Establishing the Neurotoxic Impact of Chlorpyrifos Exposure in Workers

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Credit: Dr W. Kent Anger

Scientia

ESTABLISHING THE NEUROTOXIC IMPACT OF CHLORPYRIFOS EXPOSURE IN WORKERS

Chlorpyrifos (CPF) is one of the most commonly used pesticides in the world. Agricultural workers in Egypt have relatively high levels of exposure to it when working in the cotton fields but until now, the neurotoxic impact of this has been uncertain due to a lack of evidence linking CPF dose and neurotoxicity. **Dr W. Kent Anger**, **Dr Fayssal M. Farahat**, **Dr Pamela J. Lein** and **Dr Diane S. Rohlman** have brought together their respective research expertise to collaborate on this issue. Their findings have the potential to greatly improve the long-term health of employees working with pesticides.

Chlorpyrifos: An Effective Pesticide

Organophosphorus pesticides (OPs) are the most common class of insecticides used in agriculture globally, and include a compound called chlorpyrifos (CPF). Sprayed on plants, animals and even buildings, CPF is used to kill pests by disrupting their nervous system. CPF inhibits the enzyme acetylcholinesterase (AChE), which would normally break down the neurotransmitter acetylcholine. This results in a build-up of acetylcholine in between neurons in the brain, which eventually stops the neurons from signalling. This build-up, known as acute cholinergic toxicity (or chlorpyrifos poisoning) causes the targeted insects, worms and other pests to die.

Although very effective at killing pests, CPF is also hazardous to the humans who apply it to agricultural crops. The same mechanism that causes acute toxicity in insects can also cause acute cholinergic toxicity in humans. However, a large amount of research suggests that repeated exposure to lower doses of OPs that do not cause acute cholinergic toxicity may cause a variety of neurological issues via mechanism(s) other than AChE inhibition. These range from psychiatric conditions including depression to neurodegenerative diseases like Alzheimer's disease and deficits in cognitive functioning.

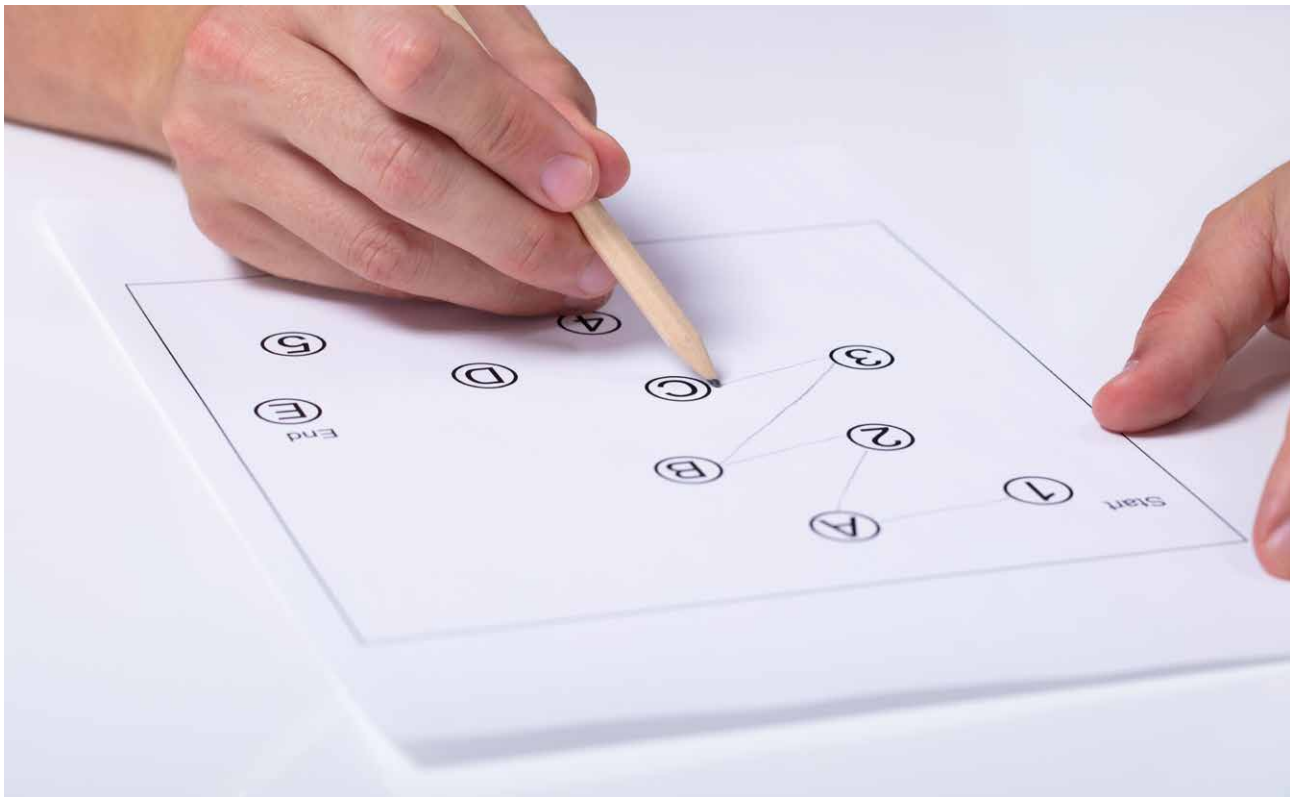
However, in the past, not all scientists have agreed that CPF is neurotoxic at doses that do not cause acute cholinergic toxicity due to a lack of evidence supporting a dose-response relationship, which is considered a central tenet of toxicology. In other words, there has been little evidence to confirm that the likelihood of a neurotoxic response to CPF increased as the levels of CPF exposure increased – a dose-response relationship. It was also unclear whether the classic biomarkers used to assess OP exposure in humans, specifically, cholinesterase activity in blood or levels of OP metabolites in urine, were relevant as biomarkers of occupationally-induced CPF neurotoxicity. Biomarkers are characteristics that can be measured to indicate biological processes occurring within the body in response to contaminants or interventions.

Cotton fields.

Establishing a definitive set of aims to investigate, Dr W. Kent Anger, Dr Fayssal M. Farahat, Dr Pamela J. Lein and Dr Diane S. Rohlman and their colleagues collaborated on an elegant set of studies to confirm the occupational health risks of CPF and to make suggestions for improvements to working conditions to mitigate health risks from CPF.

Patterns of Exposure

The first aim of the group was to establish patterns of exposure to CPF, which was achieved by Dr Farahat and his team studying different types of agricultural workers in Egypt. Based in the Nile delta region, the Ministry of Agriculture oversees cotton fields where they employ a number of different workers in different roles to ensure pest



Cognitive function assessment.

control. There are typically three groups of workers involved in the pesticide application process: applicators who spray the pesticide, technicians who walk in the fields to direct the applicators where to focus the spray, and engineers who usually stand to the side and oversee the operation.

The research team determined how much CPF each group of workers was exposed to by measuring levels of a molecule called 3,5,6-trichloro-2-pyridinol (simplified to TCPy) in their urine samples. TCPy is produced by the body during the metabolic breakdown of CPF, then eliminated in the urine. So, higher levels of TCPy in a sample indicates a higher intake of CPF. Applicators were found to have the highest levels of CPF exposure by far, followed by technicians and then engineers who had the lowest levels.

A previous study had shown that in these agricultural workers, CPF primarily enters the body through the skin (dermally), rather than from inhalation in the lungs. When the pesticide is being applied to cotton fields, the applicators walk through the plants they have just sprayed and as a result, their clothes and skin have sustained contact with the pesticides. Consequently, dermal exposure on their legs was found to be particularly high and, due to leakages on some of the applicators' backpack sprayers, some workers also experienced high exposure on their necks and backs.

The Effect of Dose on Behavioural Tests

Neurobehavioural tests provide a key tool for quantifying the biological effects of CPF on brain function in humans. In the next aim headed up by Drs Anger and Rohlman, behavioural tests were used to assess cognitive functioning and how this may be related to workers in different jobs exposed to differing levels of CPF. An established psychological assessment tool, the Trail Making Test, was used to assess participants' motor and cognitive speed, as well as their mental flexibility. The test consists of two parts, Trail Making A and B, with the latter being more mentally challenging.\ Participants included applicators, technicians and engineers as well as a control group that had experienced no occupational exposure to CPF. Participants were tested several times using Trail Making A and B throughout the summer growing season. The poorest performance was observed for the applicators who wore the backpack sprayers and who had contact with wet foliage. They were followed by the technicians who walked in the fields in front of the applicators, and then the engineers, who remained on the edges of the field during the application of CPF. The best performance throughout was observed for the control group, who had minimal exposure to CPF.

These results were clear – participants with the most contact with pesticides performed most poorly on the Trail Making Test. Testing of TCPy in the urine samples of participants further confirmed the dose-response between cognitive function and exposure to CPF based on job roles.

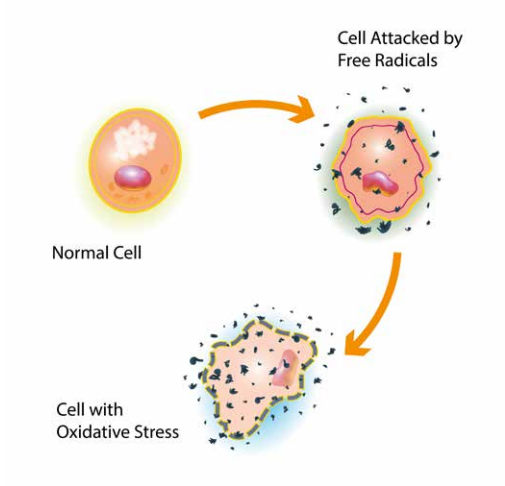


Illustration of oxidative stress.

Biomarkers

The team investigated potential biomarkers – TCPy in the urine, as well as AChE and butyl cholinesterase (BuChE) activity in the blood – and their relationship with human behavioural performance. However, they did not see a relationship between AChE and BuChE in blood samples and test performance, even though it is known that levels of these enzymes are reduced in the blood following repeated CPF exposure.

This knowledge allows us to use them as a biomarker for recent exposure, but the team concluded that they are not relevant biomarkers to determine the neurological effects of long-term, repeated exposure to CPF. That is, the repeated doses over time created damage that was measured by the behavioural test. The urinary TCPy measures reflect only the current year exposures, which accurately reflected the exposure differences between the job titles, but the behavioural test results were due to cumulative damage over many years of differing exposure levels, so any year's urinary measures would likely not, and did not, correlate directly with individual test performance in that year.

When the human CPF exposure conditions were mirrored in rat studies led by Dr Lein, similar biological effects (reduced AChE in the blood and reduced brain function as assessed by behavioural tests) were observed in the exposed rats. Using this model, Dr Lein's group identified one set of biomarkers that do appear to be linked to OP-induced neurotoxicity – those caused by oxidative stress. An imbalance between the production of reactive oxygen species in cells and tissues and the cellular mechanisms for detoxifying them causes oxidative stress. In humans, this can occur as a result of lifestyle choices like smoking, but as the team investigated here, it is also an effect of exposure to chemicals like pesticides.

Oxidative stress is frequently associated with cognitive impairment, neurodegenerative disease and now with neurotoxicity caused by OPs. Increased lipid peroxidation, protein nitration and decreased antioxidant capacity are all examples of these biomarkers. If they are proven to be indicators of OP-induced neurotoxicity in humans, this could be a useful way to diagnose neurologic damage in OP-exposed individuals and implement intervention strategies quickly.

Implementing Safer Work Practices

Gathering all of their findings over the years, Dr Anger and the team have endeavoured to use them to implement safer conditions in occupational settings. First of all, led by Dr Farahat, focus groups were held separately with applicators, technicians and engineers to provide education on their findings and discuss the use of personal protective equipment.

Employees were also given practical advice on how to better protect themselves from CPF. Rather than walking into crops that had just been sprayed with pesticide, they were encouraged to spray away from themselves and not walk into the plants. Plastic chaps made from cheap and easily accessible materials were demonstrated, which prevents CPF from coming into contact with the workers' skin on their legs. Chemical-resistant and protective clothing such as shoes and gloves have also been suggested as ways to reduce exposure, although these are expensive. During the intervention, employees were encouraged to reduce exposure, for example, by using a stick to mix pesticides.

Emphasising that these approaches are both inexpensive and simple, the researchers have helped employees working with pesticides to learn how to alter their routines to better protect themselves and their nervous systems from toxicity. As such, the dedicated work by Dr Anger, Dr Farahat, Dr Lein and Dr Rohlman on CPF exposure promises to lead to positive changes in many workers lives in the years to come.

Meet the researchers

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Dr W. Kent Anger received his PhD in Experimental and Physiological Psychology from the University of Maine. He now serves as an Associate Director for Applied Research and is a Professor at the Oregon Institute of Occupational Health Sciences at OHSU, as well as holding a Professorship at the OHSU-PSU School of Public Health.

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