Published in final edited form as:

Neurotoxicology. 2011 March ; 32(2): 268–276. doi:10.1016/j.neuro.2010.12.008.

CORRELATING NEUROBEHAVIORAL PERFORMANCE WITH BIOMARKERS OF ORGANOPHOSPHOROUS PESTICIDE EXPOSURE

Diane S. Rohlman¹, W Kent Anger¹, and Pamela J Lein²

¹Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR USA

²Department of Molecular Biosciences, UC Davis School of Veterinary Medicine, Davis, CA USA

Abstract

There is compelling evidence that adverse neurobehavioral effects are associated with occupational organophosphorous pesticide (OP) exposure in humans. Behavioral studies of pesticide applicators, greenhouse workers, agricultural workers and farm residents exposed repeatedly over months or years to low levels of OPs reveal a relatively consistent pattern of neurobehavioral deficits. However, only two studies have demonstrated a link between neurobehavioral performance and current biomarkers of OP exposure including blood cholinesterase (ChE) activity and urinary levels of OP metabolites. A variety of reasons may explain why so few studies have reported such correlations, including differing individual and group exposure histories, differing methodologies for assessing behavior and exposure, and lack of a reliable index of exposure. Alternatively, these data may suggest that current biomarkers (ChE, urine metabolites) are neither predictive nor diagnostic of the neurobehavioral effects of chronic OP pesticide exposures. This review focuses on the evidence that neurobehavioral performance deficits are associated with occupational OP pesticide exposure and concludes that research needs to return to the basics and rigorously test the relationships between neurobehavioral performance and both current (ChE and urine metabolites) and novel (eg, inflammation and oxidative stress) biomarkers using human and animal models. The results of such studies are critically important because OP pesticides are widely and extensively used throughout the world, including situations where exposure controls and personal protective equipment are not routinely used.

Keywords

Neurobehavioral Tests; Organophosphorus Pesticides; OP; Biomarkers; Urine Metabolites; Cholinesterase

© 2010 Elsevier B.V. All rights reserved

Correspondent for editorial matters: Diane S. Rohlman, Ph.D. CROET, L606 Oregon Health & Science University 3181 SW Sam Jackson Park Road Portland, OR 97239 (503) 494-2513 (503) 494-4278 FAX rohlmand@ohsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

OHSU and Drs. Rohlman and Anger have a significant financial interest in Northwest Education Training and Assessment, LLC, a company that may have a commercial interest in the results of this research and technology. This potential individual and institutional conflict of interest has been reviewed and managed by OHSU (3-2010).

INTRODUCTION

There is increasing concern regarding the widespread use of pesticides and their potential impacts on public health. Organophosphorus pesticides (OPs) are currently the most commonly utilized pesticides in the world, consisting of nearly 40 different chemical members registered by the US-EPA (www.epa.gov). About 73 million pounds of OP pesticides were used in the United States in 2001 (70% of all insecticides; Kiely, 2004). In the United States, a mixture of pesticide residues are detected in the blood and/or urine of nearly all persons sampled (Barr et al., 2005). During the 1990s some 2.5 million to 5.0 million agricultural workers were exposed to OPs, which are used as insecticides around the world (Abou-Donia, 2003, Das et al., 2001, Farahat et al., 2010, London et al., 1997). Although OPs are increasingly restricted for use in the US (EPA, 2002), many of the pesticides that are no longer available in the US and other developed countries are still being produced and used in agricultural or urban applications in developing countries.

OP pesticides were first developed during World War II as potential chemical warfare agents (Rotenberg and Newmark, 2003). They began to be widely used in agriculture to replace organochlorine pesticides, which were found to persist in the environment (Britt, 2000). Shortly after their introduction, during the 1950's and 1960s, there were reports of neurobehavioral deficits in workers following exposure to OPs, including memory impairment, anxiety, confusion, fatigue and irritability (Holmes and Gaon, 1956, Tabershaw and Cooper, 1966). In the decades following, two areas of research emerged examining exposure to OP pesticides in humans: Impairment as a consequence of single high-dose (acute) poisoning and impairment as a consequence of prolonged (chronic) exposure. Research examining effects of chronic OP exposure to adults, primarily due to occupational exposure, has identified deficits in neurobehavioral performance. However, unlike acute OP poisonings, no clear dose-response relationship between performance deficits and biomarkers of exposure has been established in studies of chronic OP exposure.

The purpose of this article is to review the neurotoxic effects associated with chronic or repeated exposure to OPs in humans, the reason why so few of these studies have reported an association between these effects and current biomarkers of OP exposure and effect, and novel biomarkers that might be more effective in predicting the neurotoxicity of OPs.

NEUROBEHAVIORAL DEFICITS FOLLOWING CHRONIC OCCUPATIONAL EXPOSURE

Neurotoxic effects of occupational and environmental chemical exposures vary along a continuum from minor subclinical deficits in sensory memory, motor or cognitive functioning to mental retardation and clinical disease (Landrigan, 2001, Mendola et al., 2002). The dose, frequency of exposure, type of OP and individual factors that influence susceptibility and sensitivity all influence the outcome of exposure (Costa et al., 2004, Rice and Barone, 2000). Although the disabling effects of high-concentration neurotoxic exposures are readily apparent, the subtle or obscure effects are not as easily detected, certainly not by standardized clinical exams. Therefore, a number of neurobehavioral tests have been used to identify these more subtle adverse health effects of a wide range of toxicants including OP pesticides in both adults (Anger, 2003) and children (Dietrich and Bellinger, 1994).

To assess the risk of occupational OP exposures it is necessary to examine neurobehavioral outcomes of extended duration (chronic) low-level exposure to these pesticides. Previous reviews of this literature, which are focused primarily on agricultural settings, have concluded that there are inconsistent findings across studies that make it difficult to

determine the impact of long-term, low-level OP exposure; and, all concluded that more research is needed to confirm and clarify the effects (Bushnell and Moser, 2006, Clegg and Van, 1999, Colosio et al., 2009, Colosio et al., 2003, Costa, 2006, Jamal et al., 2002, Kamel and Hoppin, 2004, Mearns et al., 1994) However, a different approach to evaluating the same results reveals more consistency than described in the previous reviews. Specifically, Colosio et al. (2009) evaluated the OP literature on the basis of the percent of studies that found positive and negative results for each neurobehavioral test. They concluded that the “concordances and discordances” lead to “no firm conclusion.” Perhaps the most important point that Colosio et al.'s (2009) analytic strategy misses is that the personal exposures and years of exposure are undoubtedly very different for the participants in the various studies, as exposure data are rarely recorded for a single year and never for years before the study. In contrast, we take the approach that consistencies emerging from multiple studies where the same cognitive function is shown to be adversely affected reveals an effect, and that studies that did not find the functional effect are disregarded because the lack of effect could have been due to small N or high variability within the groups, differing exposure histories across the studies, inadequate control groups, different parameters (eg, difficulty) in the tests of the same name, poor testing technique, or any number of possible confounders. Furthermore, this re-evaluation of the previous studies benefits from several new studies that have been published since all but the 2009 review, making it possible to draw conclusions about the relationship between traditional biomarkers and neurobehavioral performance and to recommend a new direction in biomarker research.

Selection of Articles: Neurobehavioral performance and OP exposure

To evaluate the weight-of-evidence regarding neurological sequelae from extended-duration OP exposure, we surveyed the peer-reviewed literature for reports of studies examining occupational exposure to pesticides in adolescents and adults. A review of PubMed and Medline from 1950 to October 2010 for articles with “pesticide” and “neurobehavioral” as keywords, and limited to “human” studies provided the initial list of articles. Circular review using the references from those articles provided the additional articles that formed the final set of publications included in this review. The articles accepted were limited to those employing neurobehavioral endpoints, asserting probable or definitive exposure to organophosphorus pesticides, and examining adults or adolescent occupationally exposed populations. Studies examining acute exposure or persistent effects of pesticide overexposure or poisoning were excluded. A total of 24 articles met these criteria (Table 1).

Study Designs

The 24 studies summarized in this review reported effects in populations of OP-exposed participants including farm workers, pesticide workers at a manufacturing plant, agricultural pesticide applicators, greenhouse workers, sheep dippers, and residential termiticide applicators; they were conducted in 10 countries around the world (Table 1). Three basic study designs were used. The design used in 22 of the 24 studies was cross-sectional, comparing performance of workers occupationally exposed to OPs to that of referents or controls who were either not occupationally exposed to an OP or were exposed to low concentrations. Of the other two studies, one classified occupationally-exposed workers into high and low exposure groups based on an exposure index (Korsak and Sato, 1977), and the other study compared performance on a neurobehavioral test battery before and after pesticide safety and use training (Cole et al., 1997). Most studies attempted to relate neurobehavioral test performance to a measure of the duration of exposure or a measure of exposure (urine metabolites) or exposure and effect (ChE activity).

Evidence of OP-induced Neurobehavioral Deficits in Humans

The prior reviews concentrated on the differences between the studies in tests and outcomes. Those reviews concluded that the findings that purported to link chronic OP exposure and neurobehavioral deficits, are inconsistent. Rather than focus on common test measures across the studies, we used an alternative approach in which we organized the measures used in the OP research into functional domains. Recognizing that the majority of tests used in these studies are complex neurocognitive tasks that call on multiple domains of function to achieve optimal and rapid performance on the tasks (Lezak et al., 2004), it is nonetheless scientifically plausible to organize tests according to the major functional domains being measured. For example, finger tapping, progressive ratio, pursuit aiming and pegboard tests assess motor speed and coordination, whereas continuous performance and selective attention assess sustained attention. We identified eight domains of neurobehavioral functioning reflected in the tests used in the 24 studies (Table 2).

The grouping of tests by function reveals similarities that are not apparent when examining the studies by test. As demonstrated in Table 2, sustained OP exposures produce adverse neurobehavioral effects across 19 studies, and there is considerable overlap in the functional areas and tests affected. Specifically, deficits reported in: motor speed and coordination (in 10 studies), information processing speed and executive functioning (10 studies), verbal abstraction (2 studies), sustained attention (3 studies), attention and short-term memory (9 studies), memory (10 studies), and perception (3 studies). The broadest range of effects were seen by Farahat et al. (2003) and Abdel Rasoul et al. (2008) in adult pesticide workers and adolescent pesticide applicators, respectively, in the same governorate in Egypt. These studies focused on complex cognitive measures and found significant deficits in the pesticide workers on: information processing speed and executive function (Digit Symbol, Trailmaking); verbal abstraction (Similarities), attention and short-term memory (Digit Span and Letter Cancellation) and memory and perception (Benton Visual Retention, Block Design). There is evidence that these pesticide workers were exposed to high concentrations of pesticides, certainly as compared to published reports of pesticide exposure measured by industrial hygiene sampling (Farahat, Fenske, 2010). While the individual epidemiological studies can be criticized and the equality of the groups compared within or between studies cannot be established, the similar findings across studies *despite these differences* support the conclusion that OPs produce a relatively consistent effect pattern of CNS dysfunction. The combination of factors seen in these studies makes it necessary to *not* rely on a single study to 'conclusively' determine health effects but rather to employ a weight of evidence approach (European Chemicals Agency, 2010, Krinsky, 2005, Weed, 2005) that synthesizes the existing research to draw more accurate conclusions.

Variation in Neurobehavioral Test Outcomes—It is important to examine the reasons that may explain the variations in neurobehavioral outcomes across the studies reported here. Reasons such differences could occur are easy to identify, however, they remain speculative for any specific OP study. Variations in test batteries used (e.g., computerized vs. non-computerized), test parameters, instructions presented in multiple languages, and populations being tested with different education levels, could all have contributed to inconsistencies between studies. For example, it is often the case that the farmworkers who are exposed to pesticides have limited education and writing skills and no computer experience. The use of tests and measures developed for English speaking, literate populations may not be appropriate in other cultures (Anger et al., 1997, Anger et al., 1993). Cross-cultural effects may have a strong influence on performance on neurobehavioral tests (London and Myers, 1997). Indeed, these confounding conditions have been reported as problematic in several studies (Cole et al., 1997, Daniell et al., 1992, Fiedler et al., 1997, London and Myers, 1997, Reidy et al., 1992, Rohlman et al., 2001). Not surprisingly, the

same tests were not used across the studies, as most were conducted by different scientists. Even studies that have used the same test may have used different test parameters (e.g., number of trials or difficulty level, parameters that are rarely reported in publications) and different administration methods, (e.g., computer-based tests vs. individually-administered paper and pencil tests) (McCauley et al., 2006). In some cases, the lack of effects could have been predicted based on the small sample sizes (Bazylwicz-Walczak et al., 1999). In sum, variations in method, procedure and population could explain the differences between the outcomes of individual OP studies, but a systematic review of the specific factors contributing to different outcomes is problematic because the impact of the variables can only be suspected, not documented.

A major tenet of our analysis, that was not considered in the prior OP reviews, is that any two studies could have found different outcomes on the same test because, for example, a suboptimal parameter was used in the test in one study but not in the other (e.g., see Colosio et al., 2009). Therefore, while what appeared to be inconsistencies in various studies could have been due to any of the reasons cited above, adverse effects are *not* subject to the same vagaries since the same tests with the same parameters were used with the exposed and control group in any given study. Of course other threats to validity could explain these differences, such as a control group with more education than their respective exposed group. The only way to address these potential threats to validity would be to identify dose response results in a study with groups from differing exposure levels and a control group or a correlation with an exposure biomarker. Unfortunately, none of the 24 studies had more than an exposed and a control group, though some had exposure biomarkers, and we turn to those next.

CORRELATION BETWEEN BIOMARKERS AND NEUROBEHAVIORAL DEFICITS

Biomarkers of exposure are indicators of how much chemical or biological agent has entered the body and does so noninvasively. Of course, the biomarkers routinely used to assess OP exposure provide information regarding exposure at the time of sampling and are not likely to reflect exposures occurring even several days prior.

Two primary biomarkers are used to assess OP exposure: Urinary OP metabolites and blood cholinesterase (ChE) activity. Blood ChE activity is a measure of not only exposure but also effect, and studies examining occupational and environmental exposure to OPs have reported reduced ChE activity in both plasma/serum and red blood cell fractions. Urinary biomarkers of OP exposure provide a non-invasive method of examining pesticide exposure. OP metabolites are detectable in urine a short time after exposure and at doses much lower than those shown to induce toxic effects (Bouchard et al., 2006). Dialkylphosphate (DAP) compounds in urine have been used as markers of OP exposure in children and adults (Azaroff, 1999, Curl et al., 2002, Koch et al., 2002, Loewenherz et al., 1997, Lu et al., 2000, Shalat et al., 2003). Other studies have examined metabolites of specific OP pesticides (e.g., 3,4,6-trichloro-2-pyridinol, TCPy as a specific metabolite of chlorpyrifos) (Farahat et al., 2010).

Because biomarkers have been sampled at only one point in time, they do not reflect a working lifetime of exposures in the workplace studies described here. In most studies, a one-time measure of exposure was combined with self-reports of years of work with pesticides (e.g., by multiplying the biomarker measure by working years), which provides a measure that is useful, though obviously subject to uncertainty. It is even possible that a high-concentration acute exposure could have occurred in some studies that would cause adverse effects, although most studies excluded people recently or ever poisoned.

Eighteen of the 24 studies report both neurobehavioral performance and a measure of a biomarker of exposure. ChE levels, treated as a measure of exposure, were quantified in 12 studies and urinary metabolites were measured in 6 studies. Seven out of twelve studies report lower ChE in exposed compared to control participants (Abdel Rasoul et al., 2008, Cole et al., 1997, Daniell et al., 1992, Farahat et al., 2003b, Gomes et al., 1998, Otto et al., 1990, Rodnitzky et al., 1975). However, only one study, that examining pesticide applicators in Egypt, reports a correlation between neurobehavioral performance and cholinesterase activity (Abdel Rasoul et al., 2008). Adolescent workers applied OP and pyrethroid insecticides, including multiple applications of chlorpyrifos to the cotton crop. Plasma BuChE activity was correlated with performance on the Digit Span, Trail Making and Information subtest from the WAIS (Table 3). The high exposures measured in pesticide applicators in Egypt (Farahat et al., 2010), may explain in part why this study found a correlation while the other 5 studies that attempted to correlate ChE level and neurobehavioral test performance did not find a significant correlation.

Six studies examining neurobehavioral deficits reported urinary metabolite levels. Five out of the six studies examined dialkylphosphate compounds in the urine (Maizlish et al., 1987, Rohlman et al., 2007, Rothlein et al., 2006, Stephens et al., 1995, Stephens and Sreenivasan, 2004). The remaining study (Steenland et al., 2000) examined TCPy, the urinary metabolite of chlorpyrifos. The Stephens et al. (1995) study used the DAP measures to confirm that their study population did not have any recent exposure to OPs. All but one of the five remaining studies (Rohlman et al., 2007) report elevated levels associated with exposure classification or pre-post application differences. Only one study reported a significant association between neurobehavioral performance and metabolite levels (Rothlein et al., 2006). Reaction time and latency scores for Symbol-Digit, Selective Attention and Continuous Performance were associated with the summed urinary methyl DAP metabolites (DMTP + DMDTP) in US orchard workers (Table 3).

Thus, 5 of 6 studies failed to find a correlation between ChE and neurobehavioral performance and 4 of 5 studies failed to find a correlation between urinary metabolites and neurobehavioral performance. In sum, most studies (9 of 11) that have attempted to correlate neurobehavioral performance with blood ChE activity or urinary metabolite levels have not uncovered an association. However, there are several factors that may have confounded the correlations between these biomarkers and the behavioral measures: There are many different OPs that may have different mechanisms of action (Pope, 1999a) and different studies were or may have been studying different OPs; some of the OPs do not metabolize to DAPs and they may not have been measured; the large degree of variability across the population and the variance in “normal” measurements across laboratories. In addition, participants in many of these studies were exposed to both OPs and other classes of pesticides, which may have led to synergies or antagonistic reactions, thus obscuring the actual effect of the OPs. These are all reasons that urinary metabolites and ChE are not ideal choices as biomarkers for human neurobehavioral studies, but their effectiveness can be judged empirically, from the results to date. Not surprisingly, this lack of association has led some to doubt the validity of the cause-effect relationship of OP exposure to the neurobehavioral effects, despite the consistency in the neurobehavioral findings

DISCUSSION

Overall, it is clear that the majority of studies report neurobehavioral changes associated with occupational OP exposure, with deficits reported in all eight functional domains. These findings are consistent and compelling since 19 of 24 studies reported significant differences between exposed and control participants, and those that did not report effects may be explained by variations in test parameters and/or small samples sizes, as outlined above.

Three studies demonstrated that years of cumulative exposure are associated with neurobehavioral deficits (Kamel et al., 2003, Rohlman et al., 2007, Roldan-Tapia et al., 2005). These findings make a compelling case that repeated or chronic OP exposure produces neurotoxic effects in human populations.

That the findings in the OP research are not perfectly consistent is not so surprising. Most of the 24 studies drew a comparison between exposed and unexposed groups defined a priori (Alavanja et al., 2004). The identification of a population as exposed to OPs has generally been based on residence or occupation with methods for documenting exposure ranging from residence in an agricultural community to occupational group to biomarkers of exposure. Beyond membership in an exposed group, it is difficult to obtain detailed exposure histories from a large number of participants. Individual job histories and exposure records do not exist in most agricultural settings where many of these studies have been conducted. Scientists often have little choice but to use surrogates of exposure that may be unreliable, including job classification, self-reported duration of work in the industry, or location of home or employment relative to sites of OP application (Ritter and Arbuckle, 2007). Often little or no information is gathered on the specific pesticide(s) used or the active ingredients in the pesticide formulations applied, or use of personal protective equipment, all factors that impact an individual's exposure to pesticides and that contribute to variation in exposure and thus presumably effect. In addition, because of the impact of demographic variables on neurobehavioral performance (Anger et al., 1997), it is important to identify a control group with similar characteristics as the exposed group but who are not exposed to pesticides. However, this approach may not take into account previous exposures of the control group or current, non-occupational exposures from living in the same community (Rohlman et al., 2007). In sum, relying on group membership for defining exposed and control groups is fraught with uncertainty, but drilling down for hard evidence on exposure history or measuring exposure itself may be equally uncertain.

Correlation of Biomarkers and Neurobehavioral Performance

Of the 24 studies reviewed, biomarkers of exposure were typically collected at a single time point, though repeated measures are *certainly* needed to improve the precision of the measurement and better characterize the range of exposure and internal dose for any work group, work season or work history. The majority of studies reviewed here (18 out of 24) incorporated at least one biomarker measure, either ChE activity or urinary metabolites. However, there is little evidence that these biomarkers are correlated with neurobehavioral performance. Only two studies demonstrated a relationship between a biomarker of OP exposure and neurobehavioral outcomes (Abdel Rasoul et al., 2008, Rothlein et al., 2006). None of the studies reported an attempt to associate a biomarker of OP exposure with a measure of the duration of exposure, personal protective equipment use (e.g., respirators), or other factors that affect exposure load and frequency. While these omissions need to be addressed in future research, it remains clear that neither ChE nor urine metabolites predict neurotoxic effects, that is they do not correlate well with neurobehavioral performance. Thus, we also need to test new biomarkers alongside the existing biomarkers to identify those that best predict neurotoxic effects and risk. It is also important, as indicated above, to measure biomarkers multiple times over a work season to provide an accurate representation of both typical exposures as well as the variability of those exposures, and if possible over multiple work years to also characterize change.

Novel Mechanistically-Based Biomarkers of OP-Induced Neurotoxicity

Several biological mechanisms have been proposed to explain the lack of association between blood ChE inhibition and OP neurotoxicity following occupational exposures. One is that genetic differences in the expression and/or activity of enzymes that metabolize OPs

(Hofmann et al., 2010, Perez-Herrera et al., 2008, Povey et al., 2007) or proteins that scavenge OPs (Lockridge and Masson, 2000) differentially influence peripheral *versus* central outcomes. Another proposal is that ChE inhibition may not be mechanistically related to chronic OP neurotoxicity. While ChE inhibition is considered the primary mechanism responsible for the acute toxicity of OPs (Echbichon and Joy, 1995), experimental evidence suggests that mechanism(s) other than or in addition to ChE inhibition mediate chronic OP neurotoxicity (Bushnell and Moser, 2006, Jett and Lein, 2006a, Pope et al., 2005, Pope, 1999b). Of the various alternative molecular targets and mechanisms proposed to mediate OP-induced neurobehavioral deficits following repeated low level exposures (Casida and Quistad, 2005, Hernandez et al., 2004, Jett and Lein, 2006b, Lockridge and Schopfer, 2010, Pancetti et al., 2007, Soltaninejad and Abdollahi, 2009), oxidative stress and inflammation are of interest because of the availability of experimentally validated quantitative peripheral biomarkers of oxidative stress and inflammation that are mechanistically linked to chronic OP neurotoxicity and correlate well with neurobehavioral deficits observed consequent to neurodegenerative disease (Dziedzic, 2006, Kadiiska et al., 2005a, Kadiiska et al., 2005b, Mrak and Griffin, 2005).

Oxidative stress has been implicated as a contributing factor in mild cognitive impairment (Montine et al., 2005) and a variety of neurodegenerative diseases (Butterfield et al., 2006, Halliwell, 2006), and a recent review of the human and animal literature concluded that oxidative stress also contributes to chronic OP neurotoxicity (Soltaninejad and Abdollahi, 2009). This conclusion was based on evidence of increased levels of protein nitration and lipid peroxidation, decreased total antioxidant capacity and protective effects of natural and synthetic antioxidants against OP-induced histopathological and biochemical alterations. The relationship between ChE inhibition and oxidative stress in OP neurotoxicity remains controversial. Biochemical studies suggest that AChE inhibition in the brain triggers oxidative stress (Milatovic et al., 2006) while other studies suggest that increased oxidative stress may contribute to (Milatovic et al., 2006) or exacerbate (Liu et al., 2006) cholinergic toxicity elicited by OPs. However, *in vitro* studies demonstrate that OP-induced oxidative stress is antagonized by co-exposure to antioxidants but not by cholinergic antagonists (Giordano et al., 2006), and a recent study of pesticide workers identified an association between chronic exposure to OP pesticides and increased levels of antioxidant enzymes and lipid peroxidation in blood leukocytes and erythrocytes in the absence of ChE inhibition (Shadnia et al., 2005), suggesting that OPs may induce oxidative stress independent of AChE inhibition. Oxidative stress and inflammation are often interrelated processes with important interactions between pro-inflammatory mediators and agents that generate reactive oxygen and nitrogen species (Chung et al., 2006, Joseph et al., 2005, Potts et al., 2006, Rahman et al., 2006, Wang et al., 2006). Experimental studies demonstrate that low-level repeated exposure to OPs induces inflammatory responses in cultured neural cells (Mense et al., 2006, Monnet-Tschudi et al., 2007) and upregulate inflammatory cytokines in an animal model (Singh and Jiang, 2003). While a functional link between inflammation and OP-induced neurobehavioral deficits has yet to be established, inflammatory cytokines have been demonstrated to cause significant impairment in spatial memory (Wenk et al., 2003). Collectively, these studies suggest the feasibility of testing the hypothesis that peripheral biomarkers of oxidative stress and inflammation either in combination with or independent of ChE inhibition may be better predictors of OP neurotoxicity than ChE inhibition alone.

CONCLUSIONS

There is clear evidence from 19 (of 24) studies that occupational exposure to OPs adversely affects neurobehavioral performance. Attempts to correlate neurobehavioral deficits with biomarkers of internal dose (urinary metabolites or ChE activity) have been generally unsuccessful, and a dose-response relationship has yet to be established. This makes

estimating risk of OP exposure to human health very difficult. Biomarkers that reliably predict or diagnose damage to the target organ significantly improve identification of at-risk individuals, the conduct of epidemiology studies and the evaluation of intervention and treatment strategies (Angerer et al., 2006). Thus we must turn to other more novel biomarkers emerging from animal research using controlled laboratory exposures to identify a convincing biomarker of effect. Two strong candidates are oxidative stress (Abdollahi et al., 2004, Abou-Donia, 2003, Banerjee et al., 2001, Dettbarn, 2006, Kovacic, 2003, Milatovic, Gupta, 2006) and inflammation (Cowan et al., 2003, Cowan et al., 2004). In sum, there is clearly a need to identify and test more mechanistically based biomarkers. Research needs to return to the basics and test the relationships in humans, physiological models and animals.

Acknowledgments

The work was supported by the National Institute of Environmental Health Sciences (NIEHS, R01ES016308, Anger & Lein, MPI and R21 ES017223, Rohlman). The content is solely the authors' responsibility and does not necessarily represent official views of NIEHS. Appreciation is extended to Fayssal M. Farahat, James R. Olson, Matthew R. Bonner, Richard A Fenske, Kit Galvin, Taghreed M. Farahat, Ahmed Ismail, Olfat Hendy and Gaafar Abdel Rasoul, members of the research teams on the grants that supported this work who contributed to the development of the information in this publication.

REFERENCES

- Abdel Rasoul GM, Abou Salem ME, Mechael AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. *Neurotoxicology* 2008;29:833–8. [PubMed: 18662718]
- Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. *Med Sci Monit* 2004;10:RA141–7. [PubMed: 15173684]
- Abou-Donia MB. Organophosphorus ester-induced chronic neurotoxicity. *Arch Environ Health* 2003;58:484–97. [PubMed: 15259428]
- Alavanja MCR, Hoppin JA, Kamel F. Health effects of chronic pesticide exposure: Cancer and neurotoxicity. *Annual Review of Public Health* 2004;25:155–97.
- Ames RG, Steenland K, Jenkins B, Chrislip D, Russo J. Chronic Neurologic Sequelae to Cholinesterase Inhibition among Agricultural Applicators. *Arch Environ Health* 1995;50:440–4. [PubMed: 8572722]
- Anger W, Sizemore O, Grossmann S, Glasser J, Letz R, Bowler R. Human neurobehavioral research methods: Impact of subject variables. *Environmental Research* 1997;73:18–41. [PubMed: 9311528]
- Anger WK. Neurobehavioral tests and systems to assess neurotoxic exposures in the workplace and community. *Occupational and Environmental Medicine* 2003;60:531–8. [PubMed: 12819291]
- Anger WK, Cassitto MG, Liang Y-X, Amador R, Hooisma J, Chrislip DW, et al. Comparison of performance from three continents on the WHO-recommended Neurobehavioral Core Test Battery (NCTB). *Environmental Research* 1993;62:837–46.
- Angerer J, Bird MG, Burke TA, Doerr NG, Needham L, Robison SH, et al. Strategic biomonitoring initiatives: moving the science forward. *Toxicol Sci* 2006;93:3–10. [PubMed: 16785253]
- Azaroff LS. Biomarkers of exposure to organophosphorus insecticides among farmers, families in rural El Salvador: Factors associated with exposure. *Environmental Research* 1999;80:138–47. [PubMed: 10092406]
- Banerjee BD, Seth V, Ahmed RS. Pesticide-induced oxidative stress: perspectives and trends. *Rev Environ Health* 2001;16:1–40. [PubMed: 11354540]
- Barr DB, Allen R, Olsson AO, Bravo R, Calabiano LM, Montesano A, et al. Concentrations of selective metabolites of organophosphorus pesticides in the United States population. *Environmental Research* 2005;99:314–26. [PubMed: 16307973]
- Bazylewicz-Walczak B, Majczakowa W, Szymczak M. Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. *Neurotoxicology* 1999;20:819–26. [PubMed: 10591517]

- Bouchard M, Carrier G, Brunet RC, Dumas P, Noisel N. Biological monitoring of exposure to organophosphorus insecticides in a group of horticultural greenhouse workers. *Ann Occup Hyg* 2006;50:505–15. [PubMed: 16510491]
- Britt, JK. Properties and Effects of Pesticides. In: Williams, PL.; James, RC., editors. *Principles of Toxicology Environmental and Industrial Applications: Environmental and Industrial Applications*. John Wiley & Sons; New York: 2000.
- Bushnell, PJ.; Moser, VC. Behavioral toxicity of cholinesterase inhibitors. In: Gupta, RC., editor. *Toxicology of Organophosphate and Carbamate Compounds*. Elsevier; San Diego, CA: 2006. p. 347-60.
- Butterfield DA, Perluigi M, Sultana R. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. *Eur J Pharmacol* 2006;545:39–50. [PubMed: 16860790]
- Casida JE, Quistad GB. Serine hydrolase targets of organophosphorus toxicants. *Chem Biol Interact* 2005;157–158:277–83.
- Chung HY, Sung B, Jung KJ, Zou Y, Yu BP. The molecular inflammatory process in aging. *Antioxid Redox Signal* 2006;8:572–81. [PubMed: 16677101]
- Clegg D, Van GM. Expert Panel Report of Human Studies on Chlorpyrifos and/or other Organophos] Exposures. *J Toxicol Environ Health B Crit Rev* 1999;2:257–79. [PubMed: 10429681]
- Cole DC, Carpio F, Julian J, Leon N, Carbotte R, De Almeida H. Neurobehavioral Outcomes Among Farm and Nonfarm Rural Ecuadorians. *Neurotoxicology and Teratology* 1997;19:277–86. [PubMed: 9253006]
- Colosio C, Tiramani M, Brambilla G, Colombi A, Moretto A. Neurobehavioural effects of pesticides with special focus on organophosphorus compounds: which is the real size of the problem? *Neurotoxicology* 2009;30:1155–61. [PubMed: 19751763]
- Colosio C, Tiramani M, Maroni M. Neurobehavioral Effects of Pesticides: State of the Art. *NeuroToxicology* 2003;24:577–91. [PubMed: 12900071]
- Costa LG. Current issues in organophosphate toxicology. *Clin Chim Acta* 2006;366:1–13. [PubMed: 16337171]
- Costa LG, Aschner M, Vitalone A, Syverson T, Soldin OP. Developmental Neuropathology of Environmental Agents. *Annual Review of Pharmacology and Toxicology* 2004;44:87–110.
- Cowan FM, Broomfield CA, Lenz DE, Smith WJ. Putative role of proteolysis and inflammatory response in the toxicity of nerve and blister chemical warfare agents: implications for multi-threat medical countermeasures. *J Appl Toxicol* 2003;23:177–86. [PubMed: 12794939]
- Cowan FM, Broomfield CA, Stojiljkovic MP, Smith WJ. A review of multi-threat medical countermeasures against chemical warfare and terrorism. *Mil Med* 2004;169:850–5. [PubMed: 15605928]
- Curl CL, Fenske RA, Kissel JC, Shirai JH, Moate TF, Griffith W, et al. Evaluation of Take-Home Organophosphorus Pesticides Exposure among Agricultural Workers and Their Children. *Environmental Health Perspectives* 2002;110:A 787–A92.
- Daniell W, Barnhart S, Demers P, Costa LG, Eaton DL, Miller M, et al. Neuropsychological performance among agricultural pesticide applicators. *Environ Res* 1992;59:217–28. [PubMed: 1425511]
- Das R, Steege A, Baron S, Beckman J, Harrison R. Pesticide-related illness among migrant farm workers in the United States. *International Journal of Occupational & Environmental Health* 2001;7:303–12. [PubMed: 11783860]
- Dettbarn, WF.; Milatovic, D.; Gupta, RC. Toxicology of Organophosphate and Carbamate Compounds. In: Gupta, RC., editor. *Oxidative stress in anticholinesterase-induced excitotoxicity*. Elsevier; Amsterdam: 2006. p. 511-32.
- Dietrich, KN.; Bellinger, D. The assessment of neurobehavioral development in studies of the effects of prenatal exposure to toxicants. In: Needleman, HL.; Bellinger, D., editors. *Prenatal Exposure to Toxicants: Developmental Consequences*. John Hopkins University Press; Baltimore: 1994. p. 57-85.
- Dziedzic T. Systemic inflammatory markers and risk of dementia. *Am J Alzheimers Dis Other Demen* 2006;21:258–62. [PubMed: 16948290]
- Echbichon, DJ.; Joy, RM. Pesticides and neurological diseases. CRG Press; Boston and London: 1995.

- EPA. Interim reregistration eligibility decision for chlorpyrifos. Agency, EP., editor. Environmental Protection Agency; 2002.
- European Chemicals Agency. Practical Guide 2: How to report weight of evidence. European Chemicals Agency; Helsinki, Finland: 2010. p. 1-22.
- Farahat FM, Fenske RA, Olson JR, Galvin K, Bonner MR, Rohlman DS, et al. Chlorpyrifos exposures in Egyptian cotton field workers. *Neurotoxicology* 2010;31:297–304. [PubMed: 20193710]
- Farahat TM, Abdel Rasoul GM, Amr MM, Shebl MM, Farahat FM, Anger WK. Neurobehavioral effects among workers occupationally exposed to organophosphorus pesticides. *Occupational and Environmental Medicine* 2003;60:279–86. [PubMed: 12660376]
- Fiedler N, Kipen H, McNeil K, Fenske R. Long-term use of organophosphates and neuropsychological performance. *American Journal of Industrial Medicine* 1997;5:487–96. [PubMed: 9327072]
- Giordano G, Afsharinejad Z, Guizzetti M, Vitalone A, Kavanagh TJ, Costa LG. Organophosphorus insecticides chlorpyrifos and diazinon and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. *Toxicol Appl Pharmacol*. 2006
- Gomes J, Lloyd O, Revitt M, Basha M. Morbidity among farm workers in a desert country in relation to long-term exposure to pesticides. *Scandinavian Journal of Work Environment and Health* 1998;24:213–9.
- Halliwell B. Oxidative stress and neurodegeneration: where are we now? *J Neurochem* 2006;97:1634–58. [PubMed: 16805774]
- Hernandez A, Gomez MA, Pena G, Gil F, Rodrigo L, Villanueva E, et al. Effect of long-term exposure to pesticides on plasma esterases from plastic greenhouse workers. *Journal of Toxicology & Environmental Health Part A* 2004;67:1095–108. [PubMed: 15205026]
- Hofmann JN, Keifer MC, Checkoway H, De Roos AJ, Farin FM, Fenske RA, et al. Biomarkers of sensitivity and exposure in washington state pesticide handlers. *Adv Exp Med Biol* 2010;660:19–27. [PubMed: 20221867]
- Holmes JH, Gaon MD. Observations on acute and multiple exposure to anticholinesterase agents. *Trans Am Clin Climatol Assoc* 1956;68:86–100. discussion 1–3. [PubMed: 13486610]
- Jamal GA, Hansen R, Pilkington A, D B, Gilham RA, Abdel-Azis M, et al. A clinical neurological, neurophysiological, and neuropsychological study of sheep farmers and dippers exposed to organophosphate pesticides. *Occupational and Environmental Medicine* 2002;59:434–41. [PubMed: 12107290]
- Jett, DA.; Lein, PJ. Non-cholinesterase mechanisms of central and peripheral neurotoxicity: Muscarinic receptors and other targets. In: Gupta, RC., editor. *Toxicology of Organophosphate and Carbamate Compounds*. Elsevier; San Diego, CA: 2006. p. 233-46.
- Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005;81:313S–6S. [PubMed: 15640496]
- Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, et al. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radic Biol Med* 2005a;38:698–710. [PubMed: 15721980]
- Kadiiska MB, Gladen BC, Baird DD, Graham LB, Parker CE, Ames BN, et al. Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl4 poisoning. *Free Radic Biol Med* 2005b;38:711–8. [PubMed: 15721981]
- Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. *Environmental Health Perspectives* 2004;112:950–8. [PubMed: 15198914]
- Kamel F, Rowland AS, Park LP, Anger WK, Baird DD, Gladen BC, et al. Neurobehavioral performance and work experience in Florida farmworkers. *Environmental Health Perspectives* 2003;111:1765–72. [PubMed: 14594629]
- Kiely, TG. A. Pesticides Industry sales and Usage: 2000 and 2001 Market Estimates. US EPA; Washington DC: 2004.
- Koch D, Lu C, Fisker-Andersen J, Jolley L, Fenske RA. Temporal association of children's pesticide exposure and agricultural spraying: report of a longitudinal biological monitoring study. *Environmental Health Perspectives* 2002;110:829–33.

- Korsak RJ, Sato MM. Effects of chronic organophosphate pesticide exposure on the Central Nervous System. *Clinical Toxicology* 1977;11:83–95. [PubMed: 872544]
- Kovacic P. Mechanism of organophosphates (nerve gases and pesticides) and antidotes: electron transfer and oxidative stress. *Curr Med Chem* 2003;10:2705–9. [PubMed: 14529460]
- Krimsky S. The weight of scientific evidence in policy and law. *Am J Public Health* 2005;95(Suppl 1):S129–36. [PubMed: 16030328]
- Landrigan PJ. Children's environmental health. *Pediatric Clinics of North America* 2001;48:1319–30. [PubMed: 11579676]
- Lezak, M.; Howieson, D.; Loring, D. *Neuropsychological Assessment*. 4th ed.. Oxford University Press; New York: 2004.
- Liu J, Gupta R, Goad JT, Karanth S, Pope C. Modulation of parathion toxicity by glucose feeding: Is nitric oxide involved? *Toxicol Appl Pharmacol*. 2006
- Lockridge O, Masson P. Pesticides and susceptible populations: people with butyrylcholinesterase genetic variants may be at risk. *Neurotoxicology* 2000;21:113–26. [PubMed: 10794391]
- Lockridge O, Schopfer LM. Review of tyrosine and lysine as new motifs for organophosphate binding to proteins that have no active site serine. *Chem Biol Interact* 2010;187:344–8. [PubMed: 20211158]
- Loewenherz C, Fenske RA, Simcox NJ, Bellamy G, Kalman D. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington state. *Environmental Health Perspectives* 1997;105:1344–53. [PubMed: 9405329]
- London L, Myers J, Nell V, Taylor T, Thompson M. An Investigation into Neurologic and Neurobehavioral Effects of Long-Term Agrichemical Use among Deciduous Fruit Farm Workers in the Western Cape, South Africa. *Environmental Research* 1997;73:132–45. [PubMed: 9311539]
- Lu C, Fenske RA, Simcox NA, Kalman D. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. *Environmental Research* 2000;84:290–302. [PubMed: 11097803]
- Maizlish N, Schenker M, Weisskopf C, Seiber J, Samuels S. A behavioral evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. *Am J Ind Med* 1987;12:153–72. [PubMed: 3661569]
- McCauley LA, Anger WK, Keifer M, Langley R, Robson MG, Rohlman D. Studying health outcomes in farmworker populations exposed to pesticides. *Environ Health Perspect* 2006;114:953–60. [PubMed: 16760000]
- Mearns J, Dunn J, Lees-Haley PR. Psychological effects of organophosphate pesticides: a review and call for research by psychologists. *J Clin Psychol* 1994;50:286–94. [PubMed: 8014255]
- Mendola P, Selevan SG, Gutter S, Rice D. Environmental factors associated with a spectrum of neurodevelopmental deficits. *Mental Retardation and Developmental Disabilities Research Reviews* 2002;8:188–97. [PubMed: 12216063]
- Mense SM, Sengupta A, Lan C, Zhou M, Bentsman G, Volsky DJ, et al. The common insecticides cyfluthrin and chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes. *Toxicol Sci* 2006;93:125–35. [PubMed: 16790487]
- Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. *ScientificWorldJournal* 2006;6:295–310. [PubMed: 16518518]
- Monnet-Tschudi F, Zurich MG, Honegger P. Neurotoxicant-induced inflammatory response in three-dimensional brain cell cultures. *Hum Exp Toxicol* 2007;26:339–46. [PubMed: 17615115]
- Montine TJ, Quinn JF, Montine KS, Kaye JA, Breitner JC. Quantitative in vivo biomarkers of oxidative damage and their application to the diagnosis and management of Alzheimer's disease. *J Alzheimers Dis* 2005;8:359–67. [PubMed: 16556967]
- Mrak RE, Griffin WS. Potential inflammatory biomarkers in Alzheimer's disease. *J Alzheimers Dis* 2005;8:369–75. [PubMed: 16556968]
- Otto, DA.; Soliman, S.; Svensgaard, D.; Soffar, A.; Ahmed, N. Neurobehavioral Assessment of Workers Exposed to Organophosphorus Pesticides. In: Johnson, BL.; Anger, WK.; Durao, A.; Xintaras, C., editors. *Advances in Neurobehavioral Toxicology: Applications in Environmental and Occupational Health*. Lewis Publishers; Chelsea, MI: 1990. p. 305-22.

- Pancetti F, Olmos C, Dagnino-Subiabre A, Rozas C, Morales B. Noncholinesterase effects induced by organophosphate pesticides and their relationship to cognitive processes: implication for the action of acylpeptide hydrolase. *J Toxicol Environ Health B Crit Rev* 2007;10:623–30. [PubMed: 18049927]
- Perez-Herrera N, Polanco-Minaya H, Salazar-Arredondo E, Solis-Heredia MJ, Hernandez-Ochoa I, Rojas-Garcia E, et al. PON1Q192R genetic polymorphism modifies organophosphorous pesticide effects on semen quality and DNA integrity in agricultural workers from southern Mexico. *Toxicol Appl Pharmacol* 2008;230:261–8. [PubMed: 18430447]
- Pope C, Karanth S, Liu J. Pharmacology and toxicology of cholinesterase inhibitors: uses and misuses of a common mechanism of action. *Environmental Toxicology and Pharmacology* 2005;12:433–46.
- Pope CN. Organophosphorus pesticides: do they all have the same mechanism of toxicity? *J Toxicol Environ Health B Crit Rev* 1999a;2:161–81. [PubMed: 10230392]
- Pope CN. Organophosphorus pesticides: do they all have the same mechanism of toxicity? *J Toxicol Environ Health B Crit Rev* 1999b;2:161–81. [PubMed: 10230392]
- Potts MB, Koh SE, Whetstone WD, Walker BA, Yoneyama T, Claus CP, et al. Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. *NeuroRx* 2006;3:143–53. [PubMed: 16554253]
- Povey AC, Jury F, Dippnall WM, Smith AE, Thomson S, Mackness B, et al. GST CYP and PON1 polymorphisms in farmers attributing ill health to organophosphate-containing sheep dip. *Biomarkers* 2007;12:188–202. [PubMed: 17536768]
- Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol* 2006;72:1439–52. [PubMed: 16920072]
- Reidy TJ, Bowler RM, Rauch SS, Pedroza GI. Pesticide exposure and neuropsychological impairment in migrant farm workers. *Archives of Clinical Neuropsychology* 1992;7:85–95. [PubMed: 14589681]
- Rice D, Barone SJ. Critical periods of vulnerability for the developing nervous system: Evidence from human and animal models. *Environmental Health Perspectives* 2000;108:511–33. [PubMed: 10852851]
- Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, Marzouk J, et al. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. *Isr J Med Sci* 1992;28:584–98. [PubMed: 1330977]
- Ritter L, Arbuckle TE. Can exposure characterization explain concurrence or discordance between toxicology and epidemiology? *Toxicological Sciences* 2007;97:241–52. [PubMed: 17234646]
- Rodnitzky R.L, Levin H.S, Mick D.L. Occupational exposure to organophosphate pesticides. *Archives of Environmental Health* 1975;30:98–103. [PubMed: 1115534]
- Rohlman D, Lasarev M, Anger W, Scherer J, Stupfel J, McCauley L. Neurobehavioral Performance of Adult and Adolescent Agricultural Workers. *Neurotoxicology* 2007;28:374–80. [PubMed: 17141876]
- Rohlman DS, Bailey SR, Anger WK, McCauley L. Assessment of neurobehavioral function with computerized tests in a population of Hispanic adolescents working in agriculture. *Environmental Research* 2001;85:14–24. [PubMed: 11161647]
- Rohlman, DS.; Lasarev, M.; Muniz, J.; Anger, WK.; McCauley, L. Environmental Exposures and Health Effects in Adolescent and Adult Farmworkers. In: Cranmer, J., editor. 23rd international Neurotoxicology Conference: Neurotoxicology in Development & Aging; Little Rock, AK. 2006.
- Roldan-Tapia L, Parron T, Sanchez-Santed F. Neuropsychological effects of long-term exposure to organophosphate pesticides. *Neurotoxicol Teratol* 2005;27:259–66. [PubMed: 15734277]
- Rotenberg JS, Newmark J. Nerve agent attacks on children: Diagnosis and management. *Pediatrics* 2003;112:648–58. [PubMed: 12949297]
- Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and non-agricultural Hispanic workers. *Environmental Health Perspectives* 2006;114:691–6. [PubMed: 16675422]

- Shadnia S, Azizi E, Hosseini R, Khoei S, Fouladdel S, Pajoumand A, et al. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. *Hum Exp Toxicol* 2005;24:439–45. [PubMed: 16235732]
- Shalat SL, Donnelly KC, Freeman NCG, Calvin JA, Ramesh S, Jimenez M, et al. Nondietary Ingestion of Pesticides by Children in an Agricultural Community on the US/Mexico border: Preliminary results. *Journal of Exposure Analysis and Environmental Epidemiology* 2003;13:42–50. [PubMed: 12595883]
- Singh AK, Jiang Y. Lipopolysaccharide (LPS) induced activation of the immune system in control rats and rats chronically exposed to a low level of the organothiophosphate insecticide, acephate. *Toxicology and industrial health* 2003;19:93–108. [PubMed: 15697179]
- Soltaninejad K, Abdollahi M. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. *Med Sci Monit* 2009;15:RA75–90. [PubMed: 19247260]
- Steenland K, Dick RB, Howell RJ, Chrislip DW, Jines CJ, Reid TM, et al. Neurologic functioning among termiticide applicators exposed to chlorpyrifos. *Environmental Health Perspectives* 2000;108:293–300. [PubMed: 10753086]
- Stephens R, Spurgeon A, Calvert IA, Beach J, Levy LS, Berry H, et al. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet* 1995;345:1135–9. [PubMed: 7723544]
- Stephens R, Sreenivasan B. Neuropsychological effects of long-term low-level organophosphate exposure in orchard sprayers in England. *Arch Environ Health* 2004;59:566–74. [PubMed: 16599004]
- Tabershaw IR, Cooper WC. Sequelae of acute organic phosphate poisoning. *J Occup Med* 1966;8:5–20. [PubMed: 5900463]
- Wang JY, Wen LL, Huang YN, Chen YT, Ku MC. Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. *Curr Pharm Des* 2006;12:3521–33. [PubMed: 17017945]
- Weed DL. Weight of evidence: a review of concept and methods. *Risk Anal* 2005;25:1545–57. [PubMed: 16506981]
- Wenk GL, McGann K, Hauss-Wegrzyniak B, Rosi S. The toxicity of tumor necrosis factor-alpha upon cholinergic neurons within the nucleus basalis and the role of norepinephrine in the regulation of inflammation: implications for Alzheimer's disease. *Neuroscience* 2003;121:719–29. [PubMed: 14568031]

Table 1

List of studies examining neurobehavioral effects in adolescent and adult populations. The table shows the population studied, whether or not neurobehavioral (NB) deficits were found, the basis for the exposure or group classification, and the country where the study was conducted.

Study	Exposed	Control	NB Deficits ³	Basis for Exposure or Group Classification	Exposure Differences ⁴	Country
Rodnitzky et al. 1975	Farmworkers (n=23) (Farmers = 12; Applicators 11)	Control (n=23)	NS	Occupation Blood cholinesterase	ChE: E < C (normal range)	US/Migrant farmworkers
Korsak et al., 1977	Workers occupationally exposed grouped into High (H) vs. Low (L) (n=59)	--	+	Exposure Index Plasma BuChE RBC AChE	ChE: H < L	US
Maizlish et al. 1987	Pest control workers (N=46)	Control (N=56)	NS	Occupation Urine metabolite (pre/post) (DETP/DMTP)		US
Otto et al. 1990	Pesticide Manufacturers (n=229)	Fertilizer workers (n=180) Textile workers (n=167)	NS	Occupation Plasma BuChE	ChE: E < C	Egypt
Daniell et al. 1992	Applicators (n=49)	Controls (n=40)	NS	Occupation RBC ChE ¹	ChE: High < Low	US
Richter et al. 1992	Kibbutz cotton and orchard workers (n=51)	Kibbutz Residents (n=39)	+	Occupation Other exposure measures not reported		Israel
Ames et al. 1995	Prior ChE inhibition (n=45)	Controls (n=90)	NS	RBC AChE Plasma BuChE		US
Stephens et al., 1995	Sheep dippers (n=146)	Control (n=143) quarry workers	+	Occupation Urine DAP – used to confirm no recent exposure		UK
Cole et al. 1997	Residents (n=23), Farmworkers (n=28), Applicators (n=123)	Controls (n=72)	+	Occupation RBC AChE	ChE: E < C	Ecuador
Fiedler et al. 1997	Farmworkers (n=57)	Controls (n=42)	+	Occupation RBC AChE Exposure Metric	ChE: w/in normal limits	US
London et al. 1997	Applicators (n=163)	Controls (n=84)	+	Occupation Plasma BuChE		South Africa
Gomes et al., 1998	Farmworkers (n=226) New farmworkers (n=92)	Controls (n=226)	+	Occupation RBC AChE	ChE: FW<C<New FW	United Arab Emirates – migrant workers
Bazylwicz-Walczak et al., 1999	Greenhouse workers (Females) N=26	Controls (N=25)	+	Occupation Dermal Respiratory		Poland
Steenland et al. 2000	Termiticide applicators (n=191)	Controls: bring a friend (n=100) blue collar state workers (n=89)	+	Occupation Metabolites (TCPy) Buccal Swab		US
Rohlman et al. 2001	Farmworkers (n=96)	Controls (n=51)	+	Occupation		US/Migrant Workers

Study	Exposed	Control	NB Deficits ³	Basis for Exposure or Group Classification	Exposure Differences ⁴	Country
Farahat et al. 2003	Pesticide workers (n=52)	Control (n=50)	+	Occupation Plasma BuChE	ChE: E < C	Egypt
Kamel et al. 2003	Farmworkers (n=288)	Controls (n=51)	+	Occupation		US/Migrant Farmworkers
Stephens et al. 2004	Orchard Sprayers (n=37)	Pig farm workers (n=26); Construction (n=31)	+	Occupation Metabolites DEP/DETP		UK
Roldan-Tapia et al. 2005	Applicators (n=40)	Controls (n=26)	+	Occupation Plasma BuChE	ChE: E < C	Spain
Rothlein et al. 2006	Farmworkers (n=92)	Controls (n=45)	+	Occupation Metabolites Residues		US/Migrant Farmworkers
Eckerman et al. 2007	Farmworkers (n=38)	Urban (n=28)	+	Occupation Exposure Index		Brazil
Rohlman et al. 2007	Farmworkers (n=119)	Controls (n=56)	+	Occupation Urine metabolites ²		US/Migrant Workers
Cole et al. 2007	Farmworkers (n=63)		+	Occupation Pre/post comparison after training		Ecuador
Abdel Rasoul et al. 2008	Pesticide Applicators (n=50)	Controls (n=50)	+	Occupation Plasma BuChE	ChE: E < C	Egypt

¹ ChE levels were reported in Karr et al. 1992.

² Metabolite levels were reported in (Rohlman et al., 2006).

³ + = statistically significant findings; NS = no significant findings.

⁴ ChE = cholinesterase; E = exposed; C = non-exposed controls

Table 2

Behavioral deficits associated with pesticide exposures.

Function and Test	Source
<u>Motor Speed/Coordination</u>	
Finger Tapping	Eckerman 07 ¹ , Rothlein 06, Kamel 03, Reidy 92
Progressive ratio	Eckerman 07 ¹
Pursuit Aiming	Bazylewicz-Walczak 99, Gomes 98, London 97
Santa Ana Pegboard	Kamel 03, London 97, Cole 97, Richter 92
Purdue/Grooved Pegboard	Reidy 92, Steenland 00
<u>Reaction Time</u>	
Simple Reaction Time	Rohlman 07, Eckerman 07 ¹ , Rohlman 01, Bazylewicz-Walczak 99, Fiedler 97, Stephens 95, Richter 92
<u>Information Processing Speed /Executive Function</u>	
Digit Symbol	Abdel Rasoul 08 ² , Farahat 03, Gomes 98, Richter 92
Symbol Digit	Rohlman 07, Rothlein 06, Rohlman 01, Stephens 95
Syntactic Reasoning	Stephens 95
Trail Making	Abdel Rasoul 08 ² , Farahat 03, Cole 97, Richter 92, Korsak 77
<u>Verbal Comprehension</u>	
Similarities	Abdel Rasoul 08 ² , Farahat 03
Information	Abdel Rasoul 08
<u>Sustained Attention</u>	
Continuous Performance	Rohlman 07 ³ , Rothlein 06, Rohlman 01
Selective Attention	Rohlman 07, Rothlein 06, Rohlman 01
<u>Attention/Short-term Memory</u>	
Digit Span	Abdel Rasoul 08 ² , Eckerman 07 ² , Cole 07 ⁴ , Rothlein 06, Farahat 03, Kamel 03, Rohlman 01, Cole 97, Richter 92
<u>Memory</u>	
Match to Sample	Rohlman 07 ³ , Eckerman 07 ²
Serial Digit Learning	Eckerman 07 ¹ , Rothlein 06, Rohlman 01
Benton Visual Retention	Abdel Rasoul 08 ² , Roldan-Tapia 05, Farahat 03, Reidy 92, Richter 92
Rey Auditory Verbal Learning	Roldan-Tapia 05
Rey-Osterrieth Complex Figure Quality	Roldan-Tapia 05 ⁵
<u>Perception</u>	
Block Design	Abdel Rasoul 08 ³ , Cole 97, Richter 92

¹ Results are of those rural 10–11 years old compared to their matched urban controls.² Results are those of applicators 15–18 years old compared to their matched controls.³ Results are for workers reporting mixing/applying pesticides in comparison to others who had not.⁴ Comparison before and after training intervention. Participants also showed improvement on composite score for visual-spatial functioning.⁵ Rey-Osterrieth Complex Figure time to completion was also significant.

Table 3

Biomarkers Associated with Neurobehavioral Effects of Pesticide Exposures

Occupational Groups with Long-Term Exposures			
Function	Test	Biomarker	Source
Motor Speed/Coordination	Finger Tapping	Urine DAP	Rothlein et al., 2006
Information Processing Speed/Executive Function	Symbol Digit	Urine DAP	Rothlein et al., 2006
	Trail Making	Plasma BuChE	Abdel Rasoul et al. 2008
Attention/Short-term Memory	Digit Span	Plasma BuChE	Abdel Rasoul et al. 2008
Sustained Attention	Continuous Performance	Urine DAP	Rothlein et al., 2006
	Selective Attention	Urine DAP	Rothlein et al., 2006
Verbal Comprehension	Information	Plasma BuChE	Abdel Rasoul et al. 2008