Practical Connections: Pesticide toxicity

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Activity Topic

Agricultural workers in the U.S. can often be exposed to chemicals that have known toxicities. If not treated immediately, these exposures can lead to death. Legal protections for these workers are in some cases minimal, and even when present, are poorly enforced. Complicating these issues is the fact that many agricultural workers have poor access to emergency services. We will focus on one specific organophosphate pesticide, chlorpyrifos, and California's January 2021 ban on use. Here we discuss topics including the underlying biology and chemistry of organophosphate toxicity, the debate over epidemiological studies, law, health equity, and ethical decision making.

Prerequisite knowledge needed:

Introductory medicinal chemistry
Introductory enzymatic reactions (biochemistry)
A&P of the Autonomic Nervous System and neurotransmitters (gen. biology/A&P)
Pharmacodynamics (enzyme dose/inhibition curves) (biochemistry)
Toxicology (biochemistry)
Basic PK of drug absorption (biochemistry)
Basics of CYP function and drug metabolism (biochemistry)

Learning objectives:

Students should:

1. Identify reactions and physiochemical properties based on molecular structures
2. Predict or identify metabolic pathways
3. Interpret scientific data, graphs, and tables
4. Predict or identify effects of modulation of the autonomic nervous system
5. Apply principles of enzymatic pharmacodynamics and
6. Apply principles of population toxicology

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Student Pre-Assignment

1. ***Read: article from US News and World Report, targeted for a general audience: Commentary: Q&A: Why California Is Banning Chlorpyrifos, A Widely Used Pesticide. Jan 23, 2020***

https://www.usnews.com/news/best-states/articles/2020-01-23/reasons-why-california-is-banning-chlorpyrifos-a-widely-used-pesticide

2. ***Read: Introduction to organophosphate (OP) insecticides***

A common class of insecticides used in the United States is the organophosphates (OPs). The OPs were initially mass produced for use as insecticides in the 1940s, and soon became the most widely used insecticides globally.[1] It was quickly recognized that these agents could produce acute toxicity in humans,[2] while long term effects continue to be studied,[3,4] and legal regulation debated.[5,6] In this module you will have a chance to apply skills and knowledge that you will use throughout your career to appraise the science and use of prescription, OTC, and illicit drugs.

Exposures to OPs can range from short-term (acute) use to chronic exposure from environmental contaminants. Acute toxicity is clearly dose-dependent, and globally, it has been estimated that each year there are approximately 3 million cases of *acute* OP poisoning, with 300,000 fatalities.[6] OP exposure is particularly high in South and South-East Asia, as seen in a study on urinary concentrations of the OP chlorpyfiros (Figure 1.),[7] and the subsequent health effects are still being investigated.[4,6]

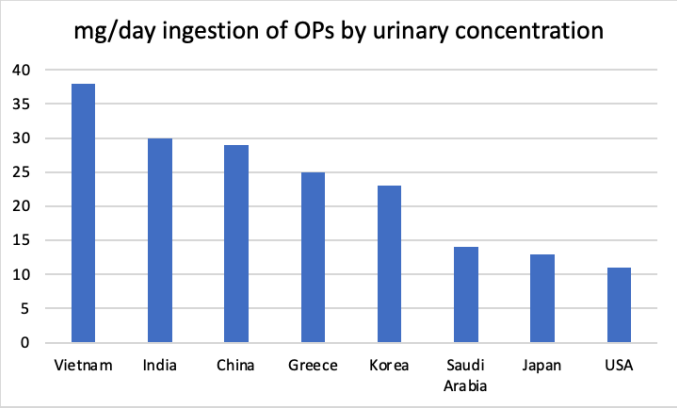
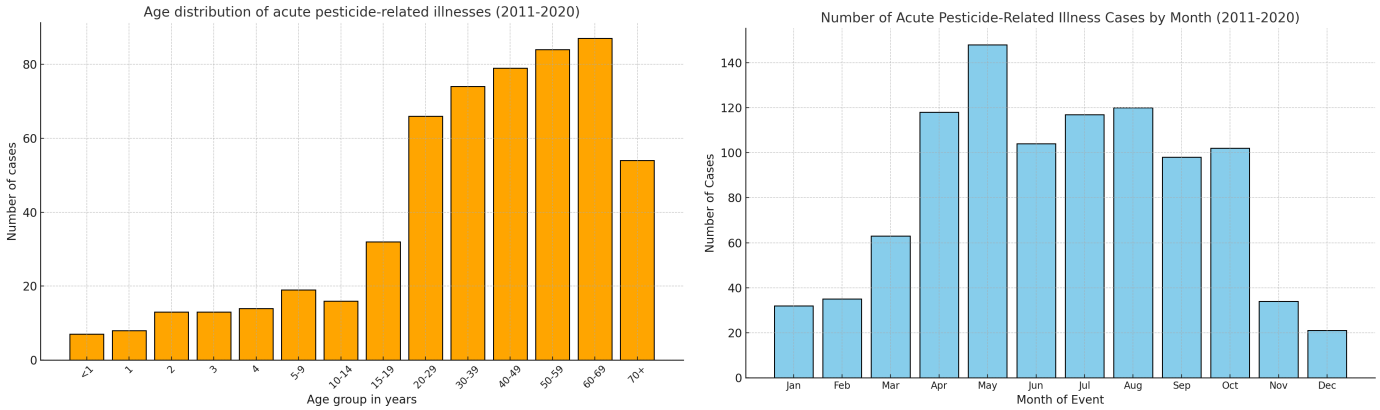


Figure 1. Median daily intake of the chlorpyrifos, parathion, diazinon, estimated from urinary metabolite concentrations. Data from Li, 2018.[7]

In the US as a whole, reported deaths from acute OP poisoning are relatively rare, possibly due to the antidote agent pralidoxime and increasing regulation.[8,9] Reliable statistics on the actual number of toxic exposures are not available as it is believed that a large percentage of poisonings are not reported due to individuals either not seeking heath care or providers not reporting to state or national databases.[9] However, some statistics are available about the demographics of persons developing acute pesticide poisoning in confirmed cases in Oregon, where the number of reported household exposures is far greater than reported work-place exposures.[10]



Figures 2 and 3. Data on acute pesticide-related cases 2011-2020, from Oregon Health Authority, 2023.[10]

Chlorpyrifos (CPF), an OP developed in the 1960s, has been a high-use insecticide in many regions of the United States (Figure 4).[11,12] It shares an acute toxicity profile with other OPs. However, low-dose exposure to this agent is also associated with chronic effects, and is widely investigated as a causative agent in neurodevelopmental toxicity. Children who were exposed to CPF *in-utero* have a higher incidence of attention disorders, lower IQ, lessened working memory, delayed motor development, and lower birth weight.[3,13] In 2021 California independently banned all use of CPF, and in December 2024 the US EPA banned all but 11 food and feed crop uses for nine brand products. The 11 uses still allowed are alfalfa, apple, asparagus, cherry (tart), citrus, cotton, peach, soybean, strawberry, sugar beets, and wheat.[5]

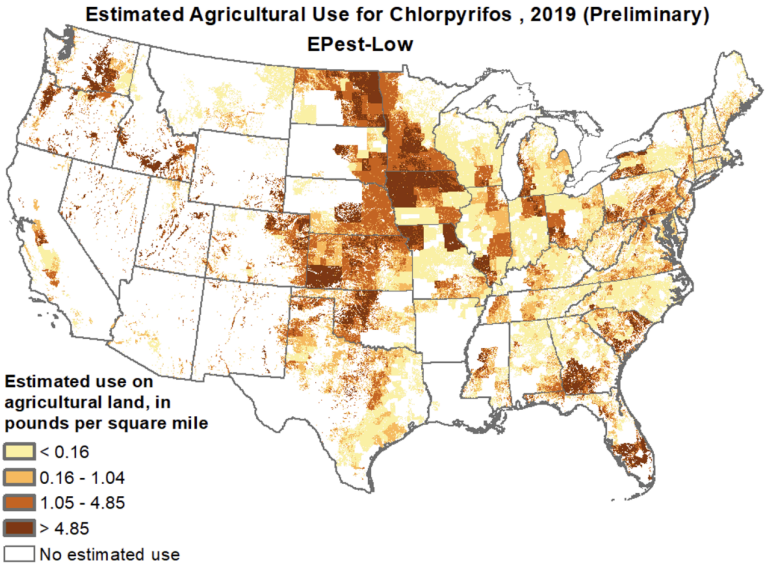


Figure 4. United States Geological Survey Pesticide National Synthesis Project, Estimated Annual Agricultural Pesticide Use, 2019.[12]

An important property of CPF is that it is not only the parent agent that is biologically active. CPF is metabolized into two compounds that have their own toxicities: 1) the active insecticide chlorpyrifos oxon (CPO), and 2) the hormone receptor disruptor 3,5,6-trichloro-2-pyridinol (TCP). These metabolites have longer half-lives in the system than the parent compound. The inhibition of estrogen and testosterone signaling by TCP is thought to be a causative factor in endocrine and reproductive toxicity seen with CPF exposure, including male and female infertility.[14,15]

3. ***Complete pre-class Moodle quiz (10pt) done individually***

Q1 Organophosphates directly inhibit:
*a. break-down of acetylcholine
b. activation of nicotinic receptors
c. synthesis of acetylcholine
d. activation of muscarinic receptors

Q2 Inhibition of AChE will produce:
a. anti-cholinergic effects
*b. pro-cholinergic effects

Q3 In the autonomic nervous system, AChEIs will increase activity of:
a. sympathetic activity
b. parasympathetic activity
*c. both sympathetic and parasympathetic activity

Q4 Using the information presented in your readings, describe factors you think may explain the demographics of OP poisoning victims. (max 3 sentences)

Students may come up with mutiple interesting possibilities that can be discussed. Students would not be expected to recognize all, and may come up with ideas not listed here:

- increased age of subjects beginning in 20s, peak reporting in summer, and the distribution of chlorpyrifos use in agricultural areas suggests occupational agricultural use.
- high ratio of household exposures and age demographics suggest some accidental household exposures in children.
- high reporting in elder subjects (60+) could be due to continued exposure or, possibly more likely, increased susceptibility to toxicity in elder adults. (The age chart reflects real numbers, not percentages, so the numerical decrease in exposures in elders 70+ would be expected based on population demographics)
- useful information: In some countries purposeful pesticide poisoning is a common method of suicide.

Q5 The relationship of CPF to the endocrine disruptor TCP is that of a/an:
*a. pro-drug
b. desensitizing agent
c. inducer

Practical Connections: Pesticide toxicity

Student Handout: In-class work

medicinal chemistry, drug metabolism, enzyme function, pharmacology, A&P, neurotransmitters, population toxicology

Photo by Leu[16]

Organophosphate medicinal chemistry

The organophosphates are a large group of agents, with some shown below. Examples of organophosphate insecticides commonly used include malathion, parathion, diazinon, fenthion, dichlorvos, chlorpyrifos, and ethion.[17]

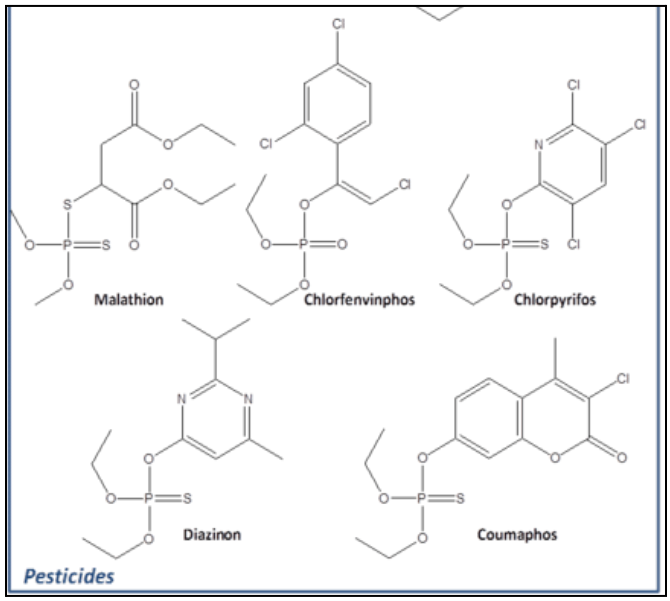


Figure 1. Structures of organophosphate pesticides, from Jacquet, 2016.[17]

1. (Fig 1) a) Are these agents generally lipophilic or hydrophilic? b) What would the major routes of absorption be during environmental exposure for unprotected farmworkers? Would these be dermally absorbed?

   They are lipophilic and are highly dermally absorbed

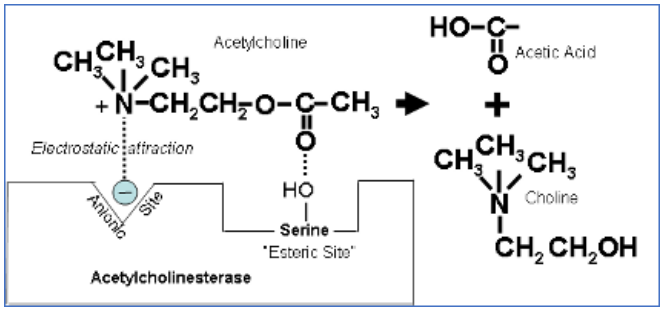


Fig 2. Enzymatic cleavage of acetylcholine from CDC Agency for Toxic Substances and Disease Registry, 2012.[18]

The figure above (Fig 2) shows the enzymatic reaction that occurs when ACh is broken down by AChE in the synaptic cleft.[18] Below (Fig 3) is the reaction that occurs when an organophosphate is acted on by AChE.[18]

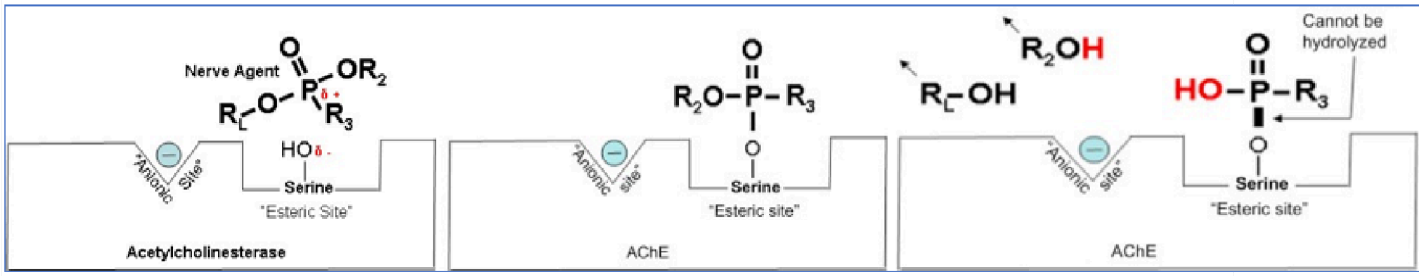


Fig 3. Suicide inhibition of acetylcholinesterase by an organophosphate from CDC Agency for Toxic Substances and Disease Registry, 2012.[18]

2. What type of reaction is occurring during ACh breakdown (Fig 2)?

   hydrolysis

3. Structurally, why isn't AChE able to break down the OP agent (Fig 3)?

   The substrate of an esterase is an ester, and this is a phosphonate therefore makes a covalent bond.

Organophosphate pharmacology and A&P

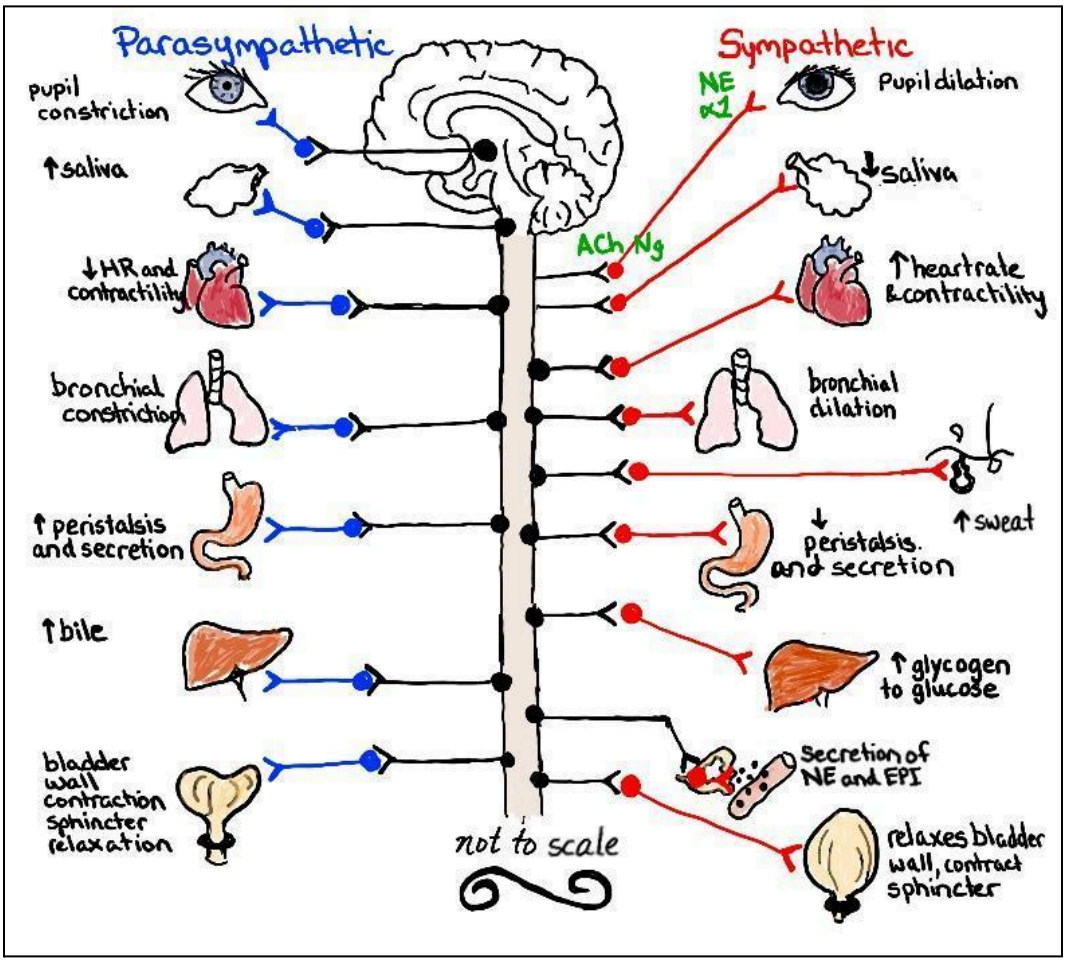


Fig 4. Conceptual representation of the ANS. The neurons are not shown exiting from specific spinal levels or ganglia. Drawing produced by author.

4. Identify the sites of cholinergic and adrenergic synapses on the diagram (Fig 4), labelling the neurotransmitter and post-synaptic receptor subtypes (Ng, M2, alpha2, etc). at each synapse and end organ.

   See example of sympathetic innervation of the pupillary muscles.

5. A child was playing in the garage with several chemicals and is now brought into the ED complaining [only] of salivation and tearing, nausea, diarrhea, and sweating. Using the information you have compiled above, was the toxic agent likely to be a/an?

   a. AChE inhibitor
   b. Nicotinic receptor agonist
   c. *Muscarinic receptor agonist
   d. Nicotinic receptor antagonist
   e. Muscarinic receptor antagonist

Cholinergic Toxidrome	
Muscarinic Symptoms	**Nicotinic Symptoms**
S – Salivation	**M** – Muscle cramps
L – Lacrimation	**T** – Tachycardia
U – Urination	**W** – Weakness
D – Defecation	**T** – Twitching
G – GI cramping	**F** - Fasciculations
E – Emesis	

table for debrief only- they should access this information online during discussion.

Organophosphate enzymology and toxicology

AChEIs have differing IC_{50}s at the AChE. You can use this information to compare physiological inhibition of several agents which have the same target. This is how you might see this information in a journal article in **table** (Table 1) or **enzyme-inhibitor dose/inhibition curve** (Fig 5) formats. (The difference between 5 and 20 minute incubation times does not impact your answers.)

Compound	IC_{50} (20 min), mol/L	Compound	IC_{50} (5 min), mol/L
Diazinon	$> 2.0 \times 10^{-4}$	Malathion	3.2×10^{-5}
Diazoxon	5.1×10^{-8}	Malaoxon	4.7×10^{-7}
Chlorpyrifos-oxon	3.0×10^{-8}	Isomalathion	6.0×10^{-7}
Chlorpyrifos	4.3×10^{-6}	Diethylmaleate	6.0×10^{-2}

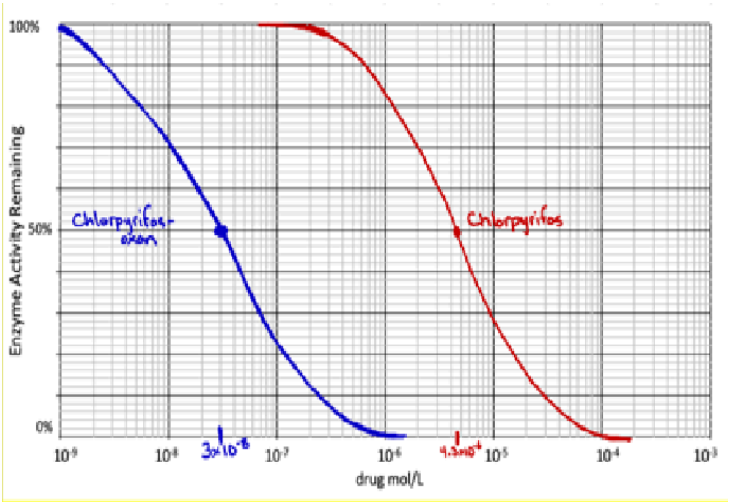


Fig. 5a and b. a)IC_{50}s for several organophosphates. Data from Colovic, 2013. b)Enzyme-inhibitor dose/inhibition curves of chlorpyrifos and chlorpyrifos-oxone at AChE. Curve by authors.

5. Using their IC_{50}s (Fig 5[19]), rank order the potency of diazoxon, chlorpyrifos, and chlorpyrifos-oxon as inhibitors of AChE. Rank from highest potency to lowest potency.

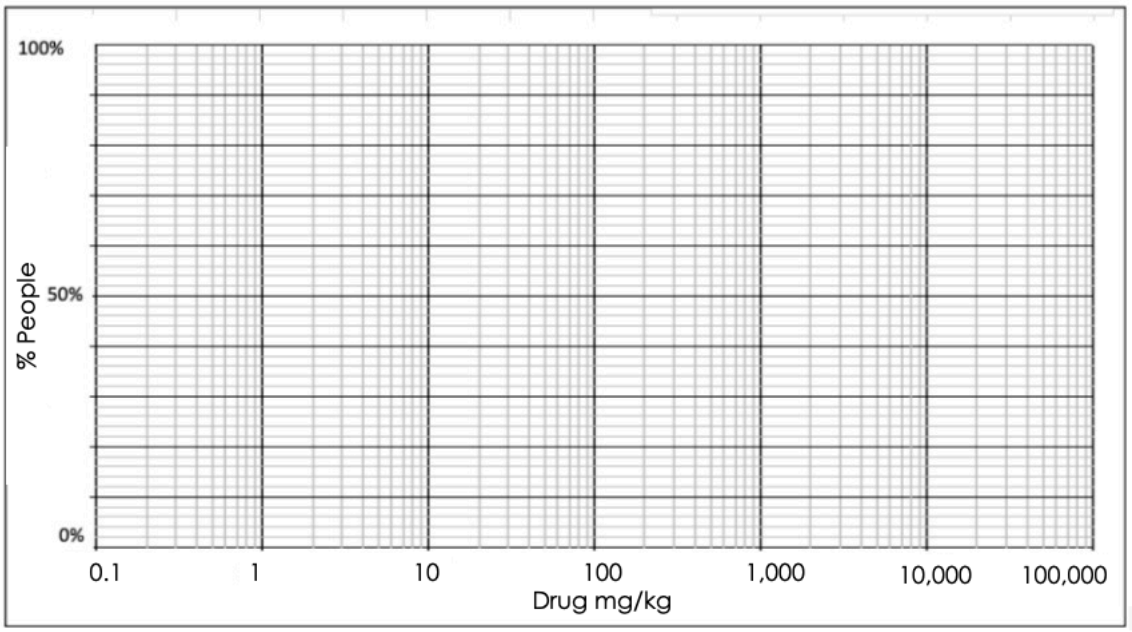
   chlorpyrifos-oxon > diazoxon > chlorpyrifos

   (Facilitator's hints: 1) ask them to define IC50 in a full sentence. 2) a more potent drug needs a lower concentration to have the same effect. 3) what is a lower concentration? 10-5 or 10-8? 0.00001 or 0.00000001?)

Mammalian toxicology	Chlorpyrifos	Parathion
Oral	LD_{50} for rats 135-163 mg/kg	LD_{50} for rats 8 mg/kg
Inhalation	LC_{50} at 4-6hr for rats >0.2 mg/l	LC_{50} at 4 hr for rats 0.03 mg/l

Fig. 6. LD_{50}s for chlorpyrifos and parathion. Data from Huang, 2009.[20]

6. Using the data from Fig. 6, draw out the LD_{50} population toxicology curves for oral chlorpyrifos and parathion administration on the graph below. (Use the entire Y-axis from 0 to 100% of population and just make up a slope and use it for both agents)



7. Explain the difference in the type of information portrayed between the graph you have just completed and the graph in Fig 5.

Fig 5 showed the amount of inhibition a drug produces at an enzyme at any particular dose. This does not require an organism and doesn't measure any downstream physiological functions. You do not know what the effect is in a person. The graph in question 6 shows the number of people dead up to and including any particular dose. This doesn't tell you the affinity of the drug at the enzyme, just the effect in a person. (suggested facilitator questions: Why does this not have an EC_{50}? Because these OPs aren't used for a therapeutic effect.)

Chlorpyrifos (CPO): pharmacodynamics and metabolism

Recall that CPF is metabolized into two compounds that have their own toxicities:

a) AChEI chlorpyrifos oxon (CPO)
b) hormone receptor disruptor 3,5,6-trichloro-2-pyridinol (TCP)

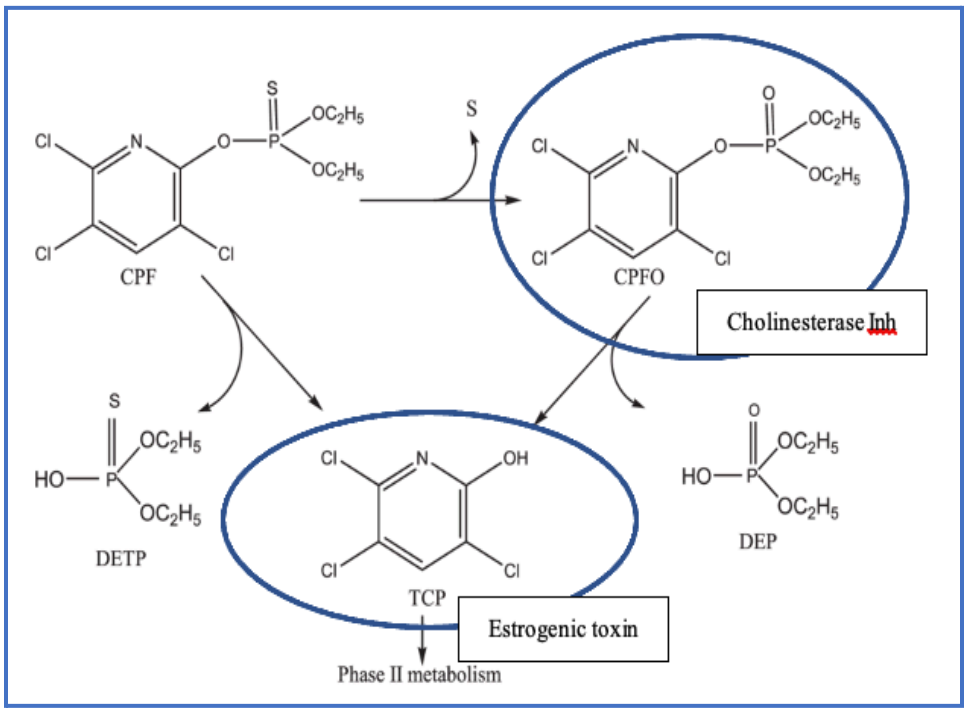


Fig 6. Metabolism of CPF modified from Wielgomas, 2006[21]

8. Which enzyme is most likely to carry out the reaction from CPF to CPO?
   a) *CYP2B6
   b) AChE
   c) UGT1B1
   d) SULT1A1

9. How might genetic factors explain the fact that there is a large variability in the amount of CPO that are formed by different people?

   CYP isoform variability (interactions with CYP inducers/inhibitors such as other toxins or drugs would be cool if mentioned, but aren't genetic)

EXTRA CREDIT IF YOU HAVE TIME:

Law and ethics

In 2018 the EPA adopted a rule that limited what evidence is allowed for consideration in their review processes. This rule entitled "Strengthening Transparency in Regulatory Science"[22] was enacted with the stated goal of ensuring that the public can evaluate the type and quality of information being used in the EPAs decision making. This rule "would require EPA to only consider studies in its rulemaking process if the underlying data can be made publicly available."[22,23] This rule was highly controversial, and scientific and medical organizations were concerned about how this relates to process and study participant privacy.[24] This rule was vacated in 2021, but similar actions may be considered in the future.[25]

10. Data collected in epidemiological or toxicology studies often include occupational histories, personal medical histories, and home locations. How does this relate to HIPAA regulations?

These studies have confidentiality agreements in which the researchers assure private information will not be made public; unless specifically agreed, this information could not be released. Under that rule, the EPA would not be able to review and regulate most new agents.

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