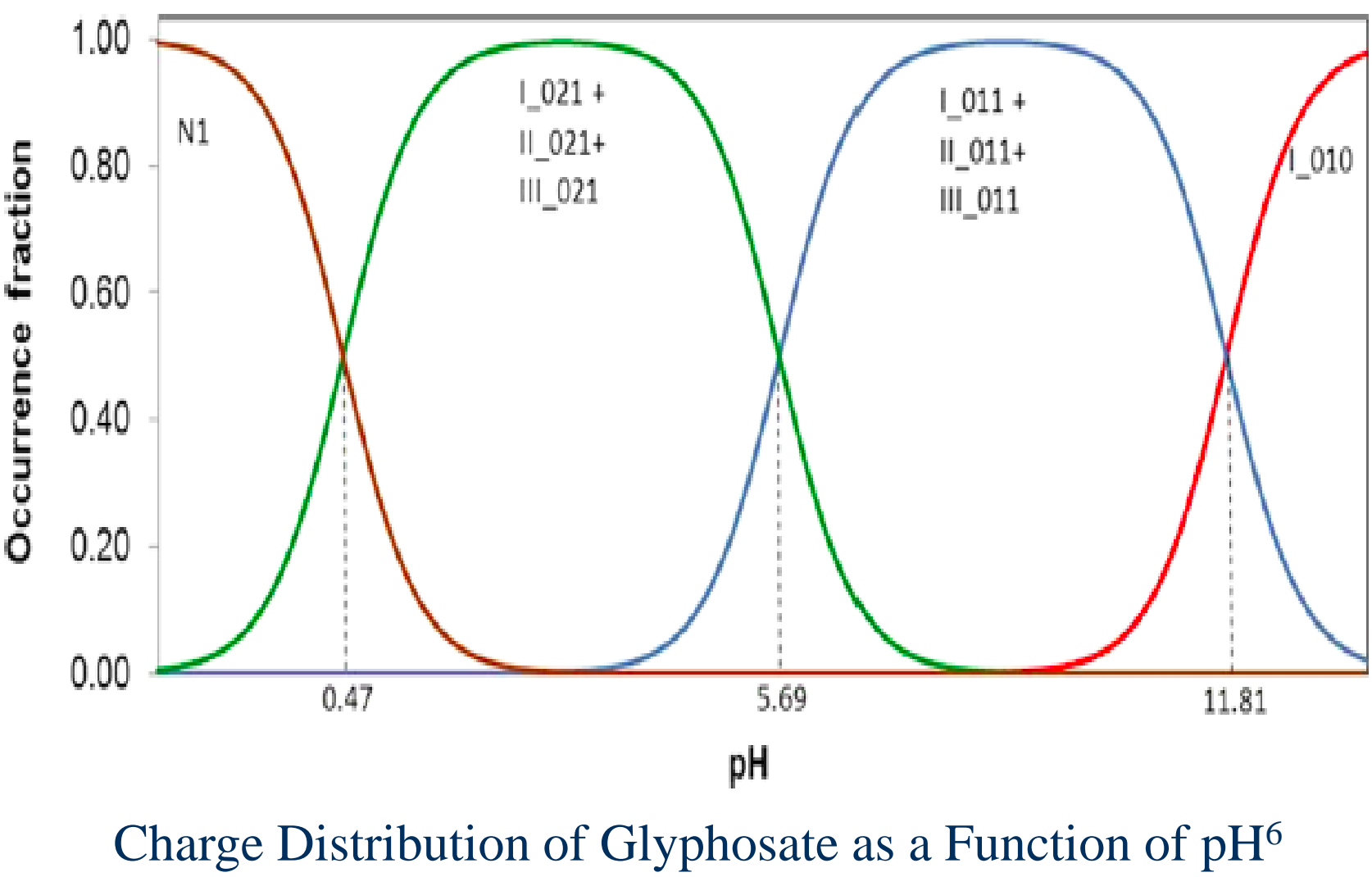
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Introduction

- Glyphosate (N-(phosphonomethyl)glycine) is arguably the most important herbicide ever discovered¹ and is the most widely used herbicide in the world with U.S. of 185 million pounds in 2007²
- Its mode of action as a herbicide is via inhibition of synthesis of aromatic amino acids in plants³
- Main breakdown product is (aminomethyl)phosphonic acid (AMPA)



- March 2015, IARC ruled glyphosate as a Group 2A carcinogen based upon a meta-analysis linking glyphosate exposure to Non-Hodgkin’s Lymphoma (OR: 1.5, 95%CI 1.1-2.0, I²=32.7%)^{4,5}
- Glyphosate is a notoriously difficult analyte
 - Small, polar, and amphoteric (pKa’s of pH 0.47, 5.69, and 11.81)⁶
 - No fluorophore or chromophore
 - Structurally similar to many small polar molecules in biological matrices (e.g. glycine)
 - Strong chelator of divalent metal cations⁷



- Aims:
 - 1.Develop a direct method for analysis of glyphosate in urine and tap water for future use in both biomonitoring studies and assessment of acute intoxication.
 - 2.Demonstrate utility of method in a public glyphosate testing in the United States.

Analysis

- LC: Agilent binary 1260 Infinity LC pump and autosampler injector
- MS: AB Sciex Triple Quad 5500 LC-MS/MS
- Column: Obelisc N column (100mm x 2.1mm x 5”m, SILIC Technologies)
- Quantitation in urine done using standard addition with IS of ¹³C-2-glyphosate with spike concentrations at 0.2, 1, and 2ppb
- Quantitation in tap water done using stable isotope dilution method using peak area ratios⁸

MRM Transitions				
Analyte	MRM Transition	DP	CE	CXP
Glyphosate	168.071/62.900	-40V	-32V	-11V
	168.071/81.000	-40V	-22V	-7V
Glyphosate-2- ¹³ C	169.400/63.000	-30V	-40V	-9V

LC-MS Conditions

LC Conditions	
Autosampler Temp.	4°C
Column Temp.	40°C
Elution Gradient	Isocratic
Mobile Phase	1% Formic Acid/HPLC-Grade H ₂ O
Mobile Phase pH	2.2
Flow Rate	1000µL/min
Injection Volume	25µL
MS Conditions	
MS Mode	Negative
Curtain Gas	20.0bar
Collision Gas	9.0bar
Ion Spray Voltage	-4500V
Temperature	700°C
Ion Source Gas 1	60.0bar
Ion Source Gas 2	60.0bar
Method Duration	6.00 min
Validation	MatrixConcentration
LOD	Urine0.2ppb
LLOQ	0.5ppb
LOD	Water0.01ppb
LLOQ	0.005ppb

Results from Public Testing[‡]

Matrix	Population	n	Avg. Glyphosate Conc. (ppb)	Standard Error
Urine	Overall	131	3.096	0.186
	Women	75	2.941	0.214
	Men	56	3.304	0.329
	Children (confirmed <18years old)*	7	3.586	0.300
	Adults*	124	3.069	0.196
	Region			
	Northeast	21	3.000	0.343
	Midwest	19	3.050	0.289
	South	16	2.313	0.828
	West	75	3.053	0.275

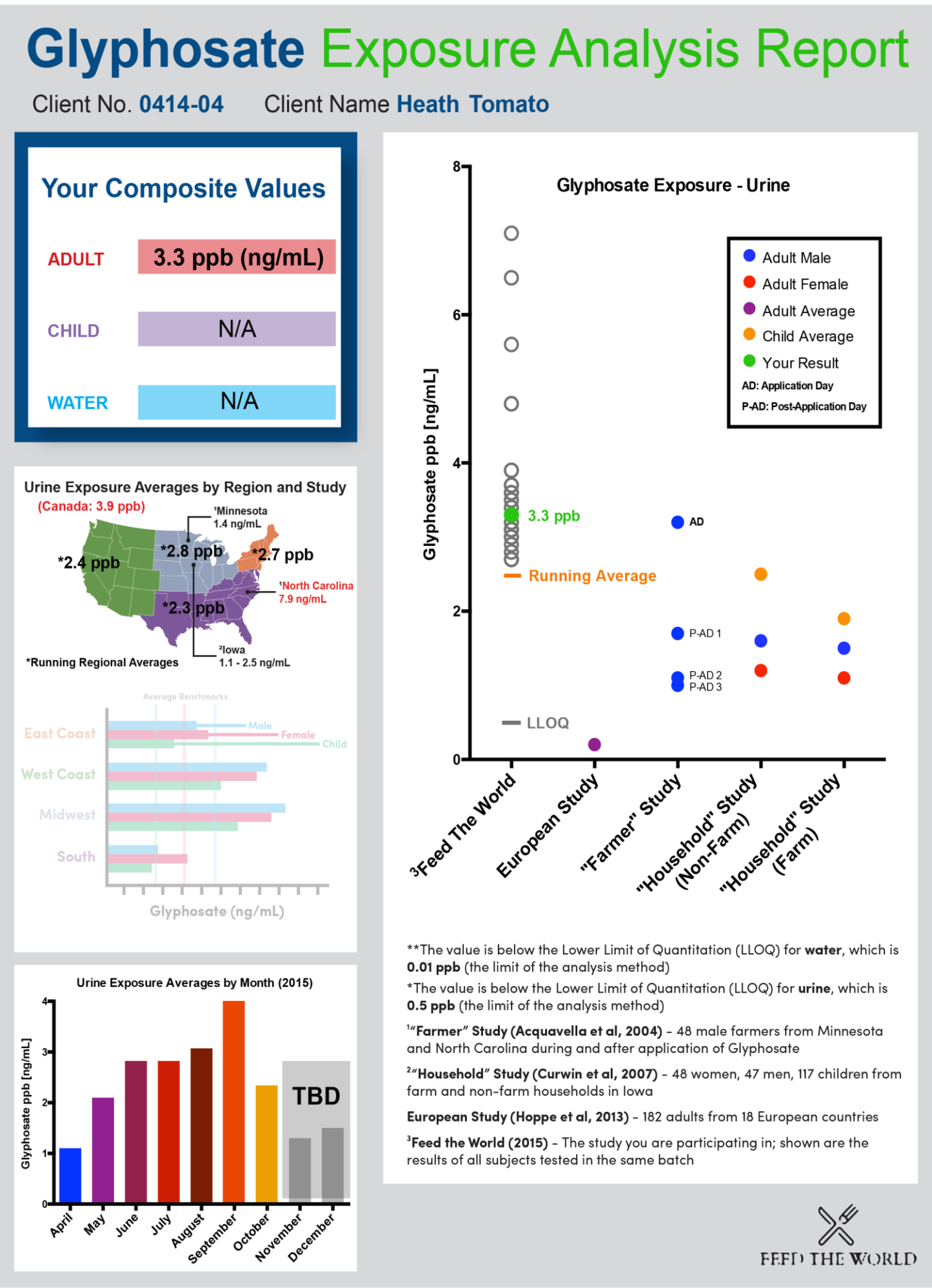
[‡]Glyphosate residues were not observed in any tap water samples – as would be expected due to phosphorus removal during water treatment
*Only 60/131 participants included age on requisition forms

Limitations:

- Selection bias: people likely to pay for this service are not representative of general U.S. population and the West is represented disproportionately
- Consistency of sample collection; urine concentrations were corrected based upon urine osmolality

Public Testing Reports

- ✧ Public Testing offered as a fee for service
- ✧ Participants received a summary report of the glyphosate levels in urine along with infographics comparing their results to results obtained for different regions of the U.S.



Conclusions:

- Glyphosate residues were observed in 93% of urine samples in voluntary public testing in the U.S. general population; this is higher than the frequency observed in Europe using GC-MS (43.9%)⁹
- Tap water obtained was free of glyphosate residues as expected; exposure is likely due to dietary intake or environmental exposure

Future Directions:

- Develop method for AMPA in urine
- Develop direct LC-MS method not requiring standard addition
- Develop method for glyphosate and AMPA in other biological matrices such as serum and breast milk

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