

*Eminent Toxicologist
Lecture Series*

Society of Toxicology

Pesticide Neurotoxicity – More or Less

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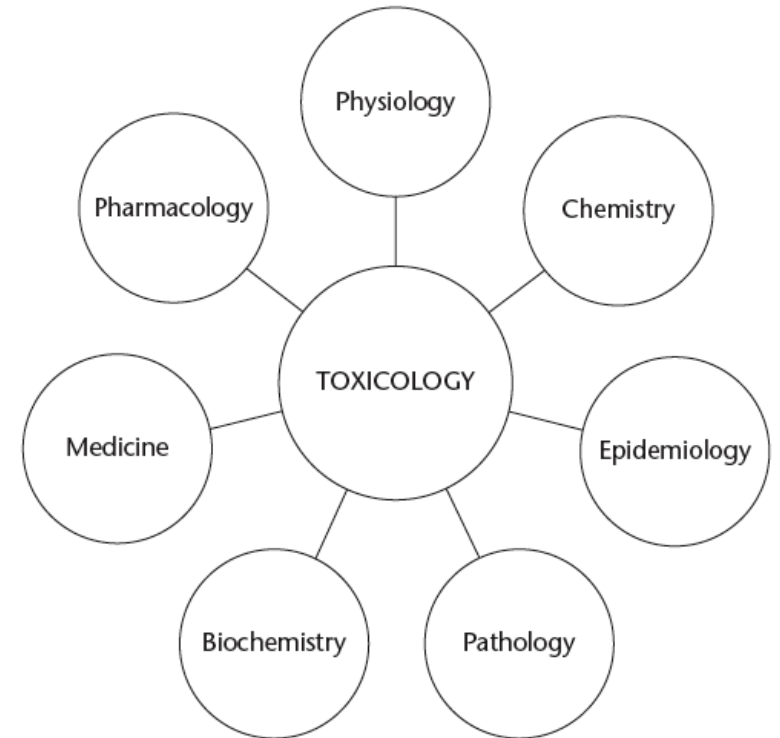
SOT president, 2003-04
Merit Award, 2010

(marion@vt.edu)



Scope of Toxicology

- Toxicology studies the effects of chemicals on biologic organisms.
- Many disciplines contribute to toxicology. There are many types of toxicologists and they have many different backgrounds.
- Toxicology can contribute to other biomedical disciplines.



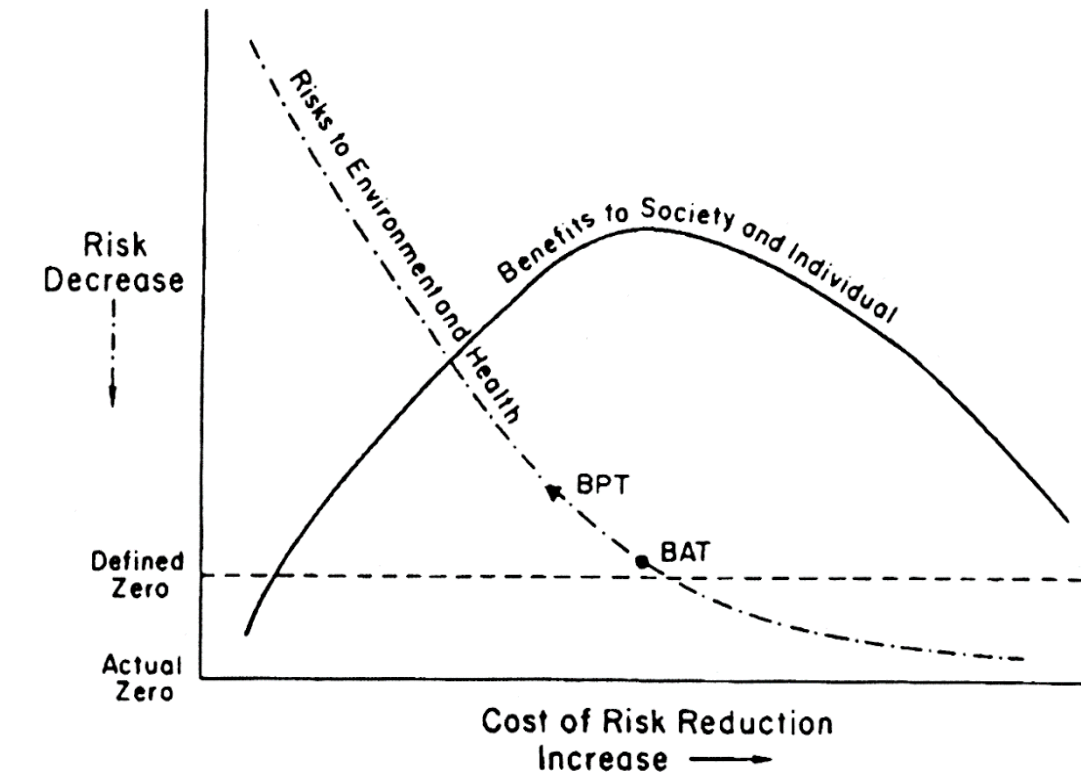
Examples of Classifications of Toxic Substances

- By chemical class and/or use
 - Solvents, pesticides, heavy metals, etc.
- By target organ or physiological system affected
 - Nervous system, immune system, liver, etc.
- By type of adverse effect
 - Neurotoxicity, developmental toxicity, etc.
- ***This presentation will deal with pesticides and the nervous system***



Defining “Pesticides”

- Agents used against any unwanted living organism
 - Insecticides
 - Rodenticides
 - Herbicides
 - Fungicides
- Beneficial: economic; nutrition and health
- Concerns: toxicities, residues



Insecticides

- This is the type of pesticide most likely to cause unintentional neurotoxicity
- Major classes of available insecticides
 - **Organophosphates** and Carbamates
 - OP compounds can be chemical warfare agents.
 - Carbamates include drugs used for treatment of myasthenia gravis and Alzheimer's Disease.
 - Neonicotinoids
 - Newer agents with recent concerns about effects on pollinating insects.
 - Pyrethrins and Pyrethroids
 - The most commonly available type of insecticide.
 - Phenylpyrazoles and others



OBJECTIVES

- To associate mechanisms of acute organophosphate (OP) toxicity with present and future prospects for treatment.
- To distinguish non-acute toxicities associated with OP exposure, including means for amelioration and prevention.
- To identify potential benefits to medical research resulting from OP compound studies.



Organophosphate and carbamate insecticides are useful but toxic

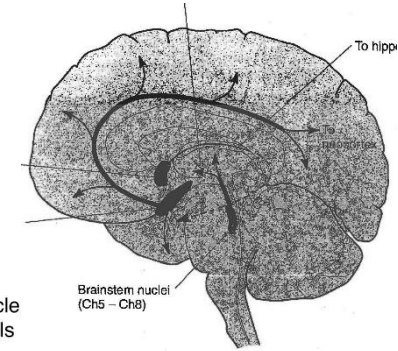
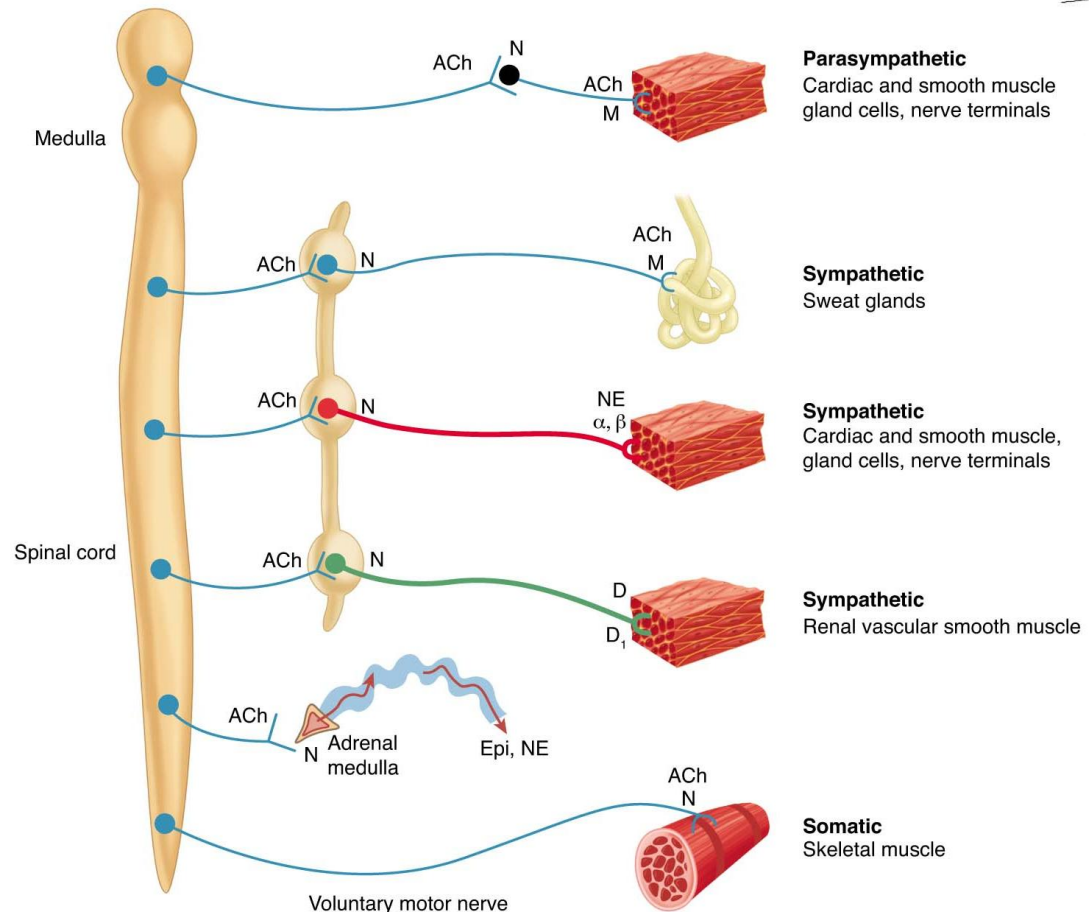


- These products may be used to protect agricultural crops from insects.
- Toxicities result from poor compliance with label directions, accessible storage or disposal, or inadvertent mix ups or spills.

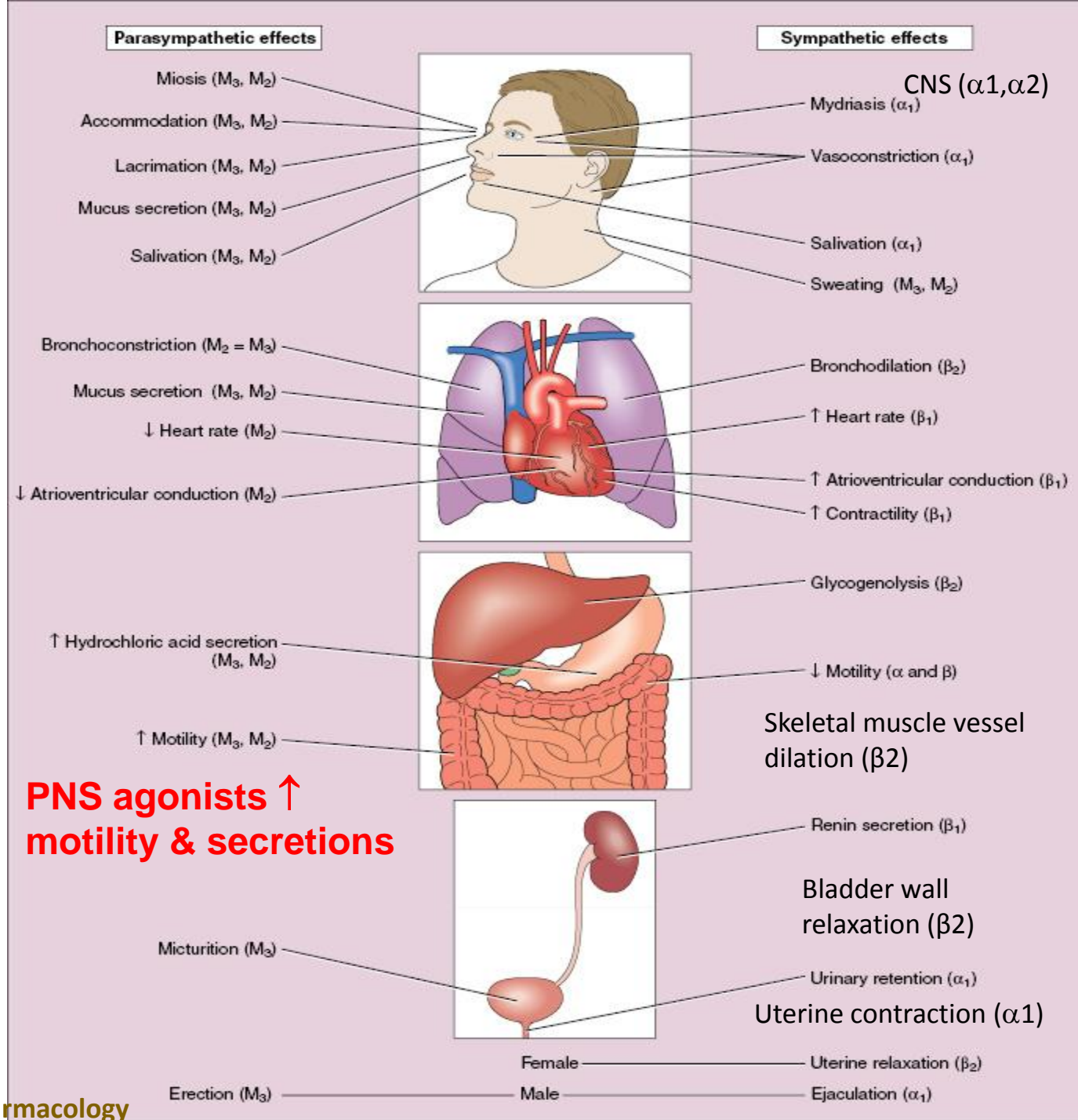


Neurotoxicity More: OPs and carbamates can cause Acute Signs Due to Excess Acetylcholine (ACh)

- **Signs occur because ACh is not degraded by acetylcholinesterase**
 - Too much neurotransmitter....
- Signs relate to sites where ACh acts
 - Central Nervous System (CNS)
 - Neuromuscular junctions
 - Ganglia of the Autonomic Nervous System (ANS)
 - Muscarinic receptors on smooth muscle of peripheral Parasympathetic Nervous System



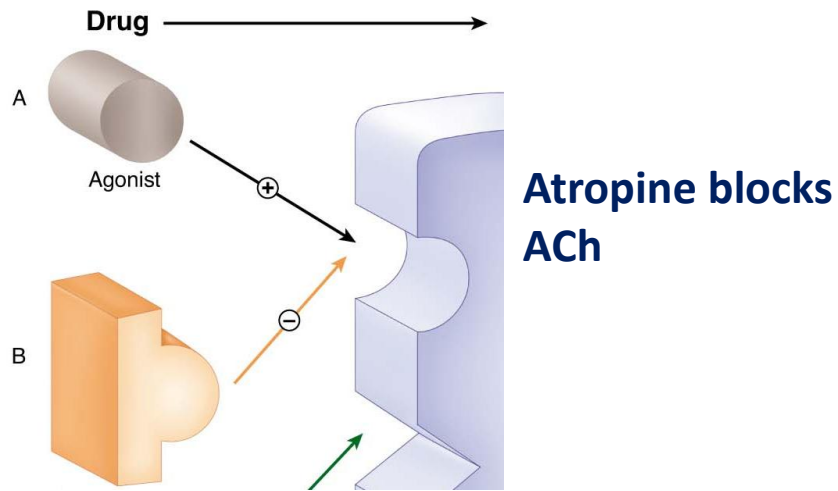
- Signs of excess ACh include agonistic effects on Parasympathetic Muscarinic Receptors
 - Atropine blocks these receptors
 - Early administration of oximes may reactivate acetylcholinesterase
- ACh is the neurotransmitter at neuromuscular junctions, causing overstimulation (tremors) and then block
 - Time needed for recovery
- ACh is a transmitter in the brain
 - Available esterase regenerating oximes not effective
 - High dose atropine only marginally effective



Neurotoxicity Less: Treatments

consider OP mechanisms:

- Atropine to block excess ACh
- Oximes to remove OP from newly inhibited enzyme
- Symptomatic treatment for convulsions
- Decrease exposure
- Time



Katzung Pharmacology

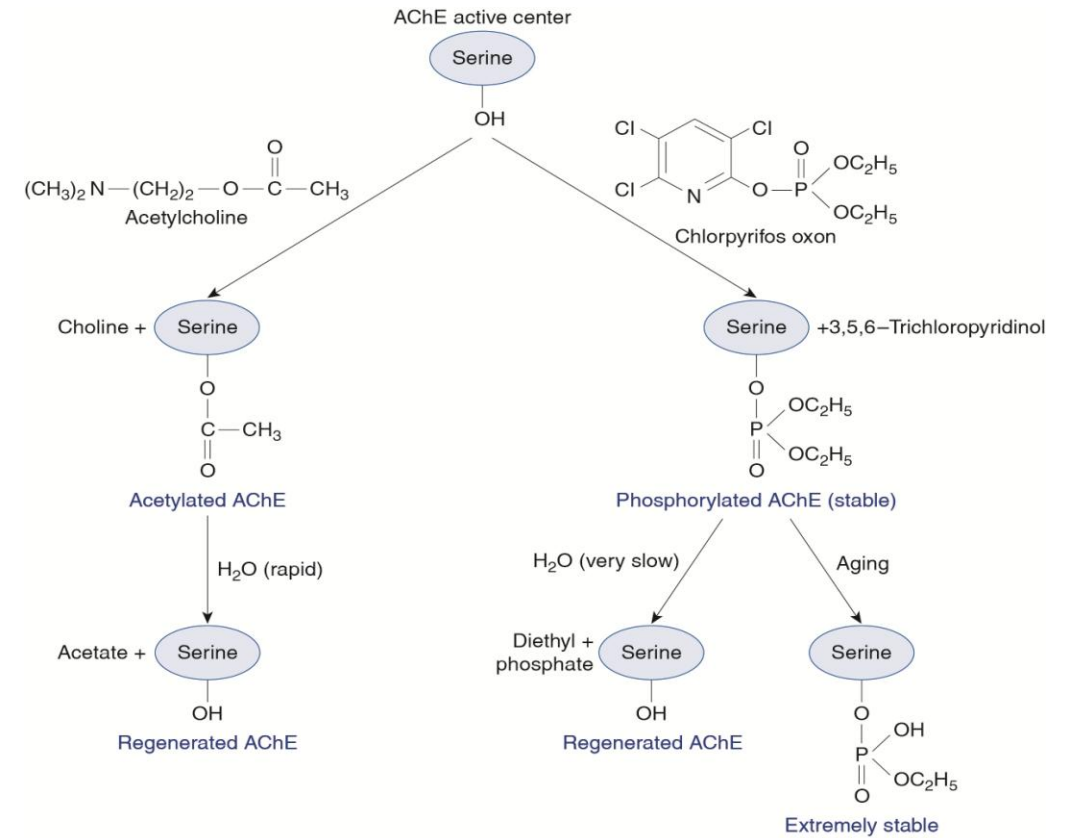


Figure 22-3. Scheme of hydrolysis of acetylcholine by acetylcholinesterase (AChE) and reaction of chlorpyrifos oxon with AChE. See text for details.

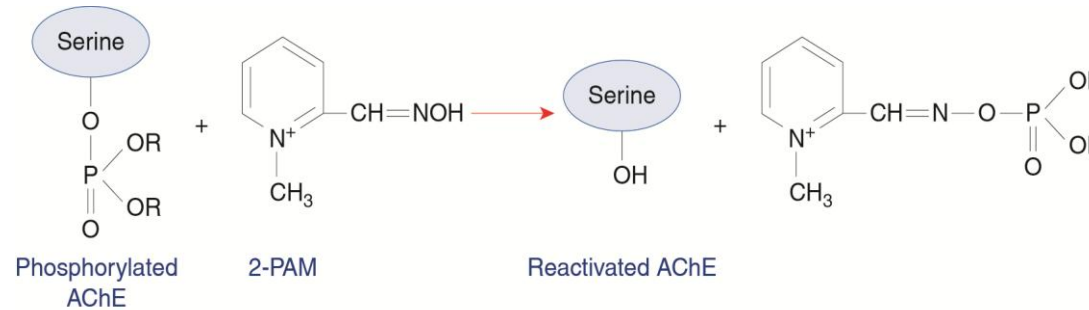


Figure 22-4. Reactivation of phosphorylated acetylcholinesterase by pralidoxime (2-PAM). Note that 2-PAM is only effective before the phosphorylated enzyme has undergone the aging reaction.

Oximes reactivate acetylcholinesterase

Casarett & Doull's Toxicology, 8th ed

Neurotoxicity More or Less:

Toxicities of Organophosphates; Treatments

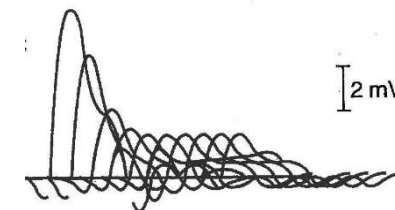
- Acute excess activity in nervous system
 - Accepted treatments: atropine, oximes (2-PAM), diazepam
 - Additional possibilities under investigation.....
 - Exposure prophylactic treatment,
 - but only for threat of OP nerve agent exposures
 - Better anticonvulsants, oximes
 - Administration of esterase enzymes
 - **Use of scavengers**
- Neuromuscular
 - Muscles no longer contract
 - Time as treatment
- Other effects appearing later.....



Nerve agents

Chemical warfare agents
Cholinesterase inhibitors
Very potent
Volatile

Muscle contraction



Later effects?



Roanoke Times, Mar 20, 1995

Investigations into Decreasing Acute OP Toxicity

- **Risk = Hazard + Exposure**

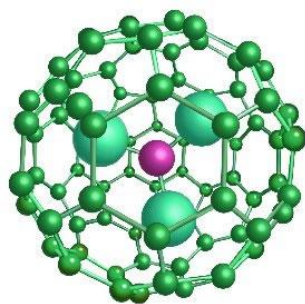
- OPs are hazardous substances
- **Use of protectants and/or scavengers to decrease exposure**

- *One Example of many possibilities:*

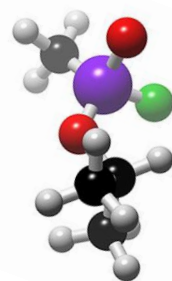
derivatized (solubilized) fullerenes

These are 'buckyball' nanoparticles

Advantages include stability and safety



C80 Trimetasphere™



OP Compounds

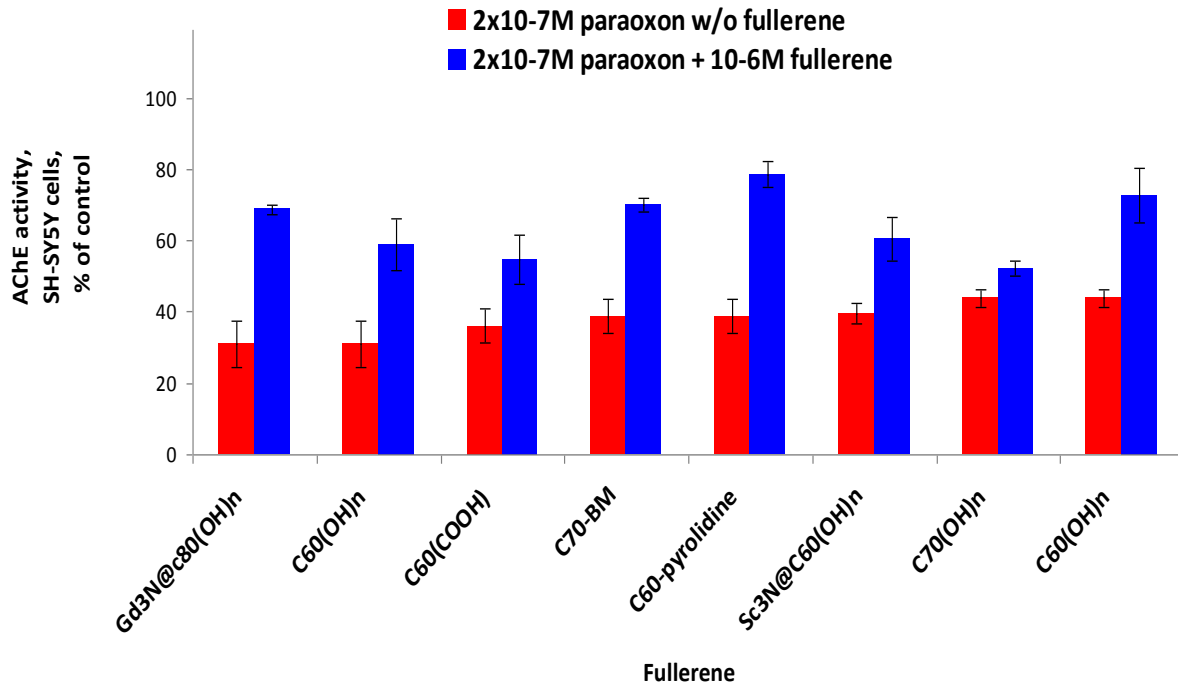


NMR demonstrated a chemical shift of the phosphorus signal, indicative of the sequestering of the OP compound by the fullerene.

Scavengers can protect from OP-induced effects

in vitro:

Fullerenes Protect From Paraoxon Induced AChE Inhibition

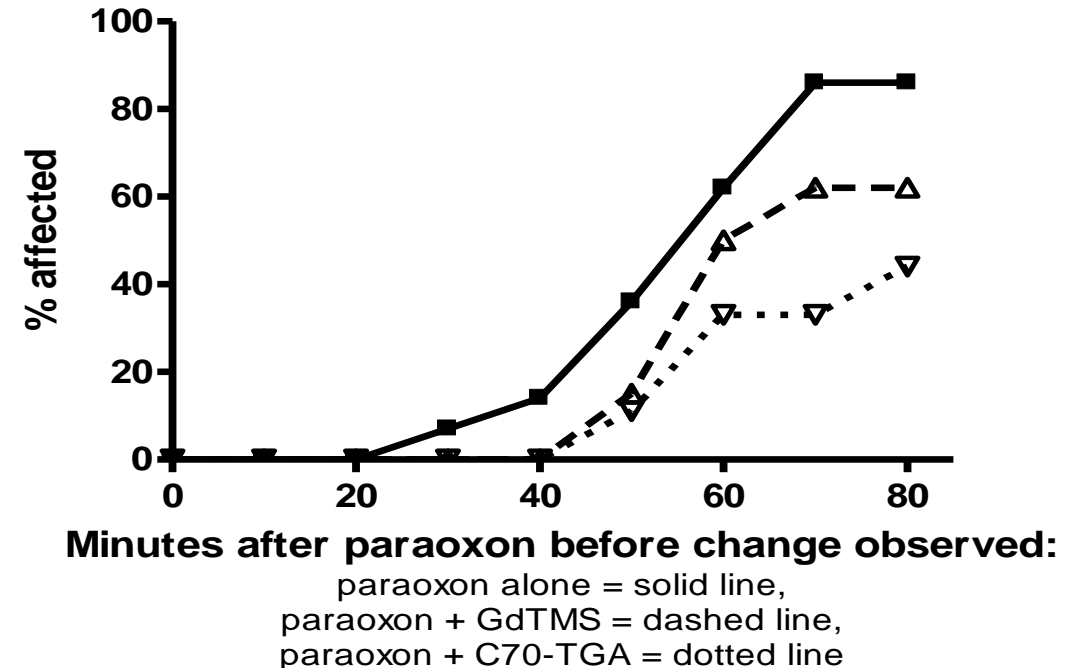


Ehrich et al, *Toxicol in Vitro* 25,301-307, 2011

in vivo results:

Topical application of solubilized fullerenes delayed onset of clinical signs caused by paraoxon that would normally appear in mice \cong 20 min after exposure.

Mobility

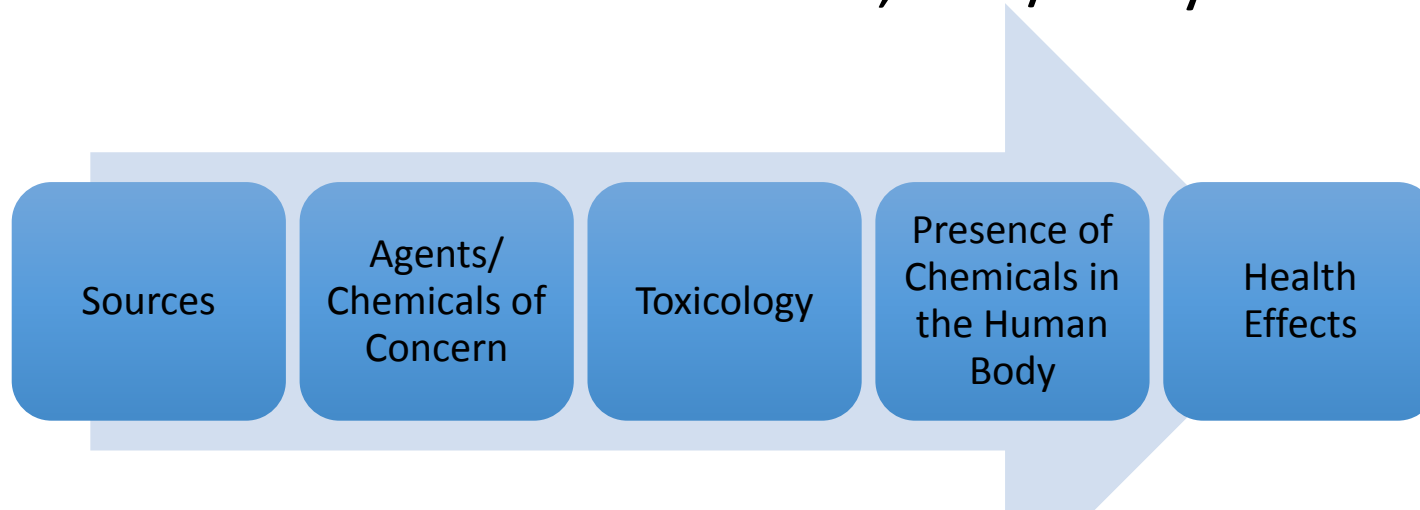


RISK OF ADVERSE EFFECTS

- Intrinsic hazard
- Dose
- Exposure



- Risk can be reduced by using lower quantities of less hazardous substances, and/or by reducing exposure.



Neurotoxicity More: Other Effects Appearing Later

1. Cognitive / Motor / Psychological

A. After recovery from serious acute toxicity

B. After long-term, low dose exposure

1. Epidemiological studies

- No direct association with esterase inhibition or acetylcholine excess
- Exposure assessment difficult
- Variable symptoms

2. Laboratory studies

- Developmental? Biochemical?
- Haven't been able to reliably reproduce in lab animals clinical effects like those of people

3. Further research needed



Roanoke Times,
Mar 20, 1995

Neurotoxicity More:

Other Effects Appearing Later (cont.)

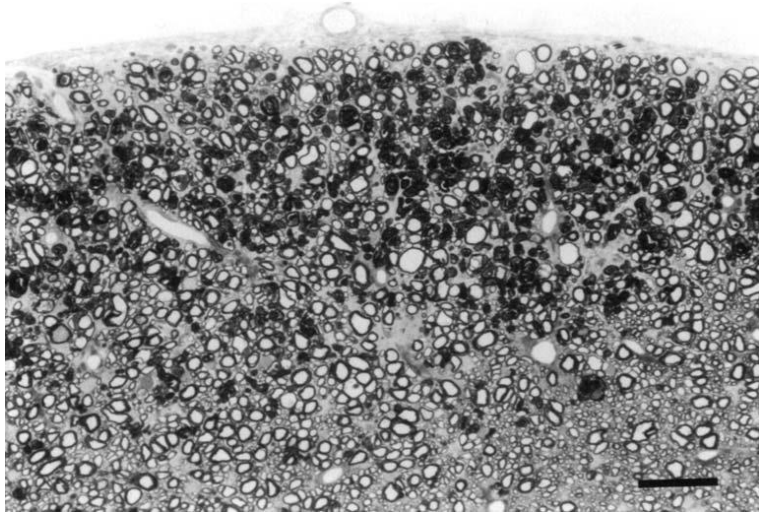
2. Organophosphate-induced *delayed neuropathy*

- Not possible except with specific OP chemistries
- Requires early significant and irreversible inhibition of an esterase different from the inhibition of AChE causing acute toxicity (*neuropathy target esterase, NTE*)
- Progressive peripheral damage to nerve axons doesn't begin until >7 days after exposure
- Does not appear in young or in all animal species
- No treatment
- Rare, because testing of potential pesticides precludes marketing
 - Some OP compounds have/had industrial uses
- Modification possible
 - Prophylaxis with reversible inhibitors of NTE
 - Neuroprotective agents decrease clinical and pathological evidence of damage
 - Exacerbated by post-exposure to inhibitors of NTE



Delayed Neuropathy:

Damage to nervous system depends on enzyme inhibition, species and time



**Neuropathy demonstrated by clinical signs and nerve damage;
Potential for damage by NTE inhibition**

Timing of effects in susceptible species



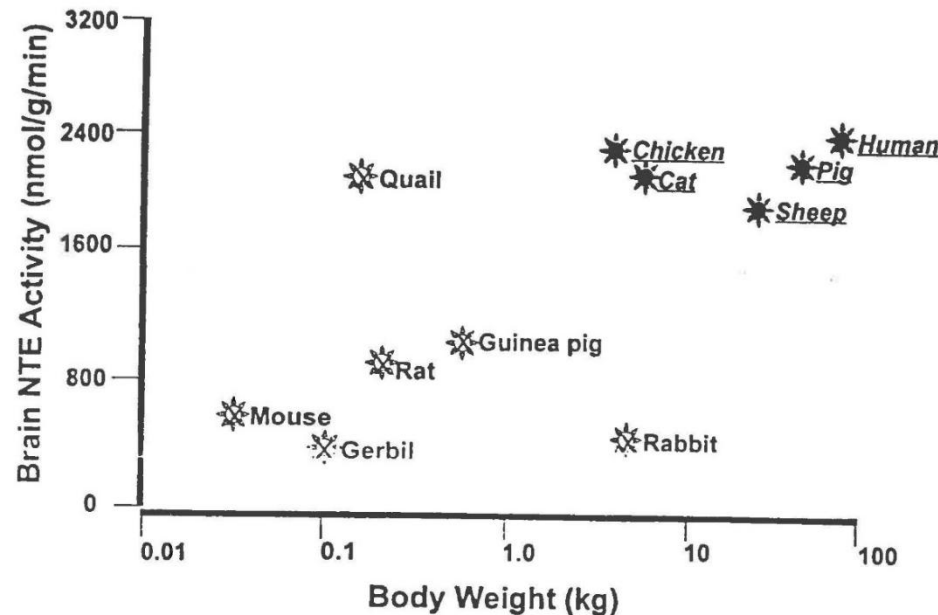
Events: ■ NTE aged (→toxic gain of function)
 ■ Aged NTE exported to axons

“Chemical transection of axons”
 Increased axonal calpain activity
 Electrophysiological deficits
 Retrograde transport deficit
 Wallerian-type degeneration

Net damage to PNS axons:

Modulation:

Ongoing repair
 (sensitive to serine hydrolase inhibitors)



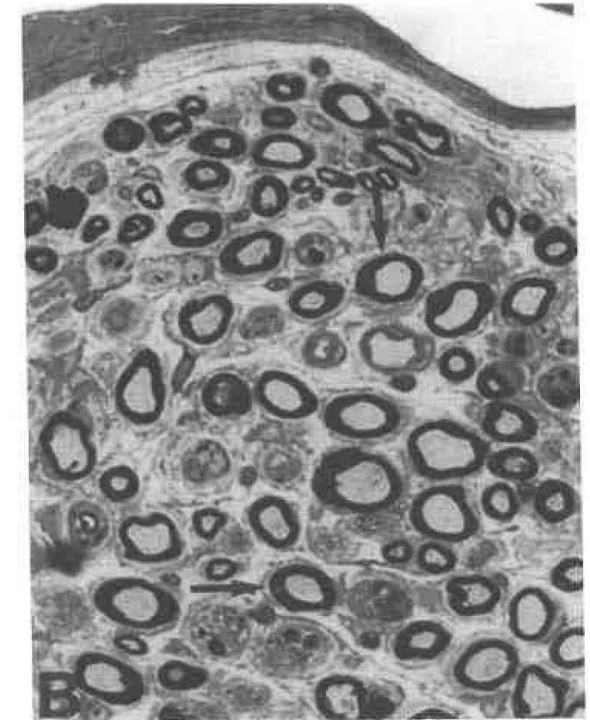
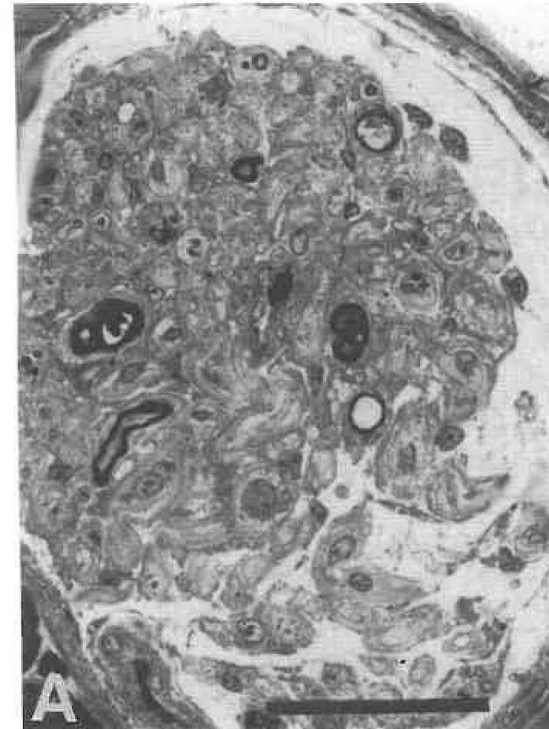
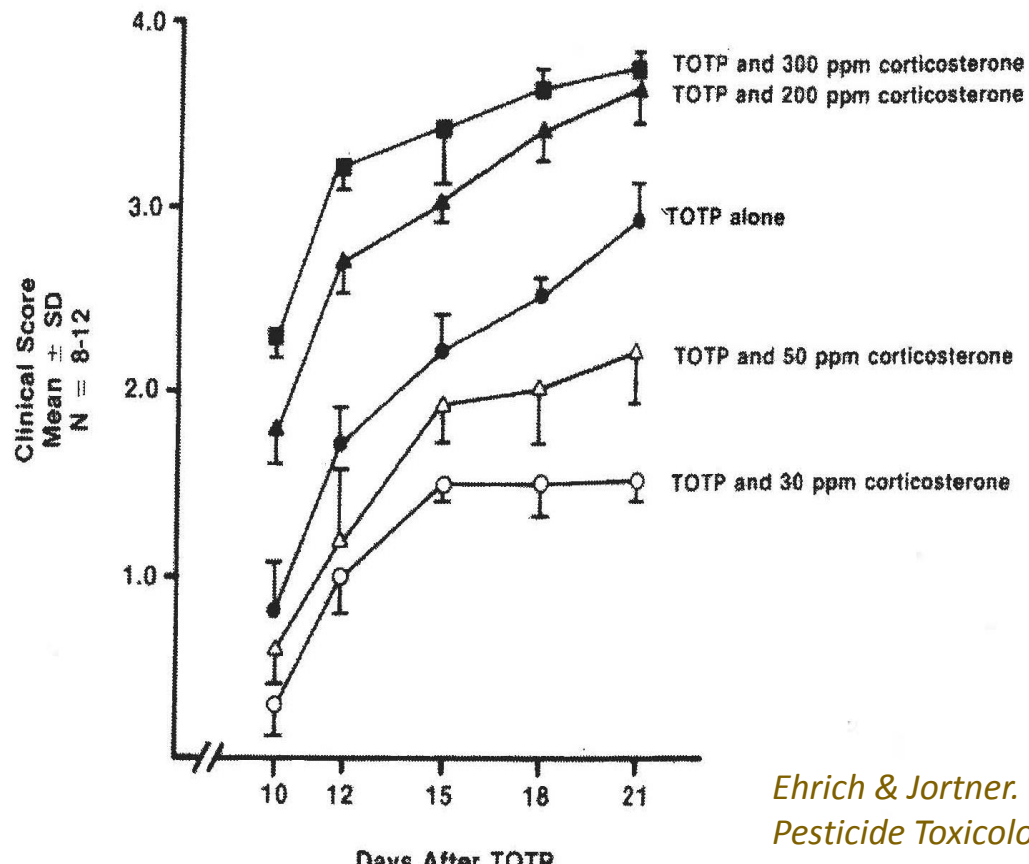
Ehrich & Jortner. Hayes Handbook of Pesticide Toxicology, 3rd ed. (R. Krieger, Ed), Chpt 69, 2010.

Glynn. 2000. Prog. Neurobiol. 61, 61-74.

Neurotoxicity Less: Nervous System Protectants as Ameliorating agents for OP-induced delayed neuropathy: corticoids and calcium channel blockers

Corticoids are neuroprotective unless dose is too high

Calcium channel blockers ameliorate delayed neuropathy



Ehrich & Jortner. Hayes Handbook of Pesticide Toxicology, 2nd ed. (R. Krieger, Ed), Chpt 49, 2001.

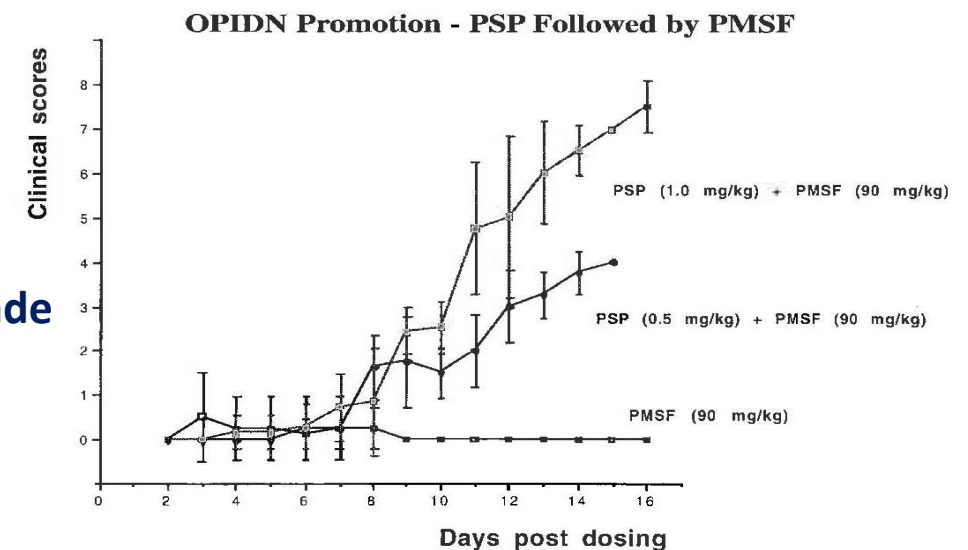
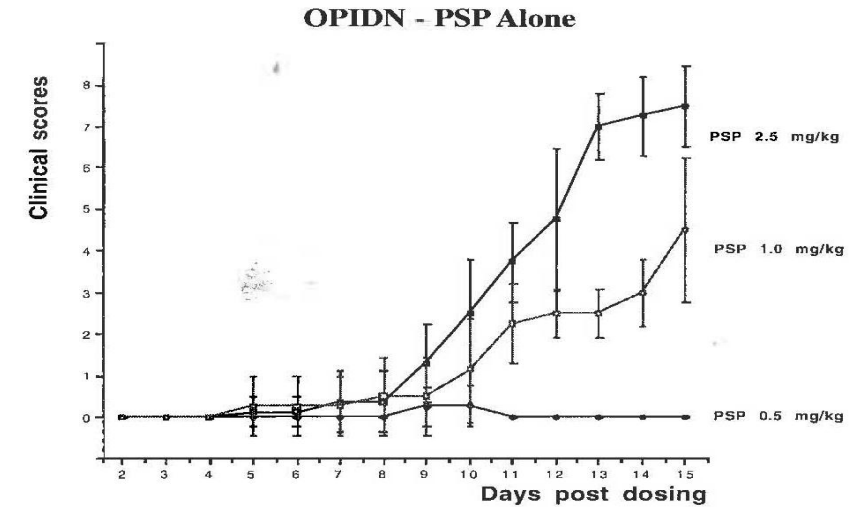
More or Less: Reversible Neuropathy Target Esterase Inhibitors Protect or Exacerbate

- Pre-exposure to reversible NTE inhibitor decreases availability of enzyme for irreversible inhibition by OP.
- Post-exposure to the same inhibitor worsens neuropathy.

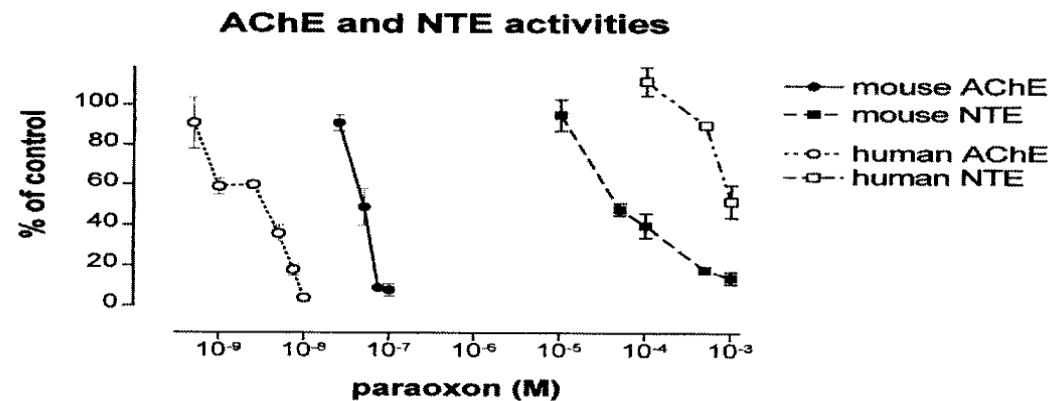


Non-neuropathic
OP doses were made
neuropathic

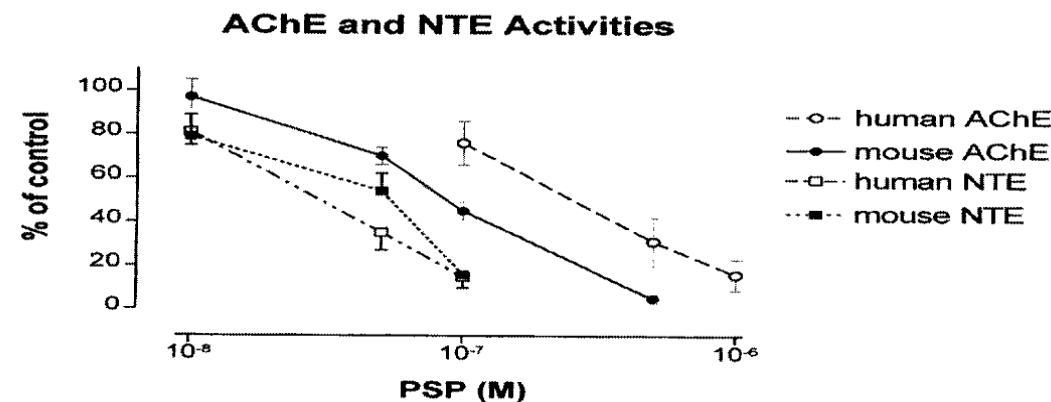
Ehrich & Jortner. Hayes Handbook of Pesticide Toxicology, 3rd ed. (R. Krieger, Ed), Chpt 69, 2010.



Neurotoxicity Less: Why Marketed Pesticides (OP Insecticides) do not cause delayed neuropathy: Comparison of target esterase inhibitions (AChE for acute effects; NTE for delayed effects)



(A)



(B)

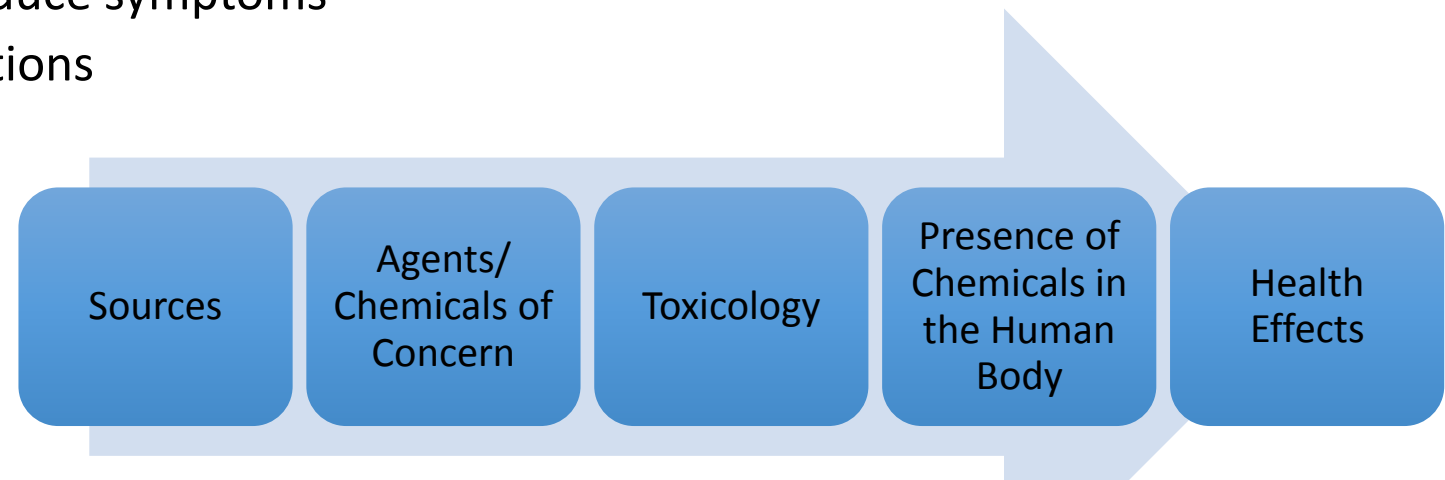
Esterases differed in sensitivity to OPs.

- Occurred with human and with rodent cells
- **AChE much more sensitive than NTE if non-neuropathic**

In vitro assays demonstrated 11/11 tested OPs had predictive NTE/AChE inhibitory ratios

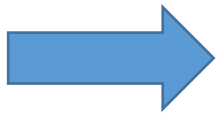
RISK OF ADVERSE EFFECTS

- Intrinsic hazard; Dose; Exposure
- Risk can be reduced by using lower quantities of less hazardous substances, and/or by reducing exposure.
 - **For OP compounds, Risk is lowered by**
 - (1) decreasing availability of the most hazard substances
 - (2) decreasing exposures with protectants/scavengers
 - (3) Prompt general measures to reduce symptoms
 - (4) Improved mechanistic interventions



OBJECTIVES

- To associate mechanisms of acute organophosphate (OP) toxicity with present and future prospects for treatment.
- To distinguish non-acute toxicities associated with OP exposure, including means for amelioration and prevention.
- To identify potential benefits to medical research resulting from OP compound studies.



OPs and potential benefits to medical research

1. Identification of mechanisms associated with neurodegenerative disorders

- Background: OP compounds can be hydrolyzed by esterases that they do not inhibit
 - A-esterases; paraoxonases
 - Paraoxonases have different subtypes; PON2 of interest
 - Work with OP compounds led to the discovery and hypothesis that PON2 is potentially neuroprotective



Paraoxonase 2 (PON2) in the mouse central nervous system: A neuroprotective role?

Gennaro Giordano^a, Toby B. Cole^{a,b,c}, Clement E. Furlong^{b,c}, Lucio G. Costa^{a,d,*}

^a Dept. of Environmental and Occupational Health Sciences, University of Massachusetts Lowell, Lowell, MA 01854

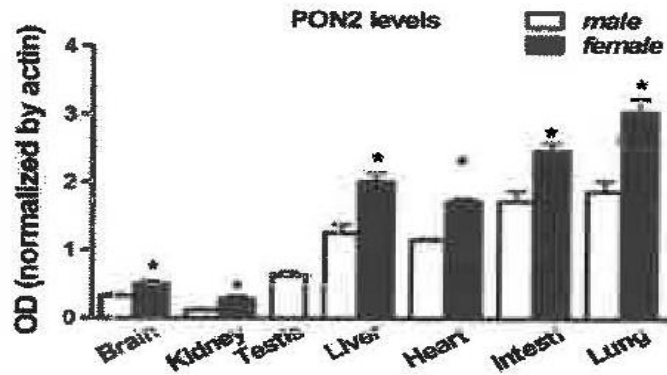
Gender differences in brain susceptibility to oxidative stress are mediated by levels of paraoxonase-2 expression

G. Giordano^a, L. Tait^a, C.E. Furlong^b, T.B. Cole^{a,b,c}, T.J. Kavanagh^a, L.G. Costa^{a,d,*}

Free Radic Biol Med 58, 98-108, 2013

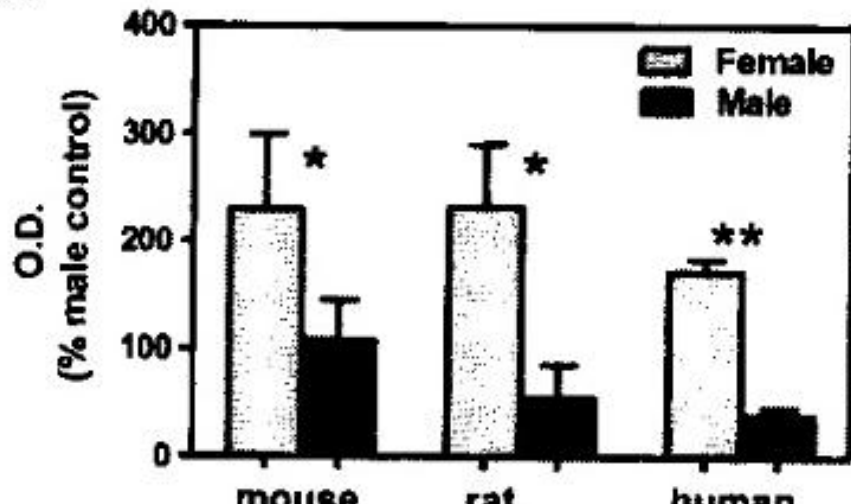
PON2, an antioxidant enzyme found in female brain > male brain tissue, declines with age

A



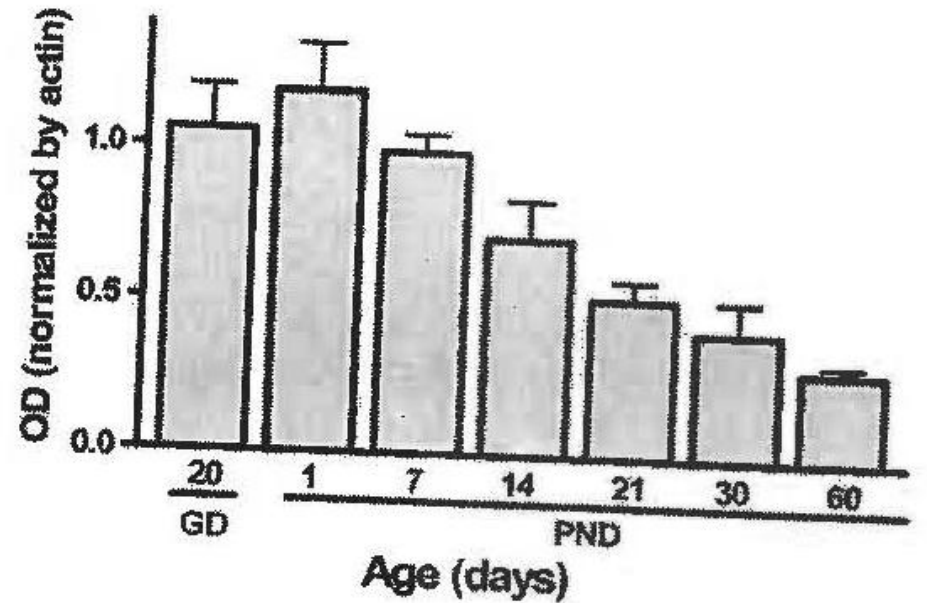
Toxicol Appl Pharmacol 256, 369-378, 2011

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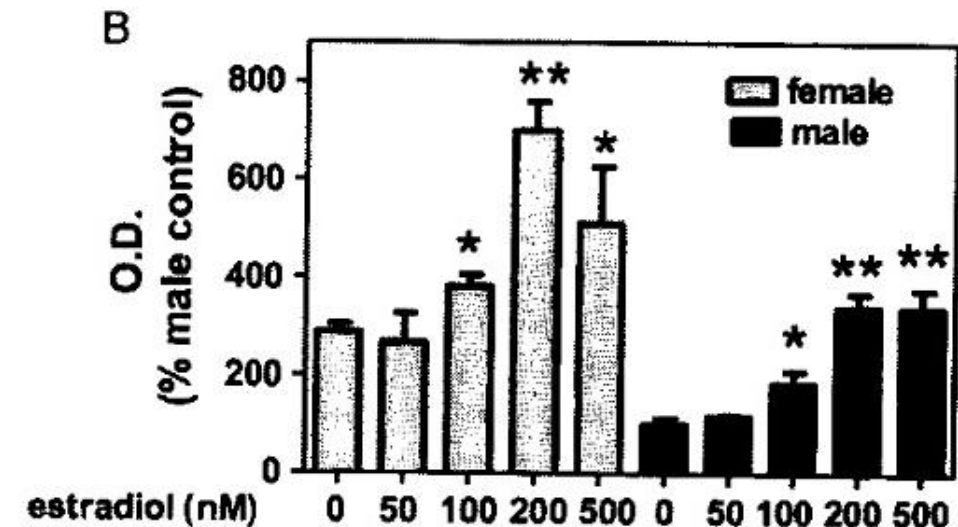
Free Radic Biol Med 58, 98-108, 2013

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Medical Research: Future prospects for PON2

- Brain antioxidant with sex difference
- Had additive protective effect with estradiol
- Role for PON2 in degenerative diseases?
- Possible reason why males often more susceptible than females to neural aging?

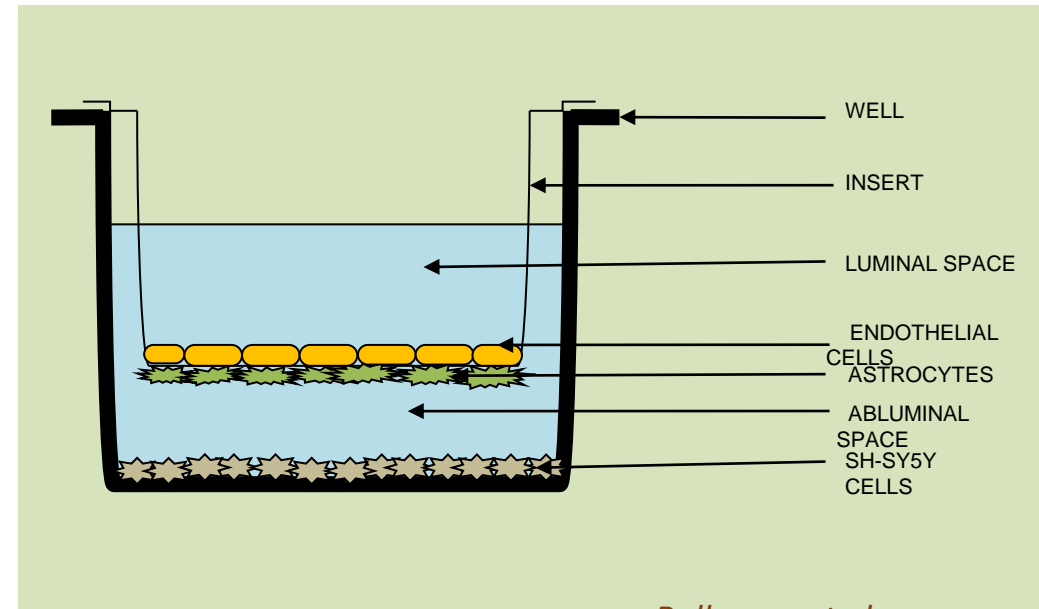
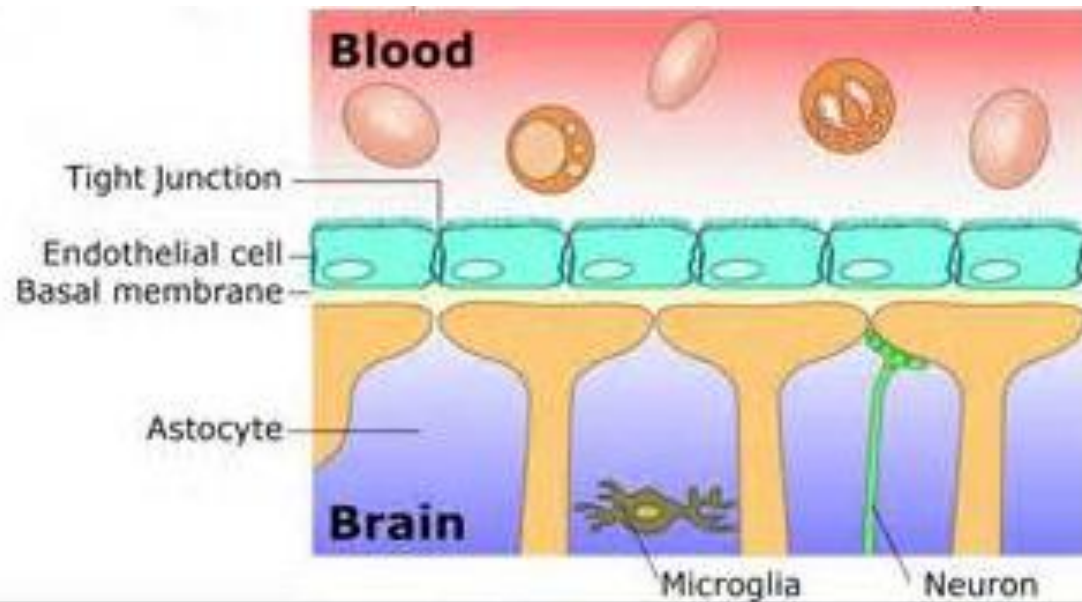


Medical Research (cont.)

2. OP compounds for study of Blood-Brain Barrier (BBB)

- BBB protects brain, but also decreases drug delivery to brain
- Disruption without destruction would be beneficial
- Need to know more about BBB function to investigate

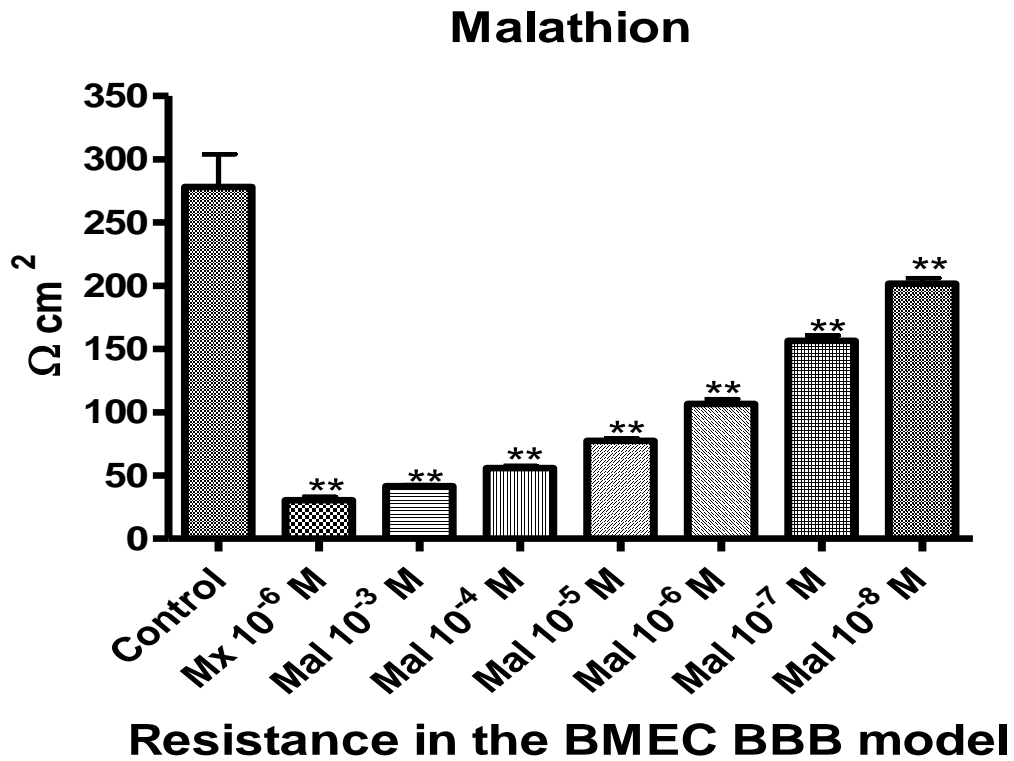
• *In vitro* BLOOD-BRAIN BARRIER



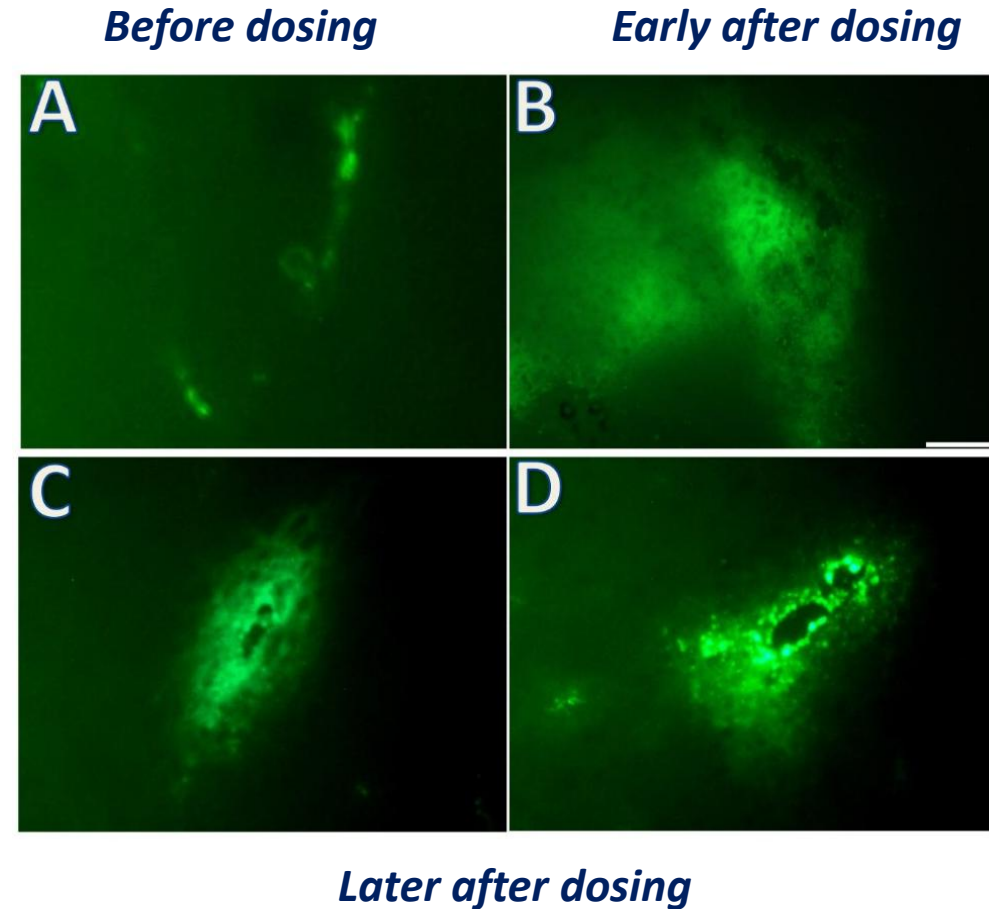
Balbuena et al.,
Toxicol Sci 114, 260-271,
2010

BBB studies (cont.): OP compounds disrupt BBB

In vitro disturbance of BBB is concentration-related

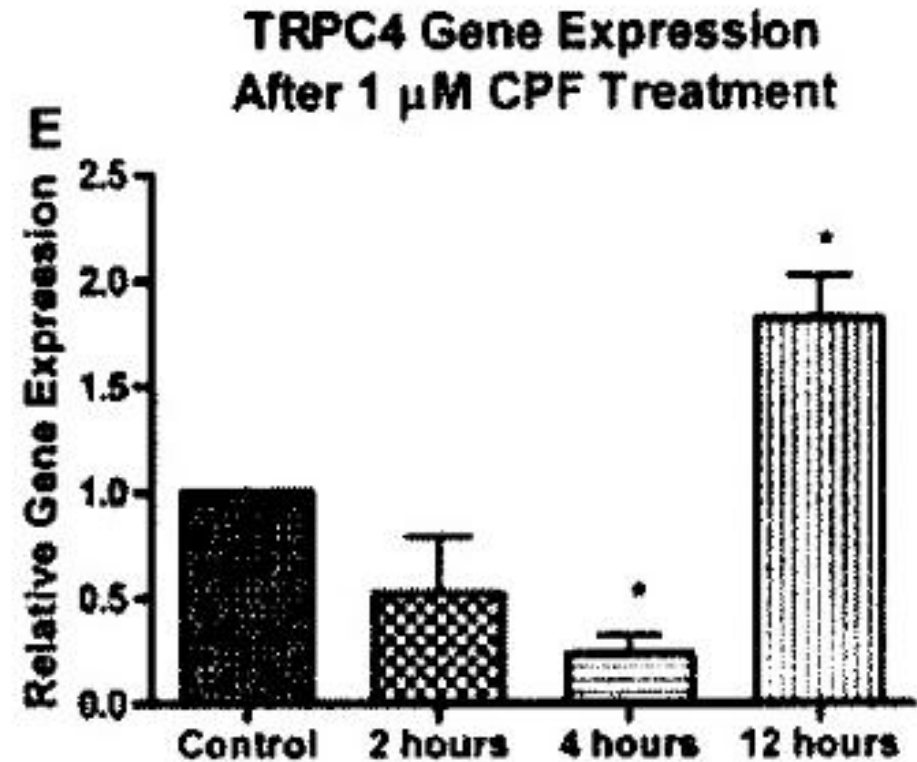
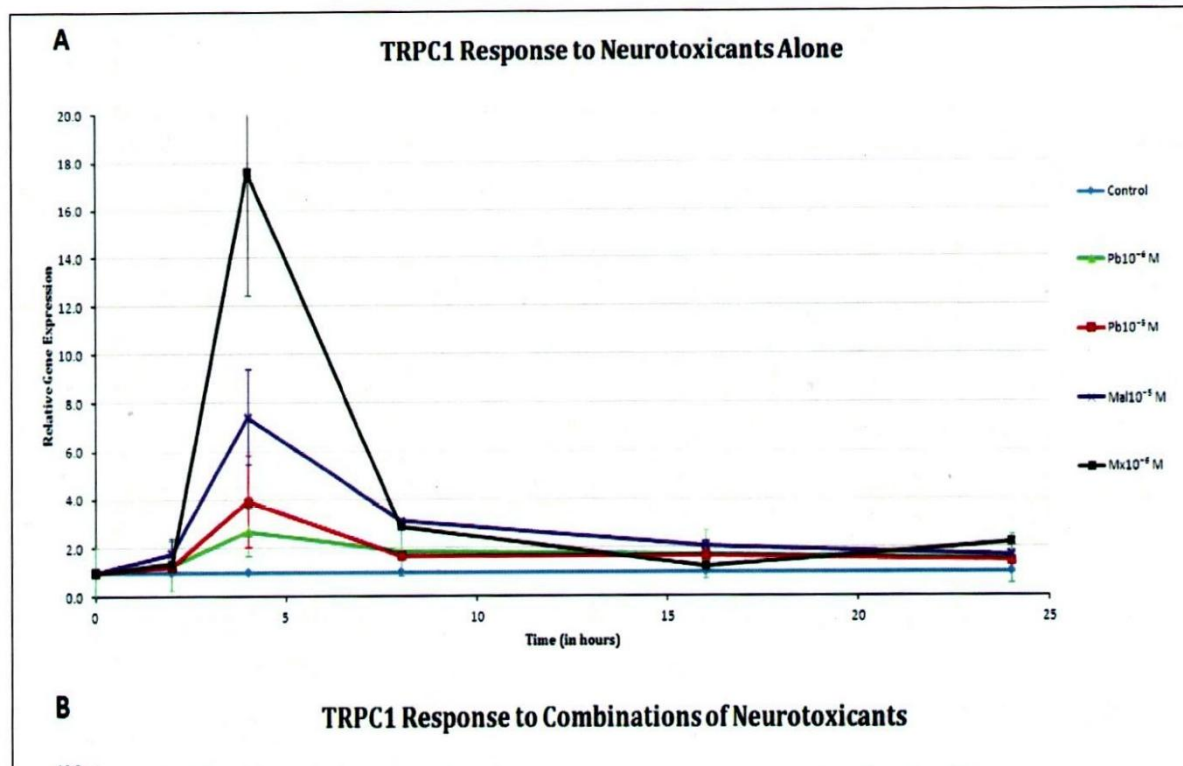


In vivo effects time-related; reversible



Learning more about the BBB: OP effect on BBB may be related to effect on transient receptor potential canonical (TRPC) channel subunits

Effect on TRPC intense but short



Medical Research: Future prospects for BBB studies

- Mechanisms of disease
 - In vitro / in vivo
- Repair
- Drug delivery
 - Treatment effectiveness

Session OBJECTIVES

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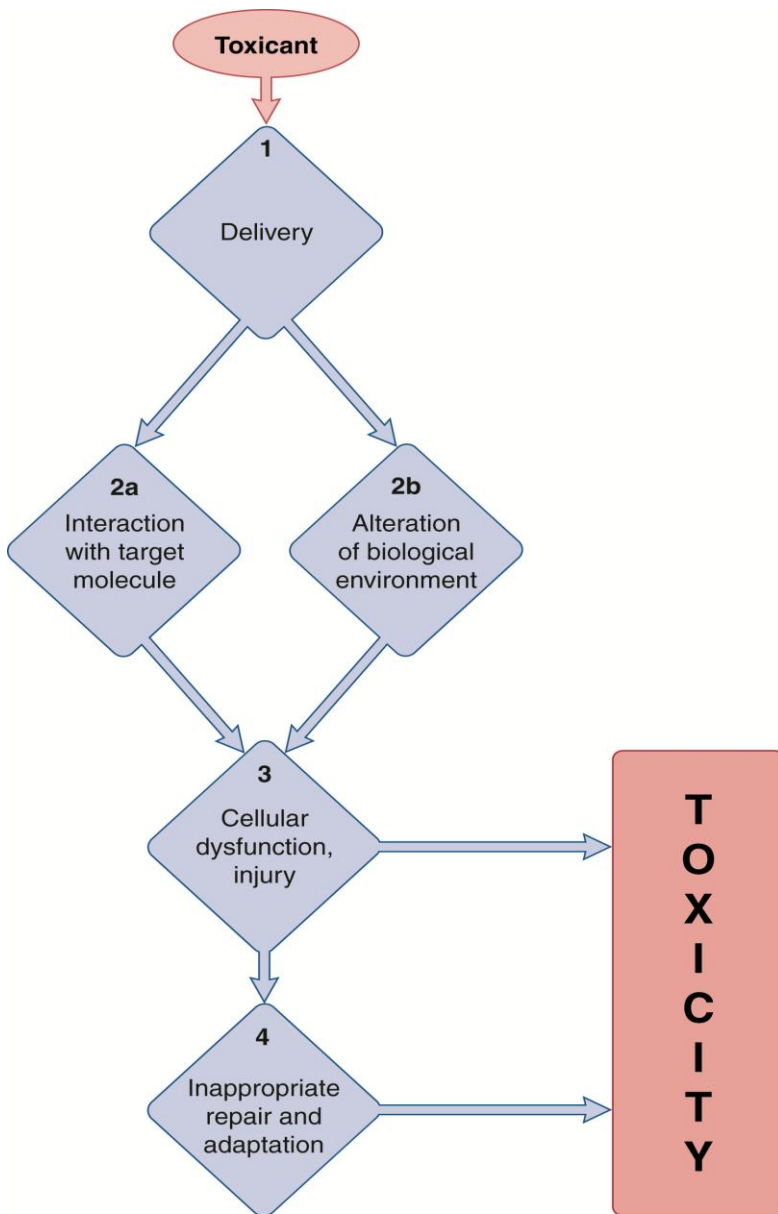


Figure 3-1. Potential stages in the development of toxicity after chemical exposure.