A Simple Spreadsheet for Estimating Low-Effect Concentrations & Associated Confidence Intervals With Logistic Dose Response Curves



Mary Barnes, Ray Correll and Daryl Stevens,

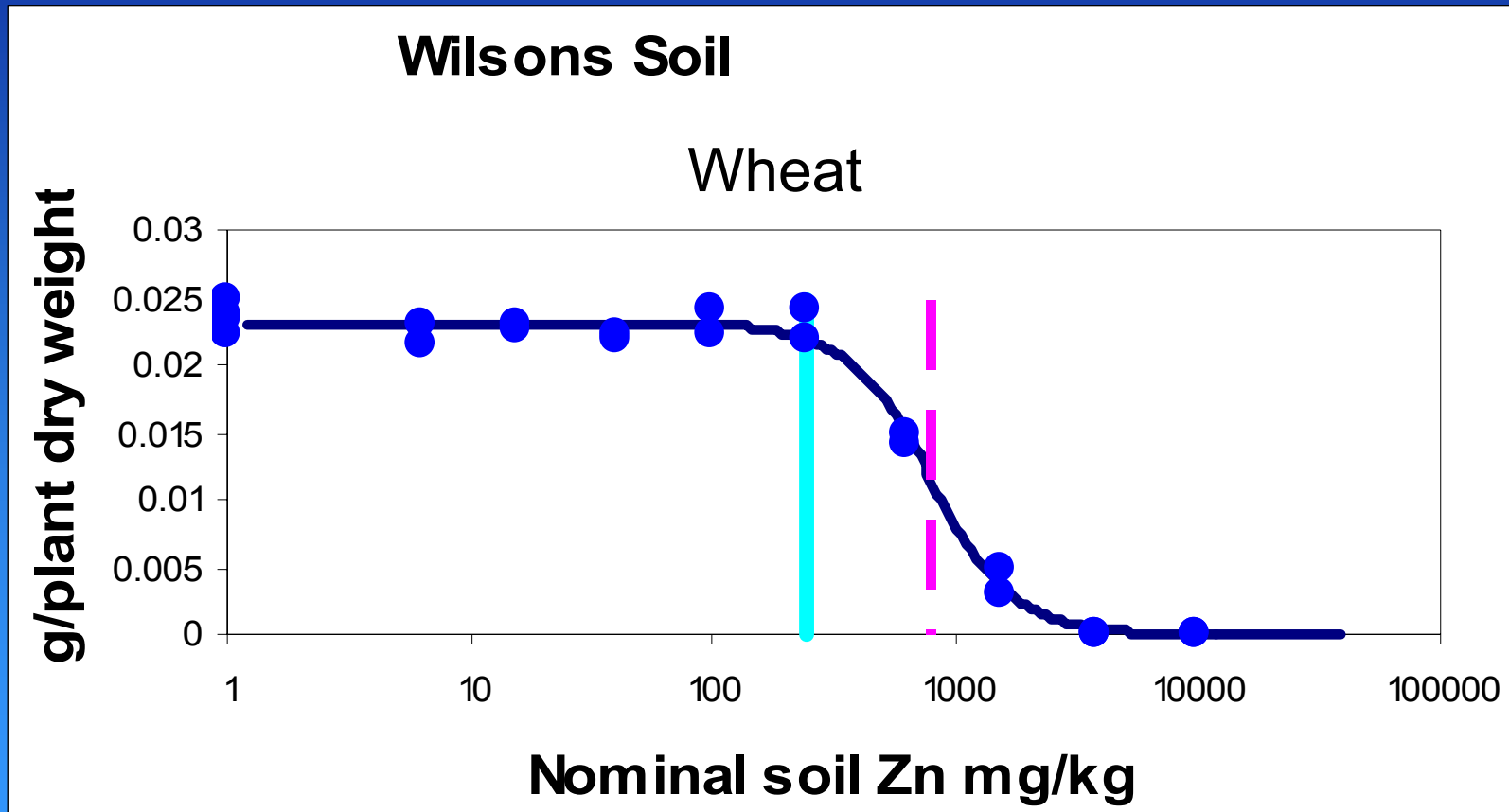
CSIRO Flinders University, Rho Environmetrics, Atura Pty Ltd

Work funded by

Australian Government

Australian Centre for International Agricultural Research

Low Effect Concentrations (EC)



Why Logistic?

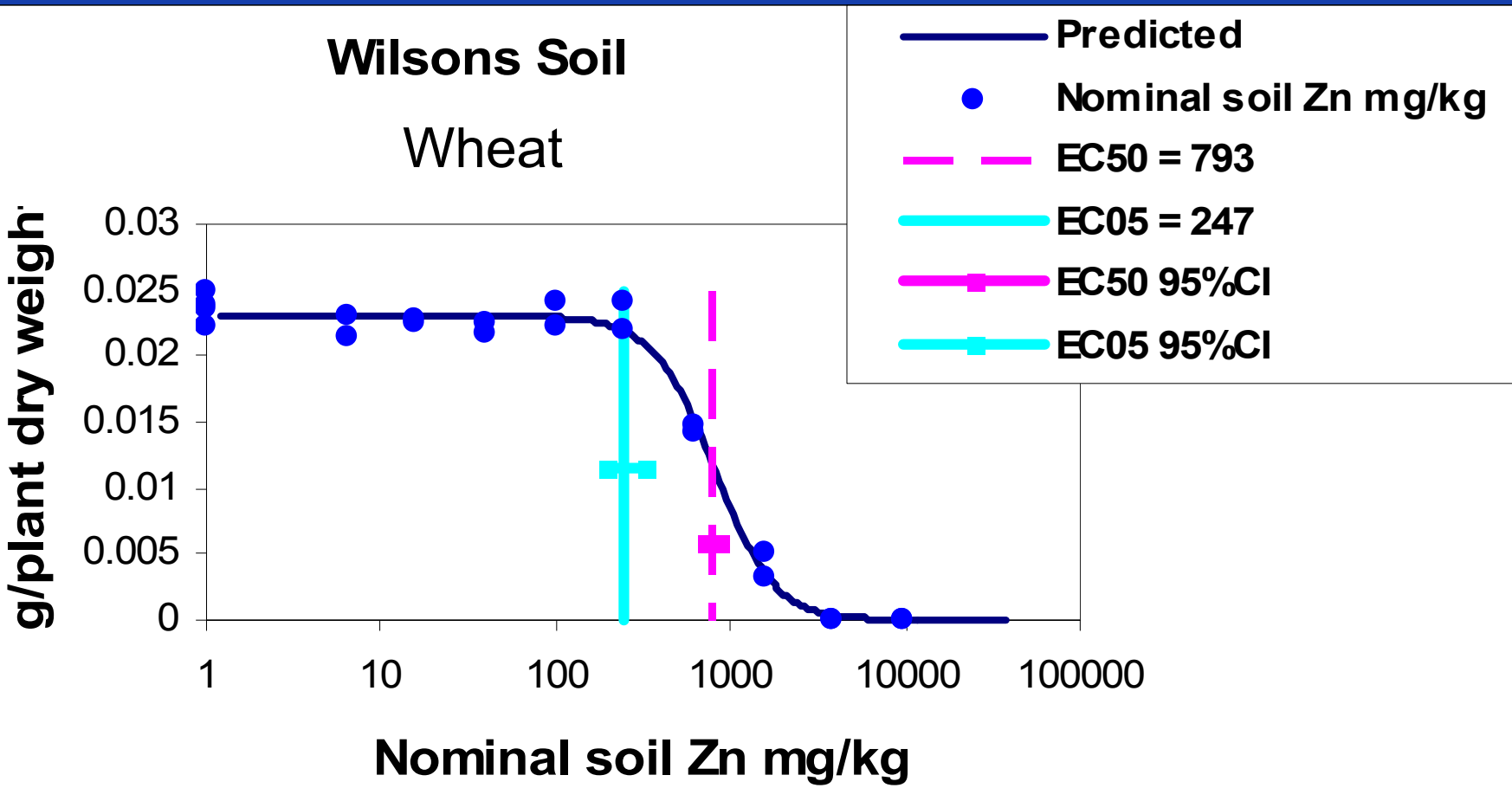
Want to estimate **EC05, EC10, NOEC**

Sigmoidal logistic curve was chosen over other models (including Probit, Weibull) because

1. is commonly used,
2. is **reasonably robust,**
3. **generally fits data reasonably well**.

Large sample sizes needed to distinguish between the different models

Low Effect Concentrations (EC)



To Weight or not to Weight?

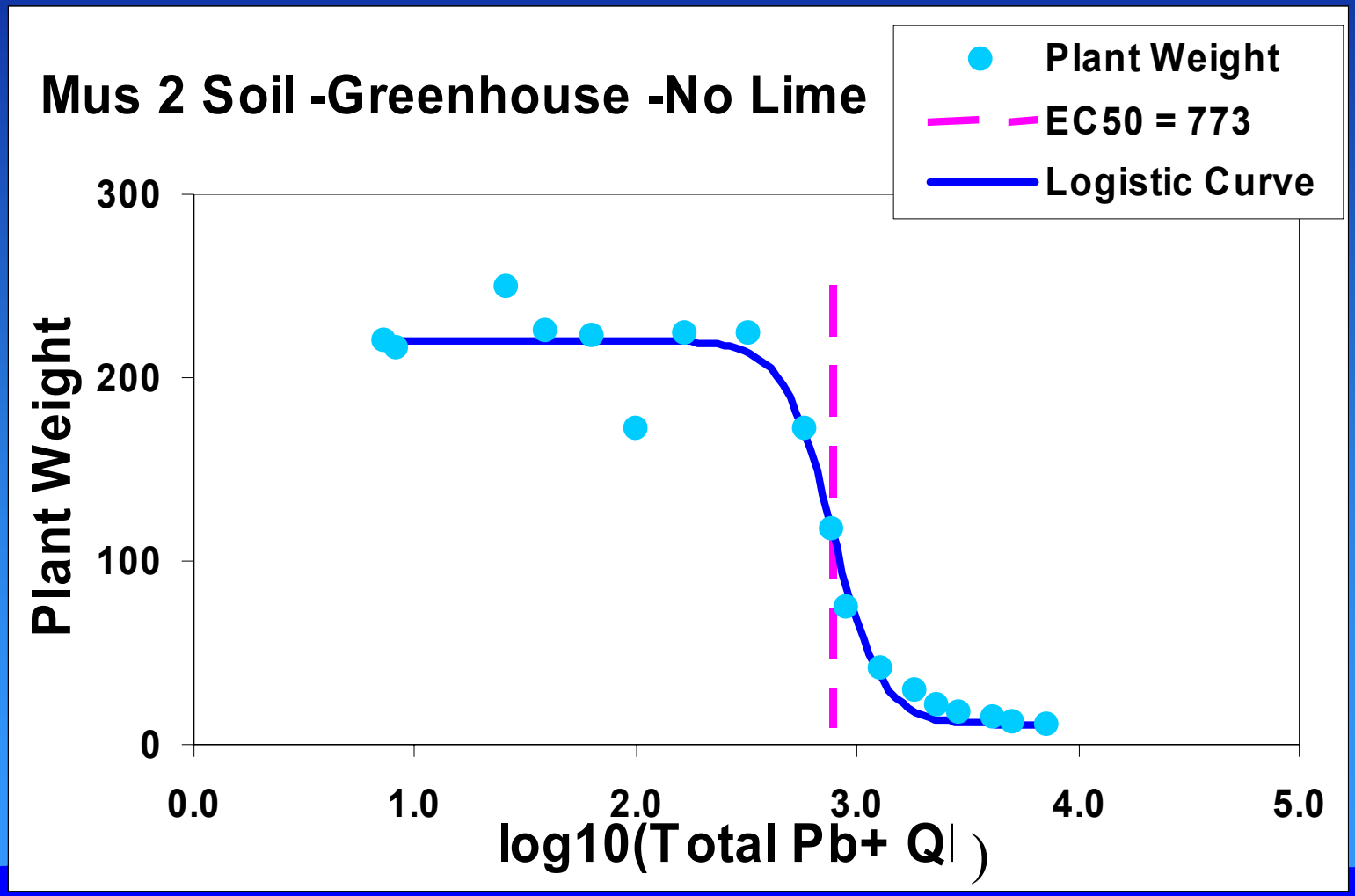
Largest variation typically observed around maximum, minimum variance around zero (lower asymptote)

Some authors weight data by inverse of variance

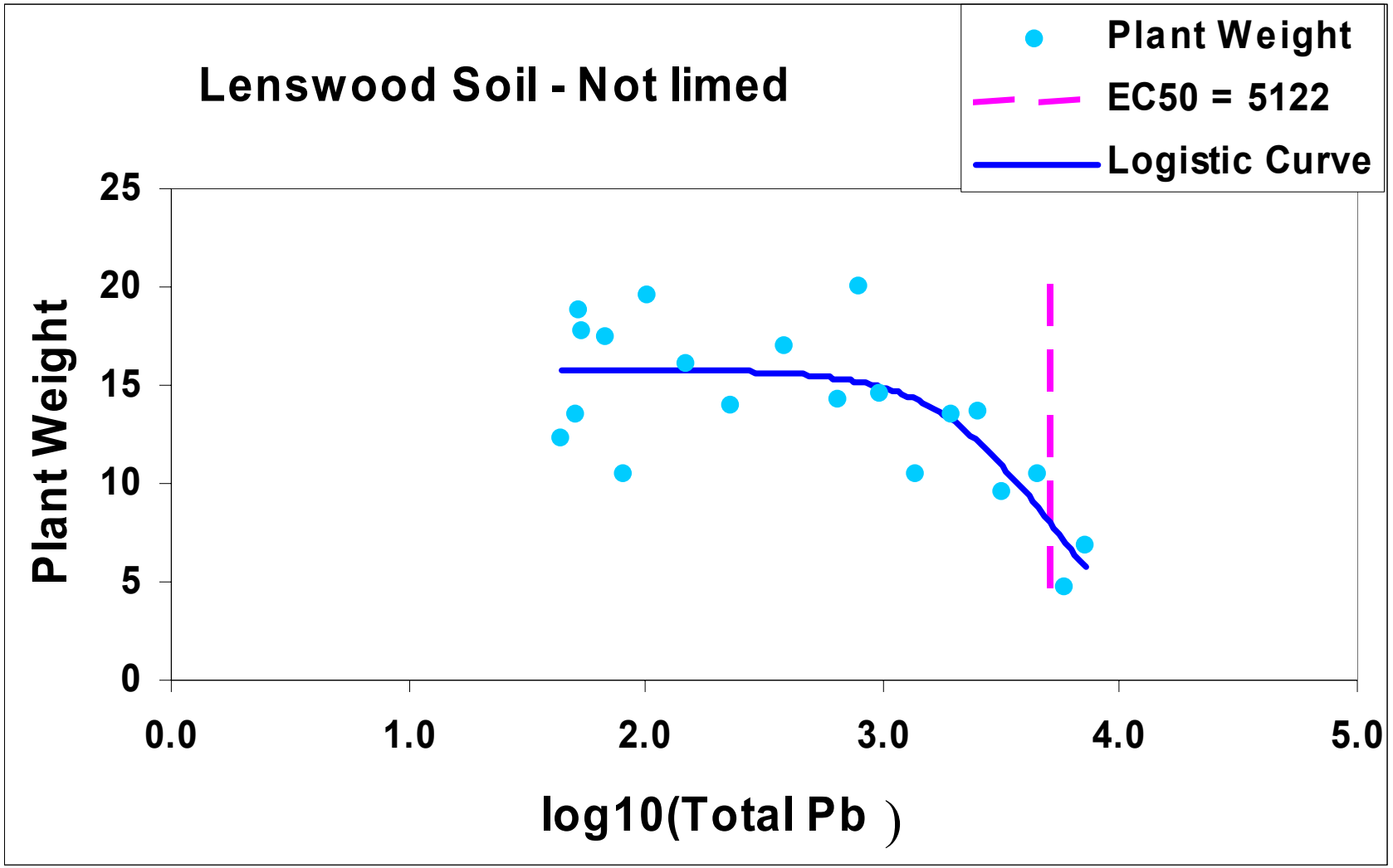
Data with minimum variance (around zero) gets largest weight. Interested in low EC's, so like to take most notice of data close to maximum

When estimating low effect concentrations, it seems more logical to NOT weight residuals. Resultant curve is strongly influenced by the observations closest to low effect concentrations (EC5 or EC10)

Another example



High Variation



Why develop Spreadsheet

Many programs exist for dichotomous (dead/alive) situations. Only a few for continuous variables

Researchers wanted something

- **Simple to use (found Genstat difficult)**
- **Reliably converges**
- **Confidence intervals around EC of interest**
- **Flexibility to easily adapt and**
- **Minimal training required**

File Edit View Insert Format Tools Data Assistants S-PLUS Window Help Acrobat

Type a question for help

E3 Wilsons

Rate	Response
Nominal soi	g/plant dw
3880	0
3880	0
9700	0
9700	0
1552	0.0031429
1552	0.005
620.8	0.0142
620.8	0.0148
6.356992	0.0213
39.7312	0.0218
248.32	0.0219
1	0.0222
99.328	0.0222
39.7312	0.0224
15.89248	0.0226
15.89248	0.0228
6.356992	0.0231
1	0.0235
1	0.0238
248.32	0.024

Effect Concentrations (EC) calculated using Logistic (Sigmoidal) curve

by minimising unweighted squared residuals sum

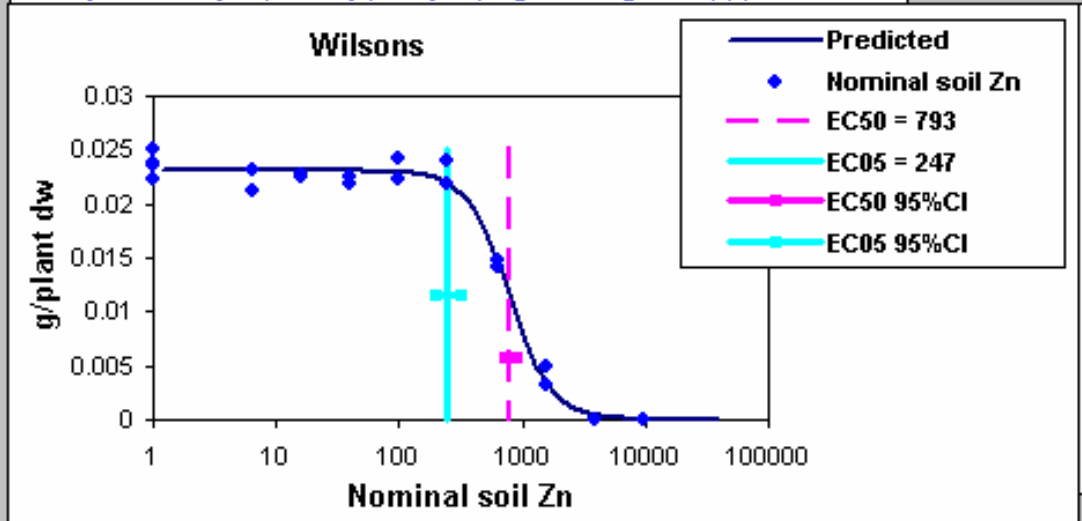
Heading	Wilsons	
EC of interest	5	

		Suggestion
Top	0.02306	0.025
log EC50	2.89918	3.19
slope	-5.8233	*Steep < -3*

Instructions (in green)

1. Paste data in columns A & B. Fill in heading & EC of interest.
   Change zero Rates to < half lowest non zero Rate. Remove blanks.
2. Guess curve parameters (E7:E9) until graph looks reasonable
2b. Sort data by g/plant dw ascending for logEC50 first guess
3. Optimise using Tools Menu, Solver, Solve, Keep if converges

Response=Top / (1+ exp(-slope*(logRate-logEC50)))



Solver Hints

Click on a cell before Solving. Solver won't work if you are on graph

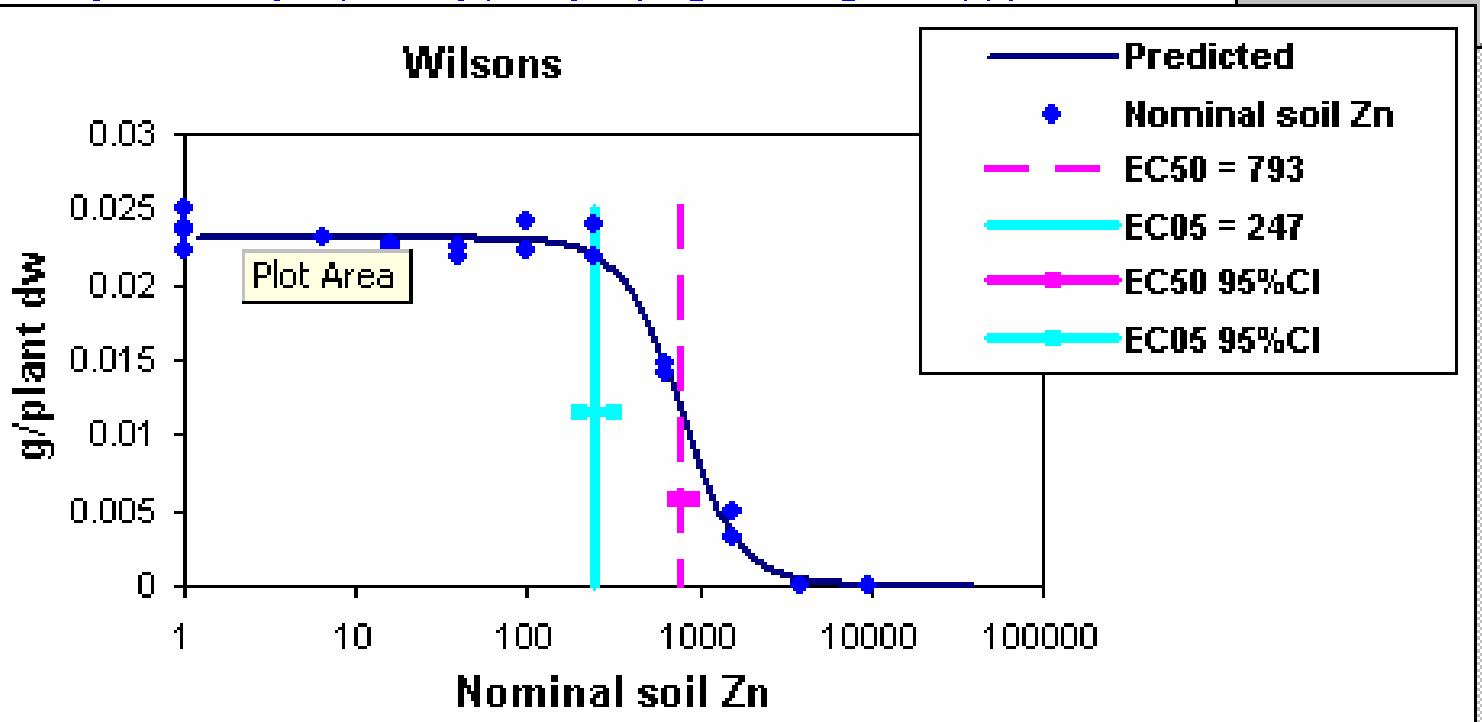
If do not have Solver in Tools Menu then get via Tools menu, Add-ins, Solver

Sum of Squared Residuals
2.03798E-05

Sheet1 Sheet2 Sheet3

ady NUM

Response=Top / (1+ exp(-slope*(logRate-logEC50)))



Results	estimate	s.error
Top	0.023	0.000
log EC50	2.899	0.022
slope	-5.823	0.577
log EC05	2.394	0.052

95% Confidence intervals on natural scale		
EC50	714.0	880.4
EC05	192.3	318.5

Sheet1 Sheet2 Sheet3

Ready NUM

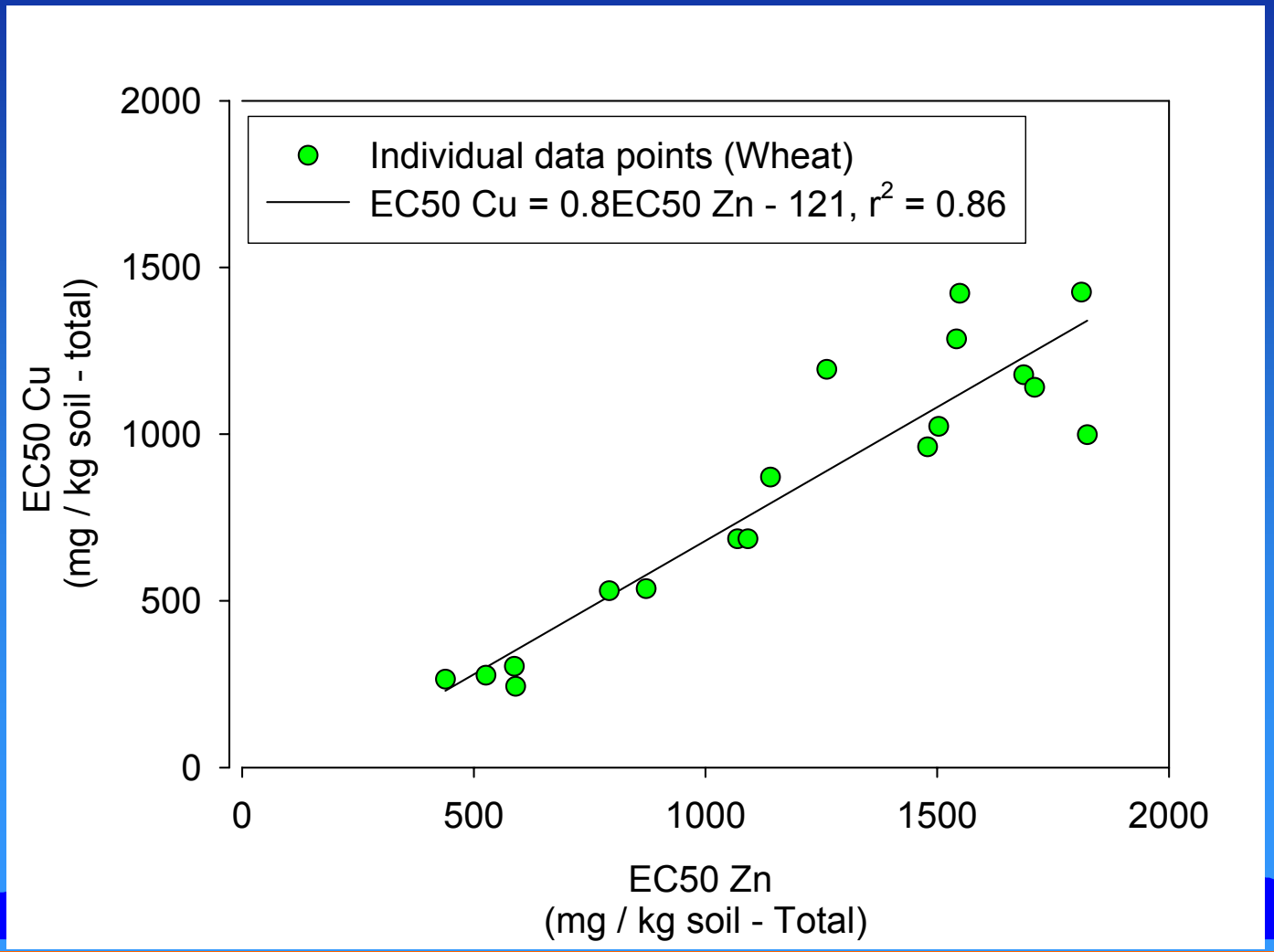
Spreadsheet Benefits

* **Simple to use**
* **Reliably converges sometimes better than other stats packages**
* **Confidence intervals around EC of interest**
* **Wide variety of uses (bio-availability & toxicity of metals or pesticides to plants, worms and other indicator organisms**
* **Flexibility to easily adapt (has been used to compare ECs between soils) and**
* **no special software is required.**

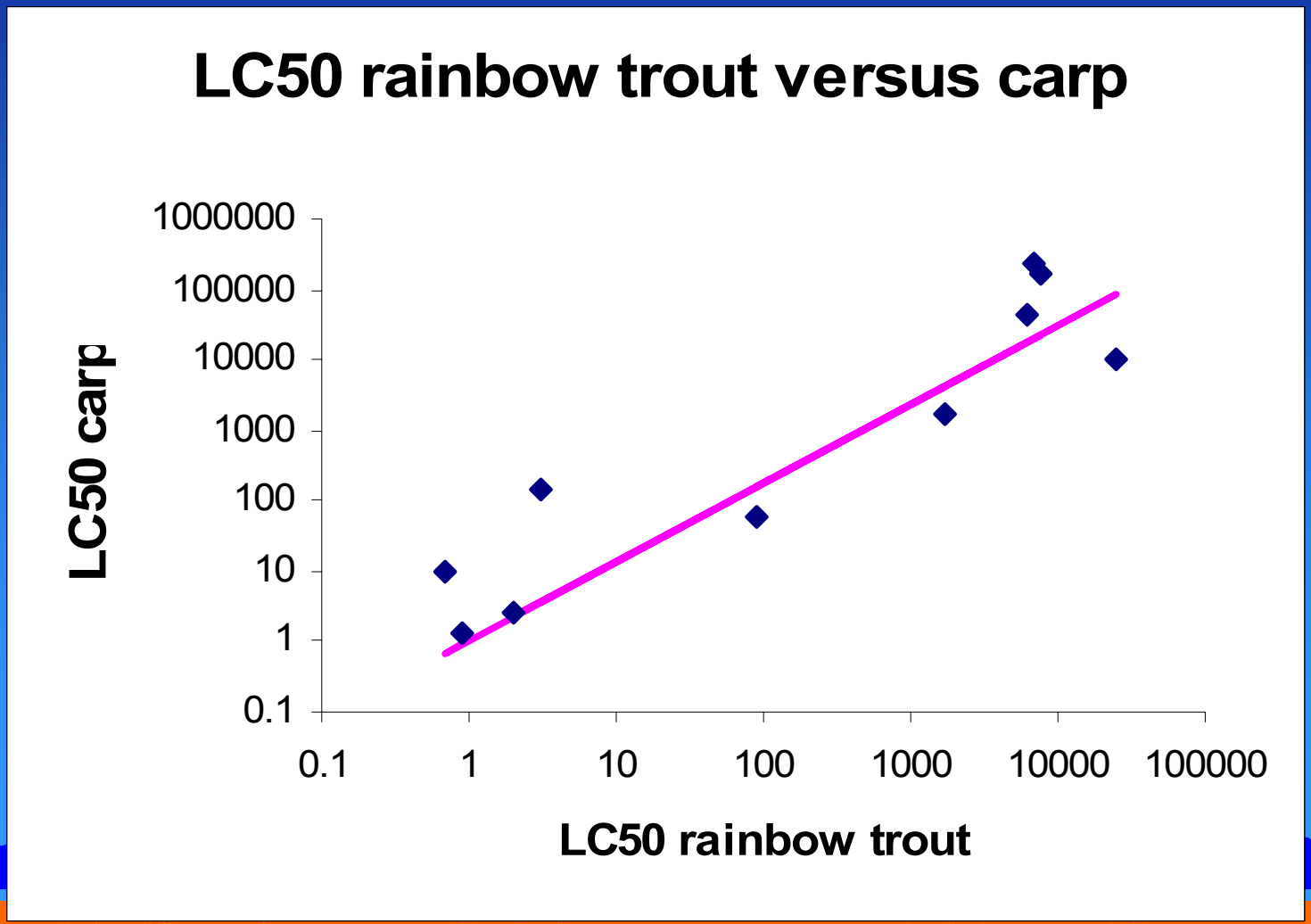
Future Work

- Bootstrap most influential points only
- Minimise residuals in both rate/response directions
- Bayesian slope estimates (similar species, toxicants) to assist in estimates of low ECs
- Fitting many curves weight by goodness of fit
- Inconsistencies between packages (US EPA)

Estimating EC50s from other toxicants/ metals



Estimating EC50 or LC50's from other species



Statistical Lessons:

Estimating Bio-availability & Toxicity of Metals to Plants

We recommend

- Estimate EC50's where possible
- Using an **asymmetric design**,
  (**more below EC50**, less toxic)
- Having some points near EC50
- Not weighting data, and
- Cautiously estimate EC10 or EC5.

Acknowledgments & Contact

Acknowlegements: ACIAR

Anne Boutros, Anu Kumar, Michael Rumsewicz

Contact: Mary Barnes

Mary.Barnes@flinders.edu.au

Solver Algorithm

The Microsoft Excel Solver tool uses the **Generalized Reduced Gradient (GRG2) nonlinear optimization code** developed by Leon Lasdon, University of Texas at Austin, and Allan Waren, Cleveland State University.

Linear and integer problems use the **simplex method with bounds on the variables, and the branch-and-bound method**, implemented by John Watson and Dan Fylstra, Frontline Systems, Inc. For more information, contact:

Frontline Systems, Inc.
Web site: http://www.frontsys.com
Electronic mail: info@frontsys.com