

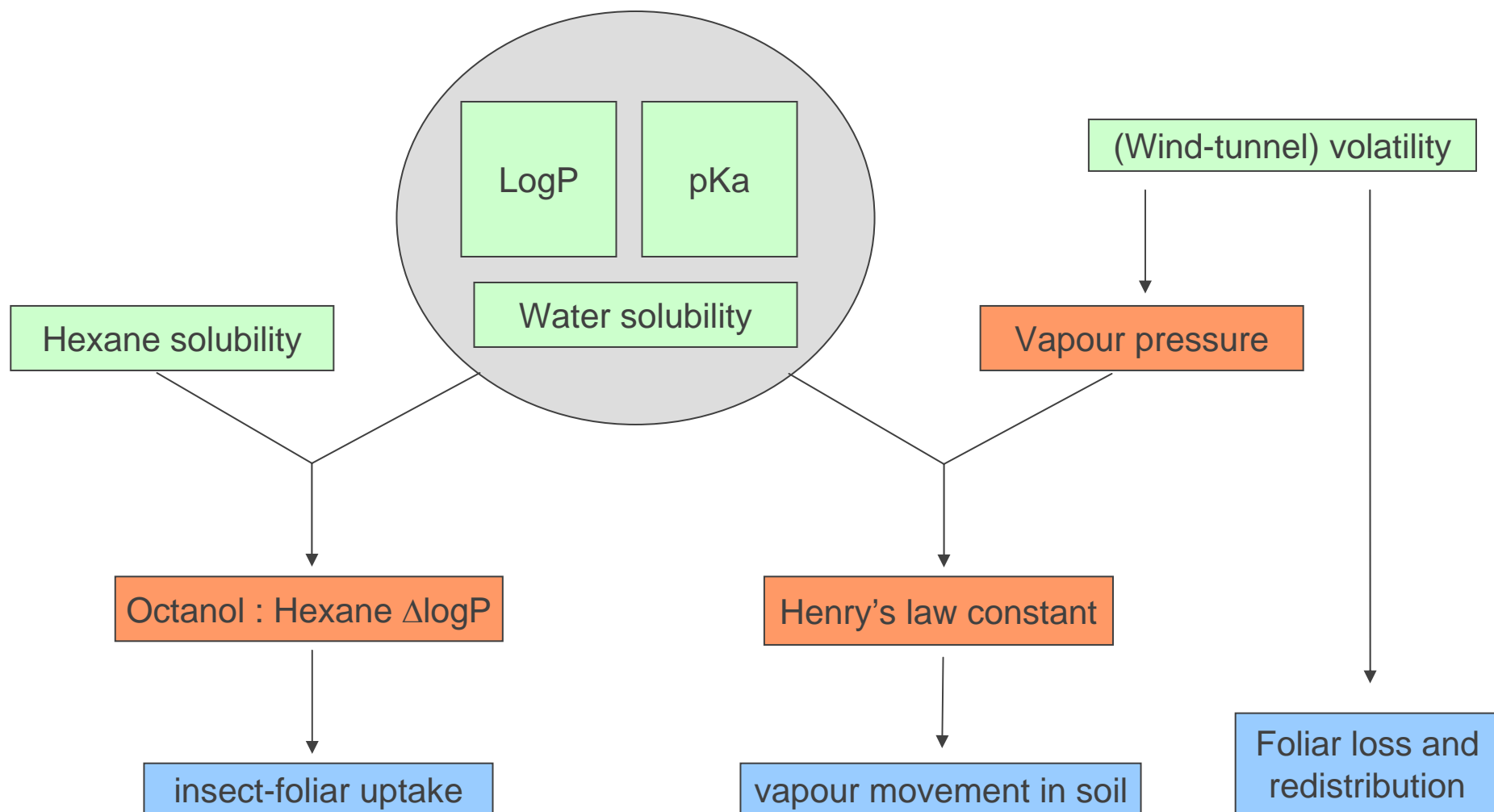


Chromatographic Hydrophobicity Index (CHI) Application to Agrochemical Research

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Properties affecting agrochemical availability and movement



Measured properties

Derived properties

Modelled properties

Direct Measurement of log P octanol

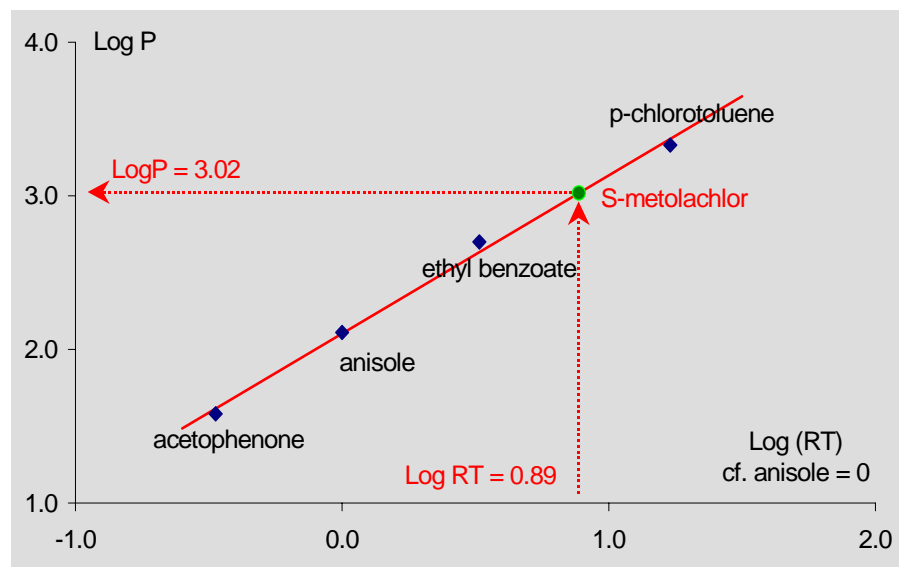
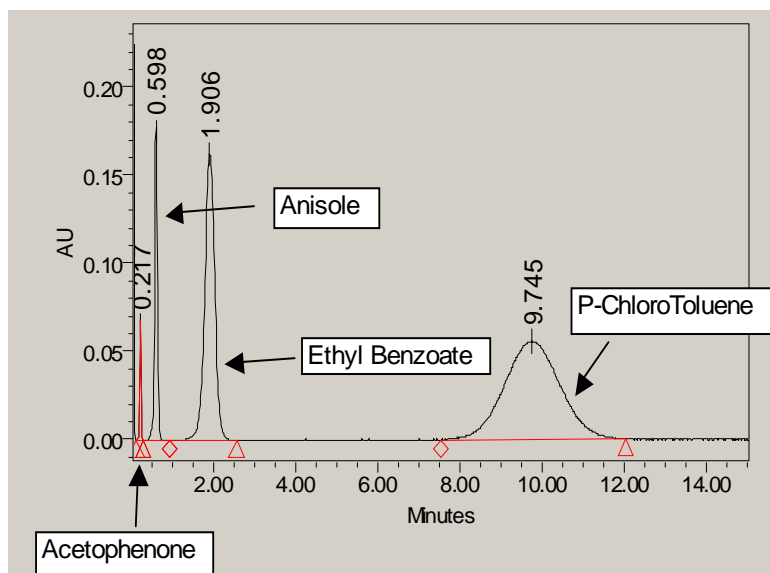
1) Shake-flask (rarely used in Syngenta)

- resource-intensive, experimentally very difficult when $\log P > 3$

2) HPLC on octanol-coated mini-columns (primary method for Syngenta)

- **not** a model system - genuine octanol-aqueous partitioning
- measurement time $\propto P$

$\log P < 3 \rightarrow < 10$ mins; $\log P > 4.5 \rightarrow$ several hours; $\log P > 5$ not possible



3) Generator column (occasional use in Syngenta)

- Can give reliable values up to $\log P = 7$ or more
- More effort and sample required, perhaps one man-week /determination

Measurement of acid-base dissociation constant (pKa)

Traditional methods :-

- UV-visible spectroscopy
- Potentiometric titration

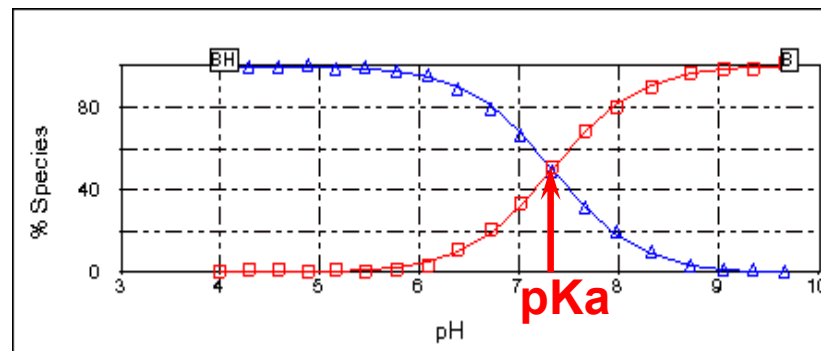
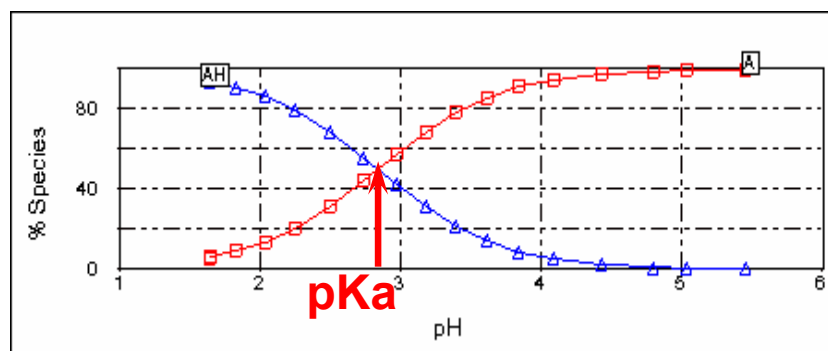
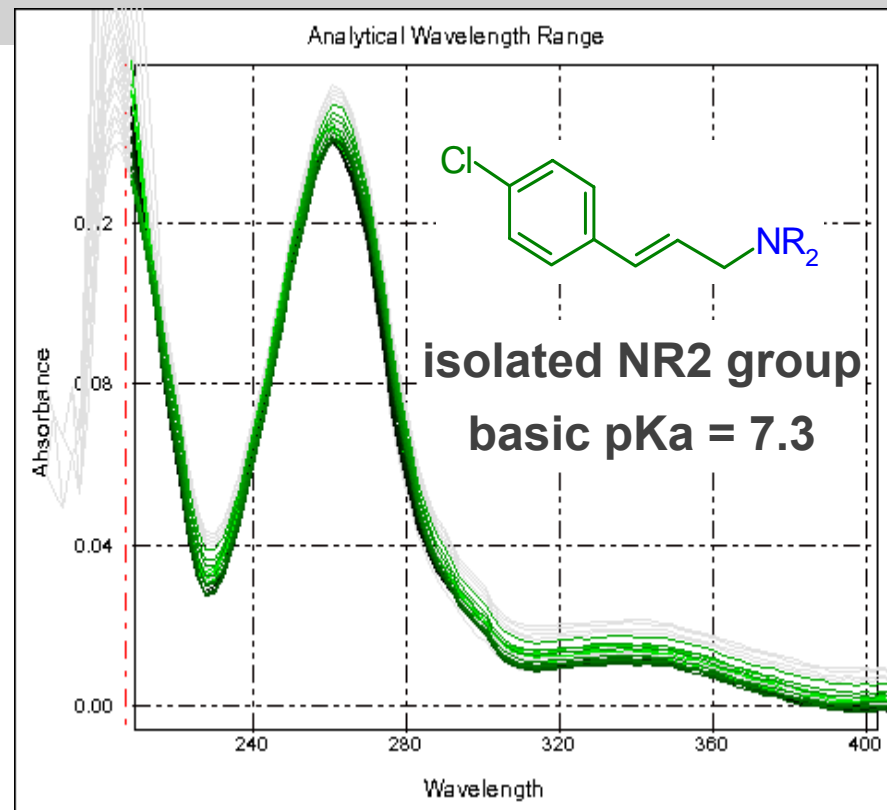
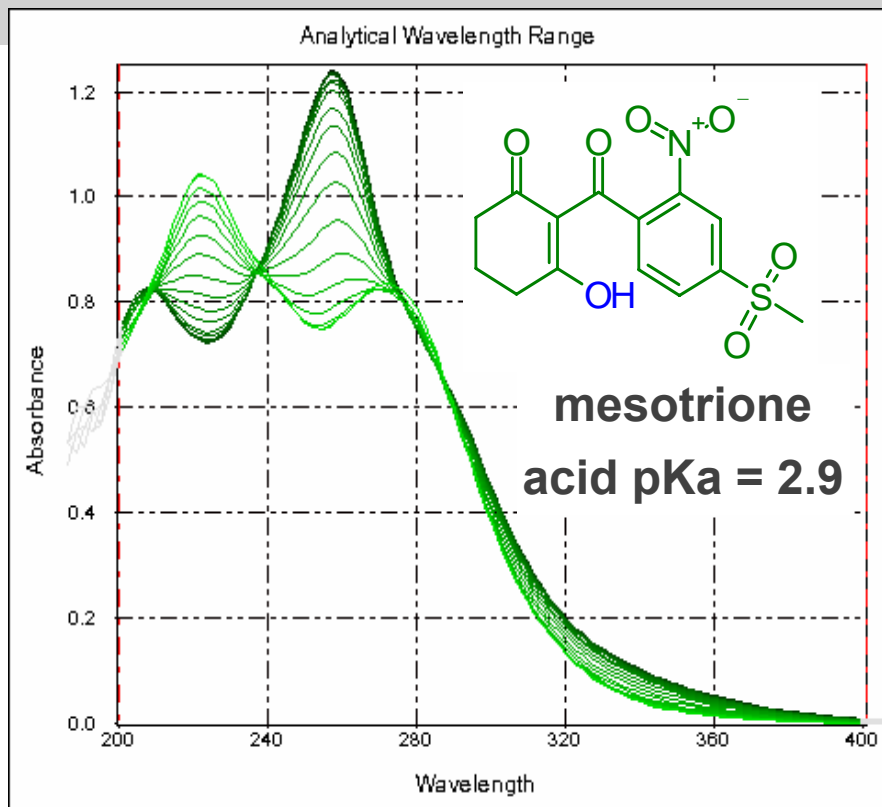
Sirius GLpKa autotitrator automates both these methods (DPAS module for UV-vis)

Use in agrochemicals (at medium throughput)

- use of **potentiometry** seriously restricted by solubility, even with co-solvent
- **UV spectroscopy** - methanol co-solvent often required (Yasuda-Shedlovsky extrapolation)
- excellent sensitivity to small spectral changes
eg $\text{PhCH}_2\text{CH}_2\text{X}$ systems where $\text{X}=\text{CO}_2\text{H}$ or NR_2

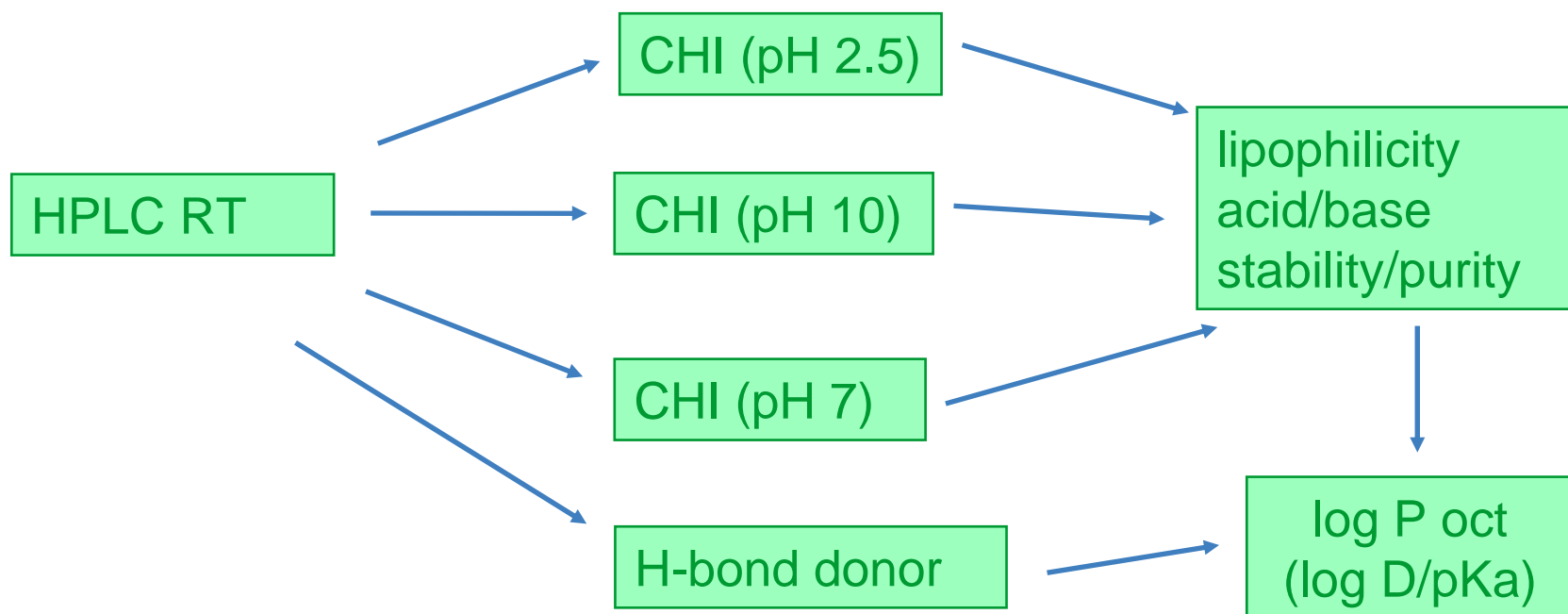
At Syngenta we often also obtain pKa data from pH dependence of logD or solubility data

Measurement of acid-base dissociation constant (pKa)



Chromatographic Hydrophobicity Index (CHI)

Fast Gradient RP-HPLC: $\text{CHI} \propto \text{RT}$: calibrated with GSK standards

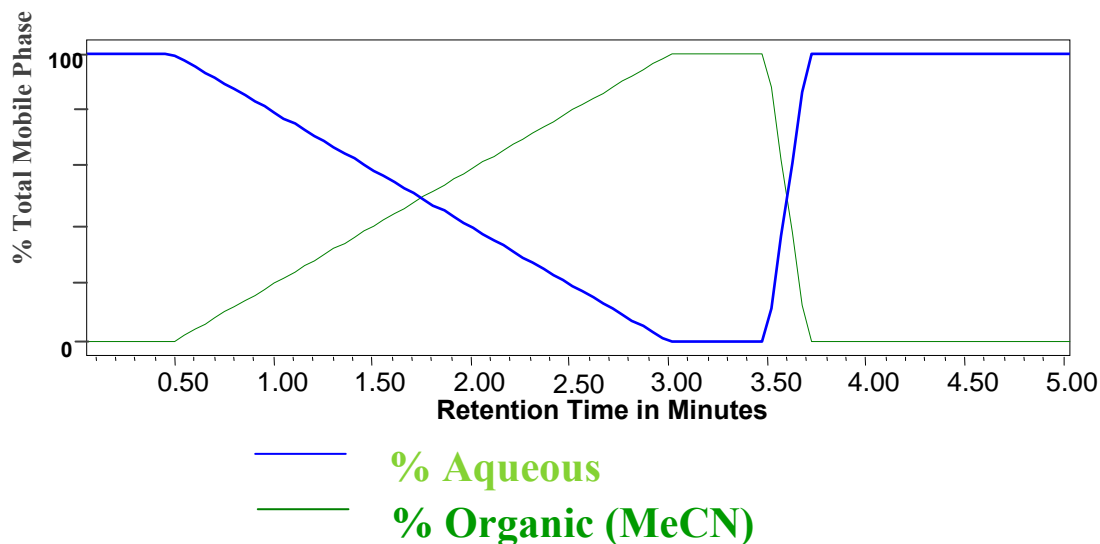


See review by K. Valko, J.Chromatography A, 1037 (2004) 299-310

Chromatographic Hydrophobicity Index (CHI)

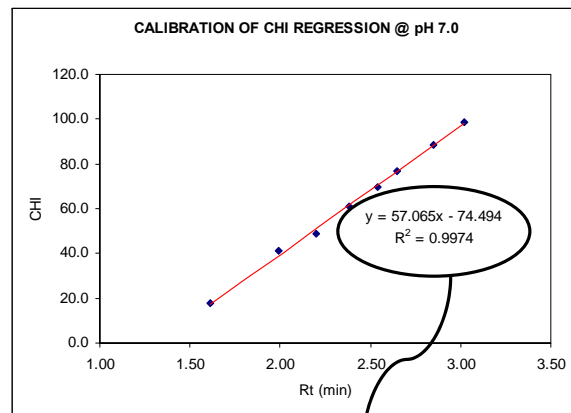
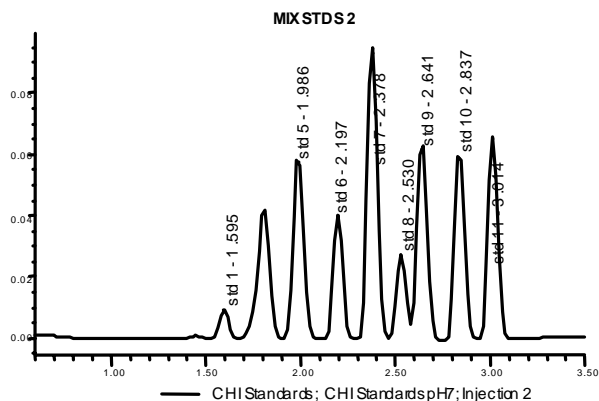
- The Chromatographic Hydrophobicity Index (CHI) is a parameter obtained via fast-gradient reversed phase HPLC with uv and/or ms detection
- The CHI value approximates to the percentage (by volume) of organic phase required to achieve an equal distribution of compound between the mobile and the stationary phases
- So, CHI is a number related to Retention Time with values often between 10 and 100, but in practice can be higher
- CHI is often determined at 3 pH's, typically with pH 2.5 and pH 10 as the extremes and it is assumed that the compound will be in its neutral form at one or more of these pH's
- Hence $\log P$ can be derived from the maximum CHI value obtained
- But how useful are CHI derived $\log P$ values?

CHI assay – HPLC method



- Sample in 50/50 MeCN/H₂O is injected into a fast flowing 100% aqueous buffer (2ml/min)
- A linear gradient changes the mobile phase to 100% organic (MeCN) to elute the compound
- The eluent then changes back to 100% aqueous in readiness for the next sample.
- The CHI value is then determined from the Retention Time of the compound, relative to a set of standards of known CHI values

CHI assay – CHI value calibration



CHI STANDARDS		RT @ pH 7.0	
NAME	STD	RT (min)	CHI pH 7.0
theophylline	1	1.61	17.8
acetanalide	5	1.99	41.2
phenyltheophylline	6	2.20	48.6
acetophenone	7	2.39	61.0
indole	8	2.54	69.7
propiofenone	9	2.65	76.7
butyrophenone	10	2.85	88.5
valerophenone	11	3.02	98.8

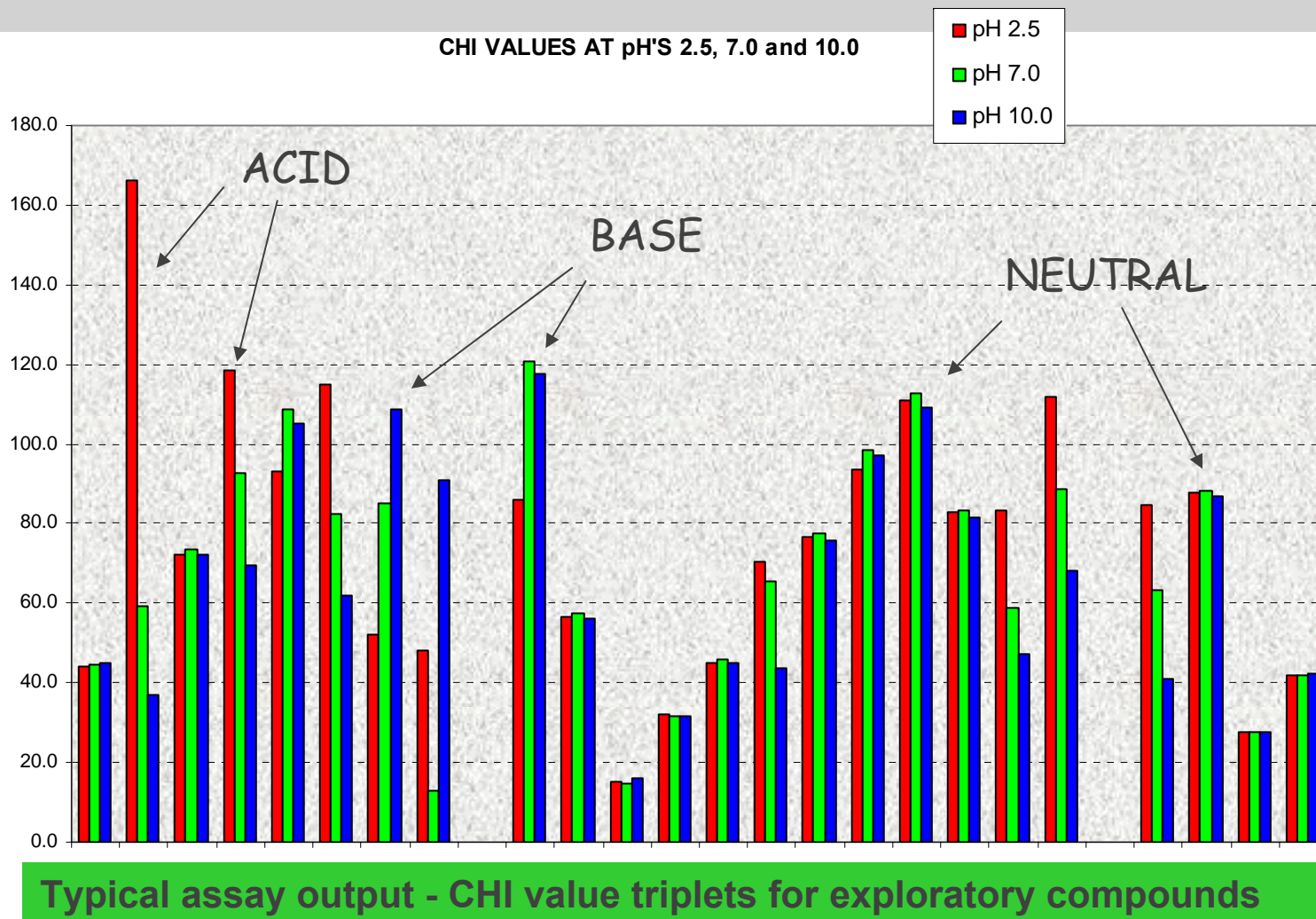
$$y = 57.065x - 74.494$$

$$R^2 = 0.9974$$

Where y = CHI value
and x = Retention Time

- The calibration curve is generated using a set of reference compounds recommended by Klara Valko (GSK)
- Then the **slope** and **intercept** from this calibration curve and the RT of the test compound used to determine its CHI value

CHI @ 3pH's – graphical representation of results



Indirect determination of log P oct from CHI

- At Syngenta we initially utilised the simple regression equation reported by Klara Valco and co-workers at GSK for the C18/MeCN system

$$(a) \text{ CHI log P} = 0.054 \times \text{CHI}_{\text{max}} - 1.467$$

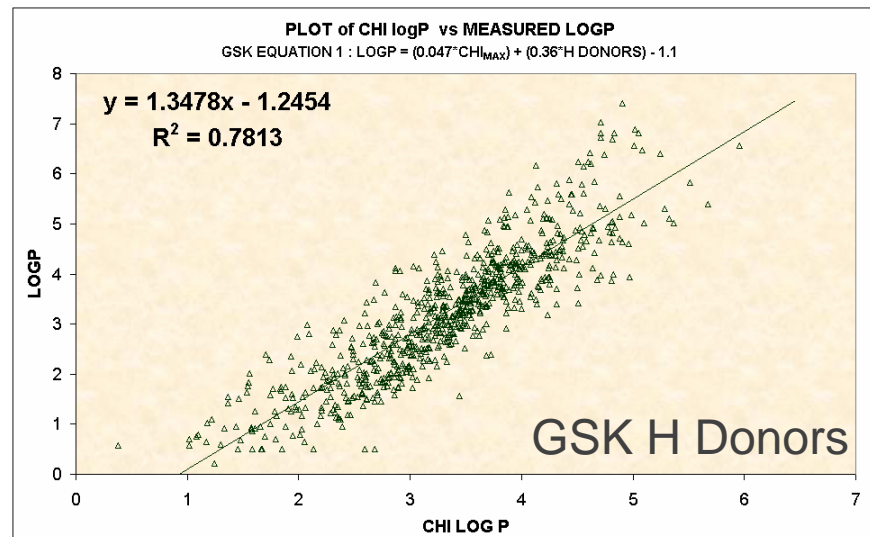
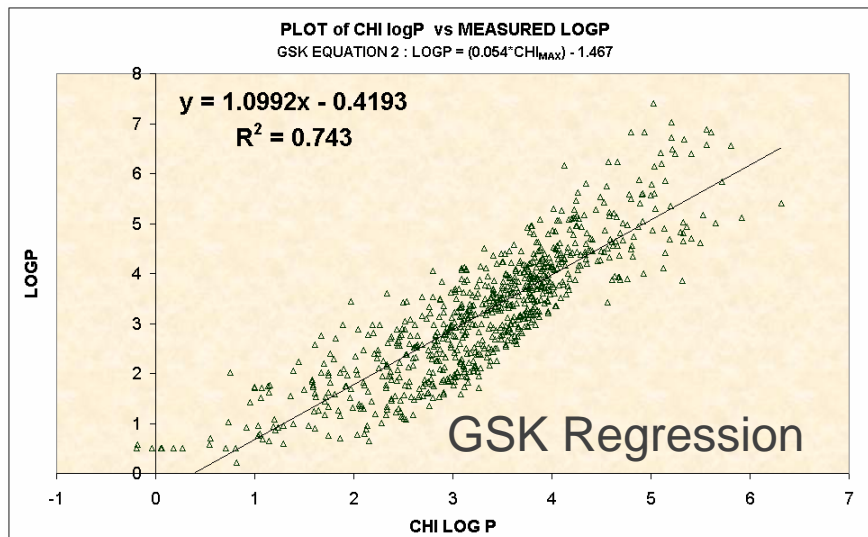
- and their equation which takes account of hydrogen bond donors

$$(b) \text{ CHI log P} = 0.047 \times \text{CHI}_{\text{max}} + 0.36 \text{ HBC} - 1.10$$

- equation (a) was also used to obtain CHI log D from CHI data at other pH's to help assess acid/base character
- Overall proved to be a useful contribution to the review of HTS hits – but can we do more with CHI data to support active projects?

CHI log P - how useful is it for agrochemicals

analysis of ~800 Syngenta compounds with measured log P & CHI values



- **'GSK Regression'**

$$\text{CHI log P} = (0.054 \cdot \text{CHI}_{\text{MAX}}) - 1.467$$

- 54% have a CHI log P within 0.5 unit of the measured log P
- 46% have a CHI log P > 0.5 unit from the measured log P
- 14% have a CHI log P > 1 unit from the measured log P.

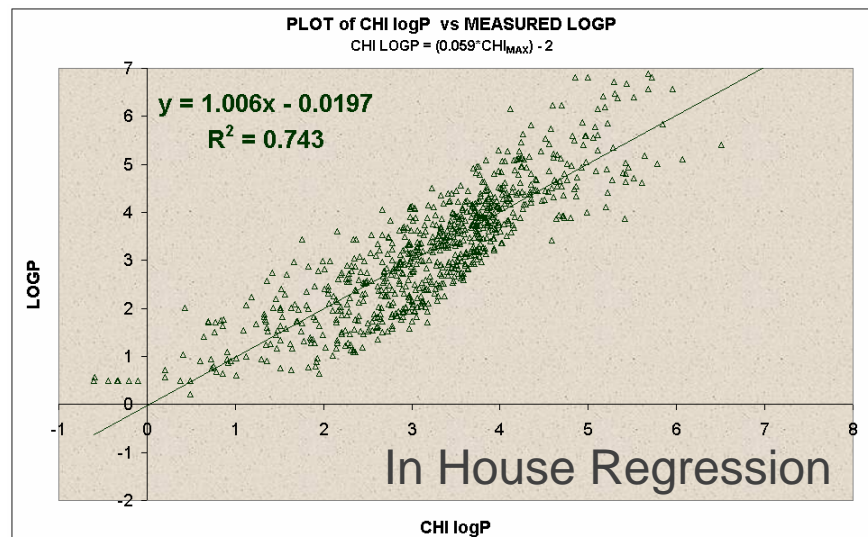
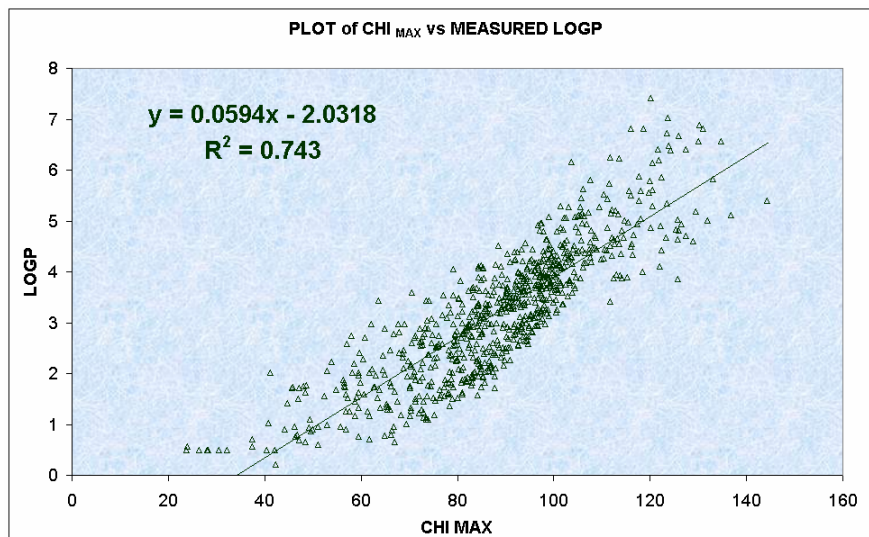
- **'GSK H Donors equation'**

$$\text{CHI log P} = (0.047 \cdot \text{CHI}_{\text{MAX}}) + (0.36 \cdot \text{H Donors}) - 1.10$$

- 58% have a CHI log P within 0.5 unit of the measured log P
- 42% have a CHI log P > 0.5 unit from the measured log P
- 14% have a CHI log P > 1 unit from the measured log P.

➤ **Both equations useful - but neither reliable enough for project support**

Correlation of CHI_{max} , CHI log P and log P for agrochemicals analysis of the ~800 compound dataset



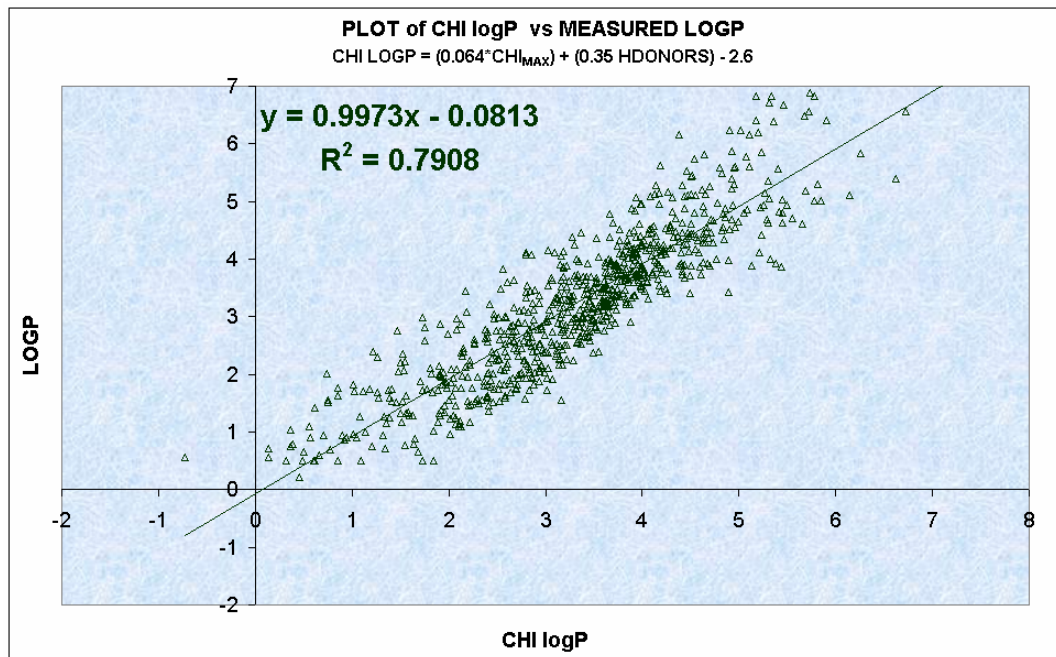
- Regression equation based on agrochemicals

$$\text{CHI log P} = 0.059 \times \text{CHI}_{\text{max}} - 2.03$$

- 53% have a CHI log P within 0.5 unit of the measured log P
- 47% have a $\text{CHI log P} > 0.5$ unit from the measured log P
- 12% have a $\text{CHI log P} > 1$ unit from the measured log P
- **Similar performance to the GSK regression equation – reassuring but little advantage for project work**

In House H Donors Equation – Is it good enough?

Analysis of ~800 agrochemical dataset

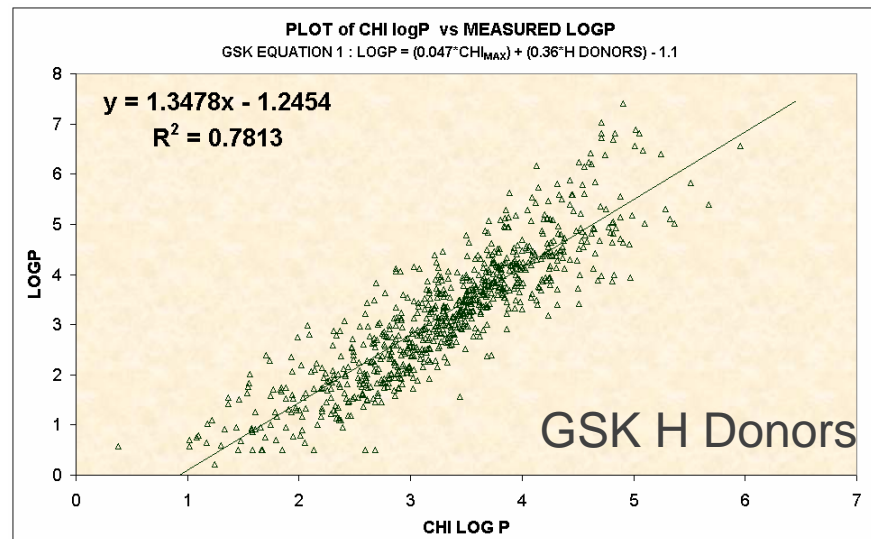
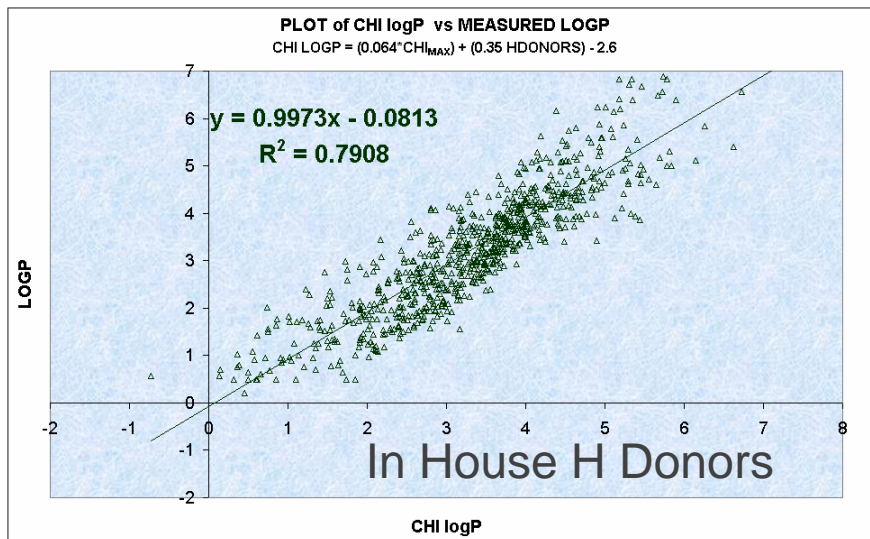
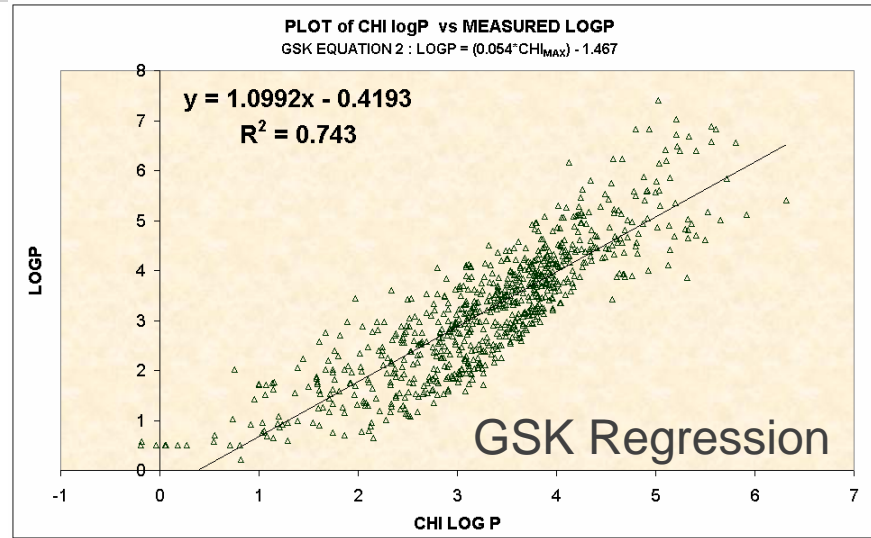
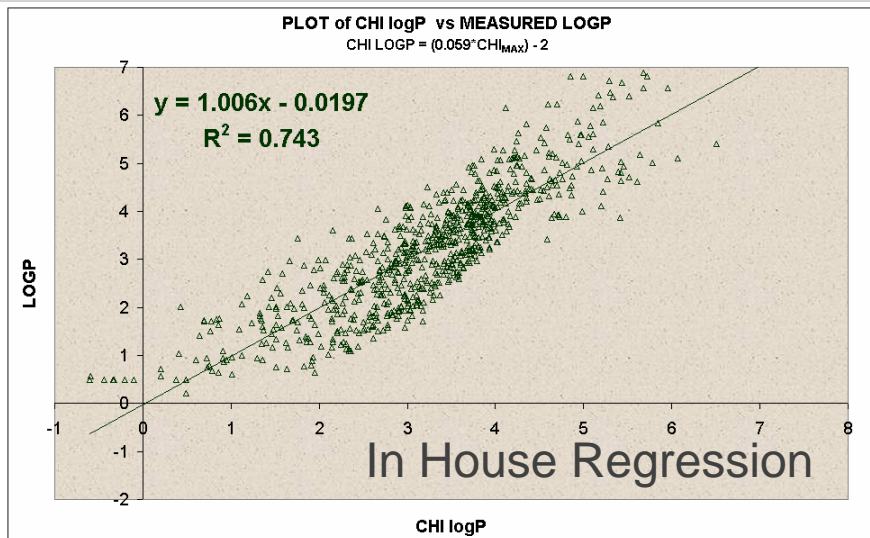


- In house H Donors

$$CHI \log P = (0.064 * CHI_{MAX}) + (0.35 H \text{ Donors}) - 2.60$$

- 59% have a CHI log P within 0.5 unit of the measured log P
- 41% have a CHI log P > 0.5 unit from the measured log P
- 8% have a CHI log P >1 unit from the measured log P.
- **Again, no significant advantage over the GSK H-bond donor equation**

Conclude that Syngenta & GSK CHI log P equations give similar predictions for agrochemicals



Improving the prediction of log P from CHI data

- ACD/Physchem provides a number of parameters which could influence the quality of log P prediction from CHI data in addition to simple counts of H- bond donors.
- The following have been examined:
 - Polar surface area (PSA)
 - Hydrogen bond acceptor count
 - Polarizability
 - Molar Refractivity
 - Molar Volume
 - Parachor (related to molar volume and surface tension)

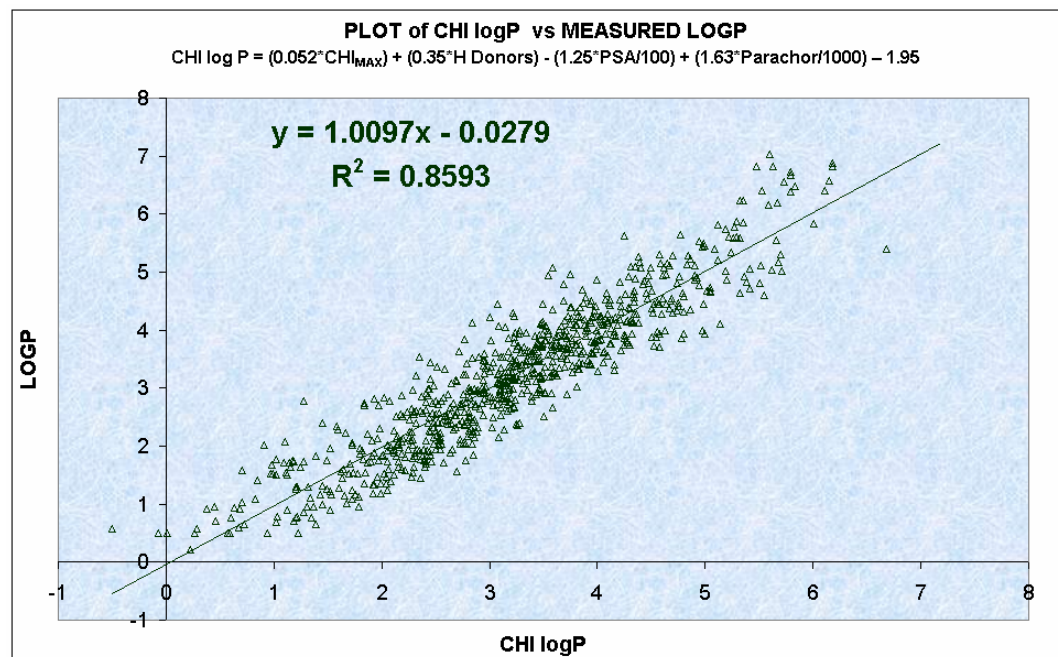
CHI log P from H Donors, PSA and Parachor

Analysis of the ~ 800 agrochemical dataset

- The best equation found utilised H- bond donor counts, PSA and Parachor.

$$\text{CHI log P} = (0.052 \cdot \text{CHI}_{\text{MAX}}) + (0.35 \cdot \text{H Donors}) - (1.25 \cdot \text{PSA}/100) + (1.63 \cdot \text{Parachor}/1000) - 1.95$$

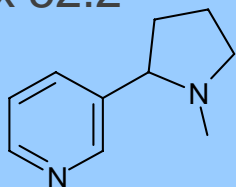
- 69% have a CHI log P within 0.5 unit of the measured log P
- 31% having a CHI log P > 0.5 unit from the measured log P
- With only 3% having a CHI log P >1 unit from the measured log P



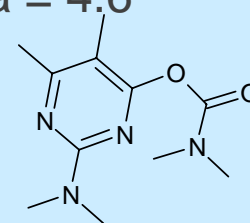
Agrochemicals (see Pesticide Manual 14th Edition 2006)

Examples: measured & CHI derived log P & pKa values

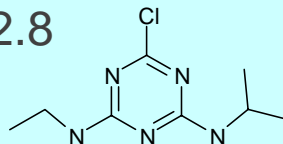
Nicotine (CAS-RN 22083-74-5)
Meas. log P = 0.9; CHI log P = 1.3
Meas. pKa = 8.2 & 3.1; CHI pKa = 7.9
CHI max 52.2



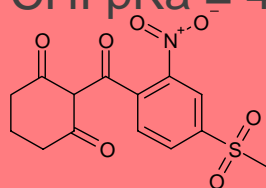
Pirimicarb (CAS-RN 23103-98-2)
Meas. log P = 1.7; CHI log P = 1.8
Meas. pKa = 4.5; CHI pKa = 4.6
CHI max 70.3



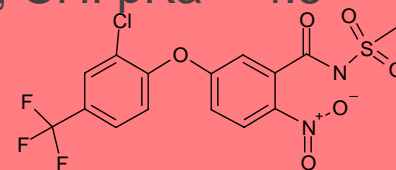
Atrazine (CAS-RN 1912-24-9)
Meas. log P = 2.6; CHI log P = 2.5
Meas. pKa = 1.7; CHI pKa – none
CHI max 72.8



Mesotrione (CAS-RN 104206-82-8)
Meas. log P = 0.8 ; CHI log P = 0.7
Meas. pKa = 2.9; CHI pKa = 4.7
CHI max 65.3



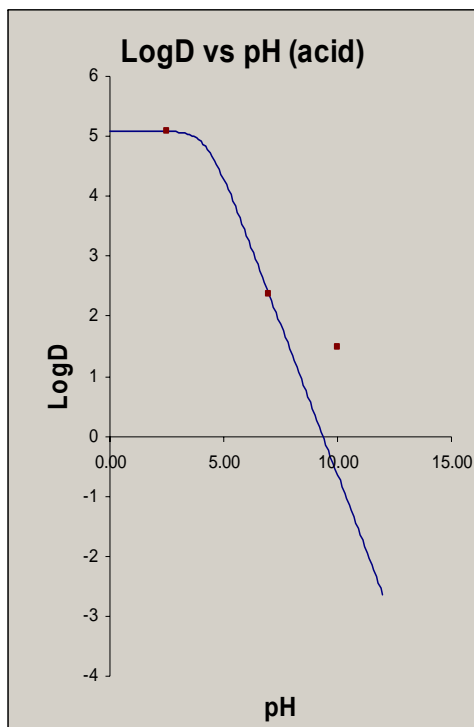
Fomesafen (CAS-RN 72178-02-0)
Meas. log P = 3.0; CHI log P = 3.7
Meas. pKa = 3.1; CHI pKa = 4.5
CHI max 110.2



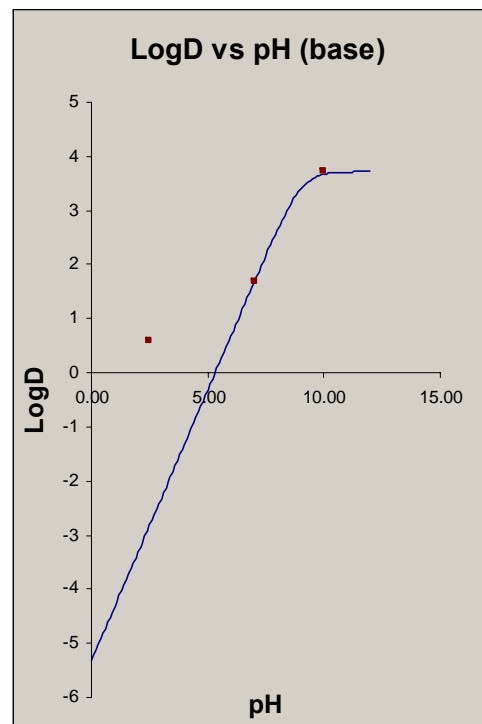
(measured values – JH or K.Chamberlain *et al*/Pestic. Sci. 1996, 47, 265)

Using CHI data to give pKa values

Acid series
GLpKa/DPAS pKa = 3.9
CHI pKa = 4.3



Base series
GLpKa/DPAS pKa = 9.4
CHI pKa = 9.0

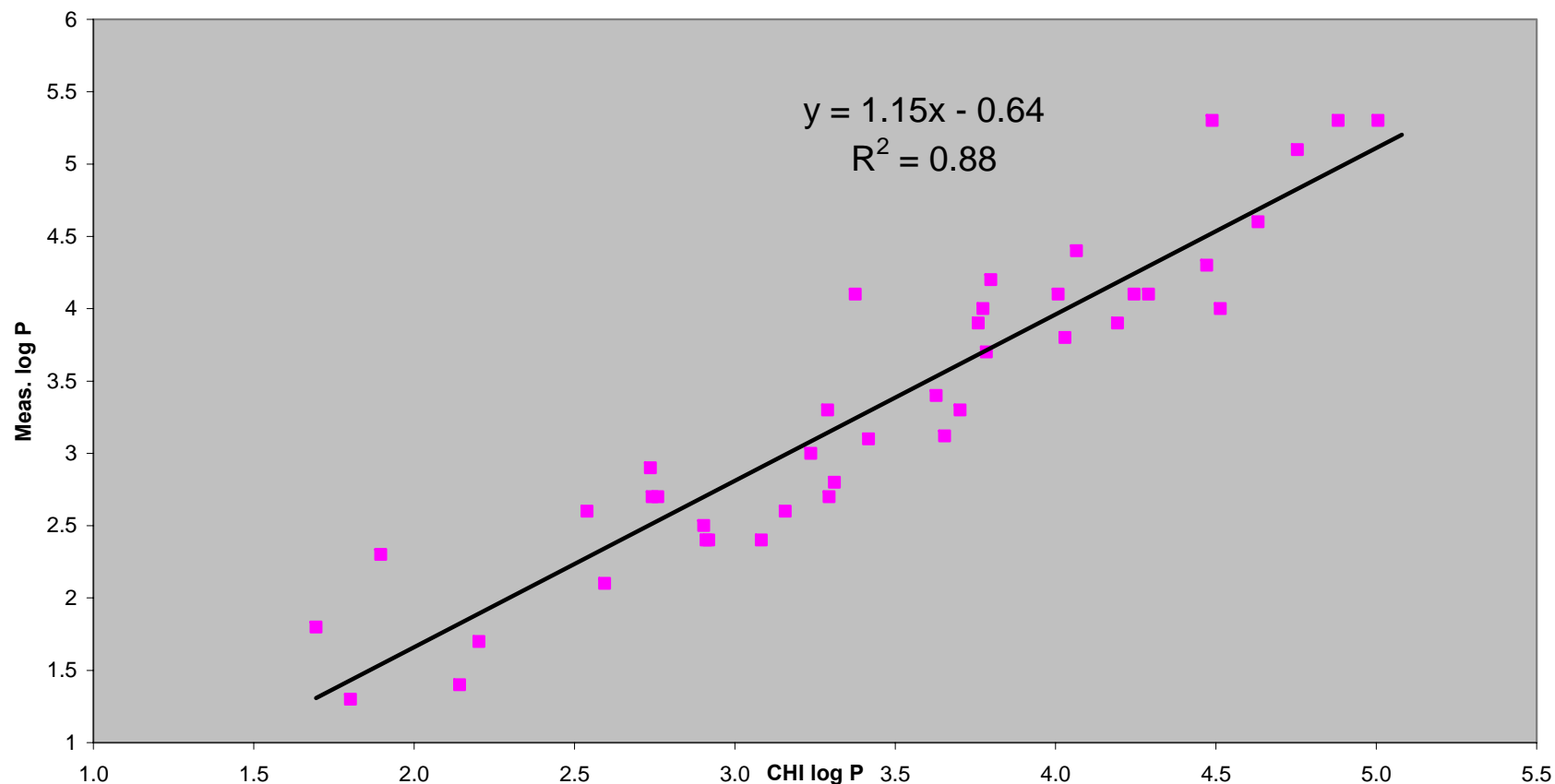


Exploratory acids: pKa, log D & log P from CHI data

	CHI @ pH 2.5	CHI @ pH 7	CHI @ pH 10	log D @ pH 7	log P	pKa
A	76	57	21	1.6 (1.2)	2.5 (2.5)	6.2 (5.7)
B	92	65	26	2.0 (1.5)	3.3 (3.2)	5.8 (5.2)
C	81	58	22	1.6 (1.4)	2.7 (3.0)	6.0 (5.4)

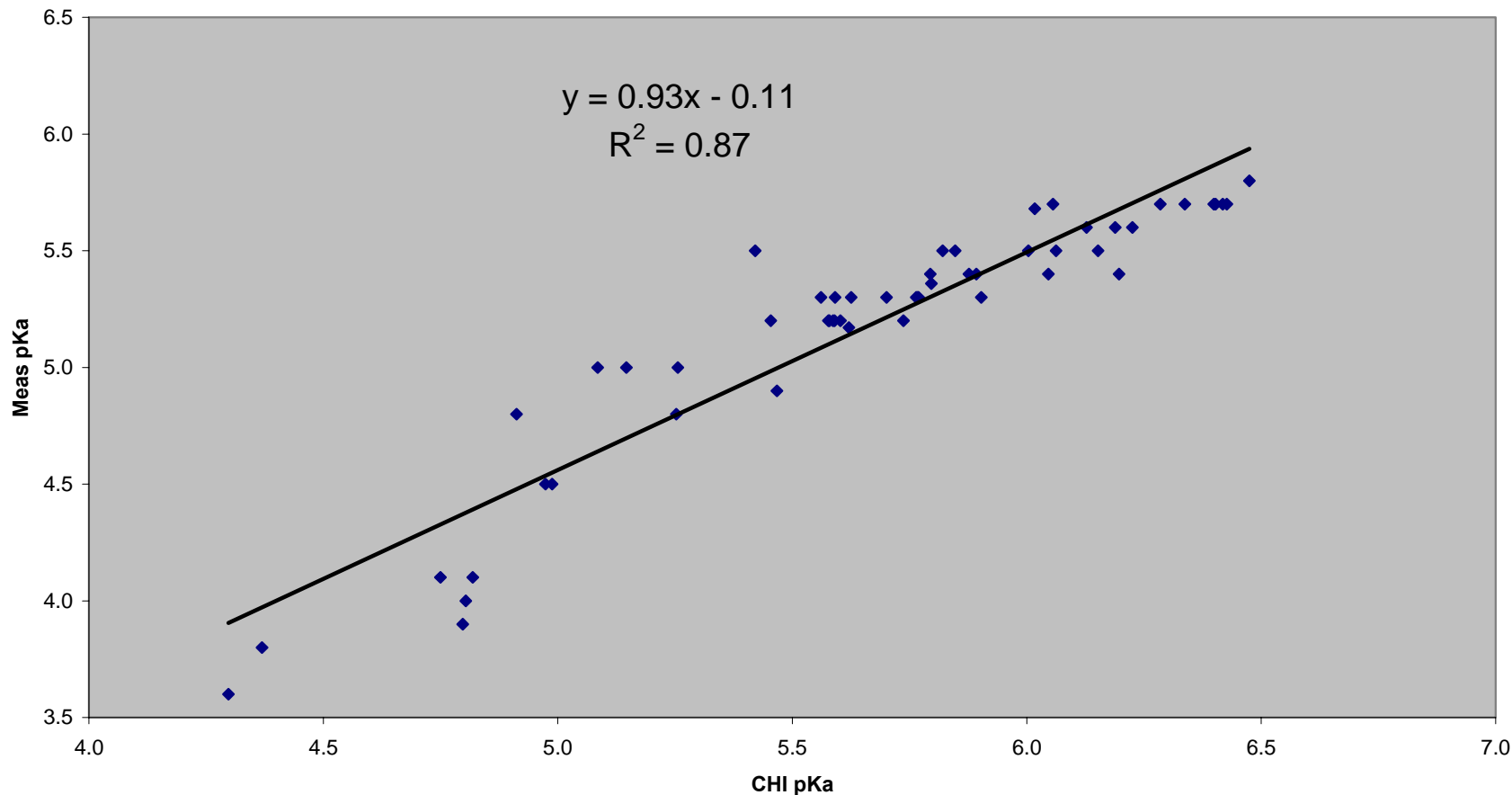
Acid series: reasonable predictions $R^2 \sim 0.9$ & MAE ~ 0.4
CHI log P values: 40% ≤ 0.2 ; 85% ≤ 0.5 ; 98% ≤ 0.7

Acid series - log P

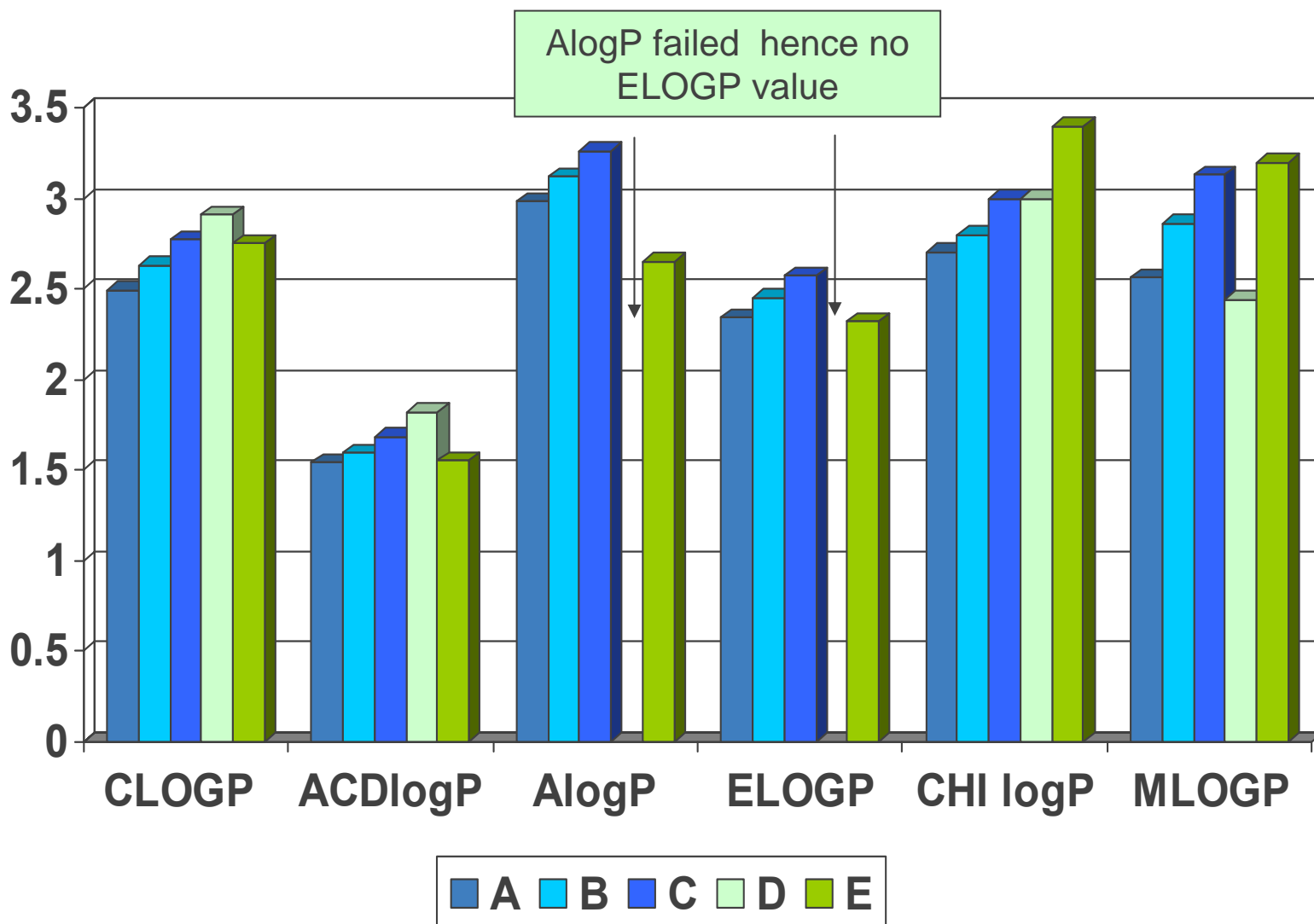


Acid series: reasonable predictions $R^2 \sim 0.9$ & MAE ~ 0.5
CHI pKa values: 22% ≤ 0.3 ; 61% ≤ 0.5 ; 94% ≤ 0.7

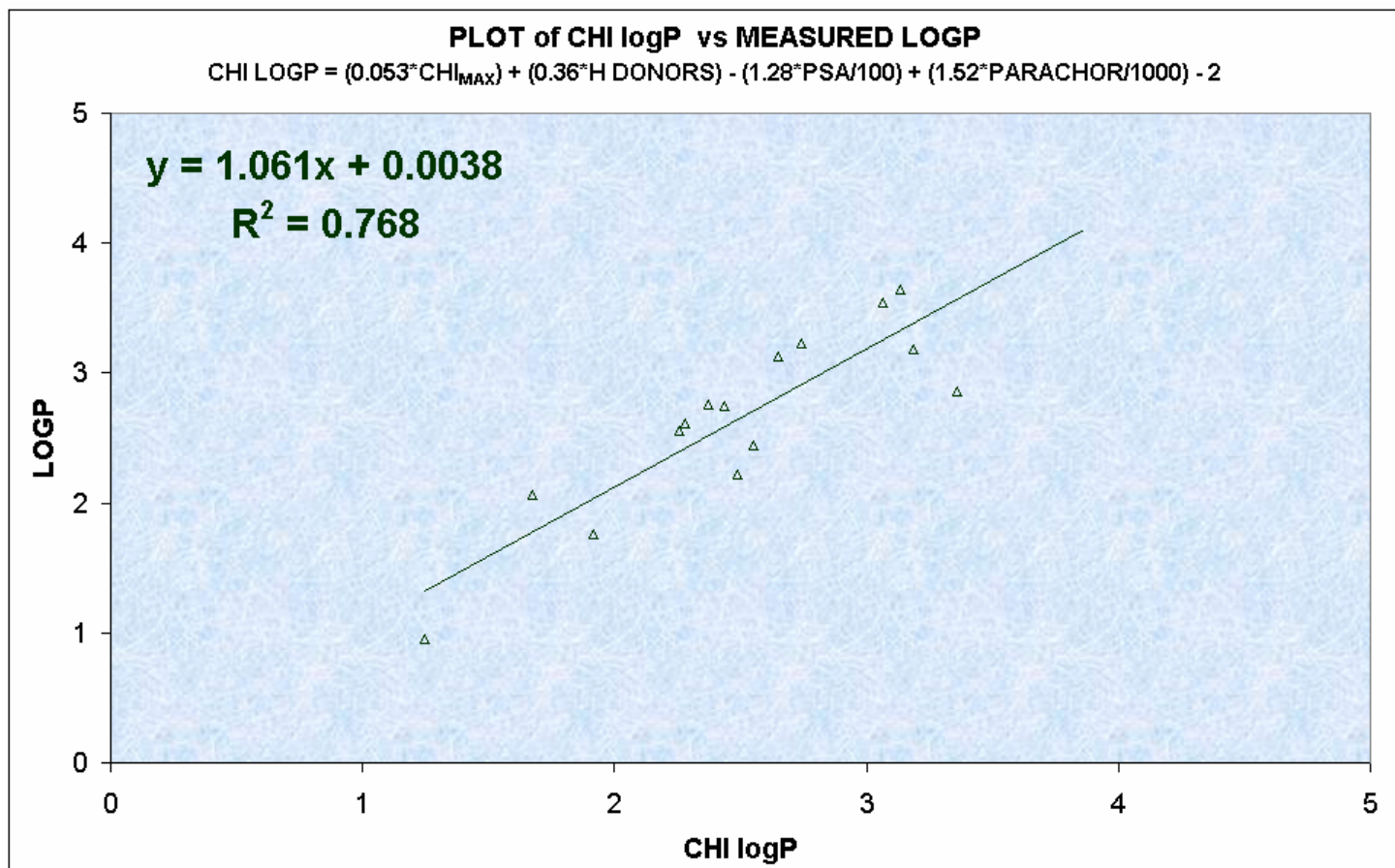
Acid series - pKa



Base series – log P determination



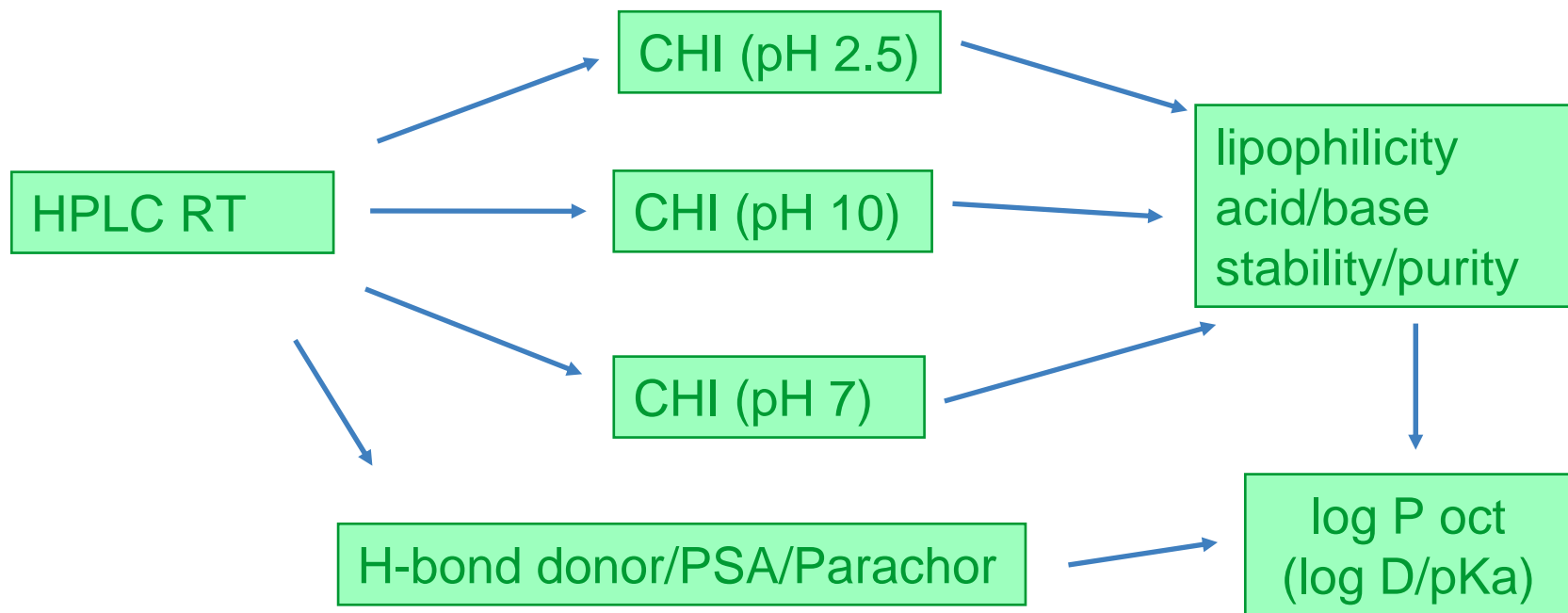
Base series – reasonable results



➤ 93% have a CHI log P within 0.5 unit of the measured log P

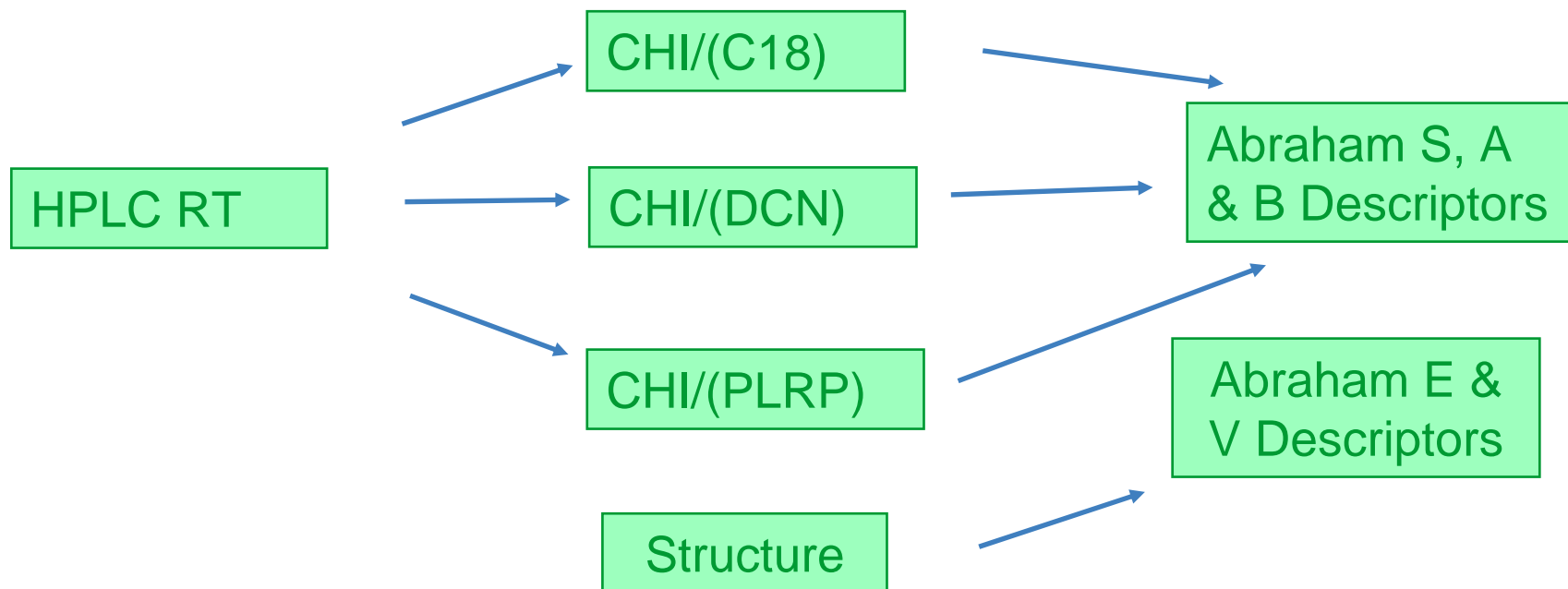
Chromatographic Hydrophobicity Index (CHI)

Fast Gradient RP-HPLC: $\text{CHI} \propto \text{RT}$: calibrated with GSK standards



Chromatographic Hydrophobicity Index (CHI)

Abraham Descriptors: Fast Gradient RP-HPLC @ pH 7: $\text{CHI} \propto \text{RT}$
(matrix of 3 column types (C18, DCN, PLRP) & 2 organic solvents (MeCN, MeOH))



See review by M.H. Abraham *et al*, J.Chromatography A, 1037 (2004) 29-47

Application of CHI to Agrochemicals - Summary

- explored column type/ organic solvent/pH parameters
- C18/MeCN system in regular moderate throughput use
- developed CHI log P equation for C18/MeCN system using CHI max, H-donor counts, PSA and parachor
- similar results if parachor replaced by molar refractivity or molar volume; and PSA by H-acceptor counts
- for project work CHI log P values are reasonable (within 0.5 log P meas.) and CHI pKa values acceptable (within 1 pKa meas.) - often better within chemical sub classes
- overall less risk in defining mobility profiles through CHI log P & pKa than current prediction methods
- but when it matters we continue to use conventional measurements!

Useful references re agrochemicals discussions at *PhysChem FORUM 4*

- Physical and molecular properties of agrochemicals: An analysis of screen inputs, hits, leads, and products. Eric D. Clarke & John S. Delaney, *Chimia* 57,(2003),731-734.
- Henry's Law constants or air to water partition coefficients for 1,3,5-triazines by an LFER method. Michael H. Abraham, Kei Enomoto, Eric D. Clarke, Marti Roses, Clara Rafols & Elisabet Fuguet. *Journal of Environmental Monitoring* 9, (2007), 234-239.
- Modern agrochemical research: a missed opportunity for drug discovery?. John Delaney, Eric Clarke, Dave Hughes & Martin Rice, *Drug Discovery Today*, 11 (2006), 839-845,
- Metabolism-related assays and their application to agrochemical research: reactivity of pesticides with glutathione and glutathione transferases. Eric D. Clarke, Daren T. Greenhow & David Adams, *Pesticide Science* 54 (1998), 385-393.