Functional Consequences of Repeated Organophosphate Exposure: Potential Non-Cholinergic Mechanisms

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Terry Lab Interests

Organophosphate Exposure
Drug Abuse
Neuropsychiatric Disorders
Cognitive Function

Novel Drug Discovery & Development

Acetylcholine

\[
\begin{align*}
&\text{CH}_3 \\
&\text{H}_3\text{C} \quad \text{N-CH}_2\text{CH}_2\text{O-C-CH}_3 \\
&\text{acetylcholine}
\end{align*}
\]
Acetylcholine (cholinergic) Synapse

Nerve Growth Factor Signaling

Adapted from Nykjaer et al., *Nature* 427:843-848, 2004

Axonal Transport

Anterograde Transport

Retrograde Transport

Adapted from Nykjaer et al., *Nature* 427:843-848, 2004
Organophosphates
Chemicals Derived From:

- O
  - HO-P-OH
  - OH
  - Phosphoric Acid
- O
  - HO-P-OH
  - H
  - Phosphonic Acid
- O
  - H-P-OH
  - H
  - Phosphinic Acid

Organophosphate-Based Chemicals Found in:

- Insecticides (e.g., malathion, parathion, diazinon, chlorpyrifos)
- Chemical Warfare (“nerve”) Agents (e.g., soman, sarin, tabun, VX)
- Ophthalmic Agents (e.g., echothiophate, isoflurophate)
- Antihelmintics (e.g., trichlorfon)
- Herbicides (e.g., tribufos, merphos)
- Solvents, Plasticizers, and Extreme Pressure Additives for Lubricants

Reviewed, Katz and Brooks, 2010
Gulf War Illness and OPs

- Exposure to one or more acetylcholinesterase inhibitors appears to offer a particularly plausible explanation for several of the neurological-based symptoms of GWI (Golomb et al., 2008).

- An estimated 41,000 military personnel in the first gulf war were exposed to insecticides that contained either carbamate or OP-based AChEIs (Fricker et al., 2000; US Department of Defense, 2003).

- As many as 100,000 military personnel may have been exposed to low (i.e., non-acutely toxic) levels of sarin/cyclosarin following the destruction of an Iraqi munitions storage complex at Khamisiyah, Iraq, in March 1991 (Berardocco, 1997).

OP-Pesticide Use in the First Gulf War

- Fly Bait
  - azamethiphos

- Pest Strips
  - dichlorvos

- Sprayed Liquids
  - chlorpyrifos, diazinon, malathion

- Fogs
  - chlorpyrifos, malathion

Organophosphate Toxicity

- **Acute**
  - **Muscarinic** (postganglionic parasympathetic) "DUMB-BELS": diaphoresis and diarrhea, urination, miosis, bradycardia, bronchospasm, emesis, lacrimation, salivation.
  - **Nicotinic** (neuromuscular junction)- muscle fasciculations, weakness, paralysis, respiratory failure; (CNS)- seizures or CNS depression/coma.

- **Chronic and/or Repeated Low-Level Exposures**
  - Anxiety, depression, psychotic symptoms, deficits in short-term memory, learning, attention, information processing, eye-hand coordination and reaction time, and extrapyramidal symptoms.

* Data primarily from case reports and retrospective epidemiological studies.
Overall Objectives

- Determine the consequences of repeated, “subthreshold” exposures to representative organophosphates on cognitive function in animal models.
  - Information processing and attention
  - Spatial Learning
  - Recognition Memory
  - Working Memory

- Determine the consequences of repeated, low-level exposures to representative organophosphates on neurobiological substrates of cognitive function
  - Cholinergic Markers
  - Neurotrophins
  - Axonal Transport

- Identify therapeutic targets for drug development

Study Algorithm

OP Exposure

OP-Free Washout

Cognitive Function
- Water Maze
- Novel Object Recognition
- Prepulse Inhibition
- 5C-SRTT
- Radial Arm Maze

Cholinergic Markers
- Enzyme Assays
- Receptor Autoradiography
- Western Blot

Neurotrophins (NGF)
- Western Blot
- Immunohistochemistry

Axonal Transport
- Mitochondrial Movement
- &APP Immunohistochemistry
- Mn²⁺ Enhanced MRI
Summary of Previous Chlorpyrifos Studies (repeated Subthreshold exposures)

- Impairments in spatial learning
- Impairments in Prepulse Inhibition of the auditory startle response
- Decreased expression of cholinergic marker proteins in the brain
- Decreased expression of neurotrophin-related proteins in the brain
- Impairments of anterograde and retrograde axonal transport ex vivo


Recently Published Studies

![Chemical structures of Chlorpyrifos and DFP](image-url)

Chlorpyrifos

\[
\begin{align*}
\text{C}_3\text{H}_7\text{O} & - \text{PO} - \text{F} \\
\text{OH}_7\text{C}_3
\end{align*}
\]

DFP
The Rat, Five Choice Serial Reaction Time Task (5C-SRTT)

Continuous Performance Task (CPT)

 AX Type

A  H  X  J  A  K  X  O  I  Y  A  U  B  A  X

5C-SRTT-Chlorpyrifos (Alternate Day Exposures)

Middlemore Risher et al., Neurotoxicology and Teratology 32: 415–424, 2010
5C-SRTT-Chlorpyrifos Experiments

Conclusion

Repeated exposures to subthreshold levels of chlorpyrifos lead to protracted impairments of sustained attention and an increase in impulsive behaviors in rats.
MitoTracker® Imaging Measurements

Movement = mean # moving/µm
Length = average length in the ROI
Number = # of mitochondria/µm

Scale bar = 100 µm

Vehicle

CPF oxon (5.0 nM)

Middlemore-Risher et al., J Pharm Exp Ther 339:341-349, 2011

Movement

Length

Number

Chlorpyrifos (µM)

Chlorpyrifos oxon (µM)

Middlemore-Risher et al., J Pharm Exp Ther 339:341-349, 2011
Summary (CPF & CPO in Neuronal Culture)

- Concentration-dependent decrease in the transport of mitochondria in axons, an increase in mitochondrial length, and a decrease in mitochondrial number (indicative of increased fusion versus fission events).

- The neuronal changes occurred at OP concentrations that did not inhibit AChE activity, they were not blocked by cholinergic antagonists, and they did not appear to be associated with directly toxic effects on mitochondria (i.e., alterations in ATP production, mitochondrial membrane potential, superoxide production).

- The results suggest that an underlying mechanism of OP-based alterations in neurological function might involve alterations in mitochondrial dynamics and/or their transport in axons.

Apoptosis
Spontaneous Novel Object Recognition

Sample Trial
Delay
Choice Trial

Spontaneous Novel Object Recognition
Spontaneous Novel Object Recognition

**Averaged Across Delays**

\[ d_2 \text{ index} = \frac{\text{nove}l - \text{familiar}}{\text{nove}l + \text{familiar}} \]

p75 neurotrophin receptor (p75\textsuperscript{NTR})


TrkA Receptor

DFP Experiments (Conclusion)

- Intermittent, subthreshold exposures nerve agent OPs can lead to protracted deficits in specific domains of cognition (i.e., spatial learning and recall, recognition memory).
- The cognitive deficits may be related to persistent functional changes in brain neurotrophin and cholinergic pathways.
Chronic impairments in spatial learning and memory in rats previously exposed to chlorpyrifos or diisopropylfluorophosphate

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Fig 1. The effects of repeated exposures to CPF 18.0 mg/kg (Left) or DFP 0.75 mg/kg (Right) on cholinesterase activity in the plasma and brain at various time points during a 45 day OP-free washout period. Data (mean ± SEM) are presented as % of vehicle-matched control levels. (N=3-6).

Terry et al., Neurotoxicology and Teratology 34:1-8, 2012
8-Arm Radial Maze
(Win-Shift Task)

8-Arm Radial Maze
(Delayed Non-Match-to Sample)

Training Phase
(Forced 4) → Delay → Test Phase
(Free 8)
Behavioral Testing Began on day 50 of the OP-washout period
Water Maze Hidden Platform Test


During Learning

After Learning

Water Maze

Phase 1

Phase 2

Latency to Platform (sec)

Swim Speeds (cm/sec)

Day of Testing

Day of Testing

Vehicle

CPF 10.0 mg/kg

CPF 18.0 mg/kg

* = p<0.05

+ = p<0.07
“Repeated, subthreshold exposures to CPF and DFP may lead to chronic deficits in spatial learning and memory (i.e., long after cholinesterase inhibition has abated) and that insecticide and nerve agent OPs may have differential effects depending on the cognitive domain evaluated”.
Current and Future Studies

- Specific Aim #1: Determine the consequences of repeated subthreshold exposures to representative OPs on axonal transport in the living rat brain.
  - Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) Studies

- Specific Aim #2: Determine the consequences of repeated subthreshold exposures to representative OPs on myelin in the living rat brain.
  - Diffusion tensor imaging (DTI)
  - Black Gold II Histology

Approved DOD-CDMRP Proposal (GW110073) “Organophosphate-Related Alterations in Myelin and Axonal Transport in the Living Mammalian Brain”

Summary/Conclusions

- Repeated, subthreshold exposures to both insecticide and nerve agent OPs lead to protracted impairments of attention and memory-related behavioral tasks in animals.

- Insecticide and nerve agent OPs may have differential effects on specific domains of cognition.

- The mechanisms underlying OP-related impairments of cognition may involve deleterious effects on mitochondrial morphology and movement, axonal transport, and neurotrophin signaling.
Potential Therapeutic Strategies

- Cholinergic-Based Compounds
- Glutamate Receptor Antagonists
- Mitochondrial-Targeted Antioxidants
- Drugs that Increase Axonal Transport?
- Drugs that Improve Neurotrophin Function
- Cytokine-Based Treatments


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