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Ontario

Glyphosate detection in water by enzyme-linked immunosorbent assay (ELISA)

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Agenda

- Background
- Objective
- ELISA
- Results
- Conclusions

Background – Glyphosate

- *N*-(phosphonomethyl)glycine
- Introduced 1974 by Monsanto
- Important and dominant herbicide worldwide
- Non-selective, broad spectrum
- Ideal herbicide
  - Unique as EPSPS inhibitor
  - Good plant uptake and translocation to sites

EPSPS = 5-enolpyruvyl-shikimate-3-phosphate synthase

Background – Glyphosate

- 2003: 1,171,000 kg used in Ontario (McGee, Berges and Callow, 2003)
- Very soluble in water
- Half life: 47 days (soil), 49–70 days (water)
- 1996: transgenic, glyphosate-resistant crops
- Evolution of glyphosate-resistant weeds

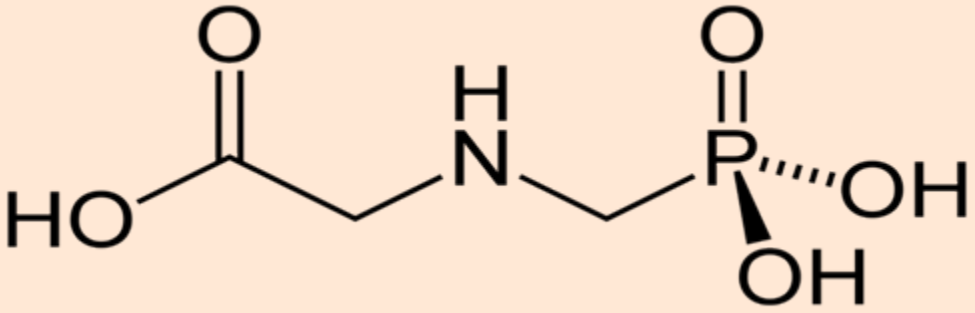
Background – Glyphosate

- Less toxic relative to other pesticides
- Formulations (e.g., Roundup®) appear to be more toxic
- Potential endocrine disruptor
  - Disrupts mammalian cytochrome P450 aromatase (estrogen synthesis) at concentrations lower than recommended for agricultural use (Richard et al. 2005)

Background – Glyphosate

Chemical Properties

- Molecular formula: $C_3H_8NO_5P$
- Molar Mass: 169.07 g/mole
- Water Solubility: 1.2 g/100 mL
- Molecular Structure:



Background – Glyphosate

Standards

- Canadian Drinking Water Quality Guideline:
  0.28 mg/L MAC (maximum acceptable concentration) or 280 ng/mL

- Ontario Drinking Water Quality Standard:
  0.28 mg/L (*SDWA, 2002*, O. Reg. 169/03)

- US EPA maximum containment level: 0.7 ppm (0.7 mg/L or 700 ng/mL)

US EPA: United States Environmental Protection Agency

Background – Glyphosate

Conventional Analysis:

High performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) or LC-MS

- Expensive

Screening Strategy:

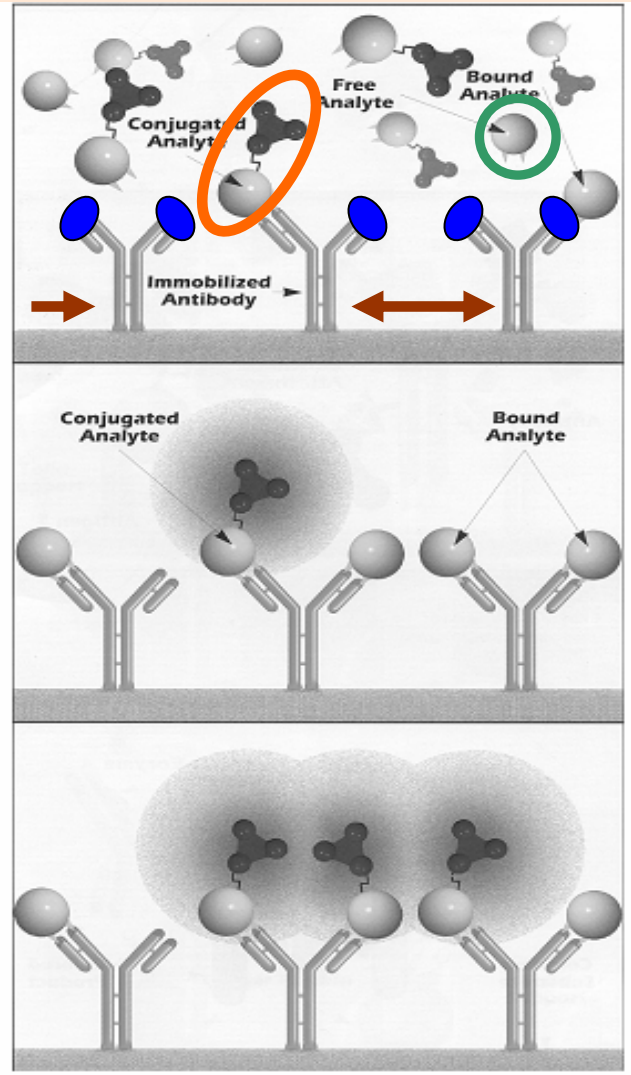
Enzyme-linked immunosorbent assay (ELISA)

- Time and cost efficient
- Presence/absence test
- Quantification by LC-MS if present

Objective

Determine suitability of ELISA for quantifying and/or qualifying glyphosate in water samples

ELISA Principle



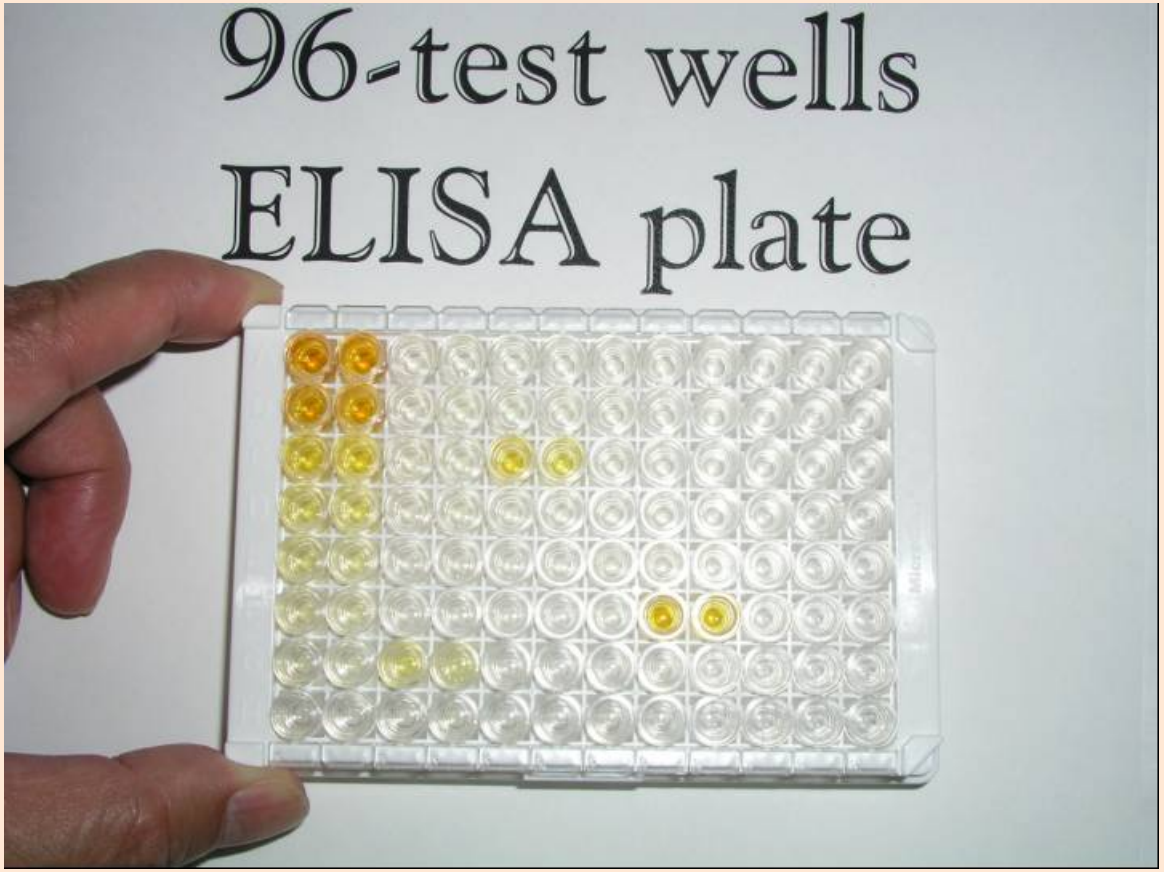
- **Glyphosate toxin** and **enzyme-labelled glyphosate** compete for **binding sites** on **immobilized antibodies**
- Unbound materials washed away
- Concentration of glyphosate is inversely proportional to colour produced

ELISA

Byer JD, Struger J, Klawunn P, Todd A and Sverko, E. 2008. Low cost monitoring of glyphosate in surface waters using the ELISA method: An evaluation. Environ. Sci. Technol. 42: 6052-6057

- ELISA can be used in conjunction with wet chemistry methods to improve temporal and/or spatial monitoring
- ELISA tube format used

ELISA microplate



ELISA Procedure

- Derivatize standards, controls and samples
- Perform ELISA:
  - Add antibody solution, derivatized standard, control, or sample, incubate 30 minutes
  - Add enzyme conjugate solution, incubate one hour
  - Discard contents, wash wells
  - Add substrate solution, incubate 30 minutes
  - Add stop solution, read absorbance at 450 nm

ELISA Procedure

- Results:
  - Calculate $\%B_0$
  - Construct standard curve: plot $\%B_0$ of standard against its glyphosate concentration
  - Inverse relationship between $\%B_0$ and concentration of glyphosate in sample

$\%B_0$: used to measure concentration; equalizes different runs of an assay

$$\%B_0 = \frac{OD\ of\ \ Sample\ \ or\ Calibrator}{OD\ of\ \ Negative\ \ Control} \times 100$$

OD = optical density

ELISA

Data processing – calculation module

The module performs data manipulations, data reductions and results interpretations

- Calculates Corrected Absorbance
- Calculates $\%B_0$
- Draws calibration curve. Plots $\%B_0$ versus concentration
- Calculates the concentration of glyphosate

Results

$\%B_0$ and Standard Curve Parameters

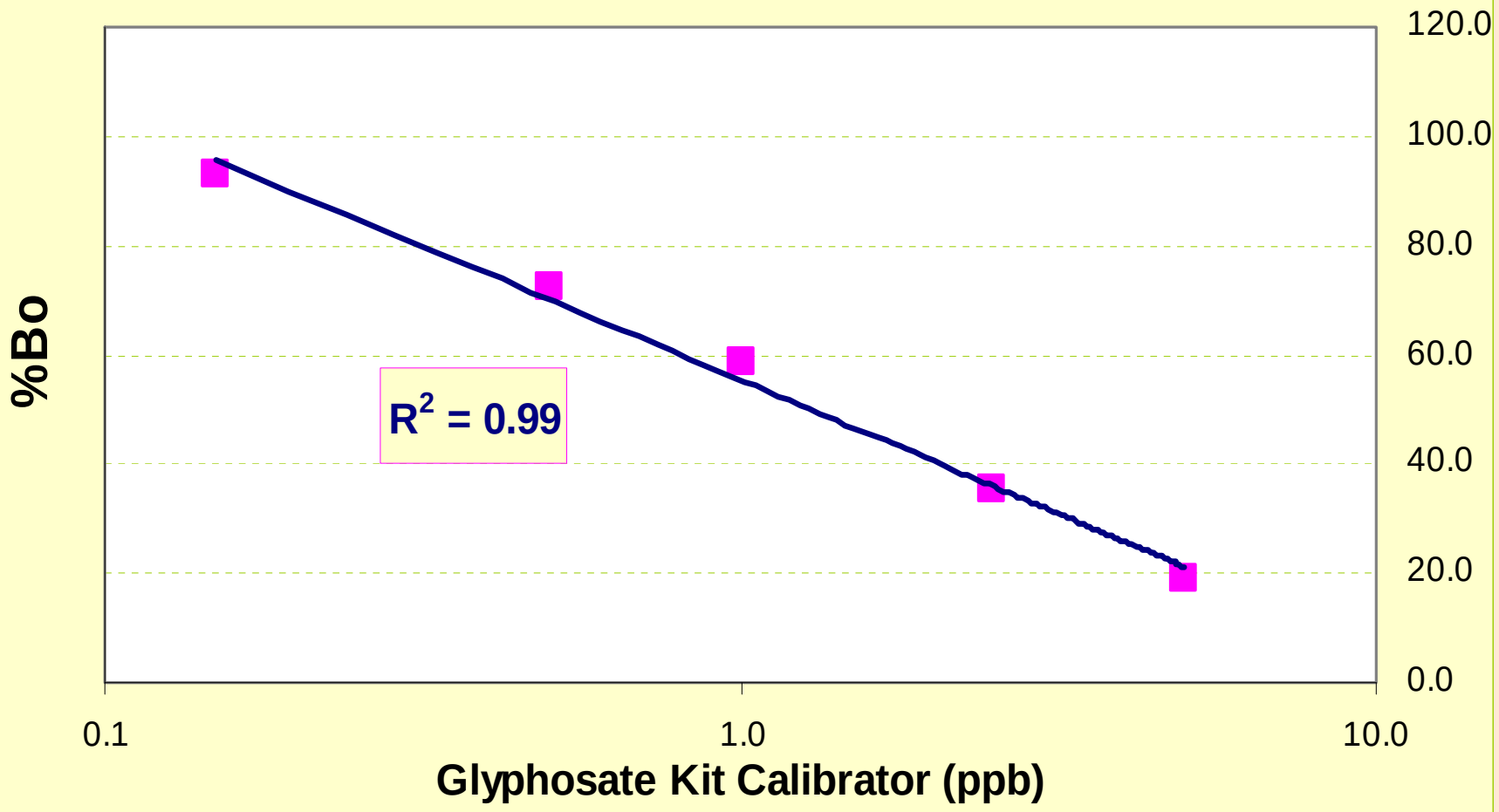
5-Point Curve	Replicates Measurements				Average	S.D.
	Expt 1 (N=2)	Expt 2 (N=2)	Expt 3 (N=2)	Expt 4 (N=2)		
Diluent	100.00	100.00	100.00	100.00	100.00	0.00
0.15 ng/mL	88.97	105.16	92.83	82.78	92.44	9.44
0.50 ng/mL	77.38	80.15	72.43	75.41	76.34	3.25
1.0 ng/mL	54.94	63.10	58.72	59.00	58.94	3.33
2.5 ng/mL	34.05	40.01	35.36	40.15	37.39	3.15
5.0 ng/mL	18.24	24.83	19.08	26.04	22.05	3.96
R^2	0.9693	0.9985	0.9914	0.9634	0.9807	0.0169
Intercept	54.44	62.35	55.41	56.11	57.08	3.58
Slope	-21.09	-23.23	-21.26	-18.22	-20.95	2.06

S.D.: Standard Deviation

0.15 ng/mL calibrator inconsistent

MAC = 280 ng/mL

Glyphosate ELISA
(5-Point Calibration Curve)



Curve Parameters: 5-point versus 4-point

	5-point Curve (0.15 ng/mL to 5 ng/mL)		4-point Curve (0.5 ng/mL to 5 ng/mL)	
	Average	S.D.	Average	S.D.
R^2	0.9807	0.02	0.9972	0.00
Intercept	57.08	3.58	59.48	2.69
Slope	-20.95	2.06	-23.57	1.67

Accuracy and Precision

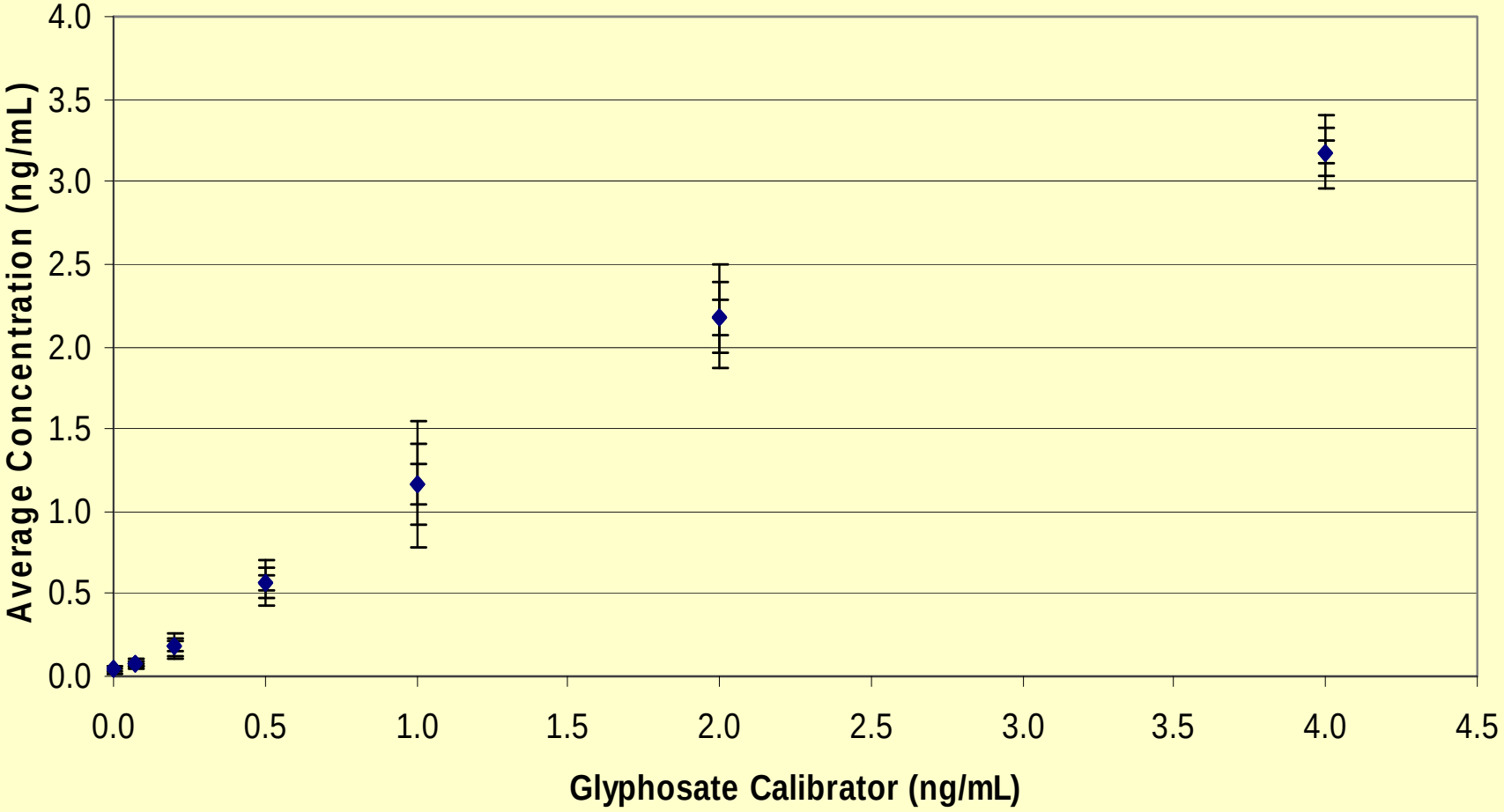
	Intra-Assay Accuracy and Precision			
	5-point Curve		4-point Curve	
	Average Recovery (%) (N=11)	CV	Average Recovery (%) (N=11)	CV
0.15 ng/mL	122.8	20.9		
0.50 ng/mL	68.3	19.6	92.8	18.3
1.0 ng/mL	86.0	28.2	100.0	23.2
2.5 ng/mL	108.8	19.6	104.8	23.2
5.0 ng/mL	100.8	9.00	87.7	7.5
Control (0.75 ng/mL)	97.8	18.8	117.0	15.9
Spike 1 (1.0 ng/mL)	76.0	14.3	92.7	18.4
Spike 2 (2.5 ng/mL)	127.7	25.8	106.8	19.7
Average	95.0	19.3	100.3	17.7

CV: Coefficient of Variation

Deleting 0.15 ng/mL calibrator improved accuracy

Accuracy and Precision (cont'd)

Intra-assay precision on 8 replicates per calibrator concentration



Accuracy and Precision (cont'd)

	Inter-assay Accuracy and Precision			
	5 Point Curve		4 Point Curve	
	Average Recovery (%) (from 4 experiments)	CV	Average Recovery (%) (from 4 experiments)	CV
0.15 ng/mL	140.7	34.9		
0.50 ng/mL	84.3	19.0	94.1	18.1
1.0 ng/mL	95.5	19.3	101.7	17.0
2.5 ng/mL	104.4	11.1	104.8	11.0
5.0 ng/mL	103.6	18.3	98.9	14.8
Control (0.75 ng/mL)	101.6	22.2	109.5	18.3
Spike 1 (1.0 ng/mL)	89.0	37.1	102.2	29.6
Spike 2 (2.5 ng/mL)	121.8	20.9	107.4	19.9
Average	105.1	22.8	102.7	18.4

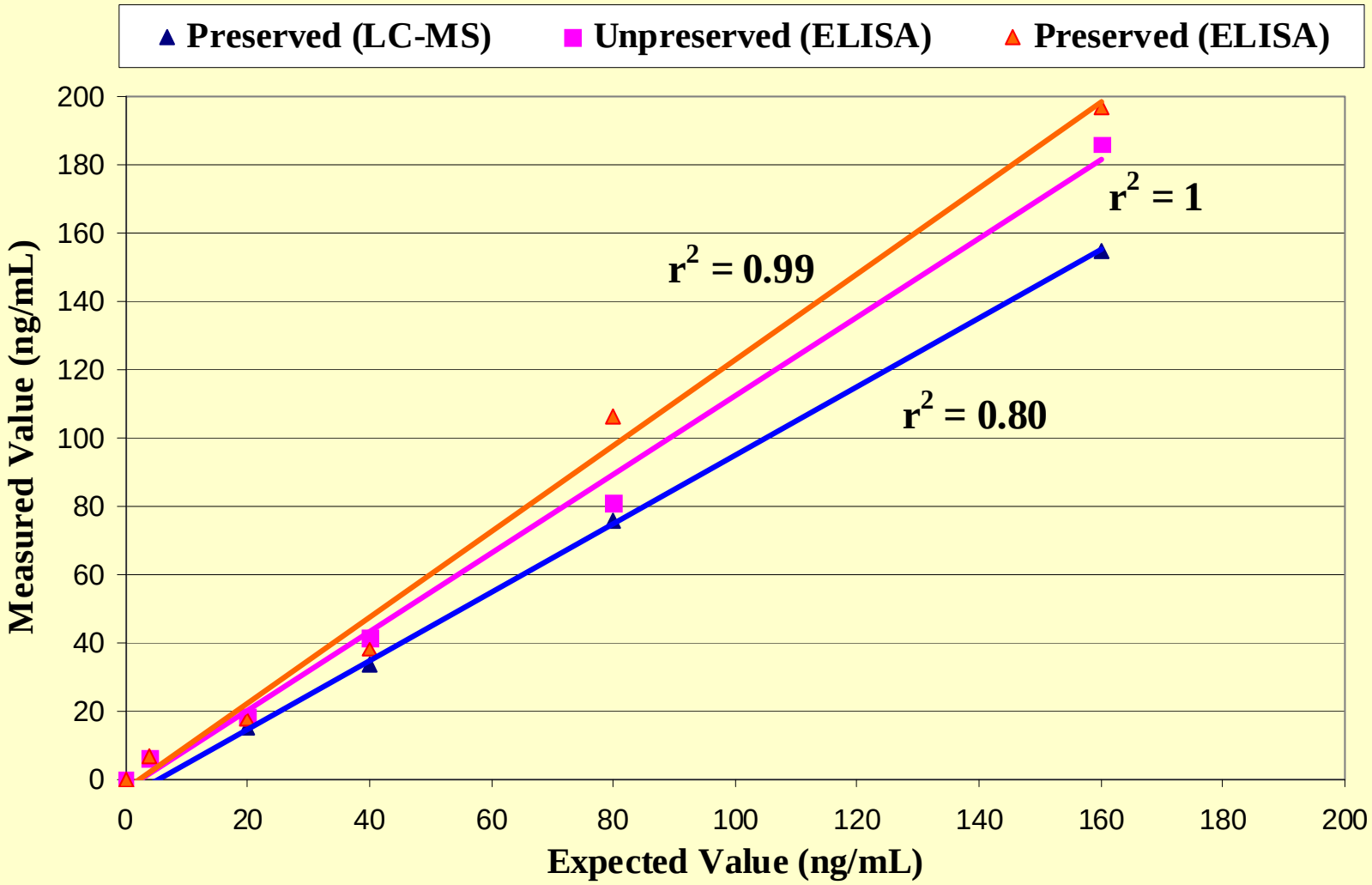
Deleting 0.15 ng/mL calibrator improved accuracy

QC samples: ELISA and LC-MS

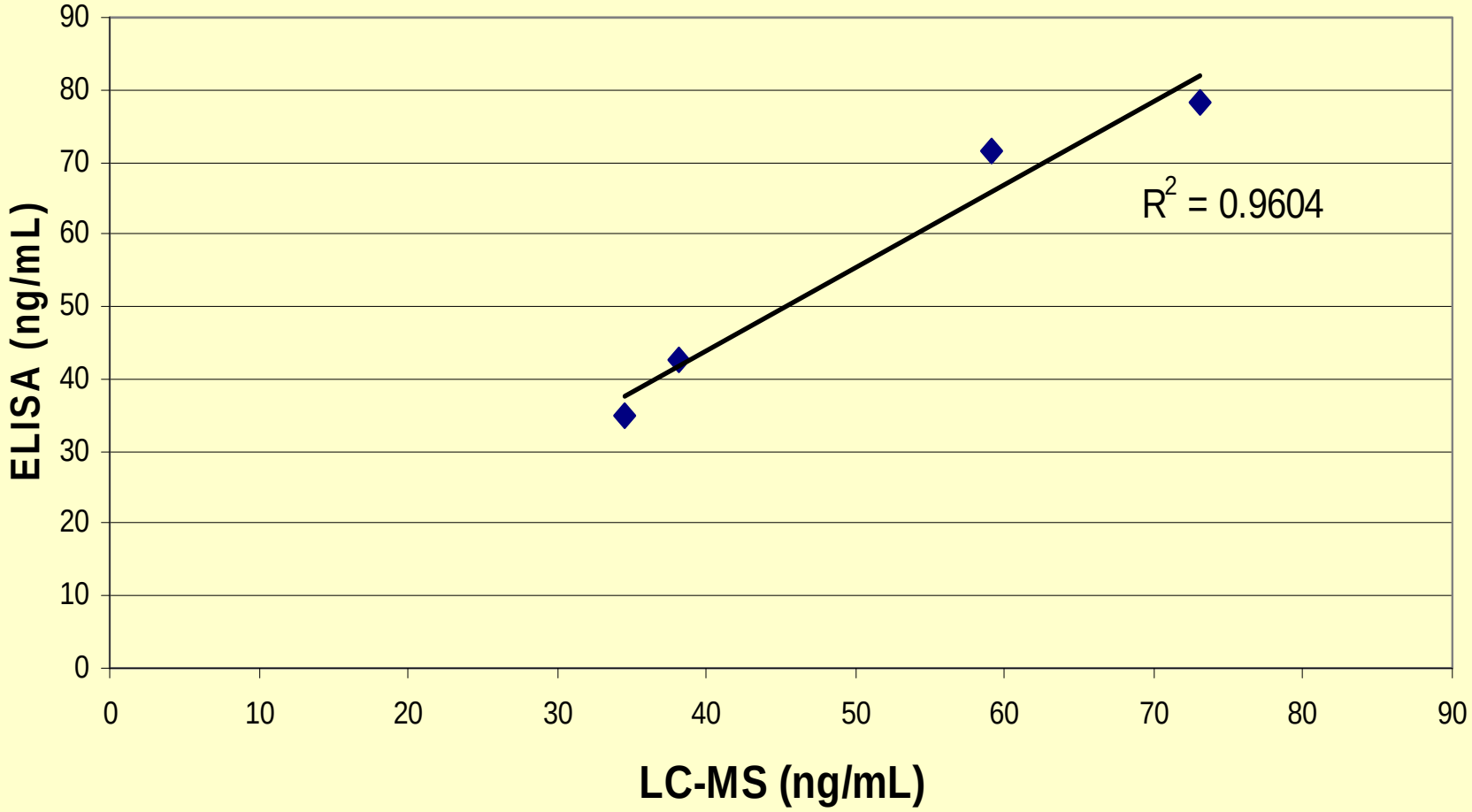
	ELISA				LC-MS	
	Unpreserved Samples (N=4, each)		Preserved Samples (N=4, each)		Preserved Samples	
Expected ng/mL	Measured (ng/mL)	% Recovery	Measured (ng/mL)	% Recovery	Measured (ng/mL)	% Recovery
0.0	0.0		0.0		0.0	
20.0	18.1	90.5	17.4	87.0	14.9	74.5
40.0	41.5	103.8	38.0	95.0	33.4	83.5
80.0	81.0	101.3	106.0	132.5	75.5	94.4
160.0	186.0	116.3	197.0	123.1	154.9	96.8
Average		102.9		109.4		87.3
S.D.		10.6		21.8		10.3

ELISA versus LC-MS

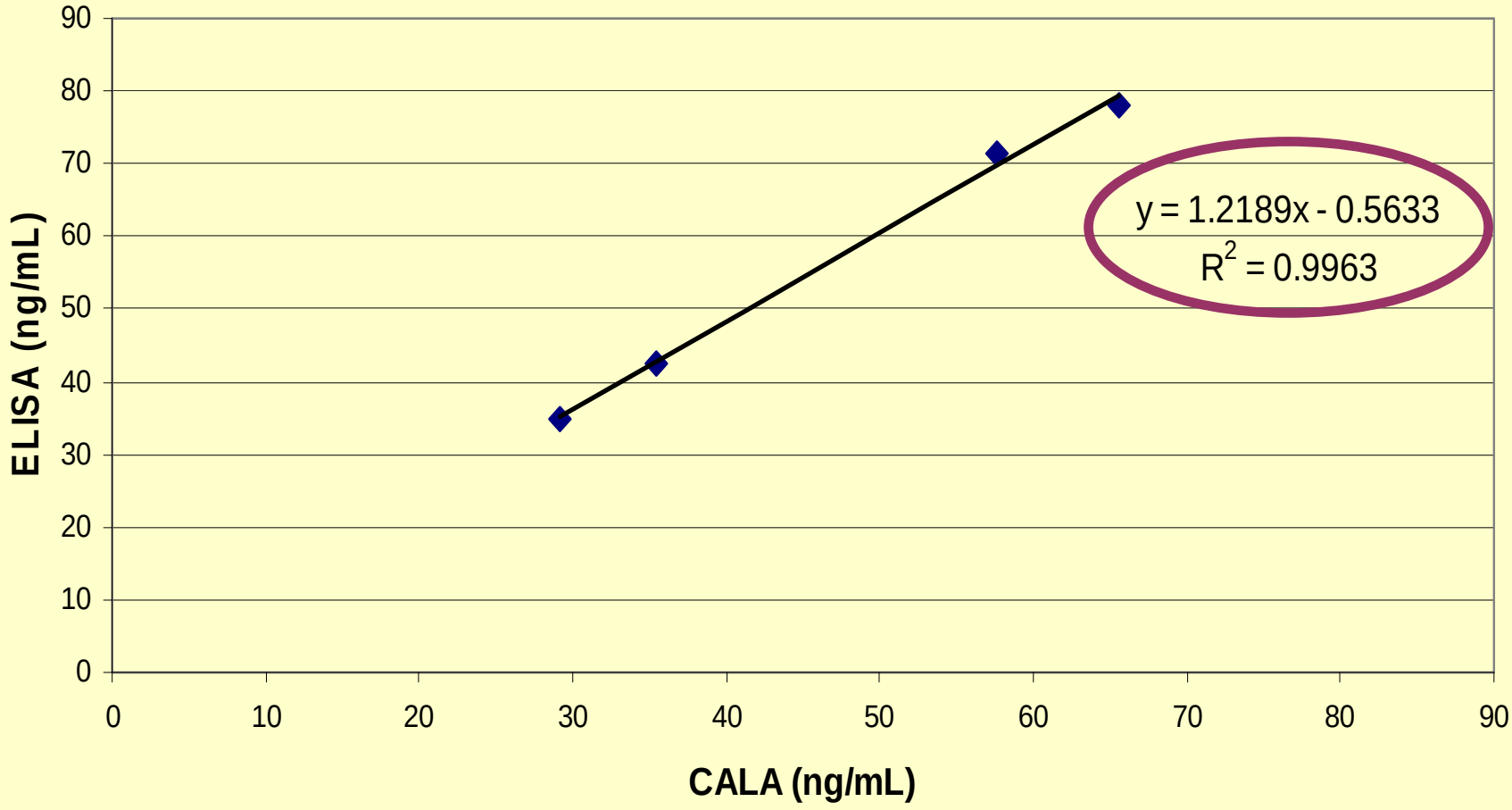
MAC = 280 ng/mL



Canadian Association for Laboratory Accreditation (CALA) QC Samples: ELISA vs. LC-MS



CALA QC Samples: ELISA vs. CALA



LC-MS Results of 502 Water Samples in 2007

	LC-MS Results	ppb (ng/mL)	Number
Positive	≥ MAC	≥ 280	3
	< MAC	21 to 279	10
Negative	< T*	2 to 20	11
	< W**	< 2	478

*T = A method attribute used to qualify low-level data

** W = A method attribute used to qualify or censor data

97% of samples glyphosate-negative

Conclusions

1. ELISA can be used as both screening and quantitative technique
2. Manufacturer: dynamic working range for ELISA is 0.15 to 5 ng/mL
3. Poor precision and accuracy at 0.15 ng/mL (MAC = 280 ng/mL)
4. Faster and less expensive than conventional analytical techniques
5. Could screen out 97% of samples (2007) with an estimated savings of $237,000

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Acknowledgements

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CALA QC Samples: LC-MS vs. CALA