Presentation 2 - John Vogel

Effects of Exposure to Multiple Chemicals at Low Dose *in vivo*:
Allowing Physiology into Toxicology

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Toxic effects of multiple pesticides are not known for low doses.

- "Insufficient data exist to determine if effects of multiple OP pesticides can be extrapolated through dose responses that :
  - ... are additive.
  - ... sum toxicologicly equivalent doses.
  - ... are synergistic through activation or detoxicfication.
  - ... are antagonistic." *
  - ... are none of the above?

- Goal: design an assumption-free test of *in vivo* interactions of co-administered compounds at low doses.

* Common Mechanism of Toxicity: A Case Study of OP Pesticides. B.E. Mileson, et al. Tox. Sci. 41:8-20 (1998)

Extrapolated high dose OP data predict no significant low dose effects.

Constraining assumptions!

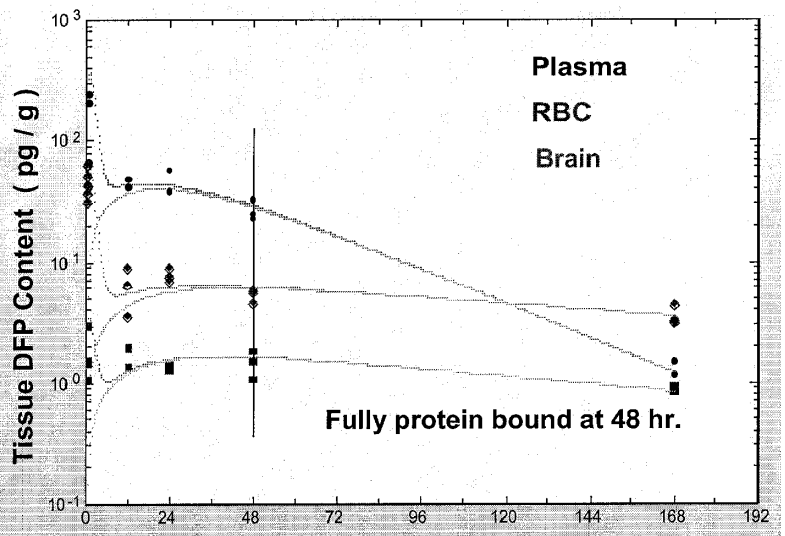
- **Dose Effects**
  - Measured index is linear with dose (e.g. AChE activity).
  - Intermediate metabolism and distribution are linear.
- **Mixture Effects**
  - Compounds act on same target molecule.
  - Compounds use the same molecular mechanism.
  - Compounds affect the same measured index.
  - Interaction is not the result of intermediate induction.

A "reporter" assay integrates physiologic and biochemical interactions.

- **Choose a quantifiable "toxic" end point.**
  - [^{14}C]-DFP binds firmly to specific enzymes.
  - Use a very sub-toxic dose.
- **Expose animals to realistic doses of compounds.**
  - Pesticides: parathion and permethrin.
  - Therapeutic: pyridostigmine bromide.
- **Control for confounding physiologic effects.**
  - Avoid stresses of handling or metabolism cages.
- **Quantify reporter in target tissues.**
  - Normalize response to reporter in plasma.

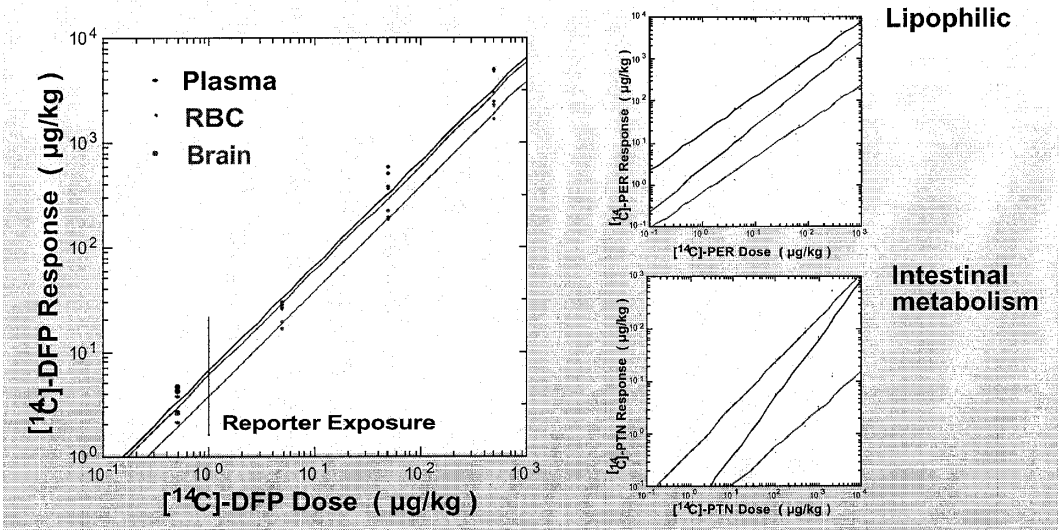
DFP - di isopropyl fluorophosphate, isofluorophate, ...
PTN - parathion
PER - permethrin
PYB - pyridostigmine bromide

DFP is quickly eliminated, but binds to exposed target proteins.



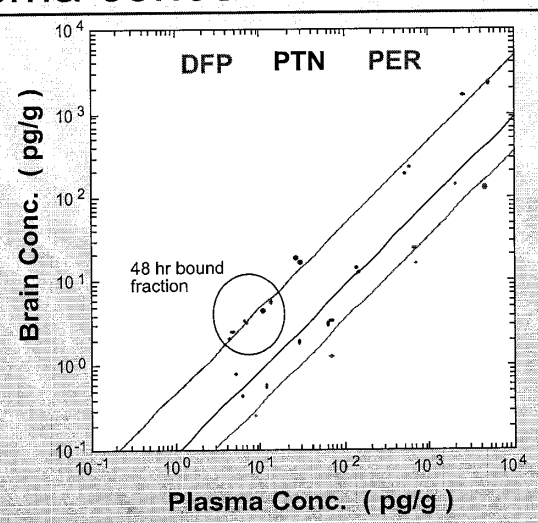
- Unbound DFP is metabolized.
- Bound DFP ^{14}C remains with the binding protein.

DFP was distributed linearly with dose by 1 hr after exposure through ingestion.



- Distribution rapidly equilibrated for DFP reporter.
- PER and PTN are not so well behaved.

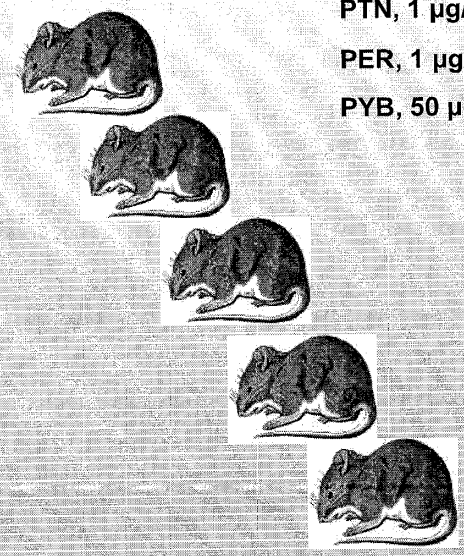
DFP brain concentrations were linear with plasma concentrations.



- Both DFP and PER are lipophilic.
- PER transfer to the brain was 10% that of DFP.
- Brain follows plasma for DFP.

Kinetics and dose response data helped design the mixture study.

CD2/F1 mice



Mixtures:

PTN, 1 µg/kg

PER, 1 µg/kg

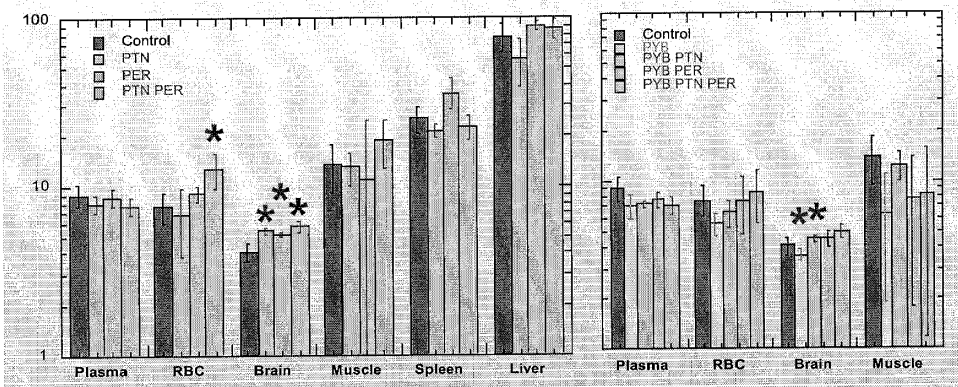
PYB, 50 µg/kg

PYB PTN PER
PYB PER
PYB PTN
PTN PER
PYB
PER
PTN

- 5 day exposure to mixture.
- in moist food, avoids dose loss
- no handling, reduces stress
- [^{14}C]-DFP reporter (5 nCi)

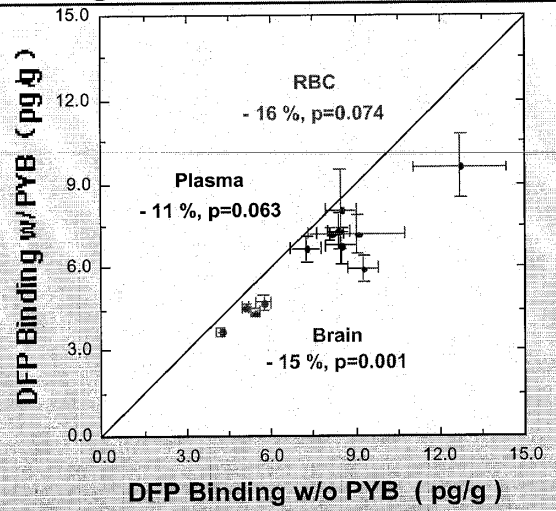
- 48 hr post DFP
  - brain
  - blood (RBC)
  - liver
  - spleen
  - muscle

Compounds increased brain DFP levels without affecting blood levels.



- Low companion doses produce no change in plasma and most tissue.
- Brain contains significantly more reporter compound.
- PYB significantly reduces reporter in brain.
- Other compounds increase reporter in brain even with PYB.

PYB provided a 15% system-wide protection against bound DFP.



- Plasma esterases are too numerous to succumb to competitive antagonism.
- Equivalence of competition across BBB is unlikely.

Mechanisms of interactions are not related to protein binding at low doses.

1. **Brain - plasma relation is linear for DFP in controls.**
   Brain increase not related to plasma concentration.
2. **Pesticides cross BBB less readily than DFP from plasma.**
   Pesticides did not induce synergistic protein binding.
3. **Pesticides increase brain DFP.**
   More plasma delivered to brain .

Hypothesis:

- PER and PTN induce nitric oxide synthase (hence, NO) through cholinergic receptors.
- NO increases brain blood flow at normal human pesticide exposures.

Mechanisms of interactions are not related to protein binding at low doses.

1. **PYB has low bioavailability and even lower brain access.**
   Plasma concentrations are low.
2. **Competitive binding in plasma would increase brain DFP.**
   Copious plasma esterases not overloaded.
3. **DFP binding decrease is systemwide at ≈ 15%.**
   Less DFP enters the system.

Hypothesis:

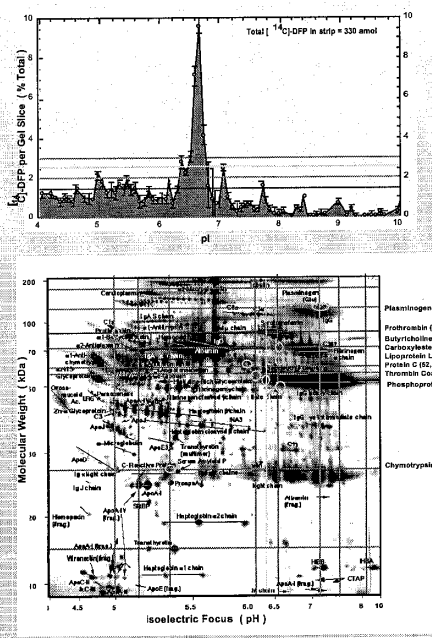
- PYB decreases intestinal peristalsis.
- Absorption of DFP also lowered by intestinal changes.

Conclusions from initial study.

- Interaction among compounds at low dose are physiological.
- Synergism /antagonism are only visible in whole animal.
- Reporter compound quantifies the effect of multiple exposures.
- Response involves a non-linear extrapolaion from high dose.

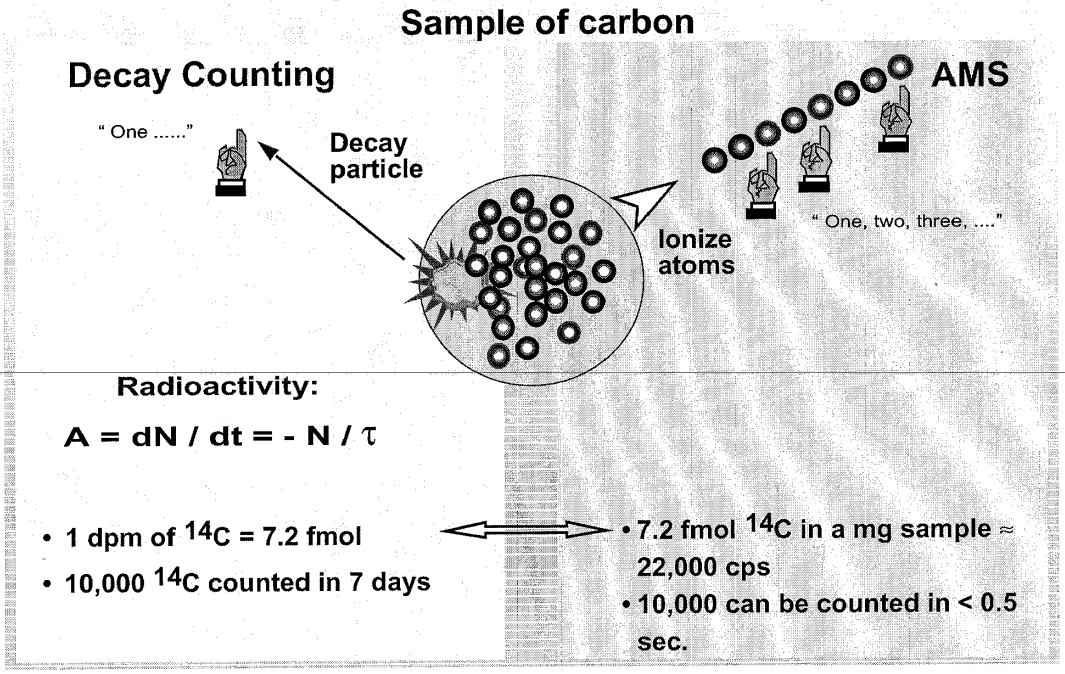
- Next Steps
  - Identify *in vivo* target proteins
  - Probe NOS levels with citruline formation from [^{14}C]-arginine

Virtual 2D gel AMS finds DFP protein targets at 1 µg/kg dose.

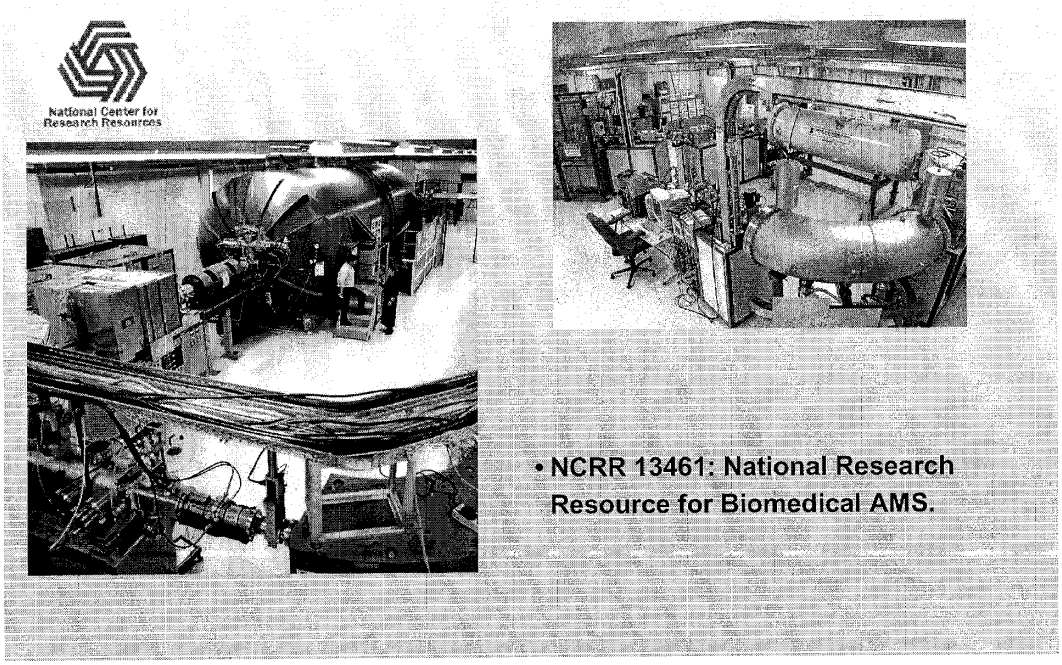


- Plasma proteins separated by molecular weight and isoelectric point.
- BChE is dominant target in plasma.

Low dose assays are made possible by high sensitivity of AMS for ^{14}C.



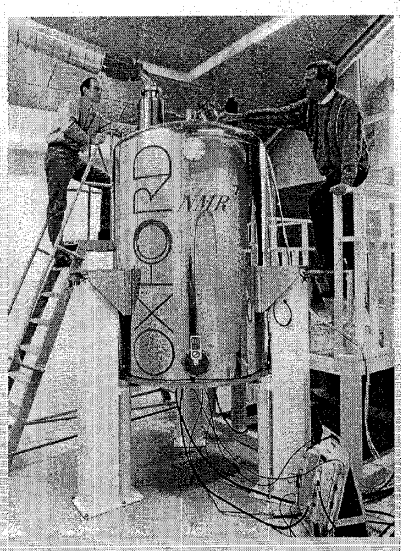
AMS is accessible through an NIH Research Resource at LLNL.



None of our animals became "radioactive waste".

- **§ 20.2005 Disposal of specific wastes.**
- **(a) A licensee may dispose of the following licensed material as if it were not radioactive:**
- **(1) 0.05 microcurie (1.85 kBq), or less, of hydrogen-3 or carbon-14 per gram of medium used for liquid scintillation counting; and**
- **(2) 0.05 microcurie (1.85 kBq), or less, of hydrogen-3 or carbon-14 per gram of animal tissue, averaged over the weight of the entire animal.**

Another large technology is being used for "hypothesis generation".

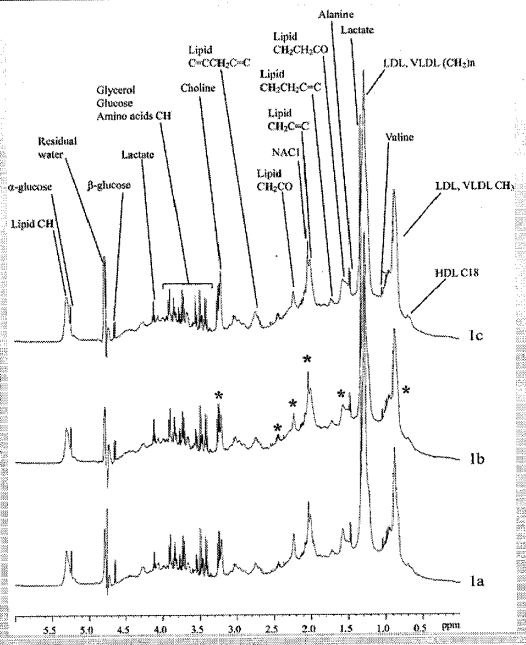


- **Nuclear Magnetic Resonance (NMR) is the basis for MRI.**
- **NMR provides exquisite resolution of chemical structures.**
- **100 µl of serum or urine can be scanned for all constituents.**

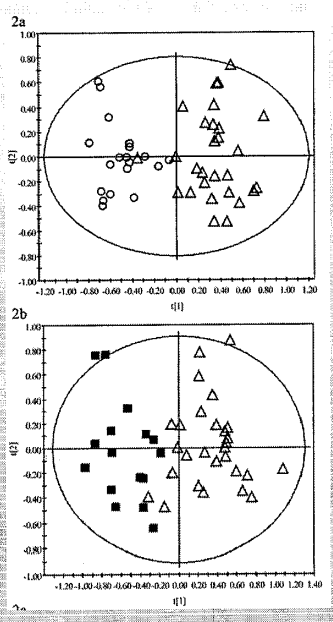
Plasma can be scanned for evidence of metabolic imbalances.

- **NMR spectra quantitate thousands of compounds.**
- **Only one scan (seconds) needed for each sample.**
- **Changes can be correlated to diagnoses.**

Metabolomics



Computers seek significant differences among defined classes of people.



- **Computers "train" on one set of diagnosed people.**
- **Find the "principal components" that distinguish the diagnosis.**
- **Principal components are the "most distinguishing" features designating membership in a class.**
- **Data are from a study of hypertensives (Imperial College London, Jeremy Nicholson)**

Acknowledgements

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