

Detection of Herbicides and Their Metabolites Using Cross Reactivity of Enzyme Immunoassays

E.M. Thurman

The 4th Pacifichem Conference

Honolulu, Hawaii

December 14-19, 2000



Literature of Immunoassay for Pesticides

- > 100 published papers in past 10 years
- Environmental Reviews and several ACS Symposia Volumes
- Many IA kits validated by US EPA

Usefulness of ELISA

- Pesticide Surveys
 - Triazines in Rainfall: 6,000 Assays
 - Alachlor in Rainfall: 6,000 Assays
 - ES&T (1997) v. 31, p. 1325, Goolsby et al.
- Value of ELISA
 - Inexpensive
 - Rapid

Concerns Using ELISA

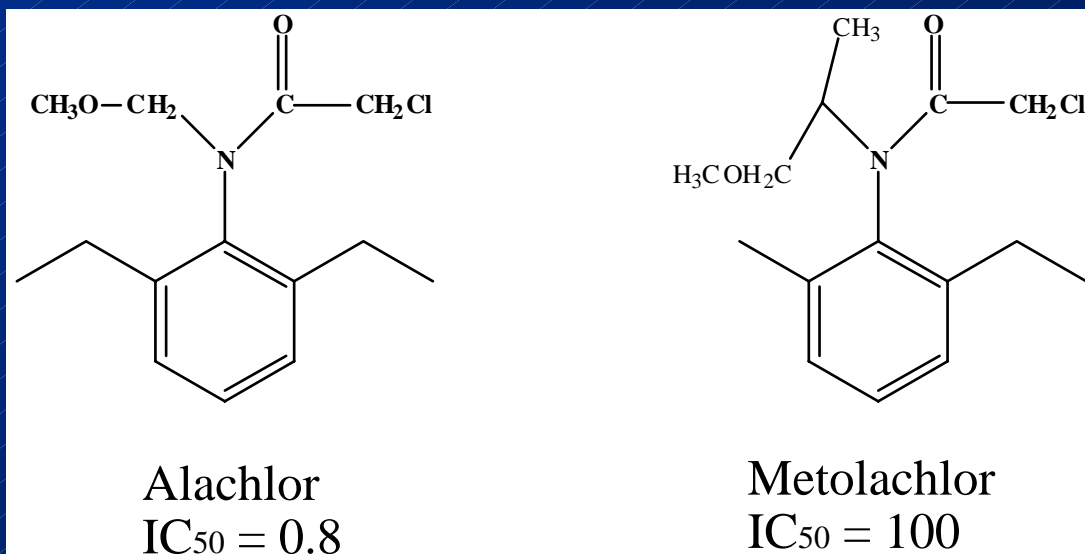
- Sensitivity or IC_{50}
- Cross reactivity to similar compounds
- Can a concern be a tool for discovery?

Water-Quality Surveys as Examples

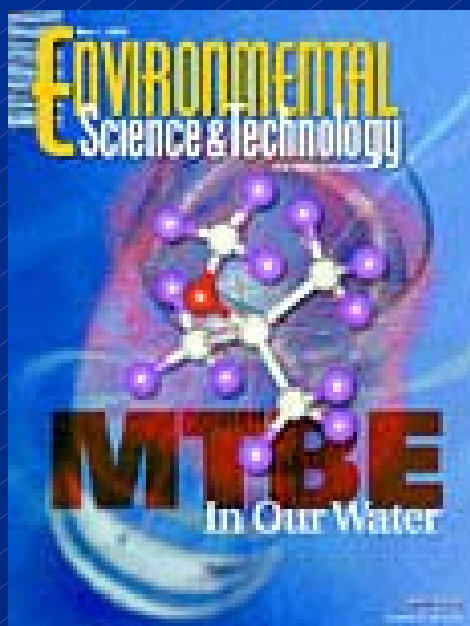
- Alachlor in Ground Water
 - Alachlor metabolite in Ohio, Illinois, and Indiana
- Atrazine in Playa Lakes
 - Atrazine and Prometryn in West Texas

ELISA Sensitivity and Cross Reactivity

- Binding of target compound and IC_{50}
- Do other similar compounds bind?



Survey of Ground Water

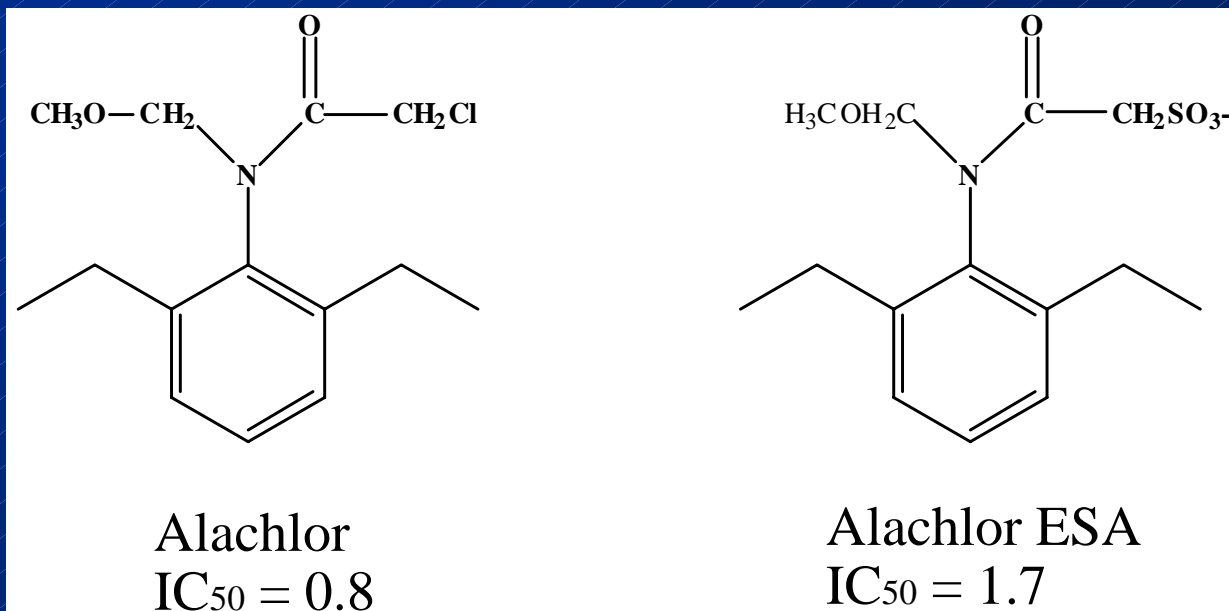


1. Survey of alachlor in GW from Ohio, Indiana, and Illinois shows many alachlor detects.
2. GC/MS does not match ELISA results with all false positives.
3. Leads to discovery of alachlor ESA by HPLC.

ES&T (1993) v. 27, p. 562, Baker et al.

Cross Reactivity Occurs

- Cross reactivity occurs with sensitivity because of similar structure.



Method Adaptation

C-18 SPE



1. Sorbalachlor andalachlor ESA on C-18.
2. Elutealachlor with ethyl acetate.
3. Elutealachlor ESA with methanol, dilute and use ELISA for analysis.

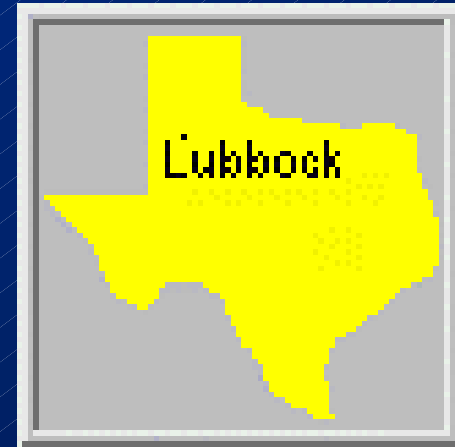
Using Cross Reactivity for Analysis



Determination of alachlor and its sulfonic acid metabolite in water by solid-phase extraction and enzyme linked immunosorbent assay:

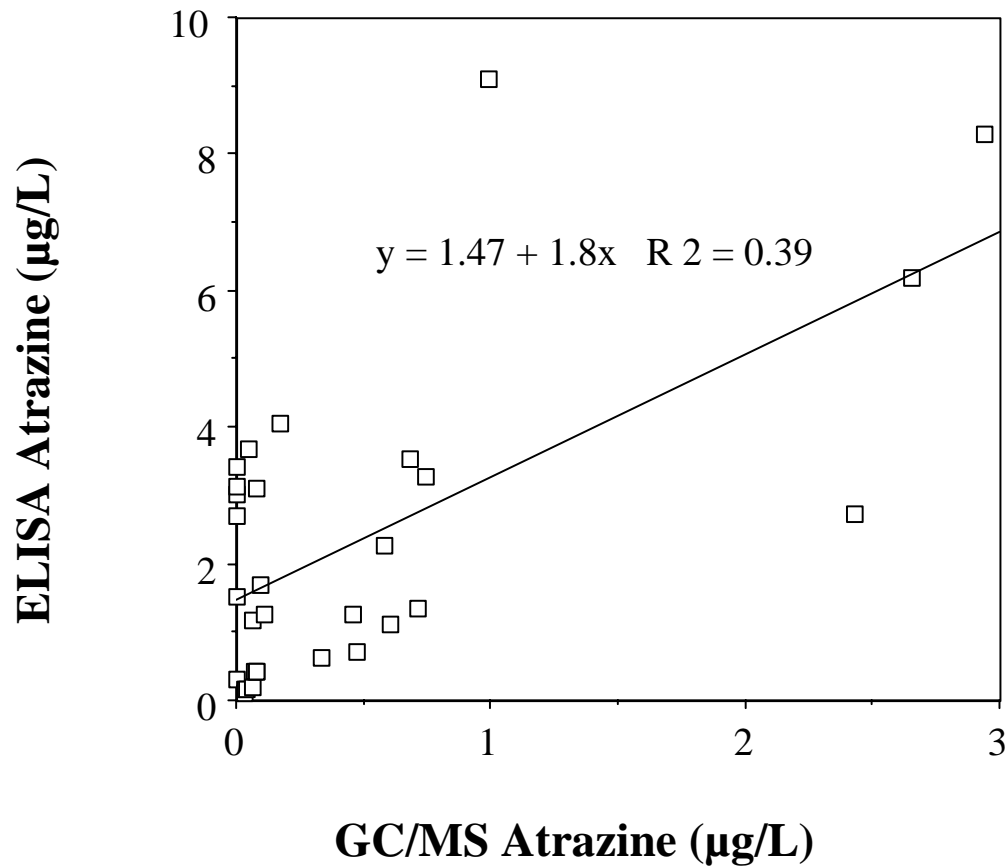
Analytical Chemistry, v.66, p. 1495 (1994), Aga and Thurman.

Atrazine in Playa Lakes, West Texas

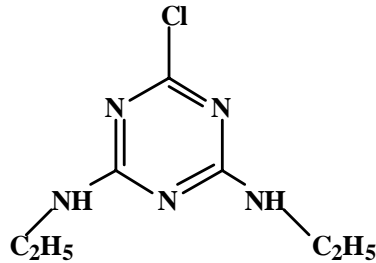


1. Survey of 30 playa lakes in West Texas for atrazine using ELISA.
2. Poor results obtained when compared to GC/MS.

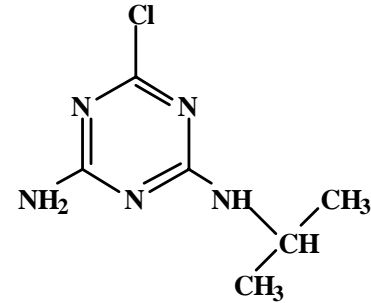
Comparison of ELISA and GC/MS for Atrazine



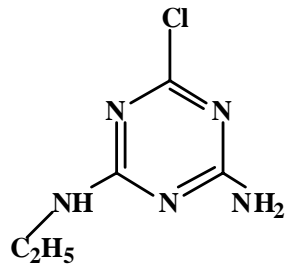
Cross Reactivity of Triazines



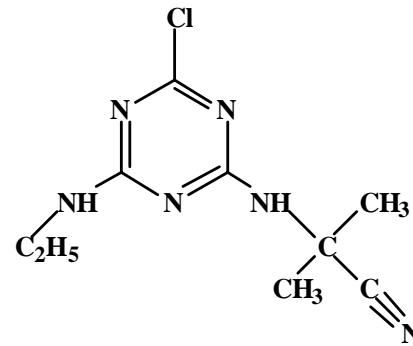
Simazine
IC₅₀ = 4.9



Deethylatrazine
IC₅₀ = 3.2

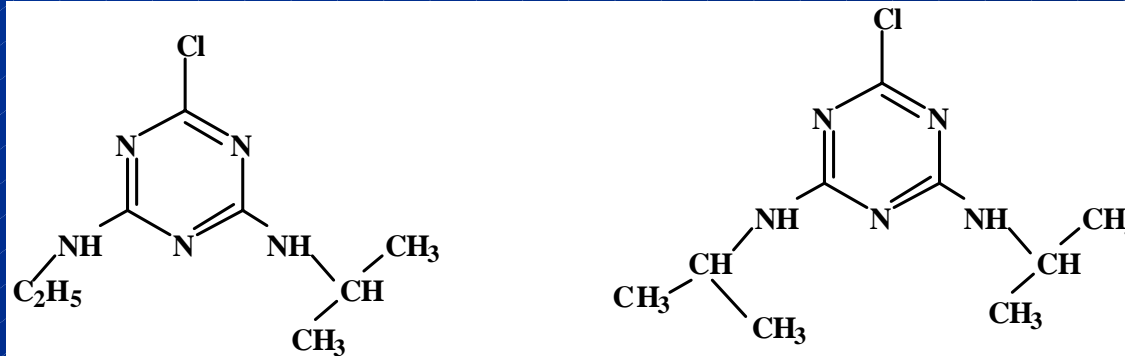


Deisopropylatrazine
IC₅₀ = 220



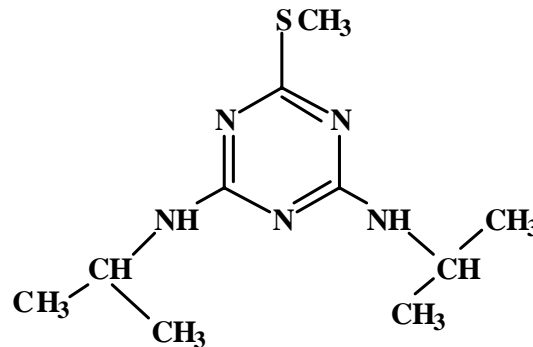
Cyanazine
IC₅₀ = 10,000

Cross Reactivity of Triazines



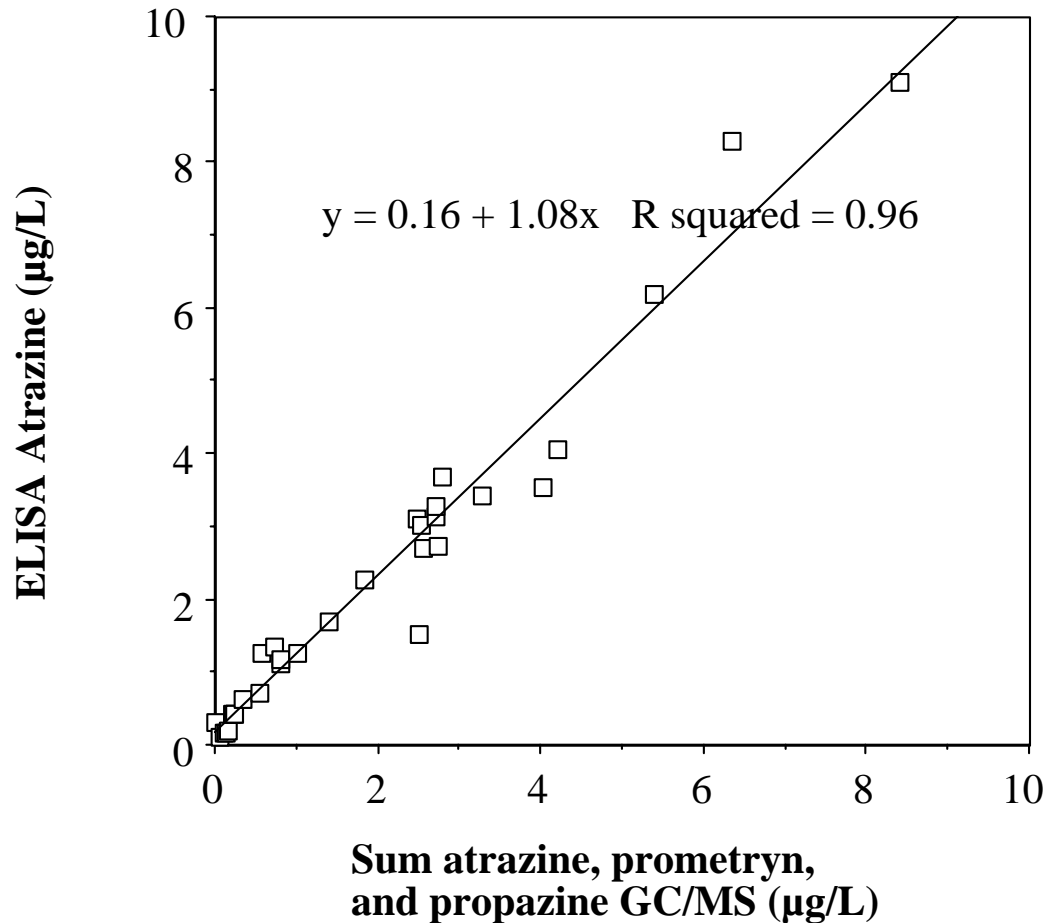
Atrazine
 $IC_{50} = 0.72$

Propazine
 $IC_{50} = 0.74$



Prometryn
 $IC_{50} = 0.64$

Comparison of ELISA and GC/MS for Triazines



Important Findings in West Texas Survey

- Prometryn was discovered as an important herbicide used on cotton in West Texas.
- Propazine, which has been off the market since 1990, is still used in West Texas on sorghum.
- Science of the Total Environment (2000), v. 248, p. 189, Thurman et al.

Conclusions

- Alachlor survey on Ground Water
 - Discovery of the alachlor ESA
- Atrazine in Playa Lakes
 - Prometryn and propazine
- Conclusion: cross reactivity is not an artifact in pesticide chemistry

Future Work

- Future work involves the cyclodiene insecticides and cross reactivity.
- Recent comparisons with GC/MS and ELISA don't match.
- A search for new degradates is underway

Acknowledgments

- Thank Diana Aga, University of Nebraska for ELISA work on alachlor
- Thank Tony Mullhagen at Texas Tech in Lubbock for work on playa lakes

