COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on long-term neurological, neuropsychological and psychiatric effects of low-level exposure to organophosphates in adults

Executive summary

i. This statement considers whether adverse neurological, neuropsychological or neuropsychiatric effects can result from exposure of adults to cholinesterase-inhibiting organophosphates at levels insufficient to cause overt acute poisoning. When the question was last considered by COT as part of a report published in 1999, the evidence was judged to be inconclusive.

ii. The statement was drafted by a working group, comprising the chairman and another member of the COT, along with four external scientists, who were invited to contribute because they provided complementary expertise in relevant scientific areas.

iii. Potentially relevant papers were identified through a computerised literature search covering the period up to September 2013, and those that provided useful information were appraised and abstracted. Where reports referenced further publications that might be relevant, these too were retrieved and evaluated. Preliminary consideration indicated that the most pertinent evidence would come from epidemiological investigations, and that toxicological studies in animals and in vitro would be less telling. The search was therefore limited to studies in humans, and reports of other types of research were not reviewed systematically.

iv. Since 1999, 13 new papers have been published on the relation of low-level exposure to organophosphates to peripheral neuropathy, adding to 13 studies that were already available at the time of the last COT report. The current balance of evidence suggests that there is no long-term risk of clearly demonstrable peripheral neuropathy from exposure to organophosphates that does not cause overt acute poisoning, a conclusion that has strengthened with the passage of time.

v. There is uncertainty as to whether long-term low level exposure to organophosphates causes detectable impairment of sensory thresholds, but if there is an effect then it is likely to be small.

vi. Studies on somatosensory evoked potentials and electromyography (EMG) have been few in number and limited in quality. Overall, they do not suggest a hazard, but the evidence base is slim.

vii. Few studies have looked at electroencephalogram (EEG) or auditory/visual event-related evoked potential (ERP) outcomes in relation to organophosphate exposures that are insufficient to cause overt acute poisoning. The evidence that is available provides little indication of adverse effects. One study has suggested impairment in the processing of auditory information, but without independent replication, little can be drawn from this isolated finding.

viii. Since the last COT review, 22 investigations have been published that looked for neuropsychological consequences of low-level exposure to organophosphates, adding to the nine that were available in 1999. Overall, there is no consistent evidence that low-level exposure to organophosphates has adverse effects on any specific aspect of cognitive function. If organophosphates do cause long-term neuropsychological impairment in the absence of overt poisoning, then the effects, at least in the large majority of cases, must be minor and subtle.

ix. Evidence on whether, in the absence of acute poisoning, low-level exposure to organophosphates can cause long-term structural changes in the brain is insufficient for any firm conclusions.

x. The overall balance of evidence from 11 studies suggests no increased risk of Parkinson's disease from exposure to organophosphates that is insufficient to cause overt acute poisoning, although a small elevation of risk cannot be ruled out.

xi. Findings from the only two studies on the relation of organophosphates to later dementia are not strongly suggestive of a hazard, but point to a need for further research.

xii. Fourteen studies (including 10 published since 1999) have investigated the association of low-level exposure to organophosphates with common mental disorders (depression and anxiety). Overall, there is no consistent evidence of a link. The balance of evidence suggests that low-level exposure to organophosphates does not lead to an increased risk of suicide.

xiii. Despite limitations of individual studies, current evidence suggests that there is an excess of multiple neuropsychiatric symptoms in people who have been exposed to organophosphates at levels insufficient to cause overt acute poisoning. However, it does not support the existence of a specific syndrome of "chronic organophosphate-induced neuropsychiatric disorder (COPIND)", as has previously been hypothesised. It is unclear whether the observed excess of symptoms is a consequence of chemical toxicity or occurs through psychological mechanisms, and it is possible that people who are aware of having been exposed to potentially toxic chemicals are more inclined to notice and report symptoms. Studies on the relationship of symptoms to polymorphisms and activity of the enzyme, paraoxonase (PON1), have not clearly established a causal link with poorer capacity to detoxify organophosphate compounds, as might be expected if the illness were a consequence of toxicity.

xiv. Collectively, the evidence reviewed is reassuring. It suggests that exposures to cholinesterase-inhibiting organophosphates that are insufficient to cause overt acute poisoning do not cause important long-term neurological toxicity in adults, and that if toxic effects on the nervous system do occur then they are minor and subtle. Moreover, it provides no basis for use of a generic outcome other than inhibition of acetylcholinesterase in regulatory risk assessment for organophosphate insecticides and medicines.

xv. The most important gap in the current evidence base concerns the relation between low-level exposure to organophosphates and later dementia. An association of such exposure with dementia has some biological plausibility, and has been suggested (although not strongly) by the two studies that have looked at the question to date. Moreover, dementia is a major and growing public health problem, and even a small increase in relative risk could have important implications at a population level. However, further research on this should be conducted only through studies with adequate rigour and statistical power.

xvi. Given the evidence that is now available, it seems unlikely that further research on neurophysiological, neuropsychological and psychiatric outcomes would identify any important hazard from low-level exposure to organophosphates. Research to explore the possibility of more subtle, minor effects would require rigorous assessment of exposure, and better methods for assessment of the health outcomes than are currently available.

Background

1. Organophosphates are esters, amides or thiol derivatives of phosphoric acid, some of which have the capacity to phosphorylate and inactivate the enzyme, acetylcholinesterase, leading to acute disruption of neurological function. This property has been exploited in their use as insecticides in agriculture and horticulture, as veterinary medicines (particularly in sheep dips used to prevent and treat ectoparasitic infestations), as human medicines (malathion only – as a treatment for head lice), and as public hygiene products (e.g. for control of cockroaches). However, it poses a hazard to people who are exposed to them inadvertently during their manufacture or as a consequence of their use. In addition, intentional human poisoning has occurred as a result of deliberate self-harm, and through the use of some organophosphates (different from those in pesticides and medicines) as chemical warfare agents.

2. The risk of acute neurotoxicity is routinely considered in regulatory risk assessment for products containing cholinesterase-inhibiting organophosphates. However, there has been concern that even where exposures are too low to cause acute toxicity, they might lead to longer term neurological injury, especially if they are prolonged or repeated.

3. In 1999, the COT published a report[1], which addressed this question. It found evidence that neuropsychological abnormalities could occur as a long-term complication of acute organophosphate poisoning, but concluded that the evidence linking neurological and neuropsychological impairment with chronic low-level exposure to organophosphates, insufficient to cause overt acute toxicity, was less convincing. Recommendations were made for further research to reduce uncertainties in the evidence base.

4. In response, a number of studies were commissioned jointly by relevant Government departments. The findings were discussed by the COT at meetings in 2007 and 2009, when the Committee concluded that they should be considered in the context of a wider review of all pertinent epidemiological studies published since 1999. In parallel with these developments, the COT received a request from the Advisory Committee on Pesticides (ACP) in December 2007 to produce an updated review of the published literature on the toxicity of organophosphates, including possible neuropsychological and neuropsychiatric effects.

5. This statement addresses the needs that were identified by the COT and ACP.

Scope

6. The statement focuses specifically on whether adverse neurological (including neuropsychological and neuropsychiatric) effects can result from exposure of adults to cholinesterase-inhibiting organophosphates at levels insufficient to cause overt

[1] http://cot.food.gov.uk/cotreports/cotwgreports/organophosphates

acute poisoning. Developmental neurotoxicity was not considered at this stage, but may be the subject of a separate statement in the future.

Methods

7. The statement was drafted by a working group, comprising the chairman and another member of the COT, along with four external scientists, who were invited to contribute because they brought special expertise which the Committee had identified as desirable. The members of the Working Group and their areas of expertise are listed in Appendix A.

8. The Working Group was greatly assisted by members of the COT secretariat from Public Health England (PHE) and of the PHE Toxicology Unit at Imperial College, who carried out an initial search of the scientific literature, abstracting and summarising relevant findings. The methods of the literature search, including the databases and search terms used, are set out in Appendix B. Preliminary consideration indicated that the most relevant evidence would come from epidemiological investigations, and that toxicological studies in animals and in vitro would be less telling. The search was therefore limited to studies in humans, and reports of other types of research were not reviewed systematically.

9. After exclusion of publications that were judged not to be relevant, those that remained were assessed, and abstracted. Where reports referenced further publications that might be relevant, these too were retrieved and evaluated. Systematic reviews and meta-analyses were identified and scrutinised as part of the literature search, but were used principally as a check that all relevant primary research had been identified.

Mechanisms of toxicity and metabolism

10. Organophosphates are reactive chemicals with a capacity to phosphorylate various proteins, either directly or (in the case of those that are not themselves oxons) through conversion by cytochromes p450 to oxon metabolites – see previous COT report on organophosphates[2]. In particular, they can bind to, and inactivate, the enzyme acetylcholinesterase, thus blocking the breakdown of the neurotransmitter, acetylcholine, at synapses in the central nervous system (CNS), in the parasympathetic autonomic nervous system, and at the neuro-muscular junction. In the first place this leads to over stimulation, which in the CNS can cause giddiness, anxiety, restlessness, headache, confusion, difficulty in concentration and respiratory depression; in the autonomic nervous system, excessive secretion (e.g. of sweat, saliva and tears), diarrhoea, miosis, involuntary micturition and bradycardia; and in muscles, fasciculation followed by weakness and paralysis.

11. The active oxon forms of organophosphates are detoxified by binding to butyrylcholinesterase as well as being hydrolysed by the enzyme, paraoxonase, which is produced by the PON1 gene. Polymorphisms of PON1 at positions 192 (R

[2] http://cot.food.gov.uk/cotreports/cotwgreports/organophosphates

form with arginine and Q form with glutamine) and 55 (L form with leucine and M form with methionine) can influence paraoxonase activity. However, the relative potency of the different forms of the enzyme differs according to the substrate (Povey, 2010). It has been reported that diazoxon (formed from diazinon, which has been widely used as a sheep dip) is metabolised less by the R than the Q isoform (Povey, 2010).

12. Paraoxonases also metabolise other chemicals, and have an antioxidant function. Thus, PON1 polymorphisms have been linked to risk of atherosclerosis and heart disease, even in people with no known exposure to organophosphates (Macharia et al., 2012).

General considerations

13. The sections that follow review evidence relating to specific categories of neurological and psychiatric illness or impairment. First, however, it is helpful to highlight a number of general considerations when interpreting such evidence.

14. Many of the epidemiological studies which provide information on possible long-term health effects of organophosphates have assessed exposures retrospectively. This often limits the extent to which exposures to specific compounds or products can be discriminated, and the accuracy with which exposures can be quantified. However, it should not prevent the detection of hazards that are common to cholinesterase-inhibiting organophosphates as a group.

15. Workers who are exposed to organophosphates may also be exposed to other hazardous pesticides or biocides, which in theory might confound associations with health outcomes. However, such confounding would occur only if the associated exposure were a cause of the health effect under study. Other potential confounders will vary according to the outcome under investigation.

16. Some of the health outcomes that have been studied are subjective (e.g. report of symptoms) or may be influenced by subjective factors (e.g. performance in some neuropsychological tests). In these circumstances, it is possible that ascertainment of the outcome could be biased if participants were aware of their exposures, and this knowledge led them to report symptoms or perform tests differently.

17. As in almost all literature reviews, there is a possibility that positive results have been published selectively. This could be because non-positive findings, especially from small studies, have not been written up, or have not been accepted for publication. In addition, where studies have investigated multiple end-points, the outcomes for which results are most interesting may have been selectively reported.

18. While the focus of this review is on long-term effects of low-level exposures to organophosphates, it is possible that some of the neurological outcomes examined (e.g. impaired performance on neuropsychological tests) could occur as acute effects of exposure. It follows that for these outcomes, greatest weight should be given to studies in which recent exposure to organophosphates can be excluded.

However, the occurrence of subtle acute effects that were not accompanied by overt toxicity would make it more plausible that long-term low-level exposures could produce chronic neurological damage. Therefore studies have been considered even where recent exposure cannot be ruled out, provided subjects had not at some time experienced clinically overt acute poisoning.

19. Another concern is that some forms of neurological impairment might cause people to be less careful or more clumsy when handling hazardous chemicals, and thereby to incur higher exposures. If so, associations with such impairment could reflect reverse causation rather than a toxic effect of exposure. However, this is likely to be more of a problem where long-term outcomes are compared between exposed workers with and without recognised episodes of acute poisoning than in studies comparing people with low-level exposures and controls who are unexposed or minimally exposed.

20. Finally, it should be noted that in addition to their generic ability to inhibit acetylcholinesterase, cholinesterase-inhibiting organophosphates can also induce compound-specific toxic effects. The possibility of compound-specific effects must be considered when findings from epidemiological studies appear discrepant.

21. In the sections that follow, evidence is reviewed in relation to specific types of long-term neurological effect that might result from low-level exposure to organophosphates.

Peripheral neuropathy and neuromuscular dysfunction

22. Peripheral neuropathy is defined as disease of the nerves of the peripheral nervous system (PNS), i.e. the electrically conducting fibres that connect brain and spinal cord to muscles, skin and other organs. These fibres transmit signals involved in movement (motor nerves), sensation (sensory nerves), and internal bodily functions (autonomic nerves). Typical symptoms of peripheral neuropathy include tingling, numbness and pain in the anatomical distribution of affected nerves (sensory effects) and weakness and paralysis of the muscles that they supply (motor effects). In some cases, the sensory impairment leads to poor balance. The symptoms may be accompanied by clinical signs such as muscle wasting, reduced sensation of pin prick and absent reflexes. The feet are often affected first and most severely, as longer nerves tend to be more vulnerable to disease.

Methods of assessment

23. Diagnosis of peripheral neuropathy poses several challenges. In particular, many tests can be influenced by the conscious brain, the patient's level of cooperation and feelings, and the expertise of the examiner. The traditional neurological examination has good negative predictive value (i.e. it is usually 'correct' where findings are normal) but poorer positive predictive value (i.e. positive findings do not necessarily imply abnormality). Assessment of muscle power and grip strength, balance, and reaction time all depend on a patient cooperating and exerting maximal effort. Sensory examination (for sense of vibration, pinprick, joint

position and light touch) requires that patients concentrate fully and report their sensations accurately. Certain 'objective' tests, such as quantitative sensory testing (QST), although more precise in their measured output, have similar limitations. And although tendon reflexes can be reliably assessed by an experienced clinician, there is wide biological variation and they may appear to be absent if patients are not fully relaxed.

24. In contrast to these partially subjective methods of assessment, some outcome measures do not depend importantly on the patient's cooperation or state of mind, or the assessor's clinical skill. These include nerve conduction studies (NCS) (generally considered the best method for detection of peripheral neuropathy), electromyography (EMG) (generally more useful for detection of myopathy than neuropathy) and somatosensory evoked potentials. However, even these have their limitations – some generic and some specific. All three tests, although reproducible, are relatively insensitive and only detect peripheral neuropathy reliably when over half of peripheral nerve fibres are damaged. NCS cannot detect abnormality of the small diameter nerve fibres which are involved in pain and temperature sensation and potentially a source of troublesome symptoms. Moreover, normal values for NCS can vary tenfold depending on patient factors (e.g. age, obesity, height, skin temperature and thickness) and test factors (e.g. electrode position, machine settings), posing a challenge in interpretation. EMG and somatosensory evoked potentials are generally less sensitive than NCS and less discriminating between different degrees of impaired function, while evoked potentials (scalp recording of cortical brain response to somatosensory stimuli) require the patient's attention and are rather non-specific (being affected also by disease in the sensory pathways of the brain and spinal cord). Small fibre neuropathy is now best assessed by counting the density of intra-epidermal nerve fibres in a 3 mm diameter skin biopsy from the lower leg. However, this is a new technique, and was not employed in any of the studies that were reviewed.

Prevalence and causes

25. Depending on exact case definition, about 2% of people in England have a peripheral neuropathy, the prevalence rising to approximately 8% above 55 years of age. Peripheral neuropathy has many causes (e.g. diabetes, alcohol, HIV, vitamin deficiencies, drugs, metabolic disorders, hereditary, cancer-related, traumatic, vascular, immunologically-related, and industrial poisons), although most are rare. In some cases, a single nerve trunk is affected (e.g. carpal tunnel syndrome due to mechanical compression of the median nerve in the carpal tunnel at the wrist), while in others, multiple nerves are involved (polyneuropathy). The most common cause of peripheral neuropathy in the general population is diabetes mellitus. It has been estimated that 10-20% of patients with newly diagnosed diabetes have a diabetic polyneuropathy and that a half of all patients with diabetes for $\geq$25 years will be affected.

Organophosphate-induced delayed polyneuropathy

26. Organophosphate-induced delayed polyneuropathy (OPIDPN) is an established complication of acute poisoning by organophosphates such as tri-ortho-cresyl phosphate (TOCP) that inhibit the enzyme neuropathy target esterase (NTE),. Thus, peripheral neuropathy occurred in an episode of TOCP poisoning in the USA, arising from contamination of the tonic Jamaica ginger (Kidd et al., 1933), and in an outbreak in Morocco caused by contaminated cooking oil (Smith and Spalding, 1959). OPIDPN typically develops two to three weeks after an episode of poisoning, following an interval of apparent recovery. The neuropathy is predominantly motor, affecting the lower more than the upper limbs. At the height of the illness, severe distal weakness and wasting can occur, together with mild proximal weakness, loss of ankle reflexes and ataxia. The clinical picture is similar to that of Guillain-Barré syndrome. Weakness often persists for many months and sometimes results in permanent foot-drop.

Low-level exposure to organophosphates

27. It has been postulated that peripheral neuropathy and long-term abnormalities of neuromuscular function can also result from exposure to organophosphates that do not inhibit NTE, and that such compounds can cause peripheral neuropathy or abnormal neuromuscular function following chronic low-level exposure, in the absence of overt acute poisoning. In the last COT report, 13 epidemiological papers were reviewed that bear on this concern.

28. Jager et al. (1970) studied neuromuscular transmission in factory workers exposed to organophosphate and organochlorine pesticides in the Netherlands. Seventeen of 36 exposed workers were classed as having abnormal muscle action potentials as compared with none of 28 controls from an oil refinery. However, studies by other investigators cast doubt on the reliability of the assessment method, while further limitations concerned the representativeness of the study sample, the adequacy of the control group and the study's capacity to distinguish long-term from short-term effects.

29. Otto et al. (1990) compared male production workers exposed to organophosphates (including some NTE inhibitors) with other men from a fertiliser plant and a textile factory in Egypt. Assessment included clinical evaluation of sensation and reflexes, measurement of tactile sensitivity, and a test for hand-eye co-ordination. The exposed workers had a higher prevalence of abnormal vibration sensation ($P<0.0001$) and were said – without supporting data – to fare worse in tests of hand co-ordination, tremor and knee reflexes, although not in touch perception or in dexterity.

30. In the US, Stokes et al. (1995) also found significantly higher mean vibration thresholds in pesticide applicators than in matched controls, but only in the hands ($p<0.04$), with no significant differences in the feet (peripheral neuropathy of the OPIDPN kind would normally affect sensation in the toes ahead of that in the fingers). Findings may have been confounded by exposure to other chemicals or differences in height, a known determinant of vibration threshold sensitivity.

31. In keeping with the report by Stokes, London et al. (1997) found no relation between long-term organophosphate exposure and sensory thresholds to vibration in the toes of 163 pesticide applicators from the Western Cape, South Africa, when compared with those in 84 controls.

32. In Ecuador, Cole et al. (1998) found a non-significant tendency to increased vibration sensory thresholds in the big toes of 144 potato farm workers as compared with 72 matched controls (P=0.087). Abnormal tendon reflexes, reduced muscle power, and poor co-ordination were also found in excess (odds ratios (ORs) 2.1 to 4.3), although deep sensation did not differ significantly. However, the COT 1999 report judged the selection of controls in this study to be seriously flawed, and noted that some of the exposed group had previously suffered acute organophosphate poisoning. Moreover, apparent effects could also have reflected recent rather than long-term exposure.

33. A large British study assessed vibration thresholds and hot and cold sensitivity (Pilkington et al., 1999, since published in peer-reviewed format with slight revision as Pilkington et al., 2001). Sheep dippers were sampled from an annual census database maintained by the Ministry of Agriculture, Foods and Fisheries. Letters were sent to some 1,000 farms in relevant postal areas of Hereford, Worcester, the Scottish Borders, Lothian and Ayrshire. Farmers who agreed to participate and who confirmed that sheep were dipped on their farms, were asked to identify recent co-workers, who were also invited to take part. A comparison group was assembled from the farmers who said that they did not dip sheep, a separate group of chicken farmers recruited by word of mouth, and brick-makers from companies known to the British Ceramics Confederation. Exposures to sheep dip were estimated from work histories and data from 20 pilot farms on levels of urinary organophosphate metabolites associated with representative tasks. From this information, a hygienist constructed several indices of cumulative exposure related to the likely degree of splashing and handling of concentrates, as well as classifying individuals simply according to whether or not they handled concentrates. In all, 772 subjects (612 sheep dippers, 53 control farmers and 107 ceramic workers) underwent quantitative sensory testing (QST) in the feet. Unfortunately, field-measured QSTs proved an unsatisfactory index of peripheral neuropathy (too often abnormal in controls when judged by clinical reference values), although an internal comparison by estimated levels of exposure was still possible.

34. After adjustment for relevant covariates, only small and non-significant differences were found in relation to cumulative exposure. Higher cold thresholds were significantly associated with ever having handled organophosphates as a concentrate, but hot and vibration thresholds were not. Only vibration threshold was significantly related to average intensity of exposure to concentrate (p=0.03). These inconsistencies on semi-objective testing contrasted with report of neurological symptoms, which was in clear excess among dippers, especially those who had handled concentrate. The authors concluded that “there was only weak evidence of a chronic [sensory] effect of low dose cumulative exposure to organophosphates”.

35. Four other studies, by Gomes et al. (1998), Maizlish et al. (1987), Daniell et al. (1992), and Fiedler et al. (1997), employed tests that had not been designed to measure peripheral nerve function per se, but which would be impaired if there were

moderate or severe peripheral neuropathy. Their reports covered farmers from the United Arab Emirates, pest control workers from Sacramento, USA, pesticide applicators from Washington State, USA, and tree fruit farmers from New Jersey, USA. Hand-eye co-ordination, finger tapping and a test of manual dexterity were assessed. Only Gomes et al. found a significant impairment in the exposed group – in an "aiming" task.

36. As mentioned above, NCS provide a more direct and objective assessment of peripheral nerve function. In 1999, findings were available from two reports. Ames et al. (1995) had compared 46 workers with a history of moderate organophosphate exposure causing asymptomatic depression of acetylcholinesterase activity and 90 unexposed controls. There were no significant differences in median, ulnar or peroneal nerve conduction velocities, muscle action potentials, or sensory action potentials, nor any in vibration thresholds in the fingers and toes. Engel et al. (1998) examined sensory and motor nerve conduction and neuromuscular transmission in 68 workers exposed to low levels of organophosphates while apple thinning. Nerve conduction was normal and similar to that in 69 non-exposed workers. No consistent or statistically significant differences were found in sensory nerve latency, sensory nerve amplitude, motor nerve conduction or function of the neuromuscular junction, and no relation of these measures to hours spent thinning over a season.

37. After weighing these 13 studies, the COT 1999 report concluded that there was no clear evidence that peripheral neuropathy could be caused by low level exposures to organophosphates that do not inhibit NTE.

New evidence

38. Since 1999, 13 new reports (plus that by Pilkington et al. (2001) summarised above in paragraphs 33-34) have been published, drawn from 10 studies. These include eight reports relating to six studies of cohort design. Eight reports (six studies) have assessed nerve conduction, three studies have employed QST and two have evaluated neuromuscular function.

39. All of these new reports are reviewed below and summarised in Table 1. For completeness, and because OPIDPN is sometimes accompanied by manifestations in the central nervous system (CNS), this section also considers – where assessed in these 13 reports – abnormalities of CNS neurophysiology such as postural sway (but excluding electroencephalography which is covered elsewhere), tests of peripheral nerve function with a CNS dimension to them (somatosensory evoked potentials), and tests which, while not seeking to assess the PNS, might nonetheless be abnormal if moderate to severe peripheral neuropathy were present (e.g. tests of manual dexterity).

Cohort studies

40. The largest of the newly available cohort studies investigated disease prevalence and various markers of function, a decade or so after a brief exposure event. McCauley et al. (2002) compared 653 Gulf War veterans potentially exposed to sarin or cyclosarin during deployment in the Gulf in 1991, with two referent groups – 610 other military personnel deployed to South-West Asia and 516 personnel on

active service but not deployed over the same timeframe. The target populations were enumerated, using duty lists of the U.S. Army and National Guard for the first quarter of 1991, held on the Operation Desert Storm Database. Eligible subjects were resident in Oregon, Washington, California, Georgia or North Carolina at the time of interview. Those with potential exposure were selected from personnel working within a 50 km radius of an Iraqi munitions site that was demolished over a few days in March 1991.

41. Inevitably there were important losses to follow-up: the databases listed 5,328, 143,910 and 814,331 personnel in each group, from which random samples were drawn, and telephone calls were attempted on 923, 927 and 1369 individuals respectively in reaching the final sample. However, an unspecified but "large" number of veterans could not be entered into the pool for random selection owing to missing telephone numbers. The study took as its outcome self-reported doctor-diagnosed peripheral neuropathy over follow-up, and this did not differ importantly between groups (2.0% in the 'exposed' vs. 2.8% and 2.1% in the comparison groups; adjusted ORs for exposed vs. non-exposed in the range 1.0 to 1.2, $p>0.05$). A complex sampling regime and problems in contact-tracing somewhat limit interpretation of the findings, but the balance of evidence from this report is against an important long-term risk of overt peripheral neuropathy – particularly as those with suspected exposure may have been more concerned to seek a medical opinion in the event of symptoms and so more likely to be diagnosed. However, many of the exposures may have been extremely low.

42. An accompanying technical report by Spencer (2001) described the measurement of somatosensory evoked potentials and neurological examination in a small subset from the McCauley study – 42 exposed subjects, with 26 and 28 from the two referent groups. No significant differences were found in lower and upper limb latencies ($p\geq0.15$) and no group differences were found on clinical assessment. However, the report gives almost no numerical data, and little information about the selection process and response rates. The representativeness of those studied and the power to detect effects are therefore unclear.

43. A second large cohort study (Starks et al., 2012b) was nested within the US Agricultural Health Survey. This enrolled private pesticide applicators during 1993-7, among whom 44% completed a baseline questionnaire. Two follow-ups were subsequently conducted at five yearly intervals, the sample for Starks' report being limited to those who had responded at all three time points, with over-sampling of those estimated to have high lifetime use of pesticides at baseline. A few participants with previously diagnosed organophosphate poisoning were excluded in sensitivity analyses, and various other exclusions relating to general risk factors for peripheral neuropathy were applied. Of 1,807 subjects with three completed questionnaires, 701 men (39%) participated, with NCS observations finally available on 544 to 664 subjects (depending on outcome). Exposure to some 50 pesticides, including 16 organophosphates, was assessed in terms of ever use and frequency and years of use. Cumulative lifetime days of exposure were estimated up to the time of the last questionnaire. The outcomes comprised NCS (motor nerve conduction velocity, distal motor latency, amplitude and F-wave latency) and certain physical signs (ankle reflexes, joint position sense, vibration sense, postural tremor,

Romberg sign and tandem gait), assessed separately for each of 16 named organophosphate pesticides.

44. Multiple comparisons were made. Of 94 risk estimates relating to clinical signs, 15 implied an elevated risk in organophosphate users (ORs increased about 2-3 fold, $p<0.05$), while four indicated a significantly decreased risk. The abnormalities most commonly associated with organophosphates related to joint position sense (6 of 16 compounds) and postural tremor (4 of 16). The most consistent positive associations were with dichlorvos (3 of 6 effect estimates), and phosmet, terbufos and tetrachlorvinphos (2 of 6 estimates for each agent). Of 128 comparisons related to nerve conduction, only eight differed significantly, seven indicating better nerve conduction in the applicators. These were for measures of distal motor amplitude, F-wave latency and motor nerve conduction velocity in users of phorate, of distal motor amplitude in users of tebupirimphos, and of F-wave latency in users of acephate.

45. This study used a large and carefully assembled cohort. Considerable effort was made to reconstruct exposure levels, and outcomes were assessed without knowledge of exposures, care having been taken to exclude subjects with previous acute poisoning. There were many losses to follow-up, however, and it is unclear whether those with more health problems participated selectively. The interval between the last phase of questioning and assessment is unclear, as is the interval between last use of organophosphates and the tests, meaning that short term effects of exposure cannot confidently be ruled out from the information given. Of note, adverse findings were almost confined to clinical examination (a semi-objective assessment method) and not apparent for NCS (a more objective method).

46. Three linked reports by Albers et al. (2004a, 2004b, 2007) compared workers manufacturing chlorpyrifos with another group manufacturing a plastic film wrapping called saran. All workers employed in chlorpyrifos manufacture at an index date in September 1999 were invited to participate, together with a random sample of the saran group. About 80% of each group took part at baseline, with only trivial losses to follow-up at one year, and analysis was based on 53 chlorpyrifos workers and 60 controls. Exposures to chlorpyrifos were quantified in terms of time spent in exposed areas (estimated by questionnaire: mean 9.7 years, range 0 – 23.7 years vs. 0.01 years for controls); cumulative airborne exposure (derived from questionnaire responses and historical hygiene records: mean 64.2 mg/m^3 days vs. 0.09 mg/m^3 days for controls); and urinary 3,5,6 trichloro-2-pyridinol (TCP), plasma butyrylcholinesterase and erythrocyte acetylcholinesterase levels between baseline and follow-up. The mean urinary TCP/creatinine ratio was 192 µg/g in chlorpyrifos workers and 6 µg/g in controls; average butyrylcholinesterase over the year of study was 7155 mu/ml in the chlorpyrifos workers and 8133 mu/ml in controls. Nerve conduction (sensory amplitude, terminal conduction velocity and distal latency; motor amplitude, conduction velocity, distal latency, and F wave latency) was measured in several nerves, and neurological examination conducted to elicit 10 physical signs (muscle strength; sensation of pin-prick, vibration, joint position, touch pressure and fine touch; von Frey monofilament test; ankle reflexes; Romberg sign; and abnormal gait). The clinical and nerve conduction measures were combined to classify subjects as having ‘definite’, ‘probable’, ‘possible’, ‘subclinical’ or no neuropathy.

47. In one report (Albers et al., 2004a), findings were compared at baseline and follow-up. No significant or consistent differences were found between exposed and non-exposed subjects in symptoms of neuropathy, signs of neuropathy, nerve conduction findings or diagnostic category, either at baseline or one year on; and the odds of newly developing these outcomes did not differ significantly either.

48. In a second report (Albers et al., 2004b), the authors explored alternative formulations of the baseline analysis (e.g. as well as individual parameters for individual nerves, they constructed composite measures such as any NCS abnormality in any single nerve, or any NCS abnormality in two or more nerves), but findings were similar.

49. In their third report, Albers et al. (2007) modelled nerve conduction as a function of past exposure to chlorpyrifos (before baseline) and 'interim' exposure (between baseline and follow-up). There were major inconsistencies in the few apparently significant findings. Parameters of nerve conduction mostly worsened slightly per unit of historical exposure, but mostly improved slightly per unit of interim exposure. In all, 80 parameter estimates and p-values were generated of which only five were significant at the 5% level (under the null hypothesis and assuming that they were mutually independent, four would be expected by chance alone). For 10 (including these five), the p-value was ≤0.10 (eight expected by chance with the same assumptions). Only one of the five significant associations and two of the 10 weakly supportive findings came from a highly exposed sub-group (32 subjects with 'high' historical exposures >20 mg/m^3 days) – both relating to exposures over follow-up, and perhaps, therefore, reflecting recent exposures. Furthermore, effects were small. For example, historical exposure above the mean (twice the cut-point for higher exposure), was associated with slowing of forearm median motor conduction velocity by less than 1 m/s (<2%) and prolongation of the median motor F wave latency by <1 ms (<4%). In summary, there were no consistent, dose-dependent associations, and the minor associations that were found could well have occurred by chance alone.

50. This investigation had several strengths, including a representative approach to sampling, well matched controls, reasonable response rates, a cohort design, better than usual information on exposure (confirmed by biomarkers), assessment of physical signs blinded to exposure status, a panel of objective outcomes and detailed exposure-response analyses. However, few subjects had 'definite' or 'probable' neuropathy, and the study lacked power to address this outcome. Also, it did not test for small fibre neuropathy by QST (a report by Jamal et al. (2002) had suggested that any impact may be predominantly on small diameter rather than large fibres). Notwithstanding these limitations, the findings argue against an important effect of chlorpyrifos on peripheral nerve function at the exposure levels studied.

51. A report by Keifer et al. (2000) complements that by Engel et al. (1998) (paragraph 36). The same population of apple thinners and referents (garment manufacturers and workers from hotels and restaurants) was studied, with analysis restricted to 87 subjects who completed a second follow-up assessment six to nine months after the spraying season had ended. Regression coefficients and p-values were presented for associations of exposure with outcomes at baseline and with changes from baseline. Among a range of tabulated outcomes, only vibration

threshold was relevant to peripheral nerve function. Findings proved inconsistent. Baseline threshold was marginally lower in apple thinners than in referents in the hand, but higher in the foot (as in Engel et al., 1998). However, thresholds worsened non-significantly over follow-up, in both the apple thinners and the controls.

52. Details of sampling, response rates and numbers of controls in the second round of testing were not reported, so the representativeness of the finally analysed groups is uncertain. Testing at two time points has scope to shed light on the impact of short-term exposures. In this case the change measure should, if low-level organophosphate exposure had an adverse short-term effect, have shown an improvement, since the second measure was several months after last exposure. The slight worsening is against this, but most probably was unrelated to organophosphates, since it was seen also in controls.

53. In Sri Lanka, Peiris-John et al. (2002) conducted NCS and neuromuscular synapse testing in 30 male farmers who regularly sprayed organophosphate pesticides and 30 fishermen living nearby. No details of sampling or response rates were reported on which to judge representativeness of the data. Tests were performed blinded, during the cultivation season and two months after it. A range of herbicides, insecticides and fungicides were applied for some 20 hours per season although no details are given of the exact formulations used. Acetylcholinesterase levels were non-significantly higher in the farmers than the fishermen. Groups were compared in terms of sensory conduction velocity and latency, and motor conduction velocity, latency, and amplitude, both during a season and between seasons. Also, changes within each group were assessed post-cultivation (in all, 20 p-values were presented). Similar assessments were made in relation to neuromuscular function (12 p-values).

54. During spraying, nerve conduction tended to be marginally and non-significantly worse in the farmers; but post-season, sensory nerve conduction was superior (by 11%) and motor conduction worse (by 4%) ($p=0.04$). After the season, sensory conduction velocities improved significantly in farmers (on average 27% faster, $p<0.01$), but also in non-farmers (14% faster, $p=0.04$). Other measures of nerve conduction changed little, with no important differences in and between groups. Neuromuscular transmission was likewise similar in all comparisons. Thus, findings were inconsistent and post-exposure improvements in some parameters in the farmers were echoed also in the controls.

Cross-sectional studies

55. In North Carolina, Steenland et al. (2000) compared current and former termiticide applicators using chlorpyrifos with control groups of friends and blue-collar workers. Applicators were identified from professional registers of general pesticide usage during 1987-1997. In all, 3,605 applicators were registered with 246 companies over the decade, but only 176 companies were contactable, of which 105 had used chlorpyrifos. These companies had employed 383 termiticide applicators with a year or more of relevant exposure, from whom 191 subjects were finally tested. One in two of the applicators were asked to enlist a friend of the same sex and rough age to serve as a control; and 2,856 blue-collar workers from four state departments were invited to form a second comparison group, the final selection being frequency-matched to applicators by age, sex and race. Response rates in

the last two groups were not reported, but 106 friends and 83 blue-collar workers were finally assessed. On average the applicators had worked with chlorpyrifos for 2.4 years.

56. A panel of outcomes was assessed, including among other things, 10 clinical signs (strength, sensation, reflexes, co-ordination, gait and station, tone, tremor, pupillary function and eye movements), six tests of sensory and motor nerve conduction in three nerves, four tests of vibrotactile threshold in the toe, 12 'sway' tests and a test of dexterity (as well as olfaction and colour vision). The applicators fared better than controls in terms of neurological signs ($p<0.0001$) and vibrotactile thresholds ($p=0.03$); similarly in terms of NCS (with no trends by duration of exposure or level of TCP in urine); and worse only in manual dexterity in the dominant hand ($p=0.07$) and one of 12 sway tests. Magnitudes of effect were not reported, but differences were described by the authors as minor.

57. This relatively large study was systematically conducted, with care taken to exclude cases of acute poisoning and blinded assessment of a broad panel of objective and semi-objective outcomes. Only a minority of the exposed population and an eclectic mix of controls were recruited, however, and 76 of 191 applicators had been exposed in the week before testing (meaning that any effects of recent exposure could not be ruled out). In the event, no significant short- or long-term effects of chlorpyrifos on peripheral nerve function were apparent at the levels studied (a caveat being that exposure durations were relatively short).

58. Less convincing is a study from Malaysia by Kimura et al. (2005). Eighty male tobacco farmers using methamidophos were identified at random from a National Tobacco Board registry, and compared with 40 office workers from the same Board. No consistent or significant differences were found in sensory or motor nerve conduction velocity. Unfortunately, only a minority of subjects were exposed to organophosphates, and results for these cannot be distinguished. Other less telling limitations included a lack of information on the sampling of controls, response rates, and exposure levels.

59. Jamal et al. (2001) compared 16 Scottish farmers who had dipped sheep without acute symptoms of poisoning, and matched healthy controls. Farmers were recruited by randomly contacting farms listed in the Yellow Pages for Glasgow North and South. In all, 129 calls identified 39 sheep farmers who had dipped sheep for four or more years using organophosphates (diazinon, propetamphos and chlorfenvinphos). Thirteen farmers declined to participate, and the 16 farmers analysed were chosen to be close in age and sex to another group of farmers who had reported acute symptoms after use of organophosphates. No details are given on the selection and recruitment of matched volunteer controls, who came from mixed populations ("mainly office staff"). Investigations included NCS (motor and sensory latencies and amplitudes plus F wave latency); EMG, somatosensory evoked potential latencies; QST (hot, cold and vibration thresholds); and clinical examination of reflexes, muscle power and sensation (pin prick, vibration, light touch).

60. In contrast to most other studies, Jamal et al. reported a very broad range of abnormality. In particular, the farmers fared worse in terms of all motor and sensory

latencies and amplitudes (in eight of 12 estimates, $p \leq 0.001$), EMG score (based on the proportion of polyphasic motor units in two muscles, $p<0.001$), median vibration and cold thresholds ($p \leq 0.004$), and clinical scores related to abnormality of reflexes and sensation ($p<0.02$). NCS abnormalities were marked, mean amplitudes of sensory and motor potentials in the farmers being approximately half those of controls, while median nerve amplitudes were affected as much as those in the sural nerve, suggesting an axonal neuropathy that was not dependent on nerve length. In comparison, abnormalities of symptoms, reflexes, sensory examination and EMG were minor.

61. Results from various parts of the assessment were obtained blind to other results by different teams. It is unclear, however, whether the testers for each component were blinded to the exposure status of those they were assessing. The representativeness of exposed and unexposed groups is also unclear, although they appear not to have been well matched by social class. A further limitation is the lack of a control group of unexposed farmers (the farmers may have had thicker skin than office-based controls, which could have reduced measured sensory NCS amplitudes in the upper limb). Findings from this very small study, if true, would point to a broad range of effects of organophosphate sheep dip on peripheral nerves. However, the impact of selection bias (if those with health concerns preferentially participated) and of diagnostic bias (for semi-objective measures such as the clinical examination and QST) may have been considerable.

62. Finally, two cross-sectional studies have explored associations with findings on clinical examination. The first by Farahat et al. (2003) in Egypt, recruited 52 men employed by the Ministry of Agriculture to spray cotton fields and 50 administrators from other ministerial departments. Groups were similar in age and years of education but less well matched by social background. Details of sampling and recruitment are limited but the response rates in each group were suitably high. The exposed group applied two of four organophosphates (profenofos, chlorpyrifos, triaziphos, phorate) using knapsack or motorised sprayers, roughly fortnightly, over a three month season, together with various “insect growth regulators”, carbamates and pyrethroids. Acetylcholinesterase levels were significantly lower in the exposed men than in the controls (mean difference –20.91 U/ml, $p<0.001$). Health assessments (muscle power, lower limb reflexes, superficial and deep sensation) were conducted during a working day of the season, with the potential that findings may have been influenced by recent rather than long-term exposures. Abnormal signs were more frequent in the applicators (ORs elevated some three- to six-fold), although adjusted p-values were not significant at the 5% level. No mention is made of blinding in the assessment of these ‘soft’ outcomes, so there may have been important potential for diagnostic bias.

63. In the second study, by Srivastava et al. (2000) in India, 59 male workers manufacturing quinalphos were compared with an eclectic control group of 17 male tea vendors and street hawkers. All of the organophosphate workers from a production unit were invited to take part, but the sampling frame and procedure for recruiting controls was not reported, and response rates are not given. Mean acetylcholinesterase levels were almost identical in the two groups. Among other items, the authors reported on lower limb reflexes. However, the criteria for abnormality are not stated and nor is the timing of assessment relative to most

recent exposure. Adverse findings were almost implausibly common in the plant workers but rare in the control group. Thus, plantar reflexes were deemed abnormal in 49% of the plant workers but in none of the controls ($p<0.05$), the corresponding figures for ankle and knee reflexes being 38% vs. 6% ($p<0.05$) and 15% vs. 0% ($p>0.05$) respectively. These figures included equivocal reflexes, which were observed in many exposed workers but surprisingly, in almost no controls. The proportions of exposed workers with absent reflexes (a more clear-cut abnormality) were 14% for plantar, 2% for knee and 7% for ankle reflexes, and these were not statistically significantly different from those in controls. Assessment of these 'soft' outcomes may well have been unblinded, with potential for diagnostic bias. In any event, as already outlined, this small study had many methodological limitations.

Overview of evidence

64. The body of evidence on organophosphates and peripheral nerve function has grown substantially since 1999. In particular, the two earlier negative nerve conduction studies by Ames et al. (1995) and Engel et al. (1998) have since been augmented by reports from Starks et al. (2012b), Albers et al. (2004a, 2004b, 2007), Peiris-John et al. (2002), Steenland et al. (2000), Kimura et al. (2005) and Jamal et al. (2001). Individually and collectively these studies have limitations, but only the last points to a significant adverse effect, while findings on NCS in the stronger, larger and more careful studies (e.g. Starks et al., Albers et al., Steenland et al.) argue against an important effect of organophosphates on nerve conduction in the absence of overt acute poisoning.

65. In keeping with this, the large study by McCauley et al. (2002) found no excess of self-reported peripheral neuropathy over long-term follow-up in veterans of the Gulf War potentially exposed to organophosphate nerve agents, while that by Albers et al. (2004a) found no evidence that work in chlorpyrifos manufacture increased risks of 'definite' or 'probable' neuropathy, although there was limited statistical power to address these outcomes. The balance of evidence appears against there being a long-term risk of clearly demonstrable neuropathy from exposure to organophosphates in the absence of overt acute poisoning, a conclusion that has strengthened with the passage of time.

66. Findings relating to QST have been more variable. Previously, Stokes et al. (1995) reported an elevated vibration threshold in workers exposed to organophosphates, but in the hands and not the toes (an unexpected pattern), while London et al. (1997) also found that vibration sense thresholds in the toes did not differ by exposure status, and Ames et al. (1995) found no differences in this outcome, either in fingers or toes. The large, complex study by Pilkington et al. (1999, 2001) explored the matter in detail but drew guarded conclusions following rather equivocal findings: little difference was found overall, with the possible exception that higher level exposures to sheep dip concentrate may have had minor effects on cold and vibration thresholds (cold threshold with ever handling concentrate and vibration threshold with intensity of handling concentrate). Steenland et al. (2000) have subsequently reported significantly better vibration thresholds in the toes of applicators using chlorpyrifos than in controls, while Keifer et al. (2000) found no significant differences in orchard workers using chlorpyrifos,

diazinon and paraoxonane. Jamal et al. (2001) found higher vibration and cold thresholds in the feet of sheep dippers than referents, but in a small study with methodological limitations. On balance, therefore, the case that long-term low level exposure to organophosphates causes detectable adverse changes in sensory thresholds remains unproven, and if there is an effect, then it is likely to be small.

67. Somatosensory potentials were evaluated in studies by Spencer (2001) and Jamal et al. (2001). Neither of these reported abnormality, and both of the reports had limitations making the evidence base on the outcome weak. Studies on EMG and neuromuscular function are similarly few in number, being described in the reports by Jager et al. (1970), Engel et al. (1998) and Jamal et al. (2001). Findings have been mixed but again some of these studies had important flaws, making for a slim evidence base.

68. Finally, the number of studies reporting findings on neurological examination has swelled, although many reports have been of limited quality. Thus, for example, small cross-sectional studies by Otto et al. (1990) and Cole et al. (1998) on lower limb reflexes have been supplemented by other small cross-sectional studies by Farahat et al. (2003) and Srivastava et al. (2000), in one case indicating implausibly high rates of abnormality. Lack of explicit blinding hinders interpretation, as does the potential for misdiagnosis of such 'soft' signs in inexperienced hands. Findings have been inconsistent. For example, muscle power was abnormal in the study of Cole et al. (1998), as against joint position sensation and postural tremor in that by Starks et al. (2012b), and these only in relation to certain organophosphates. Steenland et al. (2000), on the other hand, reported significantly better overall neurological function in exposed workers.

69. The poor positive predictive value of traditional neurological examination as a marker of peripheral nerve function bears repeating, as remarked in the introduction to this section. Findings on NCS, the best measure of peripheral neuropathy, can be contrasted with those based on softer semi-objective markers of outcome such as QST and physical examination, and also on reported symptoms. When NCS (an objective measure of large fibre function) is used, the current balance of evidence is against an important effect of organophosphates in the absence of acute poisoning, whereas findings using other measures of outcome, including several relating to large fibres (e.g. reflexes, vibration sense) and others relating to small fibres (e.g. hot and cold sensory thresholds) have been less consistent. Measurement of nerve fibre density in skin biopsies would probably improve the assessment of small fibre neuropathy, but the symptoms that have been reported in relation to low-level exposure to organophosphates do not point to impaired function of small nerve fibres specifically.

Other neurophysiological outcomes

70. Two other neurophysiological outcomes that have been studied in relation to organophosphates are electroencephalographic abnormalities and auditory/visual event-related evoked potentials (ERPs).

71. The electroencephalogram (EEG) records scalp electrical activity resulting from synchronous activity of neuronal populations in the brain, and reflects a combination of excitatory and inhibitory postsynaptic electrical potentials. Although these potentials arise from the neocortex, they are influenced by ascending pathways from limbic, thalamic, and reticular nuclei. However, because voltage falls off with the square of the distance from the source, the activity of deep sources is more difficult to detect. The typical procedure for evaluating EEG traces involves power spectral analyses, which examine the levels of electrical activity in delta, theta, alpha, and beta frequency bands and their coherence (i.e., the similarity of EEG waveform components at spatially separate regions of the scalp).

72. Whereas the EEG records spontaneous background electrical activity in the brain, ERPs are electrical potentials recorded from the scalp in response to a specified sensory stimulus (usually auditory or visual). The stimulus is delivered repeatedly, 50 or more times, and the electrical activity that is recorded in the first few hundred milliseconds (ms) after each stimulus is averaged to eliminate random background EEG activity, allowing discrimination of the electrical potentials generated by the stimulus. The technique has poor spatial resolution, but excellent temporal resolution, and has been used to study the sequence of stimulus registration and processing. Auditory ERPs are typically divided into brainstem components (which occur with a latency of <10 ms from the stimulus), middle latency components (10-90 ms), and long-latency components (>100 ms). Most studies focus on the longer latency components. Starting with a positive-polarity response at about 50 ms, the components are labelled according to their positive or negative polarity (P or N), and their latency in ms (e.g., P50, N100). The components up to 200 ms are often described as exogenous or stimulus-driven, because they primarily reflect the physical properties of the stimulus. The components after 200 ms are described as endogenous, concept-driven, or context-dependent, because they are strongly influenced by internal representations of the stimulus and its significance as interpreted by the participant.

73. At the time of the last COT review, no studies had investigated possible effects of organophosphates on EEG activity or ERPs in the absence of acute poisoning, but since 1999, four such investigations have been reported.

74. Bayrami et al. (2012) studied male farm workers (n=40) in Iran who were occupationally exposed to organophosphate pesticides, and compared them to an equal number of men from the same village who were not engaged in agricultural work and had no history of organophosphate exposure. The two groups were matched on age, years of employment, and smoking habits. Although time since last exposure was not explicitly reported, the workers were tested at the beginning of the week during the spraying season, which suggests that last exposure was likely to have been at least two days earlier. No details are given either of the method of EEG examination or of the criteria by which findings were classed as normal or abnormal. The reported prevalence of EEG abnormality was 61.5% in the farm workers and 50% in the referents, suggesting that satisfactory data were not obtained for all participants (24/40 = 60% and 25/40 = 62.5%). Whatever, the exact numbers on which the percentages were based, the difference appears to have been far from statistical significance. However, because of the poor reporting, no useful conclusions can be drawn from this study.

75. As part of a study of peripheral neuropathy, Jamal et al. (2001) assessed auditory and visual ERP latencies in 16 male Scottish farmers who had used organophosphate sheep dips over four or more years, and an equal number of controls (one of them female), who were “mainly office staff”. Little detail is given of the methods by which the tests were carried out. Visual P100 latency was significantly increased in the farmers, but not in another group of farmers with chronic neurological problems and previous acute symptoms following organophosphate exposure. Because of the incomplete reporting of methods, little can be drawn from this finding (see also paragraph 59).

76. Kimura et al. (2005) studied 76 male tobacco farmers in Malaysia (randomly selected from a register held by the National Tobacco Board), who were exposed to various pesticides including organophosphates, along with a reference group of 38 male officers from the National Tobacco Board (see also paragraph 58). The two groups appear to have been similar in age, education, and alcohol consumption. Among the farmers, the time since last exposure to pesticides ranged from 1 to 172 days (mean 19.6 days). Auditory “odd-ball” ERPs were studied using two tones (1000 Hz non-target, 2000 Hz target, 90 dB) presented binaurally through earphones at a rate of 0.5 Hz. Both the P300 component of the target tone and the N100 of the non-target tone were measured. In addition, pattern reversal visual ERPs were assessed in a darkened room. The pattern comprised white and black squares reversing at a rate of 2 Hz, and the P100 was measured. Details of the methods are not well described. No differences between the farmers and referents were reported for any of the ERP measures, but only a minority of the farmers were exposed to organophosphates.

77. As part of a study of neurophysiological and neuropsychological outcomes, Browne et al. (2006) assessed EEGs in a subset of 19 adult volunteers in Israel who lived near to fields that were sprayed with organophosphate pesticides, and a reference group of nine people living in urban environments. Among the rural residents, EEG power in the theta band (6.5-8 Hz) was significantly lower in the temporal regions, while beta 3 activity was increased in the frontal regions. Moreover, beta 3 activity in the frontal cortical regions was higher in those with the R allele of PON1. However, the functional implications of such differences are uncertain, and given that multiple EEG measures appear to have been examined, it is possible that they occurred by chance.

78. Dassanayake et al. (2009) studied male Sri Lankan vegetable farmers with no history of acute organophosphate poisoning that had required medical care. Farmers were recruited from a larger group who attended educational programmes and had been exposed to organophosphates for more than five years. However, 14 were excluded because of high alcohol consumption, diabetes or psychiatric illness, leaving a sample of 35 men aged 35-64 years, who had been exposed to pesticides for a mean of 26 years (SD 10.4 years), and who had sprayed pesticides at least three times during the past six months. Times since last exposure were not reported, but farmers were instructed to abstain from applying or handling agrochemicals in the four days preceding neurophysiological assessment. They were compared with a reference group of 38 male hospital labourers of similar age. Auditory ERPs were examined using an odd-ball task (binaurally presented through

headphones at 75 dB, 1000 Hz non-target, and 2000 Hz target), and the N100, P200, N200 and P300 components were assessed. Statistical analyses included ANCOVA to partial out the influence of the preceding ERP component. There were no delays in the N100 or P200 signals, but the exposed workers showed a significant delay in the N200 and P300 components. When adjustment was made for the timing of the preceding ERP component, the delay in the N200 signal lost significance (p=0.102), but that for the P300 peak remained significant (p=0.010). There were no significant differences in the amplitudes of either of the N200 or N300 peaks. The authors suggested that their ERP data indicated "significant impairment in active processing of information", but this assumes that the delay in ERPs reflected an effect on the P300a component. As the auditory oddball task probably relates to the P300b component, the findings are more suggestive of difficulty in resolving uncertainty about whether the stimulus was a target or non-target than in picking up that a stimulus had occurred and acting on it.

79. This was a carefully conducted study and suggests that organophosphates might have long-term impact on the speed at which people can resolve uncertainty as to whether a tone is a target or non-target. In support of this, the accuracy of detecting target tones was lower among the exposed workers (p=0.044), which also indicates difficulties in discriminating targets from non-targets. However, as there was no a priori expectation of this specific impairment, it is possible that the differences from controls occurred simply by chance, and in the absence of independent replication, it can only be regarded as hypothesis-generating.

Conclusions

80. Few studies have looked at EEG or auditory/visual ERP outcomes in relation to organophosphate exposures insufficient to cause overt acute poisoning. The evidence that is available provides little indication of adverse effects. One study has suggested impairment in the processing of auditory information, but without independent replication, little can be drawn from this isolated finding.

Neuropsychological outcomes

81. Neuropsychological outcomes are measures obtained by studying people's performance in a range of tasks designed to assess aspects of cognition such as attention, the comprehension and production of language, learning and memory, reasoning, problem-solving and thinking. Because cognitive processes occur rapidly and cannot be observed directly, they are difficult to study – the main outcome variables being the speed and accuracy with which people complete various tasks. However, the behaviour that is observed when people perform such tasks provides indirect information about the internal cognitive processes that have occurred.

General considerations

82. Most cognitive neuroscientists understand the cognitive system to comprise multiple modules which can operate with relative independence, although in normally

functioning individuals they interact closely through the interconnection of the various brain systems that subserve them. The majority of neuropsychological tests that have been employed in studies of organophosphates have been designed and developed to detect dissociations (i.e. imbalance between cognitive modules that are impaired and others that are spared) in patients with clinically apparent brain damage. Neuropsychological investigation of such patients, and especially those with discrete brain damage, has provided a wealth of information about the existence, organisation and function of different cognitive modules. However, neuropsychological tests are less informative regarding more diffuse brain damage and even less so in people who do not have clinically overt brain disorders. In this last circumstance, the typical dissociations between impaired and spared cognition that are found in clinical populations would not be expected to occur. Instead, neuropsychological test results must be analysed for more subtle patterns of impairment, reflecting effects on particular cognitive modules. However, this approach has not been widely embraced in published neurotoxicological research.

83. When looking for effects of neurotoxicants in apparently healthy populations, consideration must be given to possible confounding by other factors that can affect the efficiency of cognitive systems. These include circadian changes, exercise, caffeine, nicotine, food, sleep and anxiety, as well as sex, age, education and importantly, levels of function before exposure. In addition, interpretation must take into account that performance in neuropsychological tasks can be compromised by deficits anywhere in the chain of functions from the initial registration of a stimulus to the subsequent manifestation of behaviour. This includes non-cognitive as well as cognitive processes. For example, poor eyesight or hearing can compromise behaviour in some neuropsychological tasks, as can difficulties in the execution of responses (e.g. because of tremor, problems with the initiation of voluntary movements, or impaired motor nerve conduction). There is also a concern that subjects may underperform in some tests simply because they believe that they have suffered a potentially damaging exposure (through sub-optimal motivation or effort).

84. In assessing findings on neuropsychological outcomes following low-level exposure to organophosphates, one looks for consistent evidence that a particular set of functions is systematically affected in a way that is unlikely to be explained by bias in the selection of subjects for testing or the way in which tests are carried out.

85. Appendix C summarises the types of neuropsychological test that have been applied when looking for possible adverse effects of low-level exposure to organophosphates, and the aspects of neurological and cognitive function that might affect performance on each test.

Evidence at time of last COT review

86. The last COT review of organophosphates concluded that neuropsychological abnormalities could occur as a long-term complication of acute organophosphate poisoning, particularly if the poisoning was severe, and that abnormalities had been most evident in tests involving sustained attention and speeded flexible cognitive functioning. However, it was unclear whether long-term neuropsychological effects

could occur from exposures to organophosphates insufficient to cause overt poisoning. At that time, nine studies provided information that was relevant to this question.

87. Otto et al (1990) carried out a cross-sectional survey of 229 male production workers from a pesticide formulation plant in Egypt where four organophosphates had been handled, comparing them with 180 workers from a fertiliser plant and 167 from a textile plant. Among other things, participants undertook the block design test and the Santa Ana dexterity test of hand-eye coordination and motor performance, but no differences were observed.

88. The other eight studies were summarised as follows:

Two of these investigations by Daniell et al. and Ames et al. found little evidence of any difference between exposed and control subjects, although both employed test batteries that would be expected to be sensitive to cognitive impairments of the kind described as being characteristic of COPIND. The investigation reported by Daniell et al. studied 57 pesticide applicators involved in orchard spraying. That of Ames et al. was a follow-up study of 45 pesticide workers who had been removed from exposure to organophosphates because of low acetylcholinesterase activities, but who had not shown symptoms or signs of acute organophosphate toxicity.

Maizlisch et al. investigated 46 pesticide (diazinon) applicators before and after a working shift. They also found few significant associations with exposure, although the exposed subjects tended to perform less well than controls in the digit-symbol test.

Stephens et al. investigated 146 sheep farmers involved in the use of organophosphate sheep dips. Differences were found, after correction for a number of confounding factors, in a measure of simple reaction time, the time taken to complete a test of "syntactic reasoning" (sentence verification) and in the digit-symbol substitution test. No effects were found on measures of short-term or long-term memory.

Fiedler et al. studied 57 fruit farmers who were licensed pesticide applicators and compared them with a control group of 44 individuals. A more comprehensive test battery was employed than that of Stephens et al. They did not include either syntactic reasoning or digit-symbol substitution tests, but used other tests, for example, the "Stroop" and "Trails B" tests, that would be expected to assess some of the same processes that are assessed by the syntactic reasoning and digit-symbol substitution tests. They found that, after correction for the influence of scores on a reading test which is widely used to estimate premorbid intellectual performance, the only difference between the exposed and control groups was with respect to simple visual reaction time, the exposed group showing significantly slower responses.

Gomes et al. studied expatriate farm workers exposed to organophosphates in the United Arab Emirates. The exposed group consisted of 226 established farm workers employed for at least two years in their current jobs. They were compared to 226 matched controls, who were not employed in agriculture. In addition, a second exposed group consisted of 92 farm workers newly arrived in the country but

who had worked for at least two years in their own country. In this study, the test of cognitive function that was used was the digit-symbol substitution test. Lower test scores were reported on this test in two groups of farm workers compared to the control group. The extent of lowering was unrelated to the duration of current employment or to erythrocyte acetylcholinesterase activity, raising the possibility that it was a consequence of long-term rather than acute exposure to organophosphates. The interpretation of this finding is made difficult, however, because possible differences in literacy level and intelligence between the control group (which comprised domestic, shop, office and industrial workers) and the exposed group were neither measured nor controlled for.

Cole et al. compared three groups of individuals who had been exposed to pesticides (including organophosphates) and an unexposed control group. The exposed group consisted of 123 pesticide applicators, 28 field workers "generally" exposed to pesticides and 23 subjects exposed by consumption of local potatoes (treated with pesticide). They were compared with 72 controls from the local non-farm population, matched for age and education level but this was unlikely to have been sufficient to remove the difference between rural and urban groups. Various neuropsychological tests were employed, and several deficits were recorded in the exposed subjects. However, these were not consistent across the three groups. The study did not distinguish between exposure to organophosphates and other agents.

London et al. investigated a number (>25) of cognitive and neuropsychological measures in a sample of South African agricultural workers. These included tests such as simple reaction time and the digit-symbol substitution test that had shown sensitivity to organophosphate exposure in other studies. Two measures of motor function showed modest ($p<0.05$) relationships with estimated cumulative organophosphate exposure, as did one reaction time measure from a test of semantic memory function. As the authors themselves concluded, in view of the large number of statistical tests conducted, these positive findings could have occurred easily by chance and provide, at best, only weak evidence of an association between organophosphate exposure and cognitive function.

89. The 1999 report concluded that:

When account is taken of these limitations and of the inconsistencies between studies, the research reviewed provides little support for the hypothesis that prolonged low-level exposure to organophosphates gives rise to long-term changes in the cognitive functions that would be expected to show impairment in the postulated syndrome of COPIND. The most consistent findings are with respect to simple reaction time and a test (digit-symbol substitution) that depends on multiple cognitive functions, places individuals under time pressure, and is known to be sensitive to cognitive impairment following neurological insult such as traumatic brain injury. No study, including those with positive results on other measures, has indicated effects of organophosphate exposure on long-term memory function. It is noteworthy that the finding of positive effects on the digit-symbol substitution task, but without a decrement in long-term memory, is similar to that in people who have previously been acutely poisoned by organophosphates.

New evidence

90. Since the last COT review, a further 22 investigations have been published that looked for neuropsychological consequences of low-level exposure to organophosphates (Table 2).

91. Kilburn (1999) undertook comprehensive neuropsychological testing of 22 patients (10 self-referred, 12 referred by attorneys) with exposure to chlorpyrifos, which arose predominantly from the treatment of their homes or offices. These were a subset of 384 patients evaluated for possible neuropsychological effects of chemicals in a neurotoxicology clinic (6% response rate). The reference group comprised 264 workers with no known neurotoxic exposure (Kilburn et al., 1998). While the two groups were similar in age distribution, the patients exposed to chlorpyrifos on average had completed 2.4 more years of education ($p<0.001$). Also, their Profile of Mood States (POMS) and depression scores were elevated. In comparison with the reference group, the patient group showed impaired performance on most of the neuropsychological tests employed – simple and choice reaction time, culture fair IQ test, digit-symbol substitution, Lafayette pegboard, Trails A & B, immediate and delayed recall of stories, information (a test of general knowledge), and picture completion. There was no difference between the groups in the test of similarities and the reference group had only a marginally higher score in vocabulary ($p=0.047$). It is possible that people with neuropsychological deficits were selectively referred for investigation, making the group of patients with exposure to chlorpyrifos unrepresentative. Furthermore, the analysis did not explore the influence of possible confounding factors. This is particularly important since culture fair IQ, differed from that in the referent group. For these reasons, it is difficult to conclude much from the study.

92. Bazylewicz-Walczak et al. (1999) undertook neuropsychological testing in 26 women who had been exposed to organophosphates while carrying out gardening jobs in Poland over 1-24 years (mean 11.9 years). They were compared with a reference group of 25 women who worked in canteens, kitchens and administrative jobs at the same garden centres, and were matched for age, education, place of residence, smoking, alcohol consumption and use of “drugs” (it is unclear exactly how the exposed and control participants were selected from those eligible for study). Neuropsychological testing was carried out in the weeks before and after a two-month period of intensive application of pesticides (including dichlorvos, methamidophos, methidathion, and pirimiphos-methyl, and also carbamates, synthetic pyrethroids and dithiocarbamates). The tests administered were a Polish adaptation of the WHO Core Test battery and the Finnish Subjective Symptom Questionnaire. Analysis explored possible effects of season (after the season as compared with before) and exposure group (exposed women compared with referents). At the end of the season, there were improvements in performance on the digit-symbol, and Santa-Ana (dominant and non-dominant hands) tests in both the exposed and referent groups, probably because participants had become more familiar with the tests. In comparison with the referents, the exposed group made fewer errors in the aiming test ($p=0.02$) but tended to be slower at the task ($p=0.09$), which suggests a trade-off between speed and accuracy. The “fastest” reaction times in the exposed group were also reported to be somewhat longer, but the

definition of “fastest” was not specified. There were no significant interactions between exposure group and season. Interpretation of this study is difficult because the exposed workers had higher levels of anxiety and depression, and it is unclear whether adjustment was made for any confounding that might have resulted. The authors’ conclusion that “even low, long-term exposure may be associated with adverse behavioural effects in female greenhouse workers” is not supported by the findings. The idea that there were “chronic effects evident” relies on a lax level of statistical significance ($p<0.10$) and misinterpretation of the aiming error scores.

93. Keifer et al. (2000) used the WHO Core Test battery to investigate neuropsychological performance in Spanish speaking orchard thinners exposed to azinphos-methyl following treatment of apple trees to control codling moth. Testing was undertaken before a spraying season (to investigate the effects of earlier chronic low-level exposure), and again after the season (to investigate seasonal change). Data were obtained for 137 workers in the first examination and 87 in the second, but response rates were not reported. Each exposed farm worker was matched by age, gender and education with a non-agricultural referent employed in garment manufacturing, or a hotel or restaurant. The examination before the spraying season revealed poorer performance by the exposed workers on backward digit span, Trails A, and in a test of manual dexterity (Santa-Ana). Other tests (e.g., paired-associates, forward span, block design, Benton visual retention test) showed no significant differences from referents. After adjustment for vocabulary score (from the Peabody Picture Vocabulary Test), the only significant difference was for Trails A ($p=0.06$). The authors considered that there was no consistent effect of a season of thinning on neuropsychological test performance.

94. Steenland et al. (2000) undertook neuropsychological testing of 191 current and former termiticide applicators in North Carolina, USA, who had worked for longer than one year during 1987-1997, using chlorpyrifos. For comparison, two other groups were recruited; non-exposed friends of about the same age (n=106) and a non-exposed group of blue-collar workers (n=83). The Neurobehavioral Evaluation System (NES) was used, incorporating tests of vocabulary, digit span, continuous performance, simple reaction time, digit-symbol substitution and pattern memory, and mood scales. In addition, the Trails A and B, and grooved pegboard test were administered. Information about use of pesticides was gathered by telephone interview and recent chlorpyrifos exposure was assessed by measurement of TCP excretion in urine. The duration of chlorpyrifos use was relatively short (median 1.8 years; range 0.1-10.3 years). Creatinine-corrected values for TCP in urine from applicators exposed in the past week, applicators not exposed in the past week and unexposed controls were 331, 55 and 3 ug/g creatinine respectively. The exposed workers tended to perform less well than the non-exposed blue-collar workers on the pegboard test ($p = 0.005$), but did not differ from the non-exposed friends ($p=0.43$). Performance was worst in eight workers with a self-reported history of poisoning by chlorpyrifos, who also fared worse in continuous performance and simple reaction time. There were no significant differences between termiticide applicators and either of the two reference groups in the other neuropsychological tests. Urinary TCP did not significantly predict performance in any test except for vocabulary, in which subjects with higher TCP levels did significantly worse ($p=0.02$). There were no associations with duration of use of chlorpyrifos or other pesticides. Overall, there was no clear evidence of poorer neuropsychological performance in the

termiticide applicators. Where changes were detected, they tended to be in tests that require the rapid initiation of a response to fine discrimination of visual stimuli, but the groups did not clearly differ in conventional tests of vision. Although the non-exposed referents appear to have been volunteers from a larger pool of those who might have been eligible, it seems unlikely that their performance on tests would have been unrepresentatively poor, causing failure to detect important adverse effects.

95. Srivastava et al. (2000) studied 59 male workers engaged in the manufacture of quinalphos in India, who had no known history of acute organophosphate poisoning, and a reference group of male tea vendors and roadside hawkers (n=17) unexposed to organophosphates (it is unclear how these referents were recruited and with what response rate). Whole blood acetylcholinesterase was similar in the two groups. A limited number of neuropsychological tests were administered (forward and backward digit span, digit-symbol substitution, and the Bourdon Weirsma vigilance test) and the exposed group performed worse on all of them ($p<0.05$) (results from forward and backward digit span appear to be have been combined into a single score). However, the analysis took no account of possible confounders. For example, there was a higher proportion of graduates (47.1%) among the referents than in the exposed workers (25.5%), and the exposed group complained more of generalised weakness and fatigue.

96. Farahat et al. (2003) undertook neuropsychological testing of 52 Egyptian male cotton workers exposed to organophosphate and other pesticides and a reference group of 50 male clerks who lived and worked near to cotton fields. Most of the exposed group (88%) reported never using protective clothing, and their mean serum cholinesterase was significantly reduced, suggesting recent exposure. None had been hospitalised for acute poisoning. In comparison with the reference group, their performance was poorer in multiple neuropsychological tests (similarities, digit-symbol substitution, Trails A & B, letter cancellation, forward and backward digit span, Benton visual retention test, and delayed story recall). However, no differences were seen in the Paced Auditory Serial Attention Test (PASAT), block design, and immediate story recall (Story recall A). Comparisons were adjusted for age and years of education, but no details of this analysis were reported. While the authors concluded that moderate chronic exposure may impair attention and memory as well as visuomotor speed, this interpretation is questionable because most tests included a strong visuomotor component. The conclusions that can be drawn are limited by failure to include a test that could be indicative of functioning before exposure (e.g. of vocabulary), to explore how far the various measures of performance correlated with each other (correlations would be expected for items derived predominantly from the Wechsler Adult Intelligence Scale (WAIS)), and to consider reasons for discrepancies between tests with similar attentional demands (e.g. between (a) forward and backward span and (b) Trails A and B). A higher prevalence of reported numbness, dizziness, and (to a lesser extent) tremor might also have affected performance on tests that required visuomotor abilities. While the authors acknowledged that neurological symptoms such as numbness and dizziness might explain why there were more widespread deficits on tests involving visuomotor functioning, they made no attempt to determine whether the observed neuropsychological differences could be a consequence of peripheral impairment. It is also a concern that although none of the exposed men had required

hospitalisation for acute poisoning, some may have suffered at some time from milder acute poisoning.

97. Salvi et al. (2003) studied 37 southern Brazilian tobacco workers after they had been working with pesticides for three months, and had been exposed within the past day. A subset of 25 workers (13 females) was re-evaluated three months later, during which time they had not worked with pesticides. Of these 25 workers, 52% had a history of earlier acute poisoning. Exposure was to organophosphates, in particular chlorpyrifos. Both the Mini Mental State Examination (MMSE) and a test of "word span" were used, although the latter was not described. Performance in both tests was within the expected range for the population and no significant differences were reported between the two time points. However, because many participants had experienced acute pesticide poisoning, and there was no reference population with which to compare the exposed workers, no useful conclusions can be drawn about long-term effects of organophosphates in the absence of overt acute poisoning.

98. Stephens and Sreenivasan (2004) carried out neuropsychological testing of 37 male orchard sprayers in England who used chlorpyrifos (response rate 68%), and compared them with two referent groups: 26 pig farmers and 31 construction workers. At least two months had elapsed since last application of organophosphates, and biological monitoring confirmed that urinary levels of organophosphate metabolites were similar in all three groups. The neuropsychological tests comprised simple reaction time, digit span, and digit-symbol substitution from the Neurobehavioral Evaluation System 2 (NES2), and syntactic reasoning, location recognition, category search, and serial word learning from the Automated Cognitive Testing (ACT) system. The groups were well matched on age and educational level. However, the construction workers reported higher levels of weekly alcohol consumption. There were no significant associations of exposure with simple reaction time, forward and backward digit span, digit-symbol latency, serial word learning, speed and accuracy in the location recognition test, and speed in the category search test. Compared to construction workers, but not pig farmers, orchard sprayers had significantly slowed performance on the syntactic reasoning task for negative statements ($p<0.001$), but not for positive statements ($p=0.120$). However, except in the pig farmers, overall accuracy on the task was low and only marginally better than would be expected by chance. An equivalent analysis of the accuracy of syntactic reasoning, aside from the expected effects due to task complexity, did not indicate any particular trade-off between speed and accuracy that could account for the differences in response speed. While accuracy was also poorer among construction workers in the category search task, no analysis was undertaken to localise this poor accuracy. No exposure-response relations were detected for any of the neuropsychological tests in relation to cumulative exposure. The investigators considered the findings on syntactic reasoning for negative statements to result from the slowing of processing speed. It could be further added that the lack of effect on positively phrased reasoning items indicates that they did not result from a general slowing, and points to difficulties in conceptually manipulating material in memory.

99. Albers et al. (2004c) administered the Mini Mental State Examination (MMSE) and a qualitative questionnaire (about reading, memory, concentration and problem

solving) to 53 employees engaged in the manufacture of chlorpyrifos. They were compared with a reference group of 60 employees engaged in the manufacture of saran plastic film wrap. Exposure to cholinesterase inhibitors was confirmed by biological monitoring (urinary excretion of chlorpyrifos metabolites and decreased plasma butyrylcholinesterase activity). The two groups were matched on age, height, weight, body mass index (BMI), smoking, anxiety, and "reading scale" (which appears to have been a measure of pre-morbid IQ). No differences were observed between the exposed and reference groups. However the weight that can be given to this negative finding is limited by the low sensitivity of the MMSE in the assessment of neuropsychological function.

100. Roldan-Tapia et al. (2005) studied 40 male greenhouse sprayers from Almeria, Spain, who had used a mixture of pesticides (predominantly organophosphates and carbamates) for between 6 months and 30 years (mean 10.9 years), and a reference group of 26 male workers (drawn from a variety of occupations) who had never worked on farms or had contact with "toxic substances". Full details of the recruitment and selection procedure were not reported. Neuropsychology tests were drawn from the WHO core battery and sought to characterise measures of attention, memory, perception, praxis (execution of learned purposeful movements), expressive language, motor performance and levels of anxiety and depression. The two groups appear to have been well matched on measures of age, and educational level. For some of the neuropsychological tests scaled scores were derived, while others were analysed as raw scores or coded simply as correct/incorrect. However, it is unclear why performance on tests such as Trails A & B, and the letter cancellation task, was classified dichotomously. Multiple and logistic regression models were used to evaluate the relation of neuropsychological outcomes to years of working with pesticides (as a measure of chronic exposure) and plasma cholinesterase (as a measure of recent exposure). Age and education were considered as potential confounders. Most tests yielded negative results in comparisons of exposed workers and referents. Recent exposure was significantly related to quality of copy of Rey-Osterrieth figure ($p=0.019$), but there were no significant differences between exposed and control groups in levels of plasma cholinesterase, suggesting that recent exposures to organophosphates had not been high. Cumulative exposure was significantly associated with better recall after delay in the Rey-Auditory Verbal Learning test (RAVLT, memory for material learned following distraction), poorer quality and longer time of copy of the Rey-Osterrieth Figure, poorer Benton Visual Form Discrimination (a test of fine visual discrimination) and a tendency to lower anxiety (after 5 years of working with pesticides). There were no reported effects of exposure on forward or backward digit span, Trails A & B, digit-symbol substitution, picture completion, similarities, learning on the Rey-Auditory Verbal Learning Test, immediate or delayed recall of stories, Benton Visual Retention Test, or block design.

101. This study was limited by its small size, and the testers were not blind to the exposure status of the workers because they were tested in different locations. A major limitation was the dichotomisation of some test results when all could have been analysed as continuous measures. It is difficult to conclude much from the findings, although difficulties in copying complex drawings and making fine visual discriminations seem to characterise the "adverse" changes found. In contrast, there appeared to be better recall following distraction among those with longer exposure.

102. A series of three studies have examined Gulf War veterans potentially exposed to sarin and cyclosarin over a short period following demolition of a munitions dump at Khamisiyah in 1991 (Spencer, 2001; Proctor et al., 2006; Chao et al., 2010). However, the report by Spencer (2001) does not allow useful conclusions about neuropsychological outcomes because it contains insufficient detail of the tests employed and the results obtained.

103. Between 3.5 and 5 years after the incident, Proctor et al. (2006) undertook neuropsychological assessment of 70 veterans whom they classed as having moderate/high exposure to organophosphates, and compared them with a group of 70 veterans who were considered to have low or no exposure. No acute cholinergic symptoms had been reported after the incident. Various neuropsychological tests were administered, and importantly, the WAIS information test score was taken into account as one of several covariates. Participants with high exposure (n=23), moderate exposure (n=47) and low/no exposure (n=70) were well matched for various potentially confounding factors. As expected, the low/no exposure group had a lower "combat exposure score", but they also had a higher prevalence of current "major depression diagnosis" than the other two groups. Among the neuropsychological outcomes, exposure-response relationships were seen only for: the Purdue pegboard test (examining timed fine manual dexterity) for the dominant (p for linear trend = 0.005) and non-dominant hand (p=0.03); speed of finger tapping in both dominant (p=0.001) and non-dominant (p=0.002) hands; and the WAIS block design test – which assesses timed visuospatial constructional abilities. The high and moderate exposure groups performed better than the low/no exposure group on the finger tapping task, and the association of exposure with poorer performance on the Purdue pegboard test was strengthened after adjustment for the finger tapping measure as a covariate. Thus, manual dexterity appeared to be affected in the exposed subjects, and this was consistent with poorer scores on the block design test which also requires good manual dexterity. In contrast, there was no evidence of effects on tests examining executive function, attention, memory, and speed of performance not requiring fine manual dexterity (e.g., Trails A & B).

104. Chao et al. (2010) described a further study, 10 years after the Khamisiyah incident, which incorporated several additional neuropsychological tests, and compared 40 exposed veterans with 40 controls who had not been exposed. The groups did not differ importantly by sex, age, education, handedness, or experience of post-traumatic stress disorder (PTSD), major depression or chronic multi-symptom illness. After exclusion of four veterans who failed a test for malingering, the study did not identify any associations with performance in groove pegboard or digit-symbol tests of psychomotor performance, or in the Block Design test or tests of memory. This applied both in the full study sample, and also in the subset of subjects (32 exposed and 26 unexposed) for whom complete neuropsychological data were available.

105. While the observations in these two studies would be compatible with an initial effect on manual dexterity that subsided over time, care is need in interpretation because performance on tests may have been affected by psychological stresses arising from perceived exposure to chemical warfare agents. It is known that Gulf war veterans who report symptoms related to the conflict, perform worse in

neuropsychological tests (e.g. of memory, attention and response speed) than those who do not (Storzbach et al., 2000), and psychological stress, including perception of a chemical warfare agent, has been reported to be associated with chronic multi-symptom illness in Gulf War veterans (Riddle et al., 2003).

106. Rothlein et al. (2006) studied Hispanic immigrant workers in Oregon, USA. They compared 92 farmworkers who lived in close proximity to orchards sprayed with organophosphate pesticides and a reference population of 45 workers in a coastal town with little agriculture (response rates were not reported). Exposure to organophosphates was confirmed in dust samples from the farm workers' homes and by analysis of urine samples for thiomethyl phosphate metabolites. Sixteen tests of neuropsychological function were undertaken covering both psychomotor and cognitive function. After adjustment for age, sex and years of education, 12 of the sixteen measures showed poorer performance among those working in agriculture. However, the differences appear to have been statistically significant for only two measures – backward digit span and (in females only) finger tapping. As regards the relationship between neuropsychological performance and urinary metabolite levels, higher levels of combined thiomethyl metabolites were associated with poorer performance on digit symbol latency ($p=0.005$), selective attention latency ($p=0.011$) finger tapping (preferred $p=0.012$, alternating $p=0.029$) and perhaps hit latency on the continuous performance test ($p=0.042$) (all of these p-values are one-tailed). As the metabolites indicate recent exposure, these data suggest that recent exposure impairs measures of response speed in tasks that require the initiation of a rapid response to either the presence of an object (selective attention, digit symbol latency) or maintenance of a tapping rate. In the comparison between agricultural and non-agricultural workers, the lower backward digit span was anomalous since it resulted from the usual reduction in score from forward to backward span in the "exposed" workers, but a higher backward than forward score for the "reference" workers. Overall, the study provides little evidence of long-term effects on neuropsychological performance from low-level exposure to organophosphates.

107. Browne et al. (2006) reported data on neuropsychological testing in 23 adults in Israel, who worked in agriculture or lived near to fields that were sprayed with pesticides. A reference group (n=23), matched for age, sex and education, was taken from people living in urban environments. Exposure to cholinesterase inhibitors was confirmed by static air monitoring and demonstration of reduced "serum AChE" in the exposed agricultural workers/residents. The neuropsychological tests comprised forward and backward digit span, serial reaction time, continuous performance test, visual reproduction, and long-term verbal memory from the Rey Auditory Verbal Learning Test, and the Tower of Hanoi (a task of planning). The only test showing poorer performance in the exposed workers was the 20-minute delayed portion of the visual reproduction test ($p<0.05$); scores for immediate recall condition did not differ. This study was limited by the small group size and a failure adequately to consider potential confounders such as age. The combination of equivalent scores on visual reproduction after immediate recall with a deficit after delayed recall points to a specific effect, but despite its statistical significance, could also have been a chance finding.

108. Rohlman et al. (2007) undertook neuropsychological testing on 69 adult and 50 adolescent Hispanic farmworkers in Oregon, and 56 referents (29 adults and 27 adolescents) who had not worked on farms for at least one year, but may have carried out agriculture work before that. The adolescents were aged between 12 and 18 years (mean 15.7 years) and the adults 19-60 years. The nature of pesticide exposures was not reported. Neuropsychological performance was assessed using the Behavioral Assessment and Research System, and the tests included finger tapping, symbol-digit substitution, simple reaction time, digit span, serial digit learning, continuous performance test, selective attention, matching-to-sample, progressive ratio and reversal learning (although most participants did not complete this last test because of time constraints). Time since last exposure to organophosphates (if any) was not quantified. As many in the reference group were found to have spent at least two years working in agriculture, no comparisons were made between the exposed and reference groups. Instead, analyses were carried out according to years spent working in agriculture. The impacts of age, years of education, sex and years worked in agriculture were examined for each neuropsychological outcome measure, using multiple regression. Years worked in agriculture was associated with poorer performance on "match-to-sample" (an immediate visual recognition memory task), and (only in females) in the digit-symbol test. In males, years worked in agriculture was associated with worse performance on the selective attention measure (a go/no go task involving the speed of responding to the presence of a dot in a square). As no information was reported on exposure to specific pesticides, little can be drawn from this study regarding the effects of low-level exposure to organophosphates.

109. Abdel Rasoul et al. (2008) investigated neuropsychological performance in 20 Egyptian boys aged 16-18 years, who had been exposed to organophosphates when spraying cotton fields over an average of seven years. For each sprayer, a control who had never worked in the cotton fields was selected from friends/relatives living in the same community and attending the same school. Times since last exposure to organophosphates were not reported, but the boys who applied pesticides had lower levels of acetylcholinesterase. Several neuropsychological tests (information, similarities, arithmetic, block design, digit span, and digit-symbol from the Wechsler Intelligence Scale for Children (WISC), Trails A & B, and the Benton visual retention test) were employed. After adjustment for age, education and body mass index, significantly lower performance was observed in the exposed group on all of the neuropsychological measures. Despite differences in scores on the information subtest (general knowledge), these were not included as a covariate when analysing other measures. While not an ideal test of pre-morbid functioning, it was the best available in this study. Furthermore, while the poorer scores on digit span, both forwards and backwards, were interpreted by the authors as indicating memory impairment, this is incorrect. Deficits in forward span (which correlates highly with backward span) are extremely rare in patients with disorders of memory. That a number of significant correlations were observed with days worked in the current season, but less consistent associations with years worked, could indicate an adverse effect of recent exposure, but might also be explained by lower attendance at school. Furthermore, higher levels of neuroticism, depression, fatigue and blurred vision among the applicators may have contributed to worse performance on some tests. Given these limitations and uncertainties, little can be drawn from the study regarding neuropsychological effects of exposure to organophosphates.

110. Mackenzie Ross et al. (2010) undertook neuropsychological testing of 127 UK sheep farmers with at least 5 years exposure to organophosphate pesticides (67 working, 60 retired due to ill-health), and compared them with 78 rural policemen (38 working and 40 retired on ill-health grounds). Recruitment of the sheep farmers involved writing to farm owners listed on relevant databases (UK National Business Directory, National Farmers Union membership lists) and telephoning every fifth person on a list held by Wool Marketing Board). Of the 393 sheep farmers contacted, 59% responded. Additional farmers were recruited through advertising and articles in the media. Initially a total of 434 farmers came forward but 67% were excluded according to pre-defined criteria. Those included were aged 18-70 years, living in South-West or Northern England, currently working or retired because of ill-health, and exposed to organophosphates for a minimum of five years before 1991. Exclusion criteria included a history of psychiatric, neurological or serious medical problems, substance abuse (including alcohol) or acute organophosphate poisoning. Recruitment of controls was undertaken with the help of local constabularies and the National Association of Retired Police Officers who contacted members by email or newsletter. The study was also advertised in the police press. Initially 252 police officers came forward, but 63% were excluded (to be included, controls were required to have worked in a rural setting in South-West or Northern England, be currently working or retired on ill-health grounds, and have had no known exposure to organophosphates). Among the farmers, exposure histories (assessed by semi-structured interview) were highly variable, ranging from 8 to 49 years for those still working and 5 to 66 years in the retired group. The number of years since last dipping varied from 0-37 years in the working group (mean 9 years) and 0-42 years in the retired group (mean 11 years). The number of days per year that had been spent dipping varied from 0.5 to 30 in the working group and 1 to 21 in the retired group. On average, the farmers were three years older than the reference group.

111. A wide range of neuropsychological tests were administered (the Wechsler Adult Intelligence Scale-III (WAIS-III), Wechsler Memory Scale–III (WMS-III), verbal fluency, Trails A & B, Stroop, simple and choice reaction time, graded naming test, and grooved pegboard). The analysis focused on associations with exposure group (exposed vs referents) and working status (working vs retired – not farmers vs controls as stated in the paper). Age was analysed as a covariate when variables were not age-scaled, but it is not always clear when this was done. In addition, transformations were applied to some variables, but no information was reported on the transformations used, or the variables to which they were applied. Surprisingly, there were no significant differences in performance between the working and retired groups on any of the neuropsychological tests – perhaps due to age adjustments, and no further information was provided about the effects of working status.

112. Scores on the matrix reasoning test, a non-verbal measure of fluid IQ, were equivalent for the exposed and referent groups, as were measures of verbal ability (vocabulary, comprehension), visuospatial ability (block design, spatial span), and two other verbal/visual reasoning tasks (similarities, comprehension). However, exposure was associated with poorer performance in graded naming and picture arrangement; forward and backward digit span; the conceptually similar letter-number sequencing task; immediate and delayed verbal and visual memory tasks;

the digit-symbol substitution test; Trails A; Trails B; and the grooved pegboard tests (both dominant and non-dominant hands).

113. Unfortunately, no useful conclusions can be drawn from these findings because many of the exposed farmers, especially in the retired group, were volunteers recruited by advertisement, and therefore liable to be highly unrepresentative. There was further potential for selection bias because controls were excluded from the investigation if they had any history of psychiatric problems, whereas farmers were not excluded for this reason unless the psychiatric problems preceded their exposure to organophosphates. In addition, the methods of analysing test results were unsatisfactory. In particular, information was lost by dichotomous classification of scores as normal or abnormal, and there was insufficient attempt to explore the reasons for such wide-ranging differences between the exposed and referent groups. For example, poorer immediate memory will almost invariably lead to poor delayed memory (as initial registration of the material to remember is poor), and an appropriate analysis for delayed memory would have controlled for immediate memory.

114. As part of the US Agricultural Health Study, Starks et al. (2012a) explored impacts on neuropsychological performance of one or more episodic and unusually high pesticide exposure events (HPEEs – some of which could have involved organophosphates) that did not result in acute poisoning. A total of 693 male licensed pesticide applicators with no history of a physician-diagnosed pesticide poisoning, 156 of whom had experienced a HPEE, completed eight neuropsychological tests from the Neurobehavioural Evaluation System 3 (NES3) together with the grooved pegboard test. Associations between ever having an HPEE and neuropsychological outcomes were assessed by linear regression with adjustment for age and other possibly confounding covariates. A history of ever having an HPEE was found to be associated with worse performance on two tests: the digit-symbol substitution test and Sequences A (a touch screen version of Trails A). No effects were observed on any of the other neuropsychological tests (e.g., learning, memory, tapping, pegboard, Sequences B). The authors noted that the HPEE represented undesirable high exposure events experienced by pesticide applicators, and not overexposure to any specific pesticide. Overall, this well-conducted study suggests effects on tests that involve motor responses following visual scanning in pesticide applicators who have experienced high exposure events involving various chemicals, some of which may have been organophosphates.

115. Starks et al. (2012c) studied 701 male workers (mean age 61 years) from the US Agricultural Health Study (AHS). Eight neuropsychological tests from the Neurobehavioural Examination System 3 (continuous performance, digit-symbol, finger tapping, auditory verbal learning followed by delayed recall and recognition, and sequences A & B) were supplemented by the grooved pegboard test. Backward stepwise multiple linear regression was used to select covariates for each neuropsychological outcome measure. Scores were related to estimates of exposure (ever-used, lifetime days) to 16 different organophosphate pesticides, with adjustment for potentially relevant covariates. There were no associations with total organophosphate exposure for any of the neuropsychological tests, but associations were observed for some specific organophosphates. Some showed poorer scores with increasing exposure and others showed better scores, and some differed by

state (North Carolina or Iowa). Overall, the authors concluded that there was no consistent evidence of an association between organophosphate exposure and neuropsychological outcomes. However, they noted that the study sample was somewhat older than many which had been investigated in previous studies, and that there could have been some selection bias (only 40% of potential participants provided information on exposures and neuropsychological testing was undertaken in 701/1807 of these subjects). A major strength of the study was the large sample size and the reliability and accuracy of estimated pesticide use.

116. Bayrami et al. (2012) administered the Mini Mental State Examination (MMSE) to a group of 40 male horticulture workers from Iran who were exposed to organophosphate pesticides, and a reference group of 40 men from the same village who were not exposed to pesticides. No differences were reported in overall performance or in subscales relating to orientation, registration, attention/calculation, recall, and language. Although lower "plasma acetylcholinesterase" was reported in the horticultural workers, the assessment of longer term exposure to organophosphates was limited. Moreover, the MMSE, which is usually used as a screening test for dementia, provides only basic and relatively insensitive measures of neuropsychological function.

117. A study by Malekirad et al. (2013) investigated 187 horticultural farmers (mean age 37.9 years, range 16-80 years) in south-west Iran who were occupationally exposed to organophosphates, and a reference group of 187 unexposed workers, matched for age, from the same village. Little further information was reported about the recruitment process. A range of neuropsychological tests were conducted (psychomotor speed, selective and divided attention, verbal and non-verbal memory, prospective memory, spatial functioning), but while performance of the exposed group was reported to be significant lower ($p<0.001$) on all of the tests, a lack of detail about the methods prevents useful conclusions. Also, there were significantly higher levels of anxiety ($p=0.015$) and depression ($p<0.001$) in the farmers, but no attempt was made to control for these aspects of mental health as potential confounders.

118. In a longitudinal study of 614 agricultural workers in Gironde, France, Blanc-Lapierre et al. (2013) used detailed occupational histories elicited by questionnaire, a crop-exposure matrix, and data on dermal contamination from field studies to derive quantitative estimates of individual exposure to specific organophosphates during 1950-2003. Estimates took account of the probability, frequency and intensity of use by task. Logistic regression was used to relate exposures to performance in five neuropsychological tests (MMSE, the Benton Visual Retention Test, Stroop, Trails A and Wechsler Paired Associates), four of which were selected because they had been associated the most strongly with pesticide exposure in previous analyses of the same cohort. For each test, two outcome measures were employed – being in the worst 25% of scores at the end of follow-up (2002-2003), and being among the 25% of subjects with largest deterioration in score between baseline (1997-1998) and follow-up. Analyses were adjusted for age, sex, education and (for change in score) time interval between baseline and follow-up. Consistently positive associations were observed between all outcomes and exposure to each of 11 specific organophosphates, many being statistically significant. The strongest associations were for mevinphos, and these persisted after exclusion of subjects

who reported previous poisoning by pesticides or recent exposure at the time of neuropsychological testing. However, no clear trend in risk was observed across quartiles of exposure to mevinphos.

119. Strengths of this study include its prospective design, estimation of exposures by methods that did not rely on recall of exposure to specific chemicals, and relatively large sample size (443 subjects were classed as possibly exposed to at least one organophosphate). Against this, most of the neuropsychological tests were selected for investigation because they were already known to show associations with pesticide exposure in the study sample, which increases the possibility of false positive results by chance. Furthermore, the binary classification of outcomes makes interpretation difficult, and analyses based on continuous measures of change in score from baseline to follow-up would have been more informative. In the case of the MMSE, a poor score (lowest 25%) is specified as ≤24, a value which typically would be indicative of a diagnosis of dementia with high specificity. However, it seems extremely unlikely that there would be such a high prevalence of dementia in a working population aged 40-55 years, and the low scores may therefore reflect difficulties in understanding instructions correctly (e.g. because of reading or hearing difficulties), poor writing and drawing skills, or poor education and cultural background. On the other hand, poor literacy skills can be associated with better performance on the Stroop test, but the study found no effect on performance by nationality and poorer performance with lower levels of education.

120. These apparent anomalies and the absence of a clear exposure-response relationship limit the conclusions that can be drawn, but overall, the pattern of results is more suggestive of an effect on visual-motor performance than cognitive difficulties.

Conclusions

121. Since the last COT review in 1999, a substantial number of new studies have examined neuropsychological outcomes following exposures to organophosphate that have not resulted in recognised acute poisoning. Most have been small (fewer than 100 participants), and have relied on self-report of exposures rather than biological or environmental measurements, or other corroborative information. For some, insufficient details of the methods and statistical analyses were reported, and a number were at risk of selection bias through selective loss to follow-up or because the reference group was not ideally chosen. Important sources of measurement bias and/or confounding such as poor eyesight, poor hearing and lower premorbid intelligence were rarely taken into account. Studies by Kilburn (1999), Srivistava et al. (2000), Abdel Rasoul et al. (2008), Mackenzie Ross et al. (2010) and Malekirad et al. (2013), in which abnormalities were reported for most or all of the neuropsychological tests conducted, were all seriously flawed, principally in the methods by which participants were selected for study and by failure to address important potential for confounding.

122. The largest and best conducted investigations were based on the Agricultural Health Study (Starks et al. 2012a; Starks et al. 2012c). These found no consistent

associations between organophosphate exposures and neuropsychological outcomes on a range of tests. Performance in the digit-symbol substitution test and Sequences A was worse in men who had experienced unusually high pesticide exposure events that did not result in acute poisoning, but these exposures were not necessarily to organophosphates.

123. The French study by Blanc-Lapierre et al. (2013), which showed positive associations for multiple organophosphates and neuropsychological outcomes, incorporated a relatively strong assessment of exposure, but the abnormalities observed exhibited unusual features, and if anything suggest an effect on visual-motor performance.

124. When account is taken of the strengths and limitations of each study, there is no consistent evidence of effects of low level exposure to organophosphates on any specific aspect of cognitive function.

125. The reassurance that can be drawn from this absence of evidence is limited by (often unavoidable) weaknesses of even the strongest studies – for example in the retrospective assessment of exposures. A further problem has been the lack of well-established theoretical models by which to predict specific patterns of cognitive deficit that organophosphates might be expected to cause. Overall, however, it appears that if organophosphates do cause long-term neuropsychological impairment in the absence of overt poisoning, then the effects, at least in the large majority of cases, must be minor and subtle.

Magnetic resonance imaging

126. Magnetic Resonance Imaging (MRI) uses a strong static magnetic field and radiofrequency electromagnetic pulses to generate cross-sectional pictures of the body to an accuracy within 1 mm. It is currently the best method for structural imaging of the brain and spinal cord. Among other things, it allows visualisation of the grey matter (which contains most of the neuronal cell bodies) and white matter (which largely comprises myelinated axons).

127. MRI has been used in recent times to explore putative effects of organophosphates on brain structure, although studies have mostly related to acute poisoning and immediate hospital care. Two studies of the 1995 Tokyo subway sarin attack both indicated reduction in grey matter volume in exposed symptomatic subjects: Yamasue et al (2003) showed abnormality in the left anterior cingulate cortex, and Yamasue et al (2007) in the insular and temporal grey matter, in neighbouring temporal white matter, possibly secondary to the grey matter abnormality, and in the hippocampus (the hippocampus is a small part of the grey matter in the brain, with an important role in memory). Only two reports (Table 3) were identified on MRI findings in relation to low-level exposure to organophosphates in the absence of overt acute toxicity, both of which concern the potential exposures to sarin and cyclosarin that were investigated by McCauley et al. (2002) and Spencer (2001) (see paragraphs 40-42).

128. Chao et al. (2010) measured the white matter, grey matter, cerebrospinal fluid (CSF), intracranial and hippocampal volumes of US Gulf War veterans, potentially exposed to sarin and cyclosarin at low levels during the destruction of an Iraqi storage complex in 1991, and compared findings with those in unexposed veterans who were deployed in the same campaign. Subjects were drawn from 230 veterans who had participated in previous investigations of Gulf War Illness at a veterans' medical centre, selected from a database as potentially exposed according to plume modelling data, and from 190 veterans deemed not to have been exposed. The precise sampling method was not reported, but 40 from each group were selected, the second to match the first by age, sex, handedness, education and a score for post-traumatic stress disorder. Response rates are unclear, and MRI images were missing or inadequate for a few 'exposed' subjects. The 35 to 36 'exposed' subjects who were analysed had a lower volume of grey matter (-3.3%, p=0.007) and hippocampus (-6.5%, p=0.01) when compared with 40 unexposed controls, and these differences were even greater after adjustment for intracranial volume. However, analysis for linear trends showed no so significant exposure-response relationships. In this study the white matter volume was slightly and non-significantly greater in exposed subjects.

129. Heaton et al. (2007) reported a similar study. In this case, subjects were drawn from the Devens cohort – 2,249 deployed Gulf War veterans from New England who had been recruited into a linked study for more detailed work-up. At a third follow-up assessment, a subset of the cohort (N=220) was recruited by random sampling within strata of high and low symptom reporters, defined by a health symptom checklist administered during 1994-1996. Study subjects were a volunteer subset of this group, after exclusion of those with head injuries, loss of consciousness, substance abuse or major psychiatric illness. Response rates are unclear, but MRI findings were finally analysed on 13 potentially exposed veterans and 13 unexposed veterans (broadly following the exposure criteria in Chao et al., as described above). In a slight variation on Chao et al., assessment was made of grey matter, white matter, CSF, and right and left ventricular volumes. No statistically significant differences were found in adjusted volumes, although white matter volume declined per modelled unit of exposure to organophosphates (-4.64% (-4.79 to -4.49%) per unit, p<0.0001). In this study, the adjusted grey matter volume was non-significantly greater in exposed subjects.

Overview of evidence

130. Evidence regarding low-level exposure to organophosphates and long-term structural changes in the brain in the absence of acute poisoning is currently limited to just two reports. Both relate to short-term exposure in a combat environment, and in both, the actual exposures are unknown – only potential exposures were modelled. An advantage of the reports by Chao et al. and Heaton et al., however, is that assessments were conducted many years after the event, and so were focused on long-term outcomes.

131. Both studies were small. Over 5,000 personnel were potentially exposed to sarin or cyclosarin, according to the Operation Desert Storm Database (McCauley et al., 2002), but fewer than 40 were imaged by Chao et al. and only 13 by Heaton et

al. This limitation is understandable given the cost and effort of MRI imaging on the one hand and inevitable losses to follow-up on the other. However, questions arise regarding the representativeness of study groups, as sampling frames and response rates are unclear, and the final samples were a tiny selection of those potentially exposed. Unknown selection forces may have influenced findings. Moreover, sampling from among volunteers chosen because of high levels of post-deployment symptom-reporting is a further source of bias.

132. The results of the two studies were apparently inconsistent, Chao et al showing a loss of grey matter, Heaton et al a dose-dependent loss of white matter. However, the studies used different image analysis software to calculate grey/white matter volumes, and this methodological difference may explain the discrepancy, possibly through different labelling of grey and white matter for the same brain regions. In both studies, the combined volume of grey and white matter was lower by 2 to 3% in exposed subjects, and CSF volume non-significantly greater, suggesting a loss of total brain volume (no test of statistical significance was published for this analysis). On balance, the evidence base is insufficient to draw firm conclusions as to harmful effects, and if further research is undertaken in this area then there should be better harmonisation of methods.

Parkinson's disease and parkinsonism

133. Parkinson's disease results from degeneration of dopaminergic neurons in the substantia nigra (a part of the midbrain), which often is accompanied by the presence of protein-containing, eosinophilic inclusion bodies (Lewy bodies) in the neurons that survive. Clinical manifestations, which typically do not develop until the neuronal loss is advanced, include resting tremor, bradykinesia, rigidity and postural imbalance. The mechanisms leading to neuronal loss are not well understood, but mitochondrial dysfunction and oxidative stress are likely to have a role in the pathogenesis.

134. Diagnosis is normally based on the presence of typical symptoms and signs for which other causes can be excluded. Similar symptoms and signs (parkinsonism) can occur as a side-effect of certain drugs, from small vessel ischaemia, from occupational exposure to manganese, and following encephalitic infection. Ioflupane (^{123}I) (DaTscan ®) single-photon emission computed tomography (SPECT) is now considered the most accurate method by which to diagnose Parkinson's disease and related neurodegenerative conditions, as distinct from parkinsonism, but is generally used in only a minority of patients because of its cost and the exposure to radiation that it entails. It is a new technique, and was not used in any of the studies that are reviewed in this statement.

135. Estimates of the incidence and prevalence of Parkinson's disease depend on the exact diagnostic criteria applied, and on whether cases are ascertained through presentation to medical services or by systematic screening. However, in industrialised countries the disorder affects some 0.3% of the total population, and 1% of those above 60 years of age (de Lau and Breteler, 2006). Onset is rare before age 50, but incidence increases rapidly at older ages, and tends to be somewhat higher in men than in women.

136. Apart from sex and age, the best established risk factors are smoking and consumption of caffeine, both of which appear to protect against the disease (de Lau and Breteler, 2006; Wirdefeldt et al., 2011). In addition, there is strong evidence that its occurrence is influenced by genetic predisposition. Various specific genes have been implicated, but they appear to account for only a small proportion of the total disease burden (Wirdefeldt et al., 2011).

137. Parkinson's disease was not considered as a specific neurological outcome in the COT 1999 report. However, a body of evidence has since become available on its relation to organophosphates.

138. An important stimulus to this new research was the observation that a number of people developed acute parkinsonism following intravenous use of an illegal recreational drug contaminated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and that this was associated with selective damage to dopaminergic cells (de Lau and Breteler, 2006). This raised the possibility that other environmental toxicants, including various pesticides, might have a causal role.

139. A link with organophosphates is plausible insofar as several reports have described patients with parkinsonism following acute poisoning by organophosphates (Davis et al., 1978; Bhatt et al., 1999; Muller-Vahl et al., 1999; Shahar and Andraws 2001; Arima et al., 2003). Moreover, there is evidence from case-control studies that Parkinson's disease is associated with the M allele for the Leu54Met polymorphism of PON1 (Akhmedova et al., 2001; Carmine A et al., 2002; Zintzaras and Hadjigeorgiou, 2004), although the finding has not been entirely consistent (Carmine Belin A et al., 2012; Wingo et al., 2012).

140. Epidemiological data on the association of organophosphates with Parkinson's disease or parkinsonism are available from two cohort studies, seven case-control studies and two cross-sectional surveys (Table 4).

Cohort studies

141. In a study of chemical workers at a Dow factory in Michigan, USA, data were collected at baseline and one year later, from 53 chlorpyrifos manufacturers (mean duration of work in chlorpyrifos-exposed areas 9.72 years) and 60 controls (mean duration of work in chlorpyrifos-exposed areas 0.10 years) (Albers et al., 2004c). Biomonitoring of the metabolite, 3,5,6 trichloro-2-pyridinol (TCP) in urine indicated that levels of exposure to chlorpyrifos during the follow-up period were approximately 30% of the permissible limit for a working day (200 µg/m^3). Symptoms and signs were assessed by questionnaire and clinical examination, and no parkinsonian abnormality was detected at baseline or follow-up, either in exposed workers or the controls.

142. Limitations of this investigation include the possibility of healthy worker selection at baseline (workers with Parkinson's disease may tend to leave employment because of their illness), and the small size of the exposed cohort and

short duration of follow-up, which meant that there was low statistical power to detect unequivocal, serious health outcomes.

143. As part of the US Agricultural Health Study, baseline information was collected by self-administered questionnaires from 52,393 private pesticide applicators (84% of those eligible for inclusion) in Iowa and North Carolina, USA, along with 32,345 of their spouses (74% of those eligible) (Kamel et al., 2007a). Among other things, the questionnaires asked about pesticide usage, other relevant characteristics and exposures, and physician-diagnosed Parkinson's disease. Five years later, follow-up telephone interviews were completed by 57,251 (68%) of the original cohort, and physician-diagnosed Parkinson's disease was again ascertained. Prevalent Parkinson's disease was defined by report of the disorder at baseline (83 cases) and incident Parkinson's disease by a new report at follow-up (78 cases). More than 99% of applicators and 56% of their spouses had personally mixed or applied pesticides. Associations between Parkinson's disease and exposure to specific organophosphates (ever v never) were assessed by hierarchical logistic regression, with adjustment for age, state, and whether the subject was an applicator or spouse (effectively a surrogate for sex). Odds ratios ranged from 0.7 to 1.4 for incident Parkinson's disease, and from 0.8 to 1.3 for prevalent Parkinson's disease. None of the associations was statistically significant.

144. This study had a strong design, although healthy worker selection may have reduced the number of prevalent cases at baseline, and no account was taken of possible confounding by smoking or consumption of caffeine.

Case-control studies

145. A study carried out at nine neurology clinics in Germany compared 380 patients (251 men and 129 women) with a clinical diagnosis of Parkinson's disease (response rate 71%), 376 neighbourhood controls and 379 regional controls (Seidler et al., 1996). The controls were selected by contacting addresses according to defined algorithms, and were matched to the cases for sex and age. Information about possible risk factors was collected by standardised interviews, and their relation to Parkinson's disease was analysed by conditional logistic regression. After adjustment for smoking and education, the prevalence of reported exposure to organophosphates and/or carbamates was higher among the cases than among the neighbourhood and regional controls (ORs 1.8, 95% CI 0.9-3.3 and 2.5, 95%CI 1.3-4.6 respectively).

146. Apart from its failure to distinguish between exposure to organophosphates and carbamates, this study had several other limitations. The eligibility criteria for inclusion as a case were not clearly reported – for example, most were 65 years or younger, but four were aged 66-67, and five were recruited from neurology practices affiliated to the study clinics. The response rates among eligible controls are unclear. And most importantly, it is possible that risk estimates were inflated because cases recalled exposures more completely than controls. Thus, positive associations were observed with a wide range of environmental exposures including not only other pesticides, but also gases, vapours, solvents, and glues, paints and lacquers.

147. In another case-control study, 100 patients with Parkinson's disease who were aged 50 years or older and lived in a defined area of Texas, USA, were recruited from a neurology practice (Dhillon et al., 2008). They were compared with 84 controls selected from the same practice, who had no history of Parkinson's disease. Information about possible risk factors was collected at interview through a standardised questionnaire. Crude odds ratios for report of having personally used, mixed or applied chlorpyrifos, malathion and diazinon were 2.0 (95%CI 1.02-3.8), 1.3 (95%CI 0.7-2.4) and 0.8 (95%CI 0.4-1.4). However, because the study had major limitations, no useful conclusions can be drawn from these findings. The response rate among cases, although not stated explicitly, appears to have been low. No information is provided about the response rate for controls or their diagnostic mix. And no adjustment was made for potential confounding effects of sex, age or smoking.

148. In a study at Duke University Medical Center, North Carolina, USA, 319 patients with Parkinson's disease confirmed by a neurologist were compared with 296 unaffected relatives (Hancock et al., 2008). Application of pesticides was ascertained by telephone interviews (using an open question about specific products used), along with exposure to potential confounding factors. After adjustment for sex, age, smoking and consumption of caffeine, the odds ratio for ever having used an organophosphate relative to never having used any pesticide was 1.89 (95%CI 1.11-3.25). However, because of limitations of the study (which was designed primarily for genetic investigations), little can be drawn from this observation. The cases and controls were drawn from ill-defined and different source populations with unknown response rates. Moreover, with the method by which exposures were ascertained, there was major potential for recall bias that would tend to inflate risk estimates. In support of this, the odds ratio for exposure to any pesticide other than organophosphates was also significantly elevated (1.53, 95%CI 1.05-2.23).

149. Patients with Parkinson's disease confirmed by a neurologist (n=247, response rate 83%) were recruited from affiliates of an organisation providing health insurance to agricultural and related occupations in France, together with 676 controls, individually matched for sex, age and affiliation office (response rate 75%) (Elbaz et al., 2009). Exposures to pesticides before the onset of disease (for controls the onset of disease in the matched case) and other potential risk factors were ascertained through a combination of interviews, self-administered questionnaires, and farm visits. Analysis was by conditional logistic regression with multiple imputation for missing data. After adjustment for cigarette smoking and any cognitive impairment, there was a weak and non-significant association with exposure to organophosphates in men (OR 1.3, 95%CI 0.7-2.3) (corresponding results for women were not reported).

150. Gatto et al. (2009) studied 368 patients with incident (diagnosed in the past three years) Parkinson's disease confirmed by a neurologist, who were resident in three counties of California, USA, and 341 controls from the same population, who were selected from Medicare records and tax assessor housing unit maps. Information about residential history, type of water supply and other possible risk factors was collected by telephone interviews, and potential exposures to specific pesticides from consumption of well water were inferred from records of pesticides

that had been used within a 500 m distance of the places where participants had lived. After adjustment for sex, age, education, race and family history of Parkinson's disease, odds ratios for potential exposure (v non-exposure) to diazinon, dimethoate and chlorpyrifos in well water were 1.58 (95%CI 1.03-2.43), 1.41 (95%CI 0.94-2.11) and 1.45 (95%CI 0.94-2.24) respectively. While the use of archival data on pesticide applications was a strength of this study, the classification of exposures took no account of the movement of ground water, and therefore is of dubious validity. Also, no allowance was made for potentially higher exposures to the same compounds from their use in the participants' workplaces and homes.

151. A second report based on the same study indicated elevated risks in persons with the MM PON1-55 genotype and residence within 500 m of crops treated with diazinon (OR 2.23, 95%CI 0.89-5.62), and chlorpyrifos (OR 2.01, 95%CI 0.80-5.01), but not parathion (OR 0.94, 95%CI 0.38-2.35) (Manthripagada et al., 2010). These risk estimates were relative to persons with the LL or LM geneotype and no use of the pesticide near to their home. Exposures to diazinon and chlorpyrifos were highly correlated, and mutually adjusted risk estimates were not presented.

152. Another extension to the study, based on 287 Caucasian cases 440 controls, examined two additional functional polymorphisms of PON1 (C108T and Q192R), and took account also of occupational exposures to pesticides (Lee P-C et al., 2013). After adjustment for age, sex, smoking status, county of residence and educational level, Parkinson's disease was associated with ambient residential or workplace exposure to each of diazinon, chlorpyrifos and parathion, and more strongly in those with PON1 Q192R QQ genotype (odds ratios up to 1.95). However, there were weak associations with the same genotype even in people with no exposure to the organophosphates (ORs 1.13 to 1.14), and the statistical interaction between exposure and genotype was not formally assessed.

153. Such interactions were examined, however, in a fourth report from the study (Narayan et al., 2013). Statistically significant positive interactions were observed between PON1 Q192R QQ genotype and more frequent reported lifetime use of organophosphorus pesticides. However, the latter included glyphosate and glufosinate-ammonium, which are widely used herbicides that do not inhibit acetylcholinesterase. Also, the reliance on participants' recall of past exposures may have led to more complete ascertainment for cases than controls, although this would not be expected to have differed according to PON1 genotype.

154. Firestone et al. (2010) carried out a case-control study of Parkinson's disease among patients of a Group Health Cooperative in western Washington State, USA. A neurologist reviewed the medical records of the 404 cases to confirm their diagnosis, and the 526 controls were frequency matched to the cases by sex and age. Participation rates among eligible cases and controls were 70% and 60% respectively. Information about risk factors was elicited through structured interviews, which included questions about a checklist of toxicants. Analysis was by logistic regression, and adjusted for age, ethnicity and smoking. Among men (252 cases and 326 controls) there was a non-significant association with parathion (OR 5.8, 95%CI 0.66-50.79), but no association with malathion (OR 1.0, 95%CI 0.39-2.15) or diazinon (OR 0.8, 95%CI 0.30-2.15). Among women one case and no controls reported exposure for each of these compounds.

155. In a study carried out in two areas of British Columbia, Canada, patients likely to have Parkinson's disease were identified from prescription records, and those whose diagnoses were confirmed by neurological review completed a questionnaire at interview (Rugbjerg et al., 2011). The same questionnaire was administered to controls, who were group-matched to the cases for sex, age and geographical region, and were identified from medical insurance records that covered 97.5% of the population. The questionnaire asked about job histories, exposure to pesticides, and other possible risk factors. Completed questionnaires were reviewed by an occupational hygienist, blinded to the case/control status of the subject, who checked for possible unreported exposures (which were followed by a phone call to the participant), and classified the reported exposures as to whether or not they were likely to be above background. Analysis was based on 403 cases and 405 controls – estimated participation rates of 51% and 32% respectively. Five cases were considered to have exposure to organophosphates above background (OR 0.74, 95%CI 0.20-2.78, after adjustment for sex, age and smoking).

Cross-sectional surveys

156. A cross-sectional survey was carried out among a subset of 323 men from a cohort of 1300 pesticide-exposed workers and unexposed controls in Washington State, USA (Engel et al., 2001). The presence of parkinsonism was assessed by a nurse, who had been trained in neurological examination, using a standardised rating scale. Exposures, including to a checklist of pesticides, were ascertained through a self-administered questionnaire. Associations were summarised by prevalence ratios (PRs), adjusted for age and smoking habits. Parkinsonism, which was diagnosed in 65 (21.0%) of the 310 participants who gave a satisfactory exposure history, showed no association with reported exposure to organophosphates (PR 0.9, 95% CI 0.6-1.4). A limitation of this study was the incomplete participation of those originally recruited to the cohort, which raises the possibility that more severe cases were selectively missed (only one participant reported physician-diagnosed Parkinson's disease). Also, recall of exposures may have been incomplete, and if this occurred similarly in cases and non-cases, the effect will have been to obscure associations with disease.

157. As part of a study of agricultural workers in Brazil, Salvi et al. (2003) assessed 25 participants three months after they had last been exposed to organophosphates. Clinically significant parkinsonian symptoms, assessed by a standardised rating scale, were reported by ten subjects, which the authors considered to represent a clearly higher prevalence than would be expected in a healthy population of the same age. However, they did not study an unexposed control group, and the small size of the study sample limits the statistical confidence that can be placed in the finding.

Overview of evidence

158. Four of the reports reviewed have described significant positive associations of Parkinson's disease with organophosphates as a group (Hancock et al., 2008),

organophosphates and/or carbamates (Seidler et al., 1996), chlorpyrifos (Dhillon et al., 2008) or diazinon (Gatto et al., 2009). However, two of these investigations (Dhillon et al., 2008; Hancock et al., 2008) had major weaknesses. Moreover, the study by Seidler et al. (1996) was incompletely reported, and may have been subject to important inflationary recall bias, while that by Gatto et al. (2009) relied on a dubious index of potential exposure. In contrast, the study with the strongest design (Kamel et al., 2007a) provided no indication of a relation to any of the organophosphates. Nor were associations found in the better designed case-control studies (Elbaz et al., 2009; Firestone et al., 2010; Rugbjerg et al., 2011).

159. Several studies have indicated an association of Parkinson's disease with polymorphisms of the PON1 gene that might be expected to reduce the rate at which some organophosphate compounds are detoxified (Akhmedova et al., 2001; Carmine A et al., 2002; Zintzaras and Hadjigeorgiou, 2004; Manthripagada et al., 2010; Lee P-C et al., 2013; Narayan et al., 2013). However, findings have not been entirely consistent, and in some cases were observed in populations that would not be expected to have a high prevalence of exposure to cholinesterase-inhibiting organophosphates. This raises the possibility that the association with genotype might depend on mechanisms other than the metabolism of organophosphates. Only one report formally analysed statistical interactions between PON1 genotype and exposure to organophosphorus compounds (Narayan et al., 2013), but interpretation of the positive finding is complicated by inclusion of pesticides that do not inhibit cholinesterase within the definition of exposure.

160. The overall balance of evidence does not suggest an increased risk of Parkinson's disease from exposure to organophosphates that is insufficient to cause overt acute poisoning. However, because of the limited statistical power of the better studies that are available (which is reflected in the confidence intervals for their risk estimates), a small elevation of risk cannot be ruled out.

Dementia

161. Dementia refers to a progressive deterioration of cognitive functions, for which Alzheimer's disease is the most common cause. Alzheimer's disease is a neuorodegenerative disorder that is characterised histopathologically by the accumulation of neuritic plaques and neurofibrillary tangles in the brain. Other frequent causes of dementia include insufficiency of either large or small blood vessels in the brain (vascular dementia), fronto-temporal lobar degeneration and dementia with Lewy bodies.

162. Often it is difficult to distinguish with confidence between different causes of dementia, and many epidemiological studies therefore take all dementia as their outcome. This may be defined according to standardised criteria, based on history and clinical examination, including performance on a test of cognitive function such as the Modified Mini-Mental State Examination.

163. The prevalence of dementia rises steeply at older ages, and it tends to be higher in women than in men. Other established risk factors include genetic

predisposition (in particular the APOE ε4 allele confers increased susceptibility to Alzheimer's disease), smoking, hypertension and diabetes.

164. The COT report in 1999 did not consider the relation of organophosphates to dementia specifically. However, a link is biologically plausible given that organophosphates can cause microtubule derangements and tau hyperphosphorylation (Zaganas et al., 2013), which is a hallmark of Alzheimer's disease. Also, some studies have suggested an association of dementia with polymorphisms of the PON1 gene, although findings have not been entirely consistent (Zaganas et al., 2013). On the other hand, drugs which inhibit acetylcholinesterase have been used in the treatment of Alzheimer's disease.

165. A number of epidemiological studies have explored the association of dementia with pesticides in general, but only two provide estimates of risk for organophosphates specifically.

166. In an Australian case-control study, 170 patients (64 men and 106 women) with Alzheimer's disease clinically diagnosed at two hospitals in Sydney were compared with an equal number of controls, individually matched for sex and age, who were selected from the same or neighbouring general practices (Gun et al., 1997). Occupational histories for both cases and controls were obtained by interview of informants (mostly spouses or first degree relatives), and potential exposures were inferred by a panel of three occupational hygienists, blinded to the case/control status of the participant. Five cases were judged to have possible exposure to organophosphates (OR 2.54, 95%CI 0.41-27.06). Interpretation of this finding is limited not only by statistical uncertainty (reflected in the wide confidence interval), but also by the potential for inaccuracy in the classification of exposures from occupational histories.

167. Better information comes from a cohort study carried out in Utah, USA (Hayden et al., 2010). Residents of one county who were aged 65 years or older, were invited to complete a baseline questionnaire about possible risk factors for dementia, and to provide an occupational history, including any use of organophosphates. Assessment for Alzheimer's disease and dementia was also carried out at baseline, and again after 3, 7 and 10 years. This applied standardised criteria based on a screening questionnaire followed by full clinical evaluation where indicated. After exclusion of subjects with dementia at baseline, lost to follow-up before three years, and with missing data, analysis was based on 3084 individuals, of whom 500 developed dementia (344 with clinically diagnosed Alzheimer's disease) during follow-up. Following exclusion also of those exposed to other pesticides, and adjustment for sex, educational level, baseline Modified Mini-Mental State score and APOE ε4, Cox regression indicated modest associations of organophosphates with dementia overall (hazard ratio 1.31, 95%CI 0.96-1.78), and with Alzheimer's disease specifically (hazard ratio 1.53, 95%CI 1.05-2.23). Major strengths of this investigation were the ascertainment of exposures before the onset of disease, and the systematic method by which cases were ascertained and diagnosed. However, the association with organophosphates was not specific (similar levels of risk were observed for organochlorine insecticides), which raises the possibility of unrecognised confounding, perhaps related to agricultural work.

Overview of evidence

168. Together, these two studies point to a need for further research on the relation of dementia to organophosphates. However, because of the limitations that have been highlighted, they cannot be considered strongly suggestive of a hazard.

Psychiatric illness

169. Depression, anxiety and suicide are the main psychiatric outcomes that have been studied in relation to organophosphate exposure. A few studies have assessed symptoms of post-traumatic stress disorder following incidents in which people were acutely exposed to the cholinesterase-inhibiting nerve agent, sarin, in Japan (Ohtani et al., 2004; Tochigi et al., 2005) and during the Gulf War (McCauley et al., 2001). However, where illness occurred, it may have resulted from the psychological trauma associated with the incident, and cannot be ascribed with any confidence to a toxic effect of the organophosphate. There has also been some investigation of less specific patterns of neuropsychiatric symptoms, and these are described in the next section of this statement (paragraphs 189-207). No evidence was found linking organophosphates with psychoses such as schizophrenia or bipolar disorder.

Depression and anxiety

170. The symptoms of depression and anxiety are common, and include low mood, lack of enjoyment, altered sleep patterns, worry and weight change. The tenth revision of the International Classification of Diseases (ICD-10) sets out internationally agreed criteria for the diagnosis of depression and anxiety disorders (World Health Organisation, 1992), and in population-based studies, the two diagnoses are strongly associated. Furthermore they both occur in a continuum of severity. Thus, their prevalence depends critically upon the way in which symptoms are assessed and the thresholds of severity that are used to define symptoms. This may lead to substantial variation in quoted prevalence rates. In the UK Psychiatric Morbidity Surveys (McManus et al., 2009), the one-week prevalence of depression meeting ICD-10 criteria was approximately 3%, while 5-6% of participants had anxiety disorders. In addition, a further 6-7% had symptoms sufficient to be of clinical importance, although not meeting the internationally agreed diagnostic criteria.

171. In studies of people exposed to organophosphates, the assessment of depression and anxiety has been principally through self-administered questionnaires. Symptoms of depression and anxiety are particularly correlated when ascertained in this way, and the term, “common mental disorder” (CMD) is used to refer to a mixture of depressive and anxiety symptoms, not necessarily meeting diagnostic criteria but still indicating a clinically important degree of symptoms. There are no independent objective means of confirming CMD, and although it is regarded as good practice to compare self-administered questionnaires with longer more thorough assessments, administered either by clinicians or by lay

interviewers, these longer assessments still rely upon symptoms reported by the interviewee. As a consequence, there is potential for exaggeration, either consciously or unconsciously, which may be influenced by subjects' beliefs and expectations, or their eligibility for financial compensation because of illness.

172. Prevalence of CMD is higher in people with poorer socioeconomic status, and it is possible also that propensity to report symptoms is influenced by aspects of personality or awareness of exposures that might affect health. This potential for bias must therefore be taken into account when interpreting epidemiological findings on the relation between organophosphates and CMD.

173. At the time of the COT 1999 report, four studies provided information on the relation of CMDs to organophosphate exposures in the absence of overt acute toxicity. In a cross-sectional survey of 146 British farmers who had previously used organophosphates to dip sheep and a control group of 143 quarry workers, the farmers had an increased risk of vulnerability to psychiatric disorders as assessed by the General Health Questionnaire (Stephens et al., 1995). A study in California found no significant abnormality of mood in 45 men with a past history of documented cholinesterase inhibition but no frank toxicity (Ames et al., 1995). In Egypt, a cross-sectional comparison of formulators and applicators, who had been exposed to a wide range of pesticides, with controls from an urban textile factory indicated significantly higher frequencies of psychiatric disorders, assessed by the General Health Questionnaire, in the pesticide workers (Amr et al., 1997). In contrast, no abnormality was apparent in the emotional status of 57 licensed pesticide applicators in New Jersey, who were assessed by the Minnesota Multiphasic Personality Inventory (Fiedler et al., 1997), although this study was considered by COT to be methodologically weak.

174. Since 1999, ten further reports have addressed the association of low-level exposure to organophosphates with CMDs.

175. In Poland, Bazylewicz-Walczak et al. (1999) carried out assessments before and after a spraying season in 26 women who were exposed to organophosphates through work in greenhouses and 25 unexposed controls matched for sex, age, education and place of residence. Measurements during the spraying season indicated that exposures to organophosphates were low. Analyses of variance revealed a significant long-term excess of depression in the exposed group by one measure (the Profile of Mood States), but no increase in depression according to a second measure (the Finnish Subjective Symptoms Questionnaire).

176. Rehner (2000) carried out a survey of 115 households (response rate 76%) that had asked the Mississippi Environmental Health Department to investigate possible contamination by methyl parathion (which had been used indoors illegally), and for which some degree of contamination had been detected. The survey did not randomly select individuals from within the household, but either the head of household or spouse answered a questionnaire. Depressive symptoms were assessed using the Center for Epidemiological Studies-Depression Scale (CES-D). Within the sample there was no association between depression and the level of contamination as determined by an independent assay of surface wipes taken from the home (which was used to determine priorities for clean-up).

177. A cross-sectional survey in North Carolina, USA, compared 193 licensed pest control applicators who had been exposed to chlorpyrifos with two control groups comprising 106 friends of applicators and 83 blue-collar state employees (Steenland et al., 2000). Based on mood scales that form part of the Neurobehavioural Evaluation System, the exposed subjects had significantly more fatigue and tension than the controls, and significantly more depression than the non-exposed friends, but not in comparison with the state employees. However, it is difficult to draw conclusions from these differences because the two control groups were essentially volunteer samples, and therefore may not have provided an appropriate comparator for measures of mental health.

178. As part of a study focused mainly on neuropsychological outcomes, Stephens and Sreenivasan (2004) used the General Health Questionnaire to collect information from 37 male orchard sprayers in England who used chlorpyrifos (response rate 68%) and two referent groups, comprising 26 pig farmers and 41 construction workers. No significant increase in vulnerability to psychiatric disorder was observed in the orchard sprayers as compared with the referents, but all of the participants were in employment, and workers in the same jobs with more severe mental health problems may have left their jobs because of the illness.

179. In a cohort study of 53 workers employed in the manufacture of chlorpyrifos and 60 controls who worked in the manufacture of another chemical, levels of anxiety at baseline, assessed by the Brief Symptom Inventory, were similar in the two groups (p=0.93) (Albers et al., 2004c).

180. As part of the US Agricultural Health Study, Kamel et al. (2005) conducted a cross-sectional survey of 18,782 male licensed pesticide applicators in two states. This constituted 29% of the eligible population who had applied for a licence. After adjustment for age, state, education, smoking and use of alcohol, the prevalence of each of fatigue, tension, insomnia, irritability, depression, absent-mindedness and difficulty concentrating (ascertained through the Q16 questionnaire) was significantly elevated in participants with the highest estimated cumulative exposures to organophosphates (ORs 1.70 to 2.34). However, there was also a significantly increased prevalence of these symptoms among applicators with high cumulative exposure to organochlorines and, for most of the symptoms, to fumigants. Furthermore, these exposures were also associated with more frequent reporting of many other symptoms such as twitches in arms or legs, poor balance and poor night vision. The associations of symptoms with cumulative exposure to organophosphates and organochlorines persisted when they were mutually adjusted.

181. Using much the same study sample, Beseler et al. (2008) compared pesticide exposures in 534 men who reported a history of physician-diagnosed depression requiring medication or “shock therapy” and 17,051 controls with no past diagnosis of depression and no prominent symptoms of depression in the year before enrolment to the study. After adjustment for various potential confounders (the adjustment made little difference to risk estimates), diagnosed depression was significantly associated with ever use of organophosphates (OR1.78, 95%CI 1.27-2.50). However, positive associations were also observed with ever use of organochlorines (OR 1.32), fungicides (OR 1.24) and fumigants (OR 1.35), and non-

significantly with ever use of herbicides (OR 2.05). When risk estimates were mutually adjusted in a stepwise regression analysis, significant associations remained with organophosphates (OR 1.61) and organochlorines (OR 1.24). However, there was no significant dose-response relationship with cumulative days of using organophosphates. Limitations of this analysis include its reliance on physician diagnosis for case definition (only a small proportion of people with depression are diagnosed as depressed by a physician and access to health care may have differed in relation to exposure) and the possibility that in some cases the subject's depression preceded pesticide exposures.

182. Solomon et al. (2007) conducted a postal survey of men (N = 9844) living in three rural areas of England and Wales (response rate = 31%). Symptoms of CMD were ascertained using the HADS questionnaire, which has good agreement with longer methods of assessment. In comparison with men who had never worked with pesticides (N = 6109), and after adjustment for age, area of residence and tendency to somatise, there was no evidence that previous use of sheep dip (N = 1913) was associated with depression (prevalence ratio 1.1, 95%CI 0.9-1.3) or anxiety (prevalence ratio 1.0, 95%CI 0.9-1.2). Without adjustment for somatising tendency, prevalence ratios were slightly higher (1.2 and 1.1). The study did not attempt to ascertain exposure to organophosphates specifically, but a substantial proportion of the men who dipped sheep would be expected to have used organophosphates. Any tendency for higher response from men who had symptoms which they attributed to sheep dip would have led to inflated risk estimates, and should not have caused a true association to be missed.

183. Mackenzie Ross (2010) used the Hospital Anxiety and Depression Scale (HADS) to compare the prevalence of CMDs in 127 sheep farmers (67 working and 60 retired) who had used organophosphates for a minimum of five years before 1991, and 78 rural police workers (38 working and 40 retired). Significantly higher proportions of farmers had scores above the clinical cut-offs for depression (46.9% v 6.5%) and anxiety (41.5% v 22.1%). However, 19% of the working farmers, 68% of the retired farmers and all of the police workers were volunteers recruited by advertising. Moreover, police workers were excluded from the study if they had "a history of psychiatric ..... problems which might otherwise account for any cognitive or emotional problems identified", whereas such problems only led to exclusion of farmers if they had occurred before exposure (i.e. at least 20 years earlier). Because of these major weaknesses in the study design, and also the possibility of uncontrolled confounding, the study allows no meaningful conclusions about the relation of organophosphates to CMDs.

184. In a cross-sectional survey in Iran, Malekirad et al. (2013) assessed mental health by the General Health Questionnaire-28 (GHQ-28) in 187 horticultural farmers aged 16-80 years, and a sex- and age-matched sample of men and women from the same village who were not engaged in any agricultural work and had no history of occupational exposure to organophosphates. The extent of exposure to organophosphates in the farmers is unclear. Scores for severe depression were significantly higher in the farmers than controls (median 5.5. vs 3), but no attempt was made to adjust for possible confounding factors.

Suicide

185. If organophosphates caused severe depression, they might be associated with an increased risk of suicide. At the time of the COT 1999 report, two studies provided information that was relevant to this possibility. A case-control study nested within a cohort of Canadian farm operatives found no relation of suicide to area sprayed with insecticides, but did not look at exposure to organophosphates specifically (Pickett et al., 1998); and a case-control study of suicides among farmers in England and Wales showed no association with sheep farming (Hawton et al., 1998).

186. Since then, Beard et al. (2011) have analysed mortality from suicide during prospective follow-up of 81,988 members of the US Agricultural Health cohort (48,098 private pesticide applicators, 4,781 commercial applicators and 29,119 spouses of private applicators). A total of 110 suicides were recorded among cohort members, but after adjustment for age at enrolment, sex, number of children in family, frequency of alcohol consumption during past 12 months and smoking habits, there was no association with baseline report of exposure to organophosphate insecticides (26 deaths, hazard ratio 0.82, 95%CI 0.50-1.33).

Overview of evidence

187. Overall, there is no consistent evidence linking low-level exposure to organophosphates with CMDs. In the new research published since the last COT review, the strongest suspicion of a hazard comes from two studies based on the US Agricultural Health cohort (Kamel et al., 2005; Beseler et al., 2008). However, the associations with use of pesticides in these investigations were not entirely specific to organophosphates, raising the possibility of unrecognised bias or confounding. Three other positive studies (Steenland et al., 2000; Mackenzie Ross, 2010; Malekirad et al., 2013) may have importantly overestimated risks because of the ways in which participants were selected for study. Against this, four other investigations have found no relation between organophosphate exposure and measures of depression or anxiety (Rehner, 2000; Stephens and Sreenivasan, 2004; Albers et al., 2004c; Solomon et al., 2007), while a fifth was equivocal (Bazylewicz-Walczak et al., 1999).

188. The balance of evidence suggests that low-level exposure to organophosphates does not lead to an increased risk of suicide.

Other neuropsychiatric symptoms

189. As part of its consideration of psychiatric outcomes, the COT 1999 report noted that a syndrome of “chronic organophosphate induced neuropsychiatric disorder” (COPIND) had been proposed on the basis of observations in clinical case series. This was characterised by, among other things, personality changes, suicidal thoughts, cognitive impairment, language disorder, alcohol intolerance, heightened sense of smell, deterioration of handwriting and reduced exercise tolerance. The report also noted a survey of pesticide applicators in South Africa, in which there was

an excess of dizziness, sleepiness and headache in association with exposure to organophosphates (London et al., 1997).

190. Since 1999, further studies have generated information on the relation of organophosphates to neuropsychiatric symptoms other than those defining CMD and parkinsonism (although in some cases the symptoms investigated overlapped with those of CMDs).

191. In a longitudinal study in Poland (see paragraphs 92 and 175), analyses of variance indicated that central neurological symptoms and absent-mindedness were significantly more common in the long term, among female greenhouse workers exposed to low levels of organophosphates as compared with unexposed controls (Bazylewicz-Walczak et al., 1999). However, no significant association was found for a number of other neurological symptoms.

192. In North Carolina, USA, Steenland et al. (2000) carried out a cross-sectional survey of 193 licensed pest control applicators with exposure to chlorpyrifos and two control groups comprising 106 friends of applicators and 83 blue-collar state employees. Among other things, they used a self-administered questionnaire to collect information about experience of 24 neurological symptoms in the past month. The exposed subjects reported 16 of the symptoms significantly more often than the controls, including being tired, dizzy, confused, less able to remember, irritated and less coordinated, and having a loss of strength in the limbs, headaches, susceptibility to dizziness from chemicals, and lower tolerance of alcohol. The excess of symptoms was greater for previously exposed applicators than for those currently exposed. However, in general there was no higher risk with longer exposure to chlorpyrifos, and few significant differences from controls were found for more objective measures of neurological function (see paragraphs 55-57). Interpretation of the observed differences in symptoms is complicated by the potential for selection biases – the two control groups were essentially volunteer samples.

193. A cross-sectional study in India collected information from 59 workers employed in the manufacture of quinalphos and 17 controls unexposed to organophosphates, who were volunteers recruited from near the manufacturing plant (Srivastava et al., 2000). Although there were no differences between the two groups in acetylcholinesterase levels, the exposed group reported a significantly higher prevalence of weakness (20.7% v 0%). However, because no attempt was made to control for any potential confounding factors (it is of note that musculoskeletal disorders were also more frequent in the exposed group (33.9% v 11.7%)), no useful conclusions can be drawn from this observation.

194. In a cross-sectional survey of 612 people who had dipped sheep, 53 pig and chicken farmers who had not dipped sheep and 107 ceramics factory workers, the prevalence of autonomic symptoms such as sweating, fainting and impotence was higher in the sheep dippers (28.4%) than the ceramics workers (10.3%), but little different from that in the farmers who had not dipped sheep (Pilkington et al., 2001). The study was limited to participants who were currently in employment, and it is possible that rates of symptoms would have been higher among sheep dippers who had left work because of illness.

195. In a telephone survey of 1263 US Gulf War veterans (including 653 who may have sustained low level-exposure to sarin or cyclosarin when a chemical munitions bunker at Khamisiyah was destroyed) and 516 non-deployed veterans, carried out some nine years after the war, deployed veterans reported significantly higher rates of almost all of a long list of recent symptoms (McCauley et al., 2001). However, among the deployed veterans, the prevalence of symptoms was no higher in the potentially exposed than the non-exposed groups. Some symptoms were more frequent among 162 veterans who reported that they had been involved in or watched the destruction of the bunker, including tingling or burning sensations in the skin, changes in memory, difficulty sleeping and persistent fatigue, but these may have been provoked by anxiety about the perceived exposure rather than through toxic mechanisms.

196. Tahmaz et al. (2003) used two questionnaires to collect information about past exposure to organophosphates and symptoms of chronic fatigue from 63 subjects in the UK (mostly sheep farmers), who had reported ill-health associated with exposure to veterinary medicines (including sheep dip) to a Government reporting scheme during 1985-2001 (37% response rate). More than 85% of participants had high scores for symptoms of chronic fatigue, and the outcome was associated with higher estimated lifetime exposure to organophosphate pesticides. This survey suggests that symptoms of chronic fatigue are common among farmers who perceive illness that they attribute to their use of organophosphate sheep dips, but because of the way in which the study sample was recruited and the low response rate from those eligible to participate, no useful conclusions can be drawn about the risk of chronic fatigue from exposure to organophosphates.

197. Two studies in Egypt assessed the prevalence of neurological symptoms in adults (Farahat et al., 2003) and children (Abdel Rasoul et al., 2008) employed in the application of organophosphate pesticides to cotton crops as compared with unexposed controls. Both found that the exposed subjects had significantly more symptoms such as blurred vision, dizziness, numbness and fatigue. However, because the surveys were carried out during the spraying season, it is possible that the symptoms were a short-term effect of recent exposure. In both studies, serum cholinesterase activities were significantly lower in the exposed groups than in the controls.

198. Following an episode in which methyl parathion had been illegally sprayed in homes in Mississippi, Cox et al. (2005) carried out a survey of exposed individuals from homes that had been found to have high levels of contamination (>150 µg/100 cm^2 in wipe samples) and controls from homes in which the contamination was low (< 15 µg/100 cm^2 in wipe samples). Analysis was based on 121 exposed persons (response rate 86.4%) from 49 homes and 170 controls (response rate 86.3%) from 56 homes. Physician interviews were used to assess a long list of symptoms and reported diagnoses (the physicians were blind to the participants' exposure status, although the participants themselves were aware of their exposure level). The mean number of neuropsychiatric symptoms was not significantly higher in the exposed group (1.8) than in the controls (1.4). However, in a subgroup analysis, symptoms of weakness, insomnia, headache, depression and lack of energy were significantly

more common in subjects whose homes had the highest levels of contamination by methyl parathion (>260 µg/100 cm^2 in wipe samples).

199. Using cross-sectional data from the US Agricultural Health Study on 18,782 licensed pesticide applicators, Kamel et al. (2005) analysed the association of symptoms, ascertained using the Q16 questionnaire, with estimated cumulative exposure to various classes of pesticide. The 16 symptoms covered a wide range of neuropsychiatric complaints. Both number of symptoms (treated as a continuous variable) and report of 10 or more symptoms (a dichotomous variable) were significantly associated with cumulative exposure to organophosphates, but a similar relationship was observed with cumulative exposure to organochlorine insecticides, and rather more weakly with exposure to carbamates and pyrethroids. When use of the four categories of insecticide was considered simultaneously in mutually adjusted regression models, the associations with organophosphates, organochlorines and carbamates persisted. Each of the 16 symptoms was more common in men with the highest cumulative exposures to organophosphates and to organochlorines. Further analysis based on the same study (Kamel et al. 2007b) indicated that the elevation of risk applied similarly to each of five groupings of symptoms (affect, cognition, autonomic, motor and vision), and that it persisted after exclusion of applicators who had at some time experienced doctor-diagnosed pesticide poisoning or high exposure events.

200. In a postal survey of 9844 men from three rural areas of England and Wales (response rate = 31%), Solomon et al. (2007) collected information by postal questionnaire about the occurrence during the past month of each of seven symptoms that had been linked with COPIND (difficulty concentrating, difficulty remembering things, difficulty with handwriting, difficulty speaking, sensitivity to certain smells, increased sensitivity to the effects of alcohol, and tiredness and lack of energy). In comparison with men who had never worked with pesticides (N = 6109), those with occupational exposure to sheep dip (N =1913) had a higher prevalence of each symptom. However, symptoms were also more frequent in men who had worked with other pesticides but not with sheep dip or insecticides. Moreover, while the symptoms tended to cluster together much more frequently than would be expected by chance, there was no indication that this clustering was specific to work with sheep dip or insecticides. Associations of symptoms with COPIND symptoms tended to be highest in men with a tendency to somatise. The questionnaire also asked about past consultation with a general practitioner because of four categories of health problem that formed part of the postulated syndrome of COPIND (change in personality, difficulty with speaking, difficulty with handwriting and thoughts about self-harm or suicide). Lifetime prevalence was marginally higher among men who had worked with sheep dip than in those who had never worked with pesticides, but consultations because of thoughts about self-harm or suicide were even more frequent in men who had worked with other pesticides but not with sheep dip or insecticides. The low response rate was a limitation of this study, although it is unlikely to have importantly biased associations with different classes of pesticide differentially. Also, no attempt was made to ascertain exposure to specific pesticide products because it was considered unlikely that they would be reliably recalled. However, organophosphates had been widely used as sheep dips in the UK, and it seems likely that most men who reported dipping sheep would have been exposed to one or more organophosphates.

201. Chao et al (2010) assessed various indices of brain structure and function in 40 US Gulf War veterans who were considered potentially exposed to low levels of the nerve agents, sarin and cyclosarin, when a munitions dump at Khamisiyah was destroyed, and a control group of 40 unexposed veterans. No significant difference was found in the prevalence of chronic multi-symptom illness, but because of the small sample size and matching of controls to the exposed group for symptoms of post-traumatic distress disorder, little can be drawn from this non-positive finding.

202. Cherry et al. (2002) investigated paraoxonase (PON1) polymorphisms and diazinoxonase activities in a study of 175 farmers with ill-health that they attributed to sheep dip (cases) and 234 referent farmers who had also dipped sheep but were thought to be in good health. The cases were recruited through radio and newspaper advertisements, and the referents were nominated by the cases (up to three per case). Among those eligible for study, participation rates were high (98% for cases and 90% for referents). The detailed nature of the illnesses experienced by the cases was not described, although a later report indicated a wide range of complaints that were more common among the cases (Povey et al., 2005). Two thirds of participants (115 cases and 152 referents) reported that they had at some time used sheep dips containing diazinon, 88 had used other organophosphates but not diazinon, and 54 could not recall exactly which sheep dips they had used. Cases had a higher prevalence of RR or QR as compared with QQ at position 192 (OR 2.25, 95%CI 1.49-3.42), of LL as compared with LM or MM at position 55 (OR 1.92, 95%CI 1.26-2.93), and of diazinonoxonase activity below the median (OR 1.77, 95%CI 1.12-2.67). However, there was no clear elevation of risk for RR at position 192 as compared with QR or QQ (OR 1.11, 95%CI 0.50-2.44). When analysis was restricted to the cases and referents who had used diazinon, the odds ratio for QR or RR at position 192 was similar (2.39), whereas that for LL at position 55 was somewhat higher (3.16) (Mackness et al., 2003; Povey et al., 2007). No risk estimates were reported for the subset of participants who had worked only with organophosphates other than diazinon. An R allele at position 192 was also associated with report of acute flu-like symptoms immediately after dipping sheep (Cherry et al., 2011).

203. As part of a larger cross-sectional survey, Lee et al. (2003) compared PON1 genotypes in 82 South African farm workers, according to whether or not they reported at least two of 12 symptoms – chronic abdominal pain, nausea, rhinorrhoea, dizziness, headache, somnolence, fatigue, gait disturbance, limb numbness, paraesthesiae, limb pain or limb weakness. Only a small minority of participants had a history of acute pesticide poisoning. Report of two or more symptoms was more common in subjects with Gln/Gln (QQ) or Gln/Arg (QR) at position 192. However, this applied not only in pesticide applicators, but also in non-applicators. It is unclear to which pesticides the applicators were exposed

204. In a cross-sectional survey in India, 239 pesticide sprayers in India, who had used various pesticides including several organophosphates, were compared with 110 controls of similar socio-economic status, who were not occupationally exposed to pesticides (Fareed et al., 2012). The prevalence of various types of ocular symptom (blurred vision, lacrimation, pain in the eyes, red swollen eyes and irritation of the eyes) was higher in the exposed group. However, these symptoms occurred

mostly during spraying, and were not necessarily linked to organophosphates specifically.

Overview of evidence

205. Although many of the studies that have been reviewed in this section have important limitations, collectively they suggest that there is an excess of multiple neuropsychiatric symptoms in people who have been exposed to organophosphates at levels insufficient to cause overt acute poisoning. Particularly telling are the papers by Kamel et al. (2005 and 2007b) and Solomon et al. (2007). At the same time, Solomon's findings argue against a specific syndrome of COPIND. More difficult to determine is whether the observed excess of symptoms is a consequence of chemical toxicity or occurs through psychological mechanisms. Psychologically mediated nocebo effects have been demonstrated in relation to other environmental exposures perceived as hazardous, such as electromagnetic fields (Rubin et al., 2010), and it is possible that people who are aware of having been exposed to potentially toxic chemicals are more inclined to notice and report symptoms. This might explain the lack of specificity of associations in the studies by Kamel and Solomon. Thus, in Kamel's investigations, the same symptoms were independently associated with organochlorine insecticides, and in Solomon's survey, an excess of symptoms was observed also in men who had worked with other pesticides but not with sheep dip or insecticides. On the other hand, in both studies, the strongest associations were with organophosphates.

206. If the excess of symptoms occurred through toxic mechanisms, it might be expected that for a given level of exposure, they would occur with higher prevalence among people with lower metabolic capacity to eliminate active organophosphate moieties. With this in mind, several investigators have explored the relation of symptoms to PON1 genotype and/or activity. Both Cherry et al. (2002) and Lee et al. (2003) reported positive associations, but the patterns were inconsistent. Thus, Cherry et al. found an association with an R allele at position 192, although not with RR as compared with QR or Q, while Lee et al. found that symptoms were more common in pesticide applicators with the Q allele at position 192. This might be because the relative activity of the enzyme by genotype differs for different organophosphate substrates. However, if the observed associations reflected greater susceptibility to toxic effects of organophosphates, they would be expected to occur only in people with exposure to organophosphates. Cherry and colleagues did not assess associations with symptoms in people who were unexposed to organophosphates, but in the study by Lee et al., the association extended to subjects who were not pesticide applicators. Lower paraoxonase activity has been linked with symptoms of anxiety in the general population (Sklan et al., 2004; Bulut et al., 2013), suggesting that the association with symptoms may occur through mechanisms that do not involve organophosphates.

207. Because of this uncertainty about the mechanisms of illness, greater weight should be given to the findings on more objective neurological outcomes that are described elsewhere in this report.

Overall conclusions

208. Since the last COT report in 1999, substantial new evidence has accumulated on the relation between low-level exposure to organophosphates, insufficient to cause recognised acute poisoning, and a range of neurological, neuropsychological and neuropsychiatric outcomes.

209. The current balance of evidence suggests that there is no long-term risk of clearly demonstrable peripheral neuropathy from exposure to organophosphates that does not cause overt acute poisoning, a conclusion that has strengthened with the passage of time.

210. There is uncertainty as to whether long-term low level exposure to organophosphates causes detectable impairment of sensory thresholds, but if there is an effect then it is likely to be small.

211. Studies on evoked somatosensory potentials and EMG have been few in number and limited in quality. Overall, they do not suggest a hazard, but the evidence base is slim.

212. Few studies have looked at EEG or auditory/visual ERP outcomes in relation to organophosphate exposures insufficient to cause overt acute poisoning. The evidence that is available provides little indication of adverse effects. One study has suggested impairment in the processing of auditory information, but without independent replication, little can be drawn from this isolated finding.

213. There is no consistent evidence that low-level exposure to organophosphates has adverse effects on any specific aspect of cognitive function. If organophosphates do cause long-term neuropsychological impairment in the absence of overt poisoning, then the effects, at least in the large majority of cases, must be minor and subtle.

214. Evidence on whether low-level exposure to organophosphates can cause long-term structural changes in the brain in the absence of acute poisoning is insufficient for any firm conclusions.

215. The overall balance of evidence suggests no increased risk of Parkinson's disease from exposure to organophosphates that is insufficient to cause overt acute poisoning, although a small elevation of risk cannot be ruled out.

216. Findings from the two studies on the relation of dementia to organophosphates are not strongly suggestive of a hazard, but point to a need for further research.

217. Overall, there is no consistent evidence linking low-level exposure to organophosphates with common mental disorders (depression and anxiety). The balance of evidence suggests that low-level exposure to organophosphates does not lead to an increased risk of suicide.

218. Despite limitations of individual studies, current evidence suggests that there is an excess of multiple neuropsychiatric symptoms in people who have been exposed to organophosphates at levels insufficient to cause overt acute poisoning. However, it does not support the existence of a specific syndrome of “chronic organophosphate-induced neuropsychiatric disorder (COPIND)”, as has previously been hypothesised. It is unclear whether the observed excess of symptoms is a consequence of chemical toxicity or occurs through psychological mechanisms, and it is possible that people who are aware of having been exposed to potentially toxic chemicals are more inclined to notice and report symptoms. Studies on the relationship of symptoms to polymorphisms and activity of the enzyme, paraoxonase (PON1), have not clearly established a causal link with poorer capacity to detoxify organophosphate compounds, as might be expected if the illness were a consequence of toxicity. Because of this uncertainty about the mechanisms of illness, greater weight should be given to the findings on more objective neurological outcomes.

219. Collectively, the evidence reviewed is reassuring. It suggests that exposures to cholinesterase-inhibiting organophosphates that are insufficient to cause overt acute poisoning do not cause important long-term neurological toxicity in adults, and that if toxic effects on the nervous system do occur then they are minor and subtle. Moreover, it provides no basis for use of a generic outcome other than inhibition of acetylcholinesterase in regulatory risk assessment for organophosphate insecticides and medicines.

Recommendations for further research

220. The most important gap in the current evidence base concerns the relation between low-level exposure to organophosphates and later dementia. An association of such exposure with dementia has some biological plausibility, and has been suggested (although not strongly) by the two studies that have looked at the question to date. Moreover, dementia is a major and growing public health problem, and even a small increase in relative risk could have important implications at a population level. However, further research on this should be conducted only through studies with adequate rigour and statistical power. The method of choice would probably be a large prospective cohort investigation, such as the US Agricultural Health Study, with reliable assessment of exposures that does not depend on recall after the onset of disease, and systematic ascertainment of cases with severe and disabling illness. An investigation of this type might also include the use of MRI to assess brain volumes by standardised methods.

221. Given the evidence that is now available, it seems unlikely that further research on neurophysiological, neuropsychological and psychiatric outcomes would identify any important hazard from low-level exposure to organophosphates. Research to explore the possibility of more subtle, minor effects would require rigorous assessment of exposure, and better methods for assessment of the health outcomes than are currently available.

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References

Abdel Rasoul, G.M., Abou Salem, M.E., Mechael, A.A., Hendy, O.M., Rohlman, D.S. and Ismail, A.A. (2008) Effects of occupational pesticide exposure on children applying pesticides. *Neurotoxicology* **29**, 833-8.

Akhmedova SN, Yakimovsky AK, Schwartz EI. (2001) Paraoxonase 1 Met-Leu 54 polymorphism is associated with Parkinson's disease. *J Neurol Scie* **184**, 179-82.

Albers, J.W., Garabrant, D.H., Schweitzer, S.J., Garrison, R.P. Richardson, R.J. and Berent, S. (2004a) The effects of occupational exposure to chlorpyrifos on the peripheral nervous system function: a prospective cohort study. *Occup Environ Med* **61**, 201-11.

Albers, J.W., Garabrant, D.H., Schweitzer, S., Garrison, R.P., Richardson, R.J. and Berent, S. (2004b) Absence of sensory neuropathy among workers with occupational exposure to chlorpyrifos. *Muscle Nerve* **29**, 677-86.

Albers, J.W., Berent, S., Garabrant, D.H., Giordani, B., Schweitzer, S.J., Garrison, R.P. and Richardson, R.J. (2004c) The effects of occupational exposure to chlorpyrifos on the neurologic examination of central nervous system function: a prospective cohort study. *J Occup Environ Med* **46**, 367-78.

Albers, J.W., Garabrant, D.H., Mattsson, J.L., Burns, C.J., Cohen, S.S., Sima, C., Garrison, R.P., Richardson, R.J. and Berent, S. (2007) Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. *Toxicol Sci* **97**, 196-204.

Ames, R.G., Steenland, K., Jenkins, B., Chrislip, D. and Russo, J. (1995) Chronic neurologic sequelae to cholinesterase inhibition among agricultural pesticide applicators. *Arch Environ Health* **50**, 440-4.

Amr, M.M., Halim, Z.S. and Moussa, S.S. (1997) Psychiatric disorders among Egyptian pesticide applicators and formulators. *Environ Res* **73**, 193-9.

Arima, H., Sobue, K., So, M., Morishima, T., Ando, H. and Katsuya, H. (2003) Transient and reversible parkinsonism after acute organophosphate poisoning. *J Toxicol Clin Toxicol* **41**, 67-70.

Bayrami, M., Hashemi, T., Malekirad, A.A., Ashayeri, H., Faraji, F. and Abdollahi, M. (2012) Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. *Toxicol Ind Health* **28**, 90-6.

Bazylewicz-Walczak, B., Majczakowa, W. and Szymczak, M. (1999) Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. *Neurotoxicology* **20**, 819-26.

Beard, J.D., Umbach, D.M., Hoppin, J.A., Richards, M., Alavanja, M.C., Blair, A., Sandler, D.P. and Kamel, F. (2011) Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. *Environ Health Perspect* **119**, 1610-5.

Beseler, C.L., Stallones, L., Hoppin, J.A., Alavanja, M.C., Blair, A., Keefe, T. and Kamel, F. (2008) Depression and pesticide exposures among private pesticide applicators enrolled in the Agricultural Health Study. *Environ Health Perspect* **116**, 1713-9.

Bhatt, M.H., Elias, M.A. and Mankodi, A.K. (1999) Acute and reversible parkinsonism due to organophosphate pesticide intoxication: five cases. *Neurology* **52**, 1467-71.

Blanc-Lapierre A, Bouvier G, Gruber A. Leffonfré K, Lebailly P, Fabrigoule C, Baldi I. (2013) Cognitive disorders and occupational exposure to organophosphates: results from the PHYTONER study. *Am J Epidemiol* **177**, 1086-96.

Browne, R.O., Moyal-Segal, L.B., Zumsteg, D., David, Y., Kofman, O., Berger, A., Soreq, H. and Friedman, A. (2006) Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. *FASEB J* **20**, 1733-5.

Bulut M, Selek S, Bez Y, Karababa IF, Kaya MC, Gunes M et al. (2013) Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalised anxiety disorder. *J Affective Disorders* **150**, 829-33.

Carmine A, Buervenich S, Sydow O, Anvret M, Olson L. (2002) Further evidence for an association of the paraoxonase 1 (PON1) Met-54 allele with Parkinson's disease. *Movement Disorders* **17**, 764-6.

Carmine Belin A, Ran C, Anvret A, Paddock S, Westerlund M, Håkansson A et al. (2012) Association of protective paraoxonase 1 (PON1) polymorphism in Parkinson's disease. *Neuroscience Letters* **522**, 30-35.

Chao, L.L., Rothlind, J.C., Cardenas, V.A., Meyerhoff, D.J. and Weiner, M.W. (2010) Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology* **31**, 493-501.

Cherry, N., Mackness, M., Durrington, P., Povey, A., Dippnall, M., Smith, T. and Mackness, B. (2002) Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. *Lancet* **359**, 763-4.

Cherry, N., Mackness, M., Mackness, B., Dippnall, M. and Povey, A. (2011) 'Dippers' flu' and its relationship to PON1 polymorphisms. *Occup Environ Med* **68**, 211-7.

Cole DC, Carpio F, Julian J, Léon N. (1998) Assessment of peripheral nerve function in an Ecuadorian rural population exposed to pesticides. *J Toxicol Environ Health, Part A* **55,** 77-91.

Cox, R.D., Kolb, J.C., Galli, R.L., Carlton, F.R. and Cook, A.M. (2005) Evaluation of potential adverse health effects resulting from chronic domestic exposure to the organophosphate insecticide methyl parathion. *Clin Toxicol (Phila)* **43**, 243-53.

Daniell W, Barnhart S, Demers P, Costa LG, Eaton DL, Miller M, Rosenstock L. (1992) Neurpsychological performance among agricultural pesticide applicators. *Environ Res* **59**, 217-28.

Dassanayake, T., Gawarammana, I.B., Weerasinghe, V., Dissanayake, P.S., Pragaash, S., Dawson, A. and Senanayake, N. (2009) Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. *Clin Neurophysiol* **120**, 1693-8.

Davis KL, Yesavage JA, Berger PA. (1978) Single case study. Possible organophosphate-induced parkinsonism. *J Nerv Ment Dis* **166**, 222-5.

de Lau LML, Breteler MMB. (2006) Epidemiology of Parkinson's disease. *Lancet Neurol* **5**, 525-35.

Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, Shepherd S. (2008) Pesticide/environmental exposures and Parkinson's disease in East Texas. *J Agromed* **13**, 37-48.

Elbaz, A., Clavel, J., Rathouz, P.J., Moisan, F., Galanaud, J.P., Delemotte, B., Alperovitch, A. and Tzourio, C. (2009) Professional exposure to pesticides and Parkinson disease. *Ann Neurol* **66**, 494-504.

Engel, L.S., Keifer, M.C., Checkoway, H., Robinson, L.R. and Vaughan, T.L. (1998) Neurophysiological function in farm workers exposed to organophosphate pesticides. *Arch Environ Health* **53**, 7-14.

Engel, L.S., Checkoway, H., Keifer, M.C., Seixas, N.S., Longstreth, W.T. Jr, Scott, K.C., Hudnell, K., Anger, W.K. and Camicioli, R. (2001) Parkinsonism and occupational exposure to pesticides. *Occup Environ Med* **58**, 582-9.

Farahat, T.M., Abdelrasoul, G.M., Amr, M.M., Shebl, M.M., Farahat, F.M. and Anger, W.K. (2003) Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides. *Occup Environ Med* **60**, 279-86.

Fareed M, Kesavachandran CN, Pathak MJ, Bihari V, Kuddus M, Srivastava AK. (2012) Visual disturbances with cholinesterase depletion dues to exposure of agricultural pesticides among farm workers. *Toxicol Environ Chemistry* **94**, 1601-9.

Fiedler, N., Kipen, H., Kelly-McNeil, K. and Fenske, R. (1997) Long-term use of organophosphates and neuropsychological performance. *Am J Ind Med* **32**, 487-96.

Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Checkoway H. (2010) Occupational factors and risk of Parkinson's disease: A population-based case-control study. *Am J Indust Med* **53**, 217-23.

Gatto, N.M., Cockburn, M., Bronstein, J., Manthripragada, A.D. and Ritz, B. (2009) Well-water consumption and Parkinson's disease in rural California. *Environ Health Perspect* **117**, 1912-8.

Gomes J, Lloyd O, Revitt MD, Basha M. (1998) Morbidity among farm workers in a desert country in relation to long-term exposure to pesticides. *Scand J Work Environ Health* **24**, 213-9

Gun RT, Korten AE, Jorm AF, Henderson AS, Broe GA, Creasey H, McCusker E, Mylvaganam A. (1997) Occupational risk factors for Alzheimer disease: a case-control study. *Alzheimer Disease and Associated Disorders* **11**, 21-7.

Hancock, D.B., Martin, E.R., Mayhew, G.M., Stajich, J.M., Jewett, R., Stacy, M.A., Scott, B.L., Vance, J.M. and Scott, W.K. (2008) Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. *BMC Neurol* **8**, 6

Hawton, K., Simkin, S., Malmberg, A., Fagg, J. and Harriss, L. (1998) *Suicide and stress in farmers*, edn. London: TSO.

Hayden, K.M., Norton, M.C., Darcey, D., Ostbye, T., Zandi, P.P., Breitner, J.C. and Welsh-Bohmer, K.A. (2010) Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. *Neurology* **74**, 1524-30.

Heaton, K.J., Palumbo, C.L., Proctor, S.P., Killiany, R.J., Yurgelun-Todd, D.A. and White,

R.F. (2007) Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology* **28**, 761-9.

Jager KW, Roberts DV, Wilson A. (1970) Neuromuscular function in pesticide workers. *Br J Ind Med* **27,** 273-8.

Jamal, G.A., Hansen, S., Apartopoulos, F., Peden, A., Abdul-Aziz, M. and Ballantyne, J.P. (2001) Peripheral nerve dysfunction in farmers using organophosphate sheep dip. *J Nutr Environ Med* **11**, 9-22.

Jamal, G.A., Hansen, S., Pilkington, A., Buchanan, D., Gillham, R.A., Abdel-Azis, M., Julu, P.O., Al-Rawas, S.F., Hurley, F. and Ballantyne, J.P. (2002) A clinical neurological, neurophysiological, and neuropsychological study of sheep farmers and dippers exposed to organophosphate pesticides. *Occup Environ Med* **59**, 434-41.

Kamel, F., Engel, L.S., Gladen, B.C., Hoppin, J.A., Alavanja, M.C. and Sandler, D.P. (2005) Neurologic symptoms in licensed private pesticide applicators in the agricultural health study. *Environ Health Perspect* **113** , 877-82.

Kamel, F., Tanner, C., Umbach, D., Hoppin, J., Alavanja, M., Blair, A., Comyns, K., Goldman, S., Korell, M., Langston, J., Ross, G. and Sandler, D. (2007a) Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J Epidemiol* **165**, 364-74.

Kamel, F., Engel, L.S., Gladen, B.C., Hoppin, J.A., Alavanja, M.C. and Sandler, D.P. (2007b) Neurologic symptoms in licensed pesticide applicators in the Agricultural Health Study. *Hum Exp Toxicol* **26**, 243-50.

Keifer, M.C., Rosenstock, L., Checkoway, H., Fenske, R. and Robinson, L. (2000) Neurological Effects of Organophosphates on Farm Workers. *Govt Reports Announcements & Index* **02**,

Kidd, J.G., Langworthy, D.R. (1933) Jake paralysis. *Bull Johns Hopkins Hosp* **52**, 39-64.

Kilburn, K.H., Thornton, J.C., and Hanscom, B. (1998). Population-based prediction equations for neurobehavioural tests. *Arch Environ Res* **53**, 257-63.

Kilburn, K.H. (1999) Evidence for chronic neurobehavioral impairment from chlorpyrifos an organophosphate insecticide (Dursban) used indoors. *Environ Epidemiol Toxicol* **1**, 153-162.

Kimura, K., Yokoyama, K., Sato, H., Nordin, R.B., Naing, L., Kimura, S., Okabe, S., Maeno, T., Kobayashi, Y., Kitamura, F. and Araki, S. (2005) Effects of pesticides on the peripheral and central nervous system in tobacco farmers in Malaysia: studies on peripheral nerve conduction, brain-evoked potentials and computerized posturography. *Ind Health* **43**, 285-94.

Lee, B.W., London, L., Paulauskis, J., Myers, J. and Christiani, D.C. (2003) Association between human paraoxonase gene polymorphism and chronic symptoms in pesticide-exposed workers. *J Occup Environ Med* **45**, 118-22.

Lee P-C, Rhodes S, Sinsheimer JS, Bronstein J, Ritz B. (2013) Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. *Environment International* **56**, 42-7.

London L, Myers JE, Nell V, Taylor T, Thompson ML. (1997) An investigation into neurologic and neurobehavioral effects of long-term agrichemical use among deciduous fruit farm workers in the Western Cape, South Africa. *Environ Res* **73**, 132-45.

Macharia M, Hassan MS, Blackhurst D, Erasmus RT, Matsha TE. (2012) The growing importance of PON1 in cardiovascular health: a review. *J Cardiovasc Med* **13**, 443-53.

Mackenzie Ross, S.J., Brewin, C.R., Curran, H.V., Furlong, C.E., Abraham-Smith, K.M. and Harrison, V. (2010) Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. *Neurotoxicol Teratol* **32**, 452-9.

Mackness, B., Durrington, P., Povey, A., Thomson, S., Dippnall, M., Mackness, M., Smith, T. and Cherry, N. (2003) Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. *Pharmacogenetics* **13**, 81-8.

Maizlish N, Schenker M, Weisskopf C, Seiber J, Samuels S. (1987) A behavioural evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. *Am J Ind Med* **12**, 153-72.

Malekirad, A. A., Faghih, M., Mirabdollahi, M., Kiani, M., Fathi, A., & Abdollahi, M. (2013). Neurocognitive, mental health, and glucose disorders in farmers exposed to organophosphorus pesticides. *Archives of Industrial Hygiene and Toxicology,* ***64***, 1-8.

Manthripragada, A.D., Costello, S., Cockburn, M.G., Bronstein, J.M. and Ritz, B. (2010) Paraoxonase 1, agricultural organophosphate exposure, and Parkinson disease. *Epidemiology* **21**, 87-94.

McCauley, L.A., Rischitelli, G., Lambert, W.E., Lasarev, M., Sticker, D.L. and Spencer, P.S. (2001) Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. *Int J Occup Environ Health* **7**, 79-89.

McCauley, L.A., Lasarev, M., Sticker, D., Rischitelli, D.G. and Spencer, P.S. (2002) Illness experience of Gulf War veterans possibly exposed to chemical warfare agents. *Am J Prev Med* **23**, 200-6.

McManus, S., Meltzer, H., Brugha, T., Bebbington, P. Jenkins, R. (2009) Adult psychiatric morbidity in England 2007: results of a household survey. Leeds: NHS Information Centre

Muller-Vahl, K.R., Kolbe, H. and Dengler, R. (1999) Transient severe parkinsonism after acute organophosphate poisoning. *J Neurol Neurosurg Psychiatry* **66**, 253-4.

Narayan S, Liew Z, Paul K, Lee P-C, Sinsheimer JS, Bronstein JM, Ritz B. (2013) Household organophosphorus pesticide use and Parkinson's disease. *Int J Epidemiol* **42**, 1476-85.

Ohtani, T., Iwanami, A., Kasai, K., Yamasue, H., Kato, T., Sasaki, T. and Kato, N. (2004) Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: a 5-year follow-up study. *Psychiatry Clin Neurosci* **58**, 624-9.

Otto DA, Sollman S, Svendagaard D, Soffar A, Ahmed M. (1990) Neurobehavioral assessment of workers exposed to organophosphorus pesticides. In: Advances in neurobehavioral toxicology: applications in environmental and occupational health, edited by Johanson BL, Anger WK, Durano A, Xintaras C. Chelsea, Michigan: Lewis Publishers, pp. 306-22

Peiris-John, R.J., Ruberu, D.K., Wickremasinghe, A.R., Smit, L.A. and van der Hoek, W. (2002) Effects of occupational exposure to organophosphate pesticides on nerve and neuromuscular function. *J Occup Environ Med* **44**, 352-7.

Pickett, W., King, W.D., Lees, R.E., Bienefeld, M., Morrison, H.I. and Brison, R.J. (1998) Suicide mortality and pesticide use among Canadian farmers. *Am J Ind Med* **34**, 364-72.

Pilkington, A. , Buchanan, D., Jamal, G.A., Kidd, M., Sewell, C., Donnan, P., Hansen, S., Tannahill, S.N., Robertson, A., Hurley, J.F. and Soutar, C.A. (1999) Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers. Phase 2. Cross-sectional exposure-response study of sheep dippers. *Edinburgh Institute of Occupational Medicine* **IOM report TM/99/02b**

Pilkington, A., Buchanan, D., Jamal, G.A., Gillham, R., Hansen, S., Kidd, M., Hurley, J.F. and Soutar, C.A. (2001) An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occup Environ Med* **58**, 702-10.

Povey, A.C., Mackness, M.I., Durrington, P.N., Dippnall, M., Smith, A.E., Mackness, B. and Cherry, N.M. (2005) Paraoxonase polymorphisms and self-reported chronic ill-health in farmers dipping sheep. *Occup Med (Lond)* **55**, 282-6.

Povey, A.C., Jury, F., Dippnall, W.M., Smith, A.E., Thomson, S., Mackness, B., Mackness, M., Durrington, P. and Cherry, N.M. (2007) GST CYP and PON1 polymorphisms in farmers attributing ill health to organophosphate-containing sheep dip. *Biomarkers* **12**, 188-202.

Povey, A.C. (2010) Gene-environmental interactions and organophosphate toxicity. *Toxicology* **278**, 294-304.

Proctor, S.P., Heaton, K.J., Heeren, T. and White, R.F. (2006) Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology* **27**, 931-9.

Rehner, T.A., Kolbo, J.R., Trump, R., Smith, C. and Reid, D. (2000) Depression among victims of south Mississippi's methyl parathion disaster. *Health Soc Work* **25**, 33-40.

Riddle, J.R., Brown, M., Smith, T., Ritchie, E.C., Brix, K.A. and Romano, J. (2003) Chemical warfare and the Gulf War: a review of the impact on Gulf veterans' health. *Mil Med* **168**, 606-13.

Rohlman, D.S., Lasarev, M., Anger, W.K., Scherer, J., Stupfel, J. and McCauley, L. (2007) Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology* **28**, 374-80.

Roldan-Tapia, L., Parron, T. and Sanchez-Santed, F. (2005) Neuropsychological effects of long-term exposure to organophosphate pesticides. *Neurotoxicol Teratol* **27**, 259-66.

Rubin, G.J., Nieto-Hernandez, R., Wessely, S. (2010) Idiopathic environmental intolerance attributed to electromagnetic fields (formerly 'electromagnetic hypersensitivity'): an updated systematic review of provocation studies. *Bioelectromagnetics* **31**, 1-11.

Rothlein, J., Rohlman, D., Lasarev, M., Phillips, J., Muniz, J. and McCauley, L. (2006) Organophosphate pesticide exposure and neurobehavioral performance in agricultural and

non-agricultural Hispanic workers. *Environ Health Perspect* **114**, 691-6.

Rugbjerg K, Harris MA, Shen H, Marion SA, Tsui JKC, Teschke K. (2011) Pesticide exposure and risk of Parkinson's disease – a population-based case-control study evaluating the potential for recall bias. *Scand J Work Environ Health* **37**, 427-36.

Salvi, R.M., Lara, D.R., Ghisolfi, E.S., Portela, L.V., Dias, R.D. and Souza, D.O. (2003) Neuropsychiatric evaluation in subjects chronically exposed to organophosphate pesticides. *Toxicol Sci* **72**, 267-71.

Seidler A, Hellenbrand W, Robra B-P, Vieregge P, Nischan P, Joerg J, Oertel WH, Ulm G, Schneider E. (1996) Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case-control study in Germany. *Neurology* **46**, 1275-84.

Shahar, E. and Andraws, J. (2001) Extra-pyramidal parkinsonism complicating organophosphate insecticide poisoning. *Eur J Paediatr Neurol* **5**, 261-4.

Sklan EH, Lowenthal A, Korner M, Ritov Y, Rankinen T, Bouchard C et al. (2004) Acetylcholinesterase/paraoxonase genotype and expression predict anxiety scores in Health, Risk Factors, Exercise Training and genetics study. *Proc Natl Acad Sci USA* **101**, 5512-7.

Smith HV, Spalding JMK. (1959) Outbreak of paralysis in Morocco due to ortho-cresyl phosphate poisoning. *Lancet* **ii**, 1019-1021.

Solomon, C., Poole, J., Palmer, K.T., Peveler, R. and Coggon, D. (2007) Neuropsychiatric symptoms in past users of sheep dip and other pesticides. *Occup Environ Med* **64**, 259-66.

Spencer PS. Epidemiological and clinical evidence for residual organophosphate neurotoxicity. Accession Number ADA410490. Oregon Health Sciences University Portland, 2001.
http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA410490

Srivastava, A.K., Gupta, B.N., Bihari, V., Mathur, N., Srivastava, L.P., Pangtey, B.S., Bharti, R.S. and Kumar, P. (2000) Clinical, biochemical and neurobehavioural studies of workers engaged in the manufacture of quinalphos. *Food Chem Toxicol* **38**, 65-9.

Starks, S. E., Gerr, F., Kamel, F., Lynch, C. F., Alavanja, M. C., Sandler, D. P., & Hoppin, J. A. (2012a). High pesticide exposure events and central nervous system function among pesticide applicators in the Agricultural Health Study. *International Archives of Occupational & Environmental Health* **85**, 505-515.

Starks, S.E., Hoppin, J.A., Kamel, F., Lynch, C.F., Jones, M.P., Alavanja, M.C., Sandler, D.P. and Gerr, F. (2012b) Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect* **120**, 515-20.

Starks, S.E., Gerr, F., Kamel, F., Lynch, C.F., Jones, M.P., Alavanja, M.C., Sandler, D.P. and Hoppin, J.A. (2012c) Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the Agricultural Health Study. *Neurotoxicol Teratol* **34**, 168-76.

Steenland, K., Dick, R.B., Howell, R.J., Chrislip, D.W., Hines, C.J., Reid, T.M., Lehman, E., Laber, P., Krieg, E.F. Jr and Knott, C. (2000) Neurologic function among termiticide applicators exposed to chlorpyrifos. *Environ Health Perspect* **108**, 293-300.

Stephens, R., Spurgeon, A., Calvert, I.A., Beach, J., Levy, L.S., Berry, H. and Harrington, J.M. (1995) Neuropsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet* **345**, 1135-9.

Stephens, R. and Sreenivasan, B. (2004) Neuropsychological effects of long-term low-level organophosphate exposure in orchard sprayers in England. *Arch Environ Health* **59**, 566-74.

Stokes L, Stark A, Marshall E, Narang A. (1995) Neurotoxicity among pesticide applicators exposed to organophosphates. *Occup Environ Med* **52**, 648-53.

Storzbach, D., Campbell, K.A., Binder, L.M., McCauley, L., Anger, W.K., Rohlman, D.S. and Kovera, C.A. (2000) Psychological differences between veterans with and without Gulf War unexplained symptoms. Portland Environmental Hazards Research Center. *Psychosom Med* **62**, 726-35.

Tahmaz, N., Soutar, A. and Cherrie, J.W. (2003) Chronic fatigue and organophosphate pesticides in sheep farming: a retrospective study amongst people reporting to a UK pharmacovigilance scheme. *Ann Occup Hyg* **47**, 261-7.

Tochigi, M., Otani, T., Yamasue, H., Kasai, K., Kato, T., Iwanami, A., Kato, N. and Sasaki, T. (2005) Support for relationship between serum cholinesterase and post-traumatic stress disorder; 5-year follow-ups of victims of the Tokyo subway sarin poisoning. *Neurosci Res* **52**, 129-31.

Wingo TS, Rosen A, Cutler DJ, Lah J, Levey AI. (2012) Paraoxonase-1 polymorphisms in Alzheimer's disease, Parkinson's disease and AD-PD spectrum diseases. *Neurobiol of Aging* **33**, 204e13-204e15.

Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol* **26**, S1-S58.

World Health Organisation. (1992) The ICD-10 Classification of Mental and Behavioural Disorders. Geneva, World Health Organisation.

Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O., Kuroki, N., Fukuda, R., Tochigi, M., Furukawa, S., Sadamatsu, M., Sasaki, T., Aoki, S., Ohtomo, K., Asukai, N. and Kato, N. (2003) Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *Proc Natl Acad Sci U S A* **100**, 9039-43.

Yamasue, H., Abe, O., Kasai, K., Suga, M., Iwanami, A., Yamada, H., Tochigi, M., Ohtani, T., Rogers, M.A., Sasaki, T., Aoki, S., Kato, T. and Kato, N. (2007) Human brain structural change related to acute single exposure to sarin. *Ann Neurol* **61**, 37-46.

Zaganas I, Kapetanaki S, Masterodemos V, Kanavouras K, Colosio C, Wilks MF, Tsatsakis AM. (2013) Linking pesticide exposure and dementia: what is the evidence? *Toxicology* **307**, 3-11.

Zintzaras E, Hadjigeorgiou GM. (2004) Association of paraoxonase 1 gene polymorphisms with risk of Parkinson's disease: a meta-analysis. *J Hum Genet* **49**, 474-81.

Abbreviations

ACP	Advisory Committee on Pesticides
ACT	Automated Cognitive Testing
AHS	Agricultural Health Study
ANCOVA	Analysis of covariance
BMI	Body mass index
CES-D	Center for Epidemiological Studies-Depression Scale
CMD	Common mental disorder
CNS	Central nervous system
COPIND	Chronic organophosphate induced neuropsychiatric disorder
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CSF	Cerebrospinal fluid
CV	Conduction velocity
dB	Decibel
EEG	Electroencephalogram
EMG	Electromyography
ERP	Event-related evoked potential
GHQ	General Health Questionnaire
HADS	Hospital Anxiety and Depression Scale
HIV	Human immunodeficiency virus
HPEE	High pesticide exposure event
Hz	Herz
ICD	International Classification of Diseases
IQ	Intelligence quotient
MMSE	Mini Mental State Examination
ms	Millisecond
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NCS	Nerve conduction studies
NES	Neurobehavioral Evaluation System
NTE	Neuropathy target esterase
OPIDPN	Organophosphate-induced delayed polyneuropathy
OR	Odds ratio
PASAT	Paced Auditory Serial Attention Test
PHE	Public Health England
PNS	Peripheral nervous system
POMS	Profile of Mood States
PON1	The gene that codes for paraoxonase
PR	Prevalence ratio
PTSD	Post-traumatic stress disorder
QST	Quantitative sensory testing
RAVLT	Rey-Auditory Verbal Learning test
SD	Standard deviation
SPECT	Single-photon emission computed tomography
TCP	Trichloro-2-pyridinol
TOCP	Tri-ortho-cresyl phosphate
VBT	Vibrotactile threshold
WAIS	Wechsler Adult Intelligence Scale
WISC	Wechsler Intelligence Scale for Children
WHO	World Health Organisation

Table 1: Associations of organophosphates with peripheral neuropathy and potentially relevant central nervous system effects

Reference(s)	Country	Design	Number of subjects analysed	Exposure(s)	Outcome measure(s)	Results	Confounders considered	Comments
Chronic exposure								
Albers et al. (2004a), Albers et al. (2004b), Albers et al. (2007)	USA	Cohort	53 exposed (manufacture) vs. 58 controls (plastic film wrapping)	Chlorpyrifos	1) Clinical (10 items) 2) NCS (80 estimates in 4 nerves) 3) Diagnosis (based on 1 & 2). [*baseline and 1 year later for 2 & 3*]	No consistent and clinically important differences, overall or by exposure level	Age, sex, BMI, smoking, education, alcohol intake, anxiety	Well conducted study with detailed analyses by exposure level, historically & in the past year; but limited power to detect 'definite' neuropathy
Keifer et al. (2000)	USA	Cohort	68 exposed (apple thinning) vs. 69 controls (workers in hotels and restaurants, garment making) [*Followed for 1 year. Results given on 87, without breakdown*]	Azinphos methyl Paraoxonane Chlorpyrifos Diazinon	QST: VBTs at hand and foot [*at baseline and changes in thresholds at follow-up*]	Baseline threshold lower per hour of apple thinning in the hand, higher in the foot. Both thresholds rose over follow-up, but none of the findings was significant (P>0.05)	Age, sex, haemoglobin, seasons of thinning	The representativeness of the finally analysed groups is unclear. Baseline observation was made at the end of one season, follow-up at the start of the next, when short-term effects of exposure can be excluded. A change measure might thus manifest recovery; in fact there was a (non-significant) worsening – probably unrelated to OPs.
Farahat et al. (2003)	Egypt	Cross-sectional	52 exposed (sprayers) vs. 50 controls (office workers)	Profenofos Chlorpyrifos Triaziphos Phorate [also non-OP pesticides]	Clinical (5 items): muscle power, ankle & knee reflexes, superficial and deep sensation	ORs for signs in exposed v controls raised 3.2 to 6.4-fold; adjusted P-values all >0.05	Groups matched by sex; said to be similar in age and education, although social class differed	Relatively 'soft' outcomes, potentially assessed without blinding

Jamal et al. (2001)	Scotland	Cross-sectional	16 exposed (sheep dip) vs. 16 unexposed (mixed, but mainly office staff)	Diazinon Propetamphos Chlorfenvinphos	1) Clinical (3 scores) 2) EMG 3) NCS (12 estimates in 2 nerves) 4) QST (3 items): hot, cold, and vibration thresholds 5) ERP: visual, brainstem auditory	The exposed group performed significantly worse ($P \leq 0.02$) in 14 of 21 comparisons- viz EMG, 8 of 12 NCS, 2 of 3 clinical scores, 2 of 3 QSTs. Six of the remaining 7 effect estimates were non-significantly worse in the exposed group	Groups similar in age and sex	Small study in which representativeness and response rates cannot be judged. Groups were ill matched by social class. Possible impact of selection bias and diagnostic bias for some soft outcome measures (if assessed unblinded). If true, the study would indicate a broad range of effects on peripheral nerves
Kimura et al. (2005)	Malaysia	Cross-sectional	80 exposed (farmers) vs. 40 unexposed (office workers) [*Ns analysed 63-76 vs. 32-38; 26 farmers had used OPs*]	Tamaron (also other non-OP pesticides)	1) NCS (5 estimates): sensory CV in 2 nerves; motor CV in 2 nerves 2) ERP: auditory, visual†	No consistent or significant differences in NCS; mean ERPs delayed by <1% in exposed ($P>0.05$)	Matched on sex, age, alcohol intake; said to be similar in height, weight, smoking habits	In addition to several methodological limitations, only a minority of the exposed group (not separately analysed) were actually exposed to OPs
Peiris-John et al. (2002)	Sri Lanka	Cohort	30 exposed (sprayers) vs. 30 unexposed (fishermen) [*Ns analysed 23-29 vs. 22-26*]	OP herbicides, insecticides, fungicides	1) NCS: sensory CV and latency in 1 nerve, motor CV, latency and amplitude in 1 nerve (20 P-values) 2) Neuromuscular junction function, median nerve (12 P-values) [*assessed in season & 2 months after*]	No consistent differences in NCS; neuromuscular junction function similar.	Matched by sex; deemed similar in age, height, weight and alcohol intake	Several methodological limitations, although blinded assessment and objective outcomes. Sensory CV improved significantly in sprayers after cultivation, but significantly also in fishermen.

Srivastava et al. (2000)	India	Cross-sectional	59 exposed (manufacture) vs. 17 unexposed (tea vendors, hawkers, "etc")	Quinalphos	Clinical: Plantar reflexes Knee reflexes Ankle reflexes	'Abnormal': 49% vs 0%* 15% vs 0% 38% vs 6%* ** P<0.05*	Matched on sex; said to be similar in age, height, weight, literacy, income, smoking and alcohol intake	Small study with an eclectic group of controls and many unknowns (response rates, levels of exposure, etc). Examinations probably unblinded; 'soft' outcomes with potential for diagnostic bias
Starks et al. (2012b)	USA	Cohort	701 exposed (pesticide applicators) [*Ns analysed 544-674*]	16 named OPs (also other non-OP pesticides)	1) Clinical (6 items): ankle reflexes, proprioception, vibration sense, postural tremor†, Romberg sign†, tandem gait† (94 effect estimates) 2) NCS: 128 estimates in 1 nerve, covering motor CV, amplitude & latency [*assessed for ever use and by lifetime days*]	1) 15 of 94 ORs elevated ~2-3x & with P <0.05, most often toe proprioception (6) & postural tremor (4); 4 ORs significantly reduced; for some OPs and some outcomes, significant tests for trend with use. 2) NCV: Few significant differences (7 of 128 estimates), but *reduced* risks (P<0.05) for Phorate and several outcomes	Age, sex, BMI, height, US state	Well conducted study, nested within the respected AHS and with blinded assessment. However, limited response rate, multiple comparisons, and positive findings only for 'soft' outcomes

Steenland et al. (2000)	USA	Cross-sectional	191 exposed (applicators) vs. 189 unexposed (friends and blue-collar workers)	Chlorpyrifos	1) Clinical (10 tests): reflexes, pin prick, etc 2) NCS (6 tests) in 3 nerves 3) VBT test, toe (4 tests) 4) Sway tests† (12) 5) Pegboard tests (2) 6) Olfaction† (1 test) 7) Acquired colour vision† [*P-values given for findings jointly evaluated under these headings*]	Exposed did *better* on clinical tests (P<0.0001), VBT (P=0.03); *similarly* on NCV & acquired colour vision (P> 0.10); *worse* on peg-board test in dominant hand (P=0.07) and 1 of 12 light sway tests (P=0.04)	Age, race, education, smoking	Only a minority of the exposed population and an eclectic mix of controls were recruited; a significant minority of applicators had exposure in the past week. Magnitude of effects not given, but differences described by the authors as minor and unrelated to exposure.
Acute exposure (not known to have acute symptoms)								
McCauley et al. (2002)	USA	Cohort	653 'exposed' (GW veterans) vs. 610 deployed in SW Asia and 516 on service but not deployed	Potentially, Sarin and Cyclosarin (*chosen from workers near an Iraqi munitions site when demolished*)	Self-reported doctor-diagnosed peripheral neuropathy	2% in exposed vs. 2.8% & 2.1% in unexposed; adjusted OR exposed vs. other deployed personnel 1.0, 95%CI 0.4 to 2.6	Age, sex, race, region of residence	Comprehensive attempt to reconstruct health experience of Gulf War veterans potentially exposed to Sarin, but many losses to follow-up
Spencer 2001	USA	Cohort	43, 26, and 28 of the groups in McCauley above [*N analysed, 96 of 98*]	As in McCauley above	1) Somatosensory evoked potentials† 2) Clinical (not listed) 3) Transcranial magnetic stimulation motor responses†	1) All groups had similar lower and upper limb latencies (P≥0.15) 2) No group differences 3) Findings unstated	Age, sex, race	See McCauley above. This grey literature status report was based on a small subset of the wider study – no details of selection process and response rates

QST = Quantitative sensory testing; EMG = electromyography; NCS = nerve conduction studies; CV = conduction velocity; ERP = Event-related evoked potential; AHS = Agricultural Health Study; VBT = vibrotactile threshold

Table 2 Associations of organophosphates with performance on neuropsychological tests in studies published since last COT review

Reference	Country	Number of subjects	Exposure	Findings	Comments
Kilburn (1999)	USA	22 exposed and 264 referents	Chlorpyrifos	Exposed subjects exhibited impaired performance on most of the neuropsychological tests employed – simple and choice reaction time, culture fair IQ test, digit-symbol substitution, Lafayette pegboard, Trails A & B, immediate and delayed recall of stories, information (a test of general knowledge), and picture completion. There was no difference between the groups in the test of similarities and the reference group had only a marginally higher score in vocabulary (p=0.047).	Possibility of selection bias, and no allowance for potential confounders. There little can be concluded from study
Bazylewicz-Walczak et al. (1999)	Poland	26 women exposed to organophosphates in gardening jobs over 1-24 years and 25 controls	Included dichlorvos, methamidophos, methidathion, and pirimiphos-methyl, and also carbamates, synthetic pyrethroids and dithiocarbamates	At the end of the spraying season, there were improvements in performance on the digit-symbol, and Santa-Ana (dominant and non-dominant hands) tests in both the exposed and referent groups. In comparison with the referents, the exposed group made fewer errors in the aiming test (p=0.02) but tended to be slower at the task (p=0.09), which suggests a trade-off between speed and accuracy. The “fastest” reaction times in the exposed group were also reported to be somewhat longer	Interpretation of this study is difficult because the exposed workers had higher levels of anxiety and depression, and it is unclear whether adjustment was made for any confounding that might have resulted. The authors’ conclusion that “even low, long-term exposure may be associated with adverse behavioural effects in female greenhouse workers” is not supported by the findings.
Keifer et al. (2000)	USA	Up to 137 orchard thinners and a similar number of	Azinphos-methyl	E examination before a spraying season revealed poorer	The authors considered that there was no consistent effect of a

		matched controls		performance by the exposed workers on backward digit span, Trails A, and in a test of manual dexterity (Santa-Ana). Other tests (e.g., paired-associates, forward span, block design, Benton visual retention test) showed no significant differences from referents. After adjustment for vocabulary score (from the Peabody Picture Vocabulary Test), the only significant difference was for Trails A (p=0.06).	season of thinning on neuropsychological test performance.
Steenland et al. (2000)	USA	191 current and former termiticide applicators, 106 non-exposed friends, and 83 non-exposed blue-collar workers	Chlorpyrifos	The exposed workers tended to perform less well than the non-exposed blue-collar workers on the pegboard test (p = 0.005), but did not differ from the non-exposed friends (p=0.43). Performance was worst in eight workers with a self-reported history of poisoning by chlorpyrifos, who also fared worse in continuous performance and simple reaction time. There were no significant differences between termiticide applicators and either of the two reference groups in tests of vocabulary, digit span, digit-symbol substitution and pattern memory, mood scales, or Trails A and B.	Overall, there was no clear evidence of poorer neuropsychological performance in the termiticide applicators.
Srivastava et al. (2000)	India	59 manufactures of quinalphos with no known history of acute organophosphate poisoning, and a reference group of 17 unexposed tea vendors and roadside hawkers	Quinalphos	The exposed group performed worse on forward and backward digit span, digit-symbol substitution, and the Bourdon Wiersma vigilance test.	Analysis took no account of possible confounders.
Farahat et al.	Egypt	52 cotton workers and a	Organophosphates and	Exposed subjects performed worse	The conclusions that can be

(2003)		reference group of 50 clerks	other pesticides	in multiple neuropsychological tests (similarities, digit-symbol substitution, Trails A & B, letter cancellation, forward and backward digit span, Benton visual retention test, and delayed story recall), but there were no differences in the Paced Auditory Serial Attention Test (PASAT), block design, and immediate story recall (Story recall A).	drawn are limited by failure to include a test that could be indicative of functioning before exposure (e.g. of vocabulary), to explore how far the various measures of performance correlated with each other, and to consider reasons for discrepancies between tests with similar attentional demands (e.g. between (a) forward and backward span and (b) Trails A and B). A higher prevalence of reported numbness, dizziness, and (to a lesser extent) tremor might also have affected performance on tests that required visuomotor abilities. Although none of the exposed men had required hospitalisation for acute poisoning, some may have suffered at some time from milder acute poisoning.
Salvi et al. (2003)	Brazil	37 tobacco workers after they had been working with pesticides for three months, and had been exposed within the past day. 52% had a history of earlier acute poisoning.	Organophosphates, in particular chlorpyrifos	Both the Mini Mental State Examination (MMSE) and a test of "word span" gave results within the expected range	Because many participants had experienced acute pesticide poisoning, and there was no reference population with which to compare the exposed workers, no useful conclusions can be drawn about long-term effects of organophosphates in the absence of overt acute poisoning.
Stephens and Sreenivasan (2004)	England	37 orchard sprayers and referent groups of 26 pig farmers and 31 construction workers.	Chlorpyrifos	No significant associations of exposure with simple reaction time, forward and backward digit span, digit-symbol latency, serial word learning, accuracy, speed in the location recognition test, and speed in the category search test. Compared to construction workers,	The investigators considered the findings on syntactic reasoning for negative statements to result from the slowing of processing speed. The lack of effect on positively phrased reasoning items indicates that they did not result from a general slowing, and points to

				but not pig farmers, orchard sprayers had significantly slowed performance on the syntactic reasoning task for negative statements ($p<0.001$), but not for positive statements ($p=0.120$). However, except in the pig farmers, overall accuracy on the task was low and only marginally better than would be expected by chance. An equivalent analysis of the accuracy of syntactic reasoning, aside from the expected effects due to task complexity, did not indicate any particular trade-off between speed and accuracy that could account for the differences in response speed. No exposure-response relations were detected for any of the neuropsychological tests in relation to cumulative exposure.	difficulties in conceptually manipulating material in memory. However, the difference in syntactic reasoning was apparent only in the comparison with construction workers, raising the possibility that it occurred by chance or resulted from unrecognised selection biases.
Albers et al. (2004c)	USA	53 manufacturers of chlorpyrifos and a reference group of 60 workers making plastic film wrap	Chlorpyrifos	No differences between exposed and reference groups in Mini Mental State Examination (MMSE) and a qualitative questionnaire about reading, memory, concentration and problem solving.	Because of the low sensitivity of the neuropsychological measures, little weight can be given to these negative findings
Roldan-Tapia et al. (2005)	Spain	40 greenhouse sprayers and a reference group of 26 workers who had never had contact with “toxic substances”.	Various pesticides including organophosphates and carbamates	Cumulative exposure was significantly associated with better recall after delay in the Rey-Auditory Verbal Learning test (RAVLT, memory for material learned following distraction), poorer quality and longer time of copy of the Rey-Osterrieth Figure, poorer Benton Visual Form Discrimination (a test of fine visual discrimination) and a tendency to lower anxiety (after 5 years of	This study was limited by its small size, and the testers were not blind to the exposure status of the subjects. A major limitation was the dichotomisation of some test results when all could have been analysed as continuous measures. It is difficult to conclude much from the findings, although difficulties in copying complex drawings and making fine visual discriminations seem to characterise the

				working with pesticides). There were no reported effects of exposure on forward or backward digit span, Trails A & B, digit-symbol substitution, picture completion, similarities, learning on the Rey-Auditory Verbal Learning Test, immediate or delayed recall of stories, Benton Visual Retention Test, or block design.	"adverse" changes found. In contrast, among those with longer exposure, there appeared to be better recall following distraction.
Proctor et al. (2006)	USA	70 Gulf War veterans potentially exposed over a short period following demolition of a munitions dump and 70 veterans considered to have low or no exposure	Sarin, cyclosarin	Among the neuropsychological outcomes, exposure-response relationships were seen only for: the Purdue pegboard test for the dominant (p for linear trend = 0.005) and non-dominant hand (p=0.03); speed of finger tapping in both dominant (p=0.001) and non-dominant (p=0.002) hand; and the WAIS block design test. The exposed veterans performed better than the low/no exposure group on the finger tapping task, and the association of exposure with poorer performance on the Purdue pegboard test was strengthened after adjustment for the finger tapping measure as a covariate.	Manual dexterity appeared to be affected in the exposed subjects. However, findings could have resulted from psychological stresses rather than toxicity.
Chao et al. (2010)	USA	40 Gulf War veterans potentially exposed over a short period following demolition of a munitions dump and 40 unexposed controls	Sarin, cyclosarin	No associations with performance in groove pegboard or digit-symbol tests of psychomotor performance, or in the Block Design test or tests of memory.	This study, which was carried out later than that of Proctor et al (2006) suggests that if there was an effect of exposure on manual dexterity then it subsided over time.
Rothlein et al. (2006)	USA	92 farmworkers who lived near to orchards and a reference group of 45 workers in a coastal town with little agriculture	Organophosphates	12 of the sixteen measures showed poorer performance among those working in agriculture, but the differences appear to have been statistically significant for only two –	Measurement of urinary metabolites indicated recent exposures in the farmworkers. Overall, the study provides little evidence of long-term effects on

				backward digit span and (in females only) finger tapping.	neuropsychological performance from low-level exposure to organophosphates.
Browne et al. (2006)	Israel	23 adults who worked in agriculture or lived near to fields that were sprayed with pesticides and a reference group of 23 urban residents.	Cholinesterase-inhibiting pesticides	Neuropsychological tests comprised forward and backward digit span, serial reaction time, continuous performance test, visual reproduction, and long-term verbal memory from the Rey Auditory Verbal Learning Test, and the Tower of Hanoi (a task of planning). The only test showing poorer performance in the exposed workers was the 20-minute delayed portion of the visual reproduction test ($p<0.05$)	The study was limited by the small group size and a failure adequately to consider potential confounders such as age. The combination of equivalent scores on visual reproduction after immediate recall with a deficit after delayed recall points to a specific effect, but may have been a chance finding.
Rohlman et al. (2007)	USA	119 farmworkers and 56 referents who had not worked on farms for at least one year	Pesticides	Years worked in agriculture was associated with poorer performance on “match-to-sample” and (only in females) in the digit-symbol test. In males, years worked in agriculture was associated with worse performance on a selective attention measure.	As no information was reported on exposure to specific pesticides, little can be drawn from this study regarding the effects of low-level exposure to organophosphates.
Abdel Rasoul et al. (2008)	Egypt	20 boys aged 16-18 years, who had sprayed cotton fields over an average of seven years and 20 controls who had never worked in cotton fields.	Organophosphates	After adjustment for age, education and body mass index, significantly lower performance was observed in the exposed group on all of the neuropsychological measures (information, similarities, arithmetic, block design, digit span, and digit-symbol from the Wechsler Intelligence Scale for Children (WISC), Trails A & B, and the Benton visual retention test) that were employed	Significant correlations with days worked in the current season, but less consistent associations with years worked, could indicate an adverse effect of recent exposure, but might also be explained by lower attendance at school. Higher levels of neuroticism, depression, fatigue and blurred vision among the applicators may have contributed to worse performance on some tests. Given these limitations and uncertainties, little can be drawn from the study regarding

					neuropsychological effects of exposure to organophosphates.
Mackenzie Ross et al. (2010)	UK	127 sheep farmers with at least 5 years exposure to organophosphate pesticides (67 working, 60 retired due to ill-health), and 78 rural policemen (38 working and 40 retired on ill-health grounds)	Organophosphates	Scores on the matrix reasoning test were equivalent for the exposed and referent groups, as were measures of verbal ability (vocabulary, comprehension), visuospatial ability (block design, spatial span), and two other verbal/visual reasoning tasks (similarities, comprehension). However, exposure was associated with poorer performance in graded naming and picture arrangement; forward and backward digit span; the conceptually similar letter-number sequencing task; immediate and delayed verbal and visual memory tasks; the digit-symbol substitution test; Trails A; Trails B; and the grooved pegboard tests (both dominant and non-dominant hands).	No useful conclusions can be drawn from these findings because many of the exposed farmers, especially in the retired group, were volunteers recruited by advertisement, and therefore liable to be highly unrepresentative. There was further potential for selection bias because controls were excluded from the investigation if they had any history of psychiatric problems, whereas farmers were not excluded for this reason unless the psychiatric problems preceded their exposure to organophosphates.
Starks et al. (2012a)	USA	693 licensed pesticide applicators with no history of a physician-diagnosed pesticide poisoning, of whom 156 had experienced a high pesticide exposure event	High pesticide exposure event (HPEE)	Ever having an HPEE was associated with worse performance on two tests: the digit-symbol substitution test and Sequences A (a touch screen version of Trails A). No effects were observed in other neuropsychological tests (e.g., of learning, memory, tapping, pegboard, Sequences B).	The authors noted that the HPEEs represented undesirable high exposure events experienced by pesticide applicators, and not overexposure to any specific pesticide. Overall, this well-conducted study suggests effects on tests that involve motor responses following visual scanning in pesticide applicators who had experienced high exposure events involving various chemicals, some of which may have been organophosphates.
Starks et al. (2012c)	USA	701 male workers (mean age 61 years) from the US	16 organophosphates	There were no associations with total organophosphate exposure for	The authors concluded that there was no consistent evidence of an

		Agricultural Health Study		any of eight neuropsychological tests (continuous performance, digit-symbol, finger tapping, auditory verbal learning followed by delayed recall and recognition, and sequences A & B). Associations were observed for some specific organophosphates, some showing poorer scores with increasing exposure and others better scores.	association between organophosphate exposure and neuropsychological outcomes. A major strength of the study was the large sample size and the reliability of estimated pesticide use.
Bayrami et al. (2012)	Iran	40 horticulture workers and a reference group of 40 men from the same village who were not exposed to pesticides	Organophosphates	No differences were reported in overall performance in the Mini Mental State Examination (MMME), or in subscales relating to orientation, registration, attention/calculation, recall, and language	The assessment of longer term exposure to organophosphates was limited. The MMSE, which is usually used as a screening test for dementia, provides only basic and relatively insensitive measures of neuropsychological function.
Malekirad et al. (2013)	Iran	187 horticultural farmers and a reference group of 187 unexposed workers from the same village	Organophosphates	Performance of the exposed group was reported to be significant lower ($p<0.001$) on all of a range of neuropsychological tests (psychomotor speed, selective and divided attention, verbal and non-verbal memory, prospective memory, spatial functioning).	Lack of detail about the methods prevents useful conclusions. Also, there were significantly higher levels of anxiety ($p=0.015$) and depression ($p<0.001$) in the farmers, but no attempt was made to control for these potential confounders.
Blanc-Lapierre et al. (2013)	France	614 agricultural workers, including 443 thought to be exposed to at least one organophosphate	Organophosphates	Consistently positive associations were observed between each of five outcomes (MMSE, the Benton Visual Retention Test, Stroop, Trails A and Wechsler Paired Associates) and exposure to each of 11 specific organophosphates, many being statistically significant. The strongest associations were for mevinphos, and these persisted after exclusion of subjects who reported previous poisoning by pesticides or recent exposure at the	Strengths of this study include its prospective design, estimation of exposures by methods that did not rely on recall of exposure to specific chemicals, and relatively large sample size. Against this, most of the neuropsychological tests were selected for investigation because they were already known to show associations with pesticide exposure in the study sample, which increases the possibility of

				time of neuropsychological testing. However, no clear trend in risk was observed across quartiles of exposure to mevinphos.	false positive results by chance. Some scores in the MMSE were unusually low for a working population. This anomaly and the absence of a clear exposure-response relationship limit the conclusions that can be drawn, but overall, the pattern of results is more suggestive of an effect on visual-motor performance than cognitive difficulties.

Table 3: Associations of organophosphates with structural changes on MRI

Reference	Country	Design	Number of subjects analysed	Exposure(s)	Outcome measure(s)	Results	Confounders considered	Comments
Chao 2010	USA	Cohort	40 'exposed' subjects (GW veterans) vs. 40 unexposed (GW veterans) [*Ns analysed 35-36 vs. 40*]	Potentially, Sarin and Cyclosarin (*exposed chosen from workers near a demolished munitions site*)	MRI (5 items): grey matter, white matter, CSF, intracranial (IC) and hippocampal volumes	Exposed subjects had less grey matter and hippocampal volume (3.2% and 6.1% down), $P \leq 0.01$, after allowing for larger IC volumes in the exposed. The latter had more white matter (3.4%, $P>0.05$)	Age, sex, intracranial volume	Several methodological strengths, but only a small subset of the potentially exposed population (in all, perhaps a hundred thousand veterans) was studied
Heaton 2007	USA	Cohort	13 'exposed' subjects (GW veterans) vs. 13 unexposed (GW veterans)	Potentially, Sarin and Cyclosarin (*exposed chosen from workers near a demolished munitions site*)	MRI (5 items): grey matter, white matter, CSF, right and left ventricle volumes	No statistically significant differences in adjusted volumes; significant decline per exposure unit in white matter volume: -4.64% (-4.79 to -4.49%), $P<0.03$	Age, PTSD score, general health, unit group (in some analyses, IC volume)	The final sample was a small subset of those potentially exposed, with possible over-sampling of the symptomatic; sampling frames and response rates were unclear

Table 4 Associations of organophosphates with Parkinson's disease and parkinsonism

Reference	Country	Design	Number of subjects	Exposure	Odds ratio (95% CI)		Factors of adjustment	Comments
Albers 2004c	USA	Cohort	53 exposed and 60 controls	Chlorpyrifos in manufacture	No Parkinsonian abnormality in either group at baseline or after 1 year			Low statistical power to detect unequivocal, serious health outcomes
Kamel 2007	USA	Cohort	52,393 pesticide applicators and 32,345 spouses, with follow-up after 5 years for 57,251	Use of: Chlorpyrifos Coumaphos Diazinon Fonofos Malathion Parathion Phorate Terbufos	Prevalence at baseline 1.2 (0.7-2.1) 0.8 (0.3-1.9) 1.0 (0.5-1.8) 0.9 (0.3-1.7) 1.1 (0.6-2.0) 1.3 (0.6-2.7) 1.1 (0.6-2.0) 0.9 (0.5-1.7)	Incidence at follow-up 0.9 (0.5-1.6) 0.8 (0.4-1.9) 0.9 (0.5-1.7) 1.0 (0.5-1.8) 1.2 (0.6-2.1) 1.1 (0.6-2.2) 1.4 (0.8-2.5) 1.1 (0.6-2.0)	Age, state, type of participant (applicator or spouse)	Healthy worker selection may have reduced number of prevalent cases at baseline. No allowance for smoking.
Seidler 1996	Germany	Case-control	380 cases, 376 neighbourhood controls and 379 regional controls	Use of: Organophosphates and/or carbamates	Neighbourhood controls 1.8 (0.9-3.3)	Regional controls 2.5 (1.3-4.6)	Smoking, education	Eligibility criteria for cases unclear. Response rate for eligible controls not given. Recall bias likely to inflate risk estimates
Dhillon 2008	USA	Case-control	100 cases and 84 controls	Used, mixed or applied Chlorpyrifos Malathion Diazinon	 2.0 (1.02-3.8) 1.3 (0.7-2.4) 0.8 (0.4-1.4)			Major weaknesses (see text)

Hancock 2008	USA	Case-control	319 cases and 296 controls	Application of organophosphates	1.89 (1.11-3.25)		Sex, age, smoking, caffeine consumption	Major limitations (see text)
Elbaz 2009	France	Case-control	247 cases and 676 controls	Organophosphates	1.3 (0.7-2.3) (men only)		Smoking, mental impairment	
Gatto 2009 Manthripragada 2010	USA	Case-control	368 cases and 341 controls	Potential exposure from well water to: Diazinon Dimethoate Chlorpyrifos	 1.58 (1.03-2.43) 1.41 (0.94-2.11) 1.45 (0.94-2.24)		Sex, age, education, race, family history of Parkinson's disease	Classification of exposure based on distance of residence from treated crops and may be unreliable
Firestone 2010	USA	Case-control	404 cases and 526 controls	Occupational exposure to: Parathion Malathion Diazinon	Men 5.8 (0.66-50.8) 1.0 (0.39-2.30) 0.8 (0.30-2.15)	Women 1 case and 0 controls exposed to each	Age, ethnicity, smoking	
Rugbjerg 2011	Canada	Case-control	403 cases and 405 controls	Occupational exposures to organophosphates above background	0.74 (0.20-2.78)		Sex, age, smoking	
Engel 2001	USA	Cross-sectional	310 men assessed for Parkinsonism	Occupational use of organophosphates	Prevalence ratio 0.9 (0.6-1.4)		Age, smoking	
Salvi 2003	Brazil	Cross-sectional	25 agricultural workers assessed 3 months after last exposure to organophosphates	Occupational exposure to organophosphates in application	1025 with clinically significant Parkinsonian symptoms			No control data

Appendix A Membership of Working Group

David Coggon (Chairman)	Professor of Occupational and Environmental Medicine, University of Southampton, and Chairman of COT
Robert Hadden	Consultant Neurologist and Honorary Senior Lecturer in Neurology, Kings College Hospital, London
Anna Hansell	Senior Lecturer and Assistant Director of the Small Area Health Statistics Unit, MRC-PHE Centre for Environment and Health, Imperial College, London, and COT member
Glyn Lewis	Professor of Psychiatric Epidemiology, University College, London
Keith Palmer	Professor of Occupational Medicine, University of Southampton, and member of ACP
Brian Stollery	Senior Lecturer in Experimental Psychology, University of Bristol

Secretariat

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Appendix B Methods of literature search

The current review focused on human data concerning specific neurological health outcomes, namely peripheral neuropathy and neuromuscular dysfunction, neurophysiological effects, neuropsychological abnormalities, structural changes in the brain identified by magnetic resonance imaging (MRI), Parkinson's Disease and parkinsonism, dementia, and psychiatric illness.

A systematic search of the peer-reviewed literature relating human exposure to the above neurological outcomes was undertaken for the period January 1999 to September 2012 inclusive, using search terms set out in Table 1 below, in the PubMed, Embase, Toxline, Web of Science and CAB Abstracts databases. Relevant literature identified by the Veterinary Medicines Directorate (VMD) was also included, together with peer-reviewed references retrieved from the OrganoPhosphate Information Network (OPIN) website. An update was subsequently performed for the period September 2012 to September 2013 inclusive, using the same methods, but limited to the PubMed and Web of Science databases. Additional references identified by cross-referencing relevant papers were also included, and further references considered to be relevant by members of the working group were added.

Table 1. Database searches conducted

1. (organophosphate* OR organophosphorus*) OR diazinon OR propetamphos* OR (sheep dip*) OR parathion*
2. azamethiphos* OR azinphos methyl* OR chlorfenvinphos* OR chlorpyrifos* OR chlorpyrifos-methyl* OR demeton-S-methyl* OR dichlorvos* OR dimethoate* OR disulfoton* OR ethoprophos* OR etrimfos* OR fenitrothion* OR fonofos* OR fosthiazate* OR heptenophos* OR iodophenfos* OR malathion* OR mephosfolan* OR methyl parathion* OR phorate OR phosalone* OR phosmet OR pirimiphos-methyl* OR pyrazophos* OR quinalphos* OR thiometon* OR triazophos* OR trichlorfon* OR toclofos-methyl* OR 2-chloroethylphosphonic acid

Tables 2 (a) and (b) below indicate the inclusion and exclusion criteria applied to references retrieved from the searches.

Table 2(a) Review Inclusion Criteria

Inclusion Criteria
Health outcomes relating to the nervous system: • neuropsychological abnormalities • electroencephalographic (EEG) abnormalities • peripheral neuropathy and neuromuscular dysfunction • psychiatric illness • effects on the autonomic nervous system • focus on chronic, low-level exposure in relation to the above health outcomes • acute poisoning with persistent chronic effects, in particular memory and psychiatric illness • PON1 and polymorphism studies, if associated with organophosphate-related chronic neurological ill-health • Alzheimer's Disease (AD)

- Parkinson's Disease (PD)
- Parkinsonism
- chronic effects of Gulf War incident with low exposure at Khamisiyah, Iraq
- health effects arising from the Tokyo subway sarin episode
- Chronic Organophosphate Induced Neuropsychiatric Disorder (COPIND), which includes the symptoms: personality changes, impulsive suicidal thoughts, cognitive impairment, language disorder, alcohol intolerance, heightened sense of smell, handwriting deterioration, sensitivity to OPs, decreased exercise tolerance, and exacerbation of "dipper's flu" in sheep dippers
- symptoms most frequently mentioned by individuals who believe they suffer long-term ill-health from exposure to organophosphates, which, besides those comprising COPIND, include: anxiety, confusion, depression, headache, fatigue, impaired concentration, incoordination, irritability, memory loss, muscular pains and/or spasms, nausea, nightmares, numbness of the extremities, respiratory disease, sleep disorders
- peer-reviewed case reports
- relevant reviews

Table 2(b) Review Exclusion Criteria

Exclusion Criteria

- studies reporting animal or *in vitro* data
- studies not reporting original results: meta-analyses, comments, letters, editorials, abstracts, and most of the retrieved reviews (a few reviews thought to be particularly relevant were included)
- papers concerned only with methodology
- papers dealing with exposure or exposure monitoring only
- papers reporting on monitoring the activity of acetylcholinesterase in absence of any other health outcomes identified in the inclusion criteria
- studies dealing only with health outcomes other than those relating to the nervous system, for example, immunological or reproductive effects, mutagenicity, carcinogenicity
- studies dealing with neurodevelopmental defects in children aged 0-15 years
- papers reporting poisonings or only the acute effects of exposure to OPs
- cabin air reports previously reviewed by COT
- papers relating to medical treatment of poisoning with organophosphates
- acute poisoning giving rise to intermediate syndrome or polyneuropathy
- Gulf War Veteran studies other than those relating specifically to organophosphate exposures
- mortality studies
- Creutzfeldt-Jakob disease

Overall, in excess of 89,000 peer-reviewed references were retrieved from the database searches. After deletion of duplicates, over 5,000 references were identified and screened against the inclusion/exclusion criteria set out in Tables 2(a) and (b) above. Initially, the selection criteria were applied to the titles of retrieved papers, and references clearly unrelated or irrelevant to the review were excluded. The selection criteria were applied next to the abstracts of the remaining retrieved papers. Abstracts written in English were evaluated for papers written in other languages. Again, papers found to be irrelevant were excluded. The publications accepted after this stage in the process were screened in full.

Appendix C Commonly used neuropsychological tests

All behavioural tests require the proficient coordination of sensory-cognitive-motor functions for optimal performance. Thus, “deficits” for a particular neuropsychological test can reflect problems anywhere along the pathway from initial stimulus registration through to the final motor response. The subdivision of neuropsychological tests into the functional domains presented below generally assumes that sensory-motor deficits are not present and the classification reflects the dominant cognitive function being assessed in that particular neuropsychological test. As the assessment of any cognitive function is reliant on several cognitive domains, each neuropsychological test will be relevant to more than one domain, but only the principal domain is indicated below. Where other domains are also strongly implicated, these are noted.

WHO Neurobehavioral Core Test Battery (WHO NCTB)

Beginning in 1983, this was the first consensus battery developed by an expert panel convened by the World Health Organisation (WHO), with leadership from the US National Institute for Occupational Safety and Health, to detect deficits suggestive of neurotoxic exposures. The WHO has advocated this core battery for neurobehavioral assessment in population screening and research, to foster the development of a database of common tests shown to be sensitive to neurotoxic disorders. It comprises the seven subtests outlined below. This WHO NCTB (either in its entirety or selected subtests from it) has been used widely in the assessment of people exposed to potentially neurotoxic substances (e.g., lead, organic solvents, and pesticides). Researchers are free to supplement the core battery with more specialised tests.

Test Name	Brief Description	Main function assessed
Simple reaction time	The individual must press a blue button as rapidly as possible in response to a red light shown on a screen. The red light disappears after the response, and the individual must then release the button. The light reappears at a random interval of between 1 and 10 seconds. There are 64 presentations of the red light. Individuals use the index finger of their dominant hand to make their responses. Two minutes of practice is recommended before the main test, which lasts six minutes.	Simple detection of the presence of a visual stimulus. Incorporating the random delay renders the test sensitive to fluctuations in attention/concentration during the period between one response and presentation of the next stimulus.
Digit Span	This is the WAIS digit span test, where the individual has to repeat numbers orally, in the same order as they are given (forward), and/or in reverse order. The maximum number of digits perfectly recalled in sequence is the digit span. (See more detailed description later under attention and working memory.)	The test involves immediate rote recall for verbal material, ability to shift thought patterns from digits forwards to digits backwards, concentration and attention, and auditory sequencing.
Santa Ana Dexterity	In this test, the individual has to lift and rotate a series of 48 pegs. The numbers of pegs successfully completed in 30 seconds is the score. Separate	In addition to being sensitive to impairment of fine skilled motor movements, it also requires good eye-hand

	measurements are taken for the dominant and non-dominant hand. (See more detailed description later under visual-motor abilities.)	coordination.
Benton Visual Retention	In this test, individuals are shown a card for 10 seconds that contains one or more geometrical designs that comprise spatially separated shapes. Immediately after, the individual is shown a card containing four geometrical designs and the individual has to select the design just seen. (See more detailed description later under perception.)	The test is sensitive to problems with simple visual perception and immediate visual recognition memory.
Digit Symbol	This is the WAIS digit symbol test, in which the individual is shown an array that matches a list of digits (e.g., 1) with simple symbols (e.g.,□) shown below each digit. Underneath this "code" are a series of lines of squares with a random order of digits each shown above a blank response box. The individual must enter the associated symbol under each digit as quickly and accurately as possible for 90 seconds. A brief practice trial is recommended to be included. The usual score is the number of symbols correctly completed. (See more detailed description later under visual-motor abilities.)	The test is sensitive to impairment of complex timed fine motor abilities (writing the symbol), visual acuity, visual scanning, processing speed, and memory for the digit-symbol pairings.
Pursuit aiming II	In this test, the individual uses a pencil to place one dot inside each of a series of circles that are arranged sequentially in rows. The test is repeated twice, for 60 seconds each. The score is the number of correctly "dotted" circles. (See more detailed description later under visual-motor abilities.)	The test is sensitive to impairment of visual acuity, visual-motor coordination (e.g., tremor), and the speed of executing fine visually guided motor movements
Profile of Mood States (POMS)	In this self-report test, individuals rate a series of mood-related adjectives according to how they felt over the past week. Six mood scales are derived, reflecting the individual's degree of anxiety, depression, anger, fatigue, vigour, and confusion. (See more detailed description later under self-report scales.)	These self-reported mood states are regarded as transitory and specific to a given situation. They reflect the individual's evaluation of their subjective well-being.

Intelligence

The most commonly used intelligence test is the Wechsler Adult Intelligence Scale (WAIS). Various versions exist: the original (WAIS, 1955), its revision (WAIS-R, 1981), the WAIS-III (1997) and most recently the WAIS-IV (2008). Many of the component subtests have undergone revision over time, with some being substantially modified or dropped altogether,

and new ones introduced. Updates have been accompanied by expanded and updated norms, and have offered improved psychometric properties, better artwork and other materials, the elimination of biased items, and the provision of enlarged visual stimuli for those with reduced visual acuity. Translations from English (e.g., to Dutch and Spanish) have been undertaken, and the accompanying norms generated. Only the most commonly applied subtests are described here, and these are predominantly from the WAIS-III & WAIS-IV. Administration of the full WAIS usually takes 60-90 minutes. However, most studies employ only a subset of the component subtests. Other "general ability" scales such as the Mini Mental State Examination and Wide Range Achievement Test are also described.

Test	**Description**
WAIS III: Full scale IQ	The full WAIS-III comprises 14 subtests (see below) and by combining scores from each subtest is used to provide a measure of the individual's full scale IQ. In addition two composites of the 14 subtests are used to measure verbal IQ and performance IQ, and four indices based on verbal, comprehension, perceptual organisation and processing speed can also be derived. Each of the subtests contributing to these four indexes is described below.
WAIS III: Verbal IQ	Verbal IQ comprises 7 subtests and is characterised by a focus on verbal/language abilities, which are generally thought to be less sensitive to neurological impairment. The presence of memory tests is the exception here. This measure of verbal IQ is often subdivided into two indices: (a) a verbal comprehension index (vocabulary, similarities, information, comprehension) and (b) a working memory index (arithmetic, digit span, and letter-number sequence)
WAIS III: Performance IQ	Performance IQ comprises up to 7 subtests and is characterised by a focus on visual-spatial abilities and the need for the individual to perform timed motor activities. This measure of performance IQ is often subdivided into two indices: (a) a perceptual organisation index (picture completion, block design, matrix reasoning; with picture arrangement and object assembly sometimes used as supplementary tests) and (b) a processing speed index (digit symbol coding and symbol search).
WAIS Reading	The Wechsler Test of Adult Reading (WTAR) requires individuals to read aloud irregularly spelled words, and is used to provide a measure of premorbid intelligence – that is prior to the onset of illness or disease. It is similar in form to the National Adult Reading Test (NART) but was co-normed with the WAIS and WMS (Wechsler Memory Scale) to allow more accurate predictions of IQ scores.

WAIS Subscales

The information presented in this section refers primarily to the WAIS-IV.

Verbal Comprehension Index

Similarities: Individuals are asked an open ended question about how two seemingly dissimilar items might in fact be similar, for example, "In what way are an apple and a pear alike?" The test involves logical abstract verbal reasoning, verbal concept formation or conceptual thinking, distinguishing essential from non-essential details, and associative ability combined with language facility. Each item is scored as 0, 1 or 2.

Vocabulary: Individuals are asked for the definitions of words, for example, "What is a guitar?" The test is sensitive to the state of language development, word knowledge, and general verbal intelligence. Language usage and accumulated verbal learning ability provide a rough measure of an individual's optimal intellectual efficiency, educational background, range of ideas, and the experiences or interests that the person has acquired. Each item is scored as 0, 1 or 2.

Information*:* Individuals are asked general knowledge questions about people, places, objects and events (culture-specific), for example, "Who is the President of Russia?" The test is sensitive to the individual's range of general factual knowledge, prior learning and schooling, intellectual curiosity and desires to accumulate knowledge, alertness to the day-to-day world, and long term memory. Each item is scored as 0, 1 or 2.

Comprehension: These items focus on solutions to everyday problems, and understanding of concepts or social practices. Individuals are asked, for example, "What does, '*Kill 2 birds with 1 stone'* metaphorically mean?" The test is sensitive to a wide range of abilities that generally reflect educational level. These include the demonstration of judgement, practical knowledge, social maturity, knowledge of conventional standards of behaviour, ability to evaluate past experience, abstract thinking and generalisation, common sense and judgement in practical social situations, a grasp of one's social environment (e.g., information and knowledge of moral codes, social rules and regulations), and an understanding and alertness to the day to day world. Each item is scored as 0, 1 or 2.

Word reasoning*:* This supplemental test involves verbal comprehension. Participants are told "Let's play a guessing game. Tell me what I'm thinking of" and are asked, for example, "This is an animal that goes "woof." The test is sensitive to verbal reasoning, verbal abstraction, deductive reasoning, ability to develop alternative concepts, synthesising ability, and verbal comprehension

Perceptual Reasoning Index

Block Design: Individuals use up to nine red-and-white blocks to re-create a model or picture of a design within a specific time limit. The task requires analysis of whole into component parts, spatial visualisation, non-verbal concept formation, visual-motor spatial coordination, perceptual organisation, capacity for sustained effort (concentration), and manipulative and perceptual speed.

Matrix reasoning: Individuals look at an incomplete matrix of symbols and select the missing item from five choices. There are four kinds of reasoning examined: pattern completion, classification, analogy, and serial reasoning. The test involves visual-spatial reasoning,

abstract reasoning, visual organisation, simultaneous processing of visuospatial information, and analysis of wholes into component parts.

Visual puzzles*:* Individuals are shown a completed geometrical puzzle and asked "Which three of these pieces go together to make this puzzle?" The individual selects the three pieces from a choice of six. The test involves visual recognition and identification, perception of the parts in relation to the whole, visual spatial reasoning, analysis of wholes into component parts, capacity for sustained visual effort (concentration), and fluid reasoning. The test is similar to the block design test, but does not involve 3D manual reconstruction skills.

Figure weights*:* This is similar to the Piagetian balance beam task. Individuals are shown a balanced scale with different symbols on each side, and an unbalanced scale where one side has no symbols on it. They are asked "Which of these go here to balance the scale?" The individual selects from a choice of five what symbols are needed to balance the scale. The test involves quantitative, non-verbal, mathematical reasoning, quantitative and analogical reasoning, visual concentration combined with an ability to visually organise material, and capacity for sustained effort.

Picture completion: Individuals have to point to, or name, an important part that is missing from a picture, within a specified time. The test involves visual alertness, visual recognition and identification (long-term visual memory), awareness of environmental detail (reality contact), perception of the whole in relation to its parts (visual conceptual ability), ability to differentiate essential details form nonessential details, and visual concentration combined with ability to organise material visually.

Picture concepts*:* The individual is presented with two or three rows of pictures and chooses one picture from each row that has common characteristics. The test involves non-verbal concept formation, perceptual recognition, and abstract categorical reasoning.

Working Memory Index

Digit span: This requires the individual to do two distinct tasks: the first is to repeat orally presented numbers in the same order, and the second is to repeat the orally presented numbers in reverse order. The test involves immediate rote recall for verbal material, ability to shift thought patterns from digits forwards to digits backwards, concentration and attention, and auditory sequencing.

Arithmetic*:* Individuals solve a series of orally presented arithmetic problems within a specific time limit (e.g., Bob has ten books and Jim has twice as many. How many books does Jim have?" The test involves computational skill, auditory short-term memory, sequencing ability, numerical reasoning and speed of numerical manipulation, concentration and attention/low distractibility, reality contact and mental alertness (i.e. active relationship to outside world), school learning (acquired knowledge), logical reasoning, abstraction and analysis of numerical problems.

Letter Number Sequencing: Individuals are read a sequence of numbers and letters, and are asked to first recall the numbers in ascending order and then letters in alphabetical order. For example, "T4L5Z2H" recalled as "245 – HLTZ". The test involves auditory short-term memory, sequencing ability, concentration and attention, and the flexibility to reorganise the initial sequence according to a simple rule.

Processing Speed Index

Coding (previously called Digit Symbol)*:* Individuals copy symbols that are paired with digits or simple geometrical shapes, within a specific time period. The test involves psychomotor speed, ability to follow directions, clerical speed and accuracy, visual short-term memory, paper-pencil skills, ability to learn an unfamiliar task (capacity for learning and responding to new visual material), some degree of flexibility (ability to shift mental set), capacity for sustained effort, attention, concentration and mental efficiency, associative learning and ability to imitate newly learned visual material, and sequencing ability.

Symbol search: Individuals decide whether a set of symbols (target group) match any of the symbols in a search group, within a specific time limit. The test involves speed of visual search, speed of processing information, planning, encoding information in preparation for further processing, visual-motor coordination, and learning ability.

Cancellation: Individuals scan a structured arrangement of coloured symbols and mark all the target symbols, avoiding distracter symbols, within a specific time period. The test involves perceptual recognition, perceptual discrimination, perceptual scanning ability, speed and accuracy, attention and concentration, and visual-motor coordination.

General Ability

Culture Fair IQ test	This test is designed to test non-verbal intelligence in a way that minimises cultural and educational biases. It is based on image patterns (like the Raven's Progressive Matrices). The individual is shown a large "pattern" with a missing element and must select the missing element from a choice of six. The test is graded such that it becomes progressively more difficult to determine the missing element, which requires logical reasoning with visual-spatial problems. It contains four kinds of spatial problems (i.e., series completions, odd-man-out, matrices and topology) and is indicative of fluid IQ. The test is highly correlated with the WAIS full scale IQ and many other IQ tests.
Wide Range Achievement Test	This test has been designed to provide measures of the basic academic skills needed for effective learning. It includes subtests on reading, reading comprehension, spelling, and number skills. The main aim of the test is to assist in determining an individual's eligibility for special educational services. The reading subtest includes letter identification and word decoding, and the ability to translate sounds into written form. The reading comprehension subtest involves the ability to gain meaning from words and comprehend ideas and information contained in sentences with content drawn from a range of non-specialised topics (usually "fill in the missing word" variety). The spelling subtest involves letter writing and spelling, but also includes the ability to correctly pronounce words. The number skills subtest assesses an individual's ability to perform basic maths problems through counting, identifying numbers, solving simple oral problems, and calculating written maths problems.
Mini–Mental State	In this test the individual answers a series of questions assessing basic cognitive abilities that include orientation, language, memory, and

Examination (MMSE)	attention. It is widely used as a brief screening test for dementia. There a total of 30 questions which are divided into 11 major areas: temporal orientation (e.g., what is the date? 5 points), spatial orientation (e.g., where are we? 5 points), immediate memory (e.g., immediately repeat three words that are said: 3 points), attention/concentration (e.g., count backwards in 7's from 100 or spell WORLD backwards: 5 points), delayed recall (e.g., ask for the three words repeated earlier: 3 points), naming (e.g., point to an object and ask "What is this?": 2 points), verbal repetition (e.g., say after me – No ifs, ands, or buts: 1 point), verbal comprehension (e.g., give a three stage command: 3 points), writing (e.g., write a sentence - 1 point), read a simple sentence (e.g., close your eyes) and do what it says: 1 point), and constructional praxis (e.g., copy a pair of intersecting pentagons: 1 point). The score on the test (max = 30) is usually reported along with the scores on the five domains of (1) orientation (max=10), (2) memory (max=6), (3) attention and calculation (working memory, max=5), (4) language (max=8), and (5) design copying (max=1). Typically a score of ≤27 is suggestive of dementia, especially if score on any one of the five domains is two points below the maximum.

Language

Test	**Description**
Boston Naming Test	In this test, the individual is shown a line drawing and asked to provide the name of the item depicted (e.g., shoe, violin) within 20 seconds. There are 60 drawings which are graded in terms of difficulty (e.g., bed … wreath … protractor). This is a test of confrontation word retrieval (naming) and is frequently used with individuals who have word-finding difficulties (e.g., aphasia). In individuals with obvious word retrieval difficulties, the examiner may prompt the individual by providing various kinds of clue (e.g., initial sound, semantic category). A 15-item short form is available. There is also multiple-choice version which is used to assess comprehension of any words not generated on the confrontation naming component.
Graded Naming Test	In this test, individuals are shown black-and-white drawings of objects and asked to name them. The 30 items are presented in ascending order of difficulty. The test starts with highly familiar objects (e.g., kangaroo, scarecrow) and ends with more unusual and difficult items (e.g., cowl, retort). One mark is given for each correct name. As with the Boston Naming Test (above) it assesses confrontation word retrieval.
Peabody Picture Vocabulary Test	In this test, the individual must select and point to one of four pictures that best fits the vocabulary word they hear (e.g., "Show me the table"). The words are selected from the most recent version of Webster's dictionary, and the pictures are usually black-and-white line drawings, although more recent versions use colour pictures (e.g., PPVT-4). This is a test of receptive language (hearing vocabulary), although the norms do not include those with hearing or visual impairments (e.g., uncorrected vision),

Test	Description
	or those who have a limited proficiency in English. Scores on the test are highly correlated with the verbal IQ measure from the WAIS
WAIS III: Verbal IQ	See also the subtests associated with intelligence for other tests where language is important.
Thurstone Verbal Fluency	In this test, individuals must write as many words as possible beginning with the "S" in five minutes. In addition, individuals are then asked to write as many words as possible beginning with the letter "C" in four minutes, but the words must contain four letters only. The test is sensitive to literacy skills, vocabulary size, and the ability to organise the words into semantically related clusters. If given over 4-5 minutes, writing speed is less influential than when the generation phase is shorter (e.g., 1-minute). This is similar to the controlled oral word test, and the FAS test. Typically, the words are written down by the individual. When oral generation is requested, attention is paid to word repeats as their presence may indicate episodic memory problems.
ACTS: Syntactic Reasoning	This is a test of verbal reasoning using a linguistic manipulation and a conceptual manipulation to vary difficulty. In this test, the individual is presented with a sentence and a pair of letters and has to decide if the sentence correctly describes the order of the pair of letters (e.g., "A is followed by B. AB" or "A does not follow B. AB" - where the correct response is YES or TRUE to both statements). Eight different statement types are presented, for several replications that combine: (a) the voice of the statement (active/passive voice), (b) the presence of negation (positive/negative statement) and (c) truth (where the statement can correctly or incorrectly describe the order of the letters). The speed and accuracy of verifying the statement are the main outcome measures. In general, statement difficulty increases from positive-active, through positive-passive and negative-active, to negative-passive – as indexed by both speed and accuracy. The voice factor indexes language facility and the negation factor a working memory component associated with conceptual manipulation. The main interest is in how performance changes with statement complexity, with general sensory-motor slowing being identified as slower reaction times that are independent of statement complexity.

Perception

Test	Description
Benton Visual Discrimination Test	In this test, the individual is shown an arrangement of different geometrical items in a top panel and four choices in a bottom panel. The individual has to select from the four choices the item that matches to the top panel. Aside from the correct choice, the three erroneous choices include (a) an error in rotation or displacement of a major item, (b) an error in the rotation or placement of the peripheral item, and (c) an

Test	Description
	error in which one of the major items is “distorted” (e.g., it has an additional line or slightly different shape; pentagon or hexagon). There is no time limit for the test. The scoring system gives 2 points for a correct choice, 1 point where only the peripheral item is wrong (b above), and zero for the other two errors. There are 16 forced-choice arrangements. The test is sensitive to visual search (for comparing the top panel with the alternative), complex visual discrimination, and matching. The test does not involve memory as the item to be matched and the four choices are always present.
Poppelreuter Test	This is a simple test of visual perception. The individual is shown an image containing several partially overlapping line drawings and has to identify all the items they can see. The test is sensitive to the ability to make figure-ground discriminations and name the individual items identified (e.g., hat, glove, umbrella, etc.). The score is based on the number of items correctly identified.
Stereognosis	In this test, the individual has to recognise common objects (e.g., key, comb) using only the information conveyed through the medium of touch (e.g., edges, texture, shape, size). The test is sensitive to the ability to perceive and integrate touch information to interpret (and thereby) identify objects placed in the hand. As normally individuals can rapidly and accurately determine the identity of three dimensional objects by touch, only profound difficulties in somatosensory perception are detected.

Visual-spatial Tests

Test	Description
Hooper Visual Organisation Test	In this test, the individual is presented with 30 line drawings, one at a time, with each item shown on a single page. Each of the drawings shows a common object (e.g., football, fish, sailboat, and church) that has been cut into several pieces and the pieces are shown “scattered” across the page like parts of a puzzle. The individual is asked to say what the object would be if all the pieces were put back together correctly. There are no time limits and individuals are asked to guess when uncertain. The score is the number of drawings correctly identified. Aside from normal visual perception, the test is primarily sensitive to visual analytic and synthetic abilities (e.g., integration).
Rey-Osterrieth Complex Figure	In this test, the individual is asked to reproduce a complicated line drawing containing several component geometric shapes in an unusual arrangement. Individuals are first asked to copy the figure (which remains on view). After a delay the individual is asked to draw the figure again from memory – usually immediately after the drawing is removed from view and again some 20-30 minutes later. This widely used

Test	Description
	neuropsychological test was developed by Rey (1941) and standardised by Osterrieth (1944) as a test of visual-spatial drawing ability and visual memory. There are a range of scoring systems in place for evaluating the accuracy of the drawing (e.g., the Boston system), although common components relate to the location, accuracy and organisation of the 18 component parts. The test is sensitive to impairments of visual-spatial perception, reconstruction and memory, with poor performance being associated with increasing age and poorer education. Impairments in higher level executive functions (e.g., planning) are also associated with poorer scores.
WAIS: Block Design	Individuals use up to nine red-and-white blocks to re-create a model or picture of a design, within a specific time limit. The task requires analysis of whole into component parts, spatial visualisation, non-verbal concept formation, visual-motor spatial coordination, perceptual organisation, capacity for sustained effort (concentration), and manipulative and perceptual speed.
WAIS: Spatial span	In this task, the individual is shown a spatially arranged series of blocks and has to repeat, in the correct order, the sequence of taps performed by the examiner. The sequence of taps increases across trials. The task is similar to that of the digit span task, but in this case the span length concerns the reproduction of locations in space rather than digits. Also like the digit span task, both forward and backward sequences can be used. The test involves immediate rote recall, ability to shift thought patterns from sequences forwards to sequences backwards, concentration or attention, and spatial location sequencing. The task is also known as the Corsi blocks test.

Attention and Working Memory

Test	Description
'A' Cancellation test	Letter cancellation tasks are paper and pencil tests widely used in clinical and research settings. In the "A" cancellation test, the individual has to striking through all occurrences of "A" and avoid striking through non-A letters. Variations include striking through other letters, and sometimes increasing the number of letters held in short-term memory (e.g., strike through occurrences of "A, C and X"). It is sensitive to attention/concentration, visual-spatial scanning abilities, motor response speed, and visual-spatial dysfunctions such as visual neglect.
Auditory consonant trigrams	The individual is presented with three consonants (e.g., B-D-S) and has to remember the consonants. Various delays are used (e.g., 0, 9, 18, and 36 seconds) and during this delay period the individual is required to count backwards (aloud) in three's (e.g., 100-97-94); different

Test	Description
	numbers are used in each trial. Among other things, the test is sensitive to the ability to retain information following a period of distraction.
Bourdon-Wiersma Vigilance	This is a dot cancellation test. Individuals are asked to cross out all groups of four dots in a large array (37 x 25) containing groups of three, four and five dots. The dots are crossed through with a pen to provide visual feedback on the current location. The number of “four dots” detected in a fixed period of time is the usual measure of performance, although the number of omission and intrusion errors is also sometimes used. As the number of “correct” dots on each line is the same, time spent on each line of the matrix is used to provide an index of variability (vigilance). Among other things, the test involves fine visual perception, vigilance, sustained concentration, and visual-motor response speed.
Continuous Performance	There are many variations in the format of this test, but all involve individuals being presented with a repetitive "boring" task for which they must maintain their focus over a period of time. They respond to targets (which could be visual or auditory, numbers, letters, symbols) and inhibit response to non-targets. Typically, target frequency is low, so that individuals only respond occasionally, but need to remain alert throughout. Performance is scored as correct detections, detection reaction time, omission, and intrusion errors. Sometimes the task is made more difficult by having individuals respond only to certain sequences (e.g., respond when you see the number two, but only when it follows another even number). For errors, typically omissions index inattention and intrusions index impulsivity.
Digit span (e.g., WAIS: Digit span)	This requires the individual to do two distinct tasks: the first is to repeat orally presented numbers in the same order, and the second is to repeat the orally presented numbers in reverse order. The test involves immediate rote recall, reversibility, ability to shift thought patterns from digits forwards to digits backwards, concentration and attention, auditory sequencing, and rote learning. See WAIS description above.
Digit Vigilance Test (DVT)	Individuals are asked to find and cross out a number (e.g., 6 or 9) which appear randomly within 59 rows of single digits on two separate pages. The test requires rapid visual tracking, vigilance, accurate target selection, and speed of responding. This simple task is designed to be sensitive to remaining vigilant during rapid visual tracking and accurately select target stimuli from among distractors.
FTMS (Failure to Maintain set)	FTMS is a subscore of the Wisconsin card sort tests. It measures the loss of the correct sorting rule during performance (see Wisconsin Card Sorting Test).

Test	Description
Letter Cancellation	The letter cancellation test is a variation of the 'A' cancellation test (see earlier) with a single 8.5"x11" sheet of paper containing 6 lines with 52 letters per line, and the target letter (e.g., H) presented 104 times. As a test designed to look at hemispheric neglect, in this particular test the stimulus letter occurs equally often on the left and right hand side of the paper.
Paced Auditory Serial Addition Test (PASAT)	The PASAT is a test of immediate memory and sustained attention. In PASAT, individuals are presented with a sequence of single digit numbers where the two most recent digits have to be summed. For example if the three digits were "3", "5" and "7", the individual would respond "8" and "12". The individual must respond before the presentation of the next digit before an answer is classified as correct. Typically a series of practice trials are given before the collection of data, to familiarise the individual with the test requirements. In the auditory version the digits are presented by audiotape, first at a rate of 3 seconds per digit (PASAT3), then, in a second trial, at a rate of 2 seconds per digit (PASAT2). Although scores are usually reported separately for the two presentation rates, sometimes the sums of correct responses for the 3- and 2-second forms of the task are combined. The task can also be presented visually (PVSAT), in which case, scores are generally higher. The test is sensitive to immediate short-term working memory (for one item) and speed of encoding and adding the digits.
Seashore Rhythm (Rhythm test)	In this test, thirty pairs of tape-recorded, non-verbal sounds are presented and for each pair the individual decides if the two sounds are the same or different (e.g., by marking "S" or "D" on an answer sheet). The pairs are grouped into three subtests. This test is also called the Seashore Rhythm Test, and is based on the Seashore Tests of Musical Ability. The test is sensitive to auditory discrimination between non-verbal sounds and assumes normal hearing.
Selective Attention	A generic term. Typically these tests can use auditory or visual stimuli, and they require the individual to focus on one aspect of a stimulus (e.g., identity) and to ignore another aspect of the stimulus (e.g., colour, size, position). One example of this is the Stroop test where, in the "interference" condition, the individual has to focus on the colour in which the word is printed, and ignore the meaning of the word.
Stroop Test	In this test the individual scans a printed page containing the names of colour words that are printed in a different colour ink to the colour word, They must say aloud, as quickly and accurately as possible, the ink colour in which the word is written. The individual must therefore selectively attend to the colour of the font and ignore the colour meaning (e.g., RED written with GREEN ink, where the correct response is GREEN). As the meaning of the colour word is typically activated faster than the colour of the ink, the individual must inhibit the response based

Test	Description
	on the word's meaning. The time taken to read the list of words is the usual outcome measure with slower times indicating poorer selective attention to the font colour. However, to assess appropriately the selective attention aspect, two additional tests are usually also administered. The first is to ask the individual to read aloud a list of colour words written in BLACK ink (i.e., speed of activating the word meaning). The second is to ask the individual to say aloud the names of the colours in which a list of non-words (usually XXXX) is printed (i.e., speed of accessing the names of colours). Together they allow for the separation of the speed of naming (a) colour words and (b) colours. However, these two control conditions are rarely employed in neurotoxicological studies, only the incongruent colour-word condition being used. The test is sensitive principally to selective attention (to font colour), inhibition of dominant responses (word meanings) and mental flexibility. Poor literacy skills and education generally produce less interference as the word meaning is less strongly activated. Similarly, poor visual acuity, which interferes with the ability to read the word, produces less interference.
Sequence A and B	The same as Trails A and B or Trailmaking A and B (see below).
Trailmaking A and B	Both parts of the Trailmaking Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the individual draws lines to connect the numbers in ascending order as quickly as possible without lifting the pen or pencil from the paper. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the individual draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The time to make the complete "trail" is recorded. If an error is made, then this is pointed out immediately and needs to be corrected. Thus, errors affect the score as they are included in the completion time for the task. It is unnecessary to continue the test if the individual has not completed both parts after five minutes have elapsed. The test is sensitive to motor speed, visual scanning and letter/number detection. The Trails B is additionally sensitive to "set-shifting" (as the individual must switch between letters and numbers) and working memory (as they need to remember where in the letter-number sequence they are).
WAIS: Arithmetic	The individual is given 14 mental arithmetic brief story type problems to solve (see above). It is sensitive to short-term working memory and distractibility as well as numerical reasoning.
Word Memory Test	This computer-based test employs a series of verbal memory tasks that most individuals can perform without error. Individuals are presented with 20 word pairs (e.g., rat-tail). The words appear in pairs: one word is presented followed by the next 1 second later. The pair disappears and another set is presented 2 seconds later. The list is presented twice and

Test	Description
	then the individual is asked to recall as many word pairs as possible. Various assessments of memory can be undertaken including: immediate and delayed recognition memory, a multiple choice assessment with five choices. The test is designed to be virtually insensitive to all but the most extreme forms of impairment of learning and memory, and the range of genuine scores is very narrow. Thus, poor performance on this test is usually taken to imply low effort or malingering.

Other Memory Tests

Test	Description
ACTS: Category Search	In this ACT System subtest, individuals are shown 40 words sequentially and for each word have to decide, as quickly and accurately as possible, whether each word describes a member of a semantic category (e.g., birds). There are 20 words that belong to the category and 20 words that are not members of the category. For category members, there are 10 words that are good examples of the category (e.g., robin), and 10 words that are poor examples of the category (e.g., penguin). For the non-category words, there are 10 that are related to the category through a common superordinate (e.g., lion) and 10 words that are unrelated to the category (e.g., rose). In this part of the test, the speed and accuracy of decisions are assessed. Typically, good examples of the category are classified faster than poor examples, and words related to the category are classified slower than words unrelated to the category. The test is designed to look at the relative difficulty individuals have with access to semantic memory for common categories. General slowness of sensory-motor abilities show as slowing that is unrelated to the type of decision. Typically, the initial “category search” serves as the encoding phase for later memory – either by recall or recognition – as the task ensures that words are encoded with various degrees of elaboration and this influences later memory. For example, more elaborately encoded items (e.g., related no-category members) are better remembered than less elaborately encoded items (e.g., unrelated non-category members). However, none of these features has been used in published studies of pesticides. Furthermore, when used in the study by Stephens et al. (2004) no distinction was made between the four different decision types and therefore cognitive processing (i.e., semantic) factors cannot be distinguished from sensory-motor factors.
ACTS: Location recognition	In this test, the individual is shown a series of circles on the computer screen that are in different locations. Immediate recognition memory is then probed for one of the locations shown. There are three different memory loads (2, 4 or 6 locations to remember) and the recognition probe occurs equally often in a valid and invalid location. The test is scored in terms of the speed and accuracy of the recognition judgement.

Test	Description
	Typically, reaction times increase, and accuracy declines, with increasing memory load. The test requires good visual acuity and assesses immediate recognition memory for absolute locations as a function of increasing the number of locations to remember. General slowness of responding is indicated by slower reaction times that are independent of the number of locations to remember. In the study reported by Stephens et al. (2004), as only overall speed and accuracy were reported, it is not possible to determine whether changes were due to sensory-motor slowing or impairment of spatial memory.
ACTS: Serial Word Learning	In this ACT System subtest, individuals are presented with a series of 15 words that they have to remember in the correct sequence. Immediately after seeing the words, participants are asked to write down, in the correct sequence, all the words they can remember. They are allowed two minutes to do this. If some of the words are omitted, wrong, or in the incorrect sequence the words are shown again in the original sequence. This presentation-recall sequence continues until all the 15 words are remembered in the correct order or five presentation trials have been given – whichever comes first. The test assesses initial recall ability (first trial) and subsequent learning of the word list (trial-by-trial improvement). As the test requires the correct sequence of words to be learned, it is sensitive to the ability to recall the next word, given the previous word as a recall cue. Typically, recall is again requested after a delay, to assess forgetting from a common criterion (perfect recall), but this has not been implemented in studies of pesticides. In addition, rather than reporting initial memory followed by the rate of learning over five trials, Stephens et al. (2004) reported only the sum of words successfully recalled over three learning trials.
Benton Visual Retention test	In this test individuals are shown a geometrical design comprising spatially separated shapes (typically for 10 seconds). In some studies, the individual is required to recall the design immediately and draw it on a piece of paper (more accurately described as the Benton Visual Reproduction Test). However, the test is most commonly administered in the forced-choice recognition format, in which the individual has to select (from four different spatial arrangements of the shapes) which design they saw. The test is untimed. The test is sensitive to problems with visual perception, visual recognition memory, and in the case of recall (via drawing) visual-constructive abilities. If the recall (drawing) of the design is used, the design produced is professionally scored with reference to the quality and accuracy of the form, shape, pattern, and arrangement of shapes on the paper.
Brief Visual Memory Test	The Brief Visuospatial Memory Test (BVMT) exists in two forms. In the original form, individuals are required immediately to draw (reproduce) as many geometrical designs as possible from an array of six presented for 10 seconds. Often a delay recall trial (e.g., 25 minutes later) is also administered. In the revised version (BVMT-R), six designs are shown in a grid containing six locations (as in the original) and the individual draws

Test	Description
	the designs in the correct location with no time pressure. This initial trial is then followed by two additional learning trials, using the same designs in the same locations, to assess learning. This is followed by a 25 minute free recall test, and a final yes-no recognition memory test is then conducted to assess retention of the shapes (12 items: six targets and six distractors). The test is scored by giving one point for the correct shape and two points for the correct shape in the correct location. There are six alternative forms of the test, using different designs. The test requires good visual acuity and is sensitive to memory for visually presented information. In the revised version, visual learning is also assessed.
California Verbal Learning Test	In this test, individuals are orally presented with 16 words (four words from each of four different semantic categories such as fruit, herbs, etc.) over five trials. After each presentation, the individual is required to freely recall as many words as possible. Following these five trials, a second list (interference list) is given for a single trial. Free and category-cued recall is then requested (short-delay) for the first list. After 20-minutes (long delay), during which non-verbal testing occurs, free recall and category-cued recall are again requested for the first list. Finally, the ability to recognise the words on the first list is tested. The CVLT is a commonly used neuropsychological test, and there are many measures of performance that can be derived from it. Although a number of process measures can be derived from the test (e.g., semantic clustering, speed of learning, learning consistency, intrusion from the second list), the five measures most often reported are the cumulative free recall measure from trials 1-5, the short and long-term free recall and category-cued recall scores, and the long term recognition memory score.
MMSE: recall	This is a component of the Mini Mental State Examination (MMSE), described earlier. In this subtest, the individual must remember the names of three unrelated common objects. While the three words (e.g., comb, book, glass) can be repeated up to six times until the individual recalls them all, only recall following the first presentation is scored. This simple test is insensitive to all but a profound impairment in short-term auditory memory.
Oregon Dual Task	Individuals are presented with a five-digit number, followed by a distractor task (counting backwards) for 5, 15 or 25 seconds, followed by a forced-choice decision between the original number and an incorrect five-digit number. Speed and accuracy are measured for the forced-choice recognition memory decision and the distractor task. As virtually all individuals can do this task perfectly, it is used primarily as a test of malingering or lack of motivation to perform.
Paired Associates	Paired associate tasks come in many forms, but all require individuals to associate two items together, and then recall one item given the other item as a cue. In verbal paired associates, two words are presented together (e.g., house-fire), with typically between 5 and 10 pairs being presented during the learning phase. After each learning phase, the first word of each pair is presented (e.g., house) and the individual has to

Test	Description
	recall which word it was paired with it (e.g., fire). To assess learning, usually 3 to 10 presentations of the paired associates are given and the pairs are presented in a new random order. The presentation can be auditory or visual, and recall is either oral or written. Another variation involves spatial-pattern pairing. During learning, a number of boxes are displayed on the screen. Each box is then "opened" to reveal a pattern and then "closed". The patterns are then displayed in the middle of the screen, one at a time, and the individual must touch the box in which the pattern was originally located. Another variation is called the selective reminding paradigm. Here, when the pairs are re-presented, not all pairs are shown (standard version). Rather, only those pairs that that were incorrect are presented. However, all pairings are assessed during the cued recall phase. The number of pairings correct on each learning trial, and occasionally, the nature of the errors (e.g., omissions or intrusions) are recorded. In computer based variations, the time needed to make the response is recorded.
Rey Auditory Verbal Learning Test (RAVLT)	Individuals are given a list of 15 unrelated words (usually verbal, but can be visual) and asked to repeat them. This presentation-recall procedure is repeated over five trials. Then a second list of 15 unrelated words is given (distractor list) and the participant must recall this list. Participants are then asked to recall the original list of 15 words. For delayed recall, the recall of the first list is requested after 30 minutes. This is similar to the California Verbal Learning Test (CALT), but the words do not comprise distinct semantic categories. The test provides scores for assessing immediate memory, learning, susceptibility to interference (proactive and retroactive), and retention of information after a period of distraction. Memory recognition can be assessed with a suitable recognition test – this includes old words (the first, and sometimes second, list items) together with new words.
Story Recall A and B	The story recall test (SRT) is a variation on the WMS-III logical memory test. It can also be referred to as the Auditory Comprehension Test. In this test the individual is read up to 30 short stories, which have been recorded in a professional studio. The stories can be played via computer speakers or headphones. Reliable measures of immediate and delayed recall require more than one story to be presented (e.g., Story A and Story B), with the presentation of five stories being recommended. Standard procedures are applied to score the information recalled.
Wechsler Memory Scale III (WMS III)	This is an updated version of the Wechsler Memory Scale Revised (WMS-R) and has now been superseded by the WMS-IV. A full description is beyond the scope of this summary, but a good review of the development of the various versions can be found in Kent, P. (2013). The evolution of the Wechsler Memory Scale: A selective review. *Applied Neuropsychology: Adult, 20*(4), 277-291. The WMS-III provides subtest and composite scores that assess memory and attention functions, using both auditory and visual stimuli. Six

Test	Description
	subtests are used to provide information on eight primary indexes. Each subtest is briefly described below: Auditory Memory Index: Two tests (1) the logical memory test – immediate and delayed recall of a simple story (see earlier description) and (2) verbal paired associates – immediate and delayed recall of paired associates (learn eight pairs of unrelated words across four learning trials). In the latter, the word pairs are read (e.g., house-paper) and the individual responds verbally with the second word (e.g., paper) given the first word (e.g., house) as a recall cue. Visual Memory Index: Two tests (1) the Faces test – immediate and delayed recognition of 24 colour pictures of human faces and (2) the Family Pictures test – immediate and delayed recall of four pictures (each seen for 10 seconds). For each picture, the individual has to recall (a) which family members are in a scene (characters), (b) where they are located in that scene (location), and (c) what they are doing (activity). Working Memory Index: Two tests (1) Letter-Number sequencing and (b) Spatial Span (both subtests have been described earlier). The eight indexes are: auditory immediate, visual immediate, immediate memory; auditory delayed, verbal delayed, auditory recognition delayed; general delayed memory; working memory.
WMS-III: Logical memory	This is a subtest of the WMS-III. The individual hears a short story and is asked to recount the story immediately after it is read to them (immediate memory), and after a 1-hour delay (delayed memory). The story incorporates 25 specific points or "story elements" and 1 point is given for each element recalled. To assess forgetting, the percentage of information retained from the immediate to the delayed recall can also be calculated. The test is sensitive to the individual's immediate memory for the 25 elements of the simple story, with the percentage of information retained being sensitive to the ability to retain this information following a period when it cannot be rehearsed (i.e., forgetting).While sensitive to problems in understanding auditory information, integrating this into the gist of the story, is not sensitive to sensory-motor deficits as the story is recalled orally.
WMS-III: Visual Memory	This is an optional subtest of WMS-III. In this test the individual is shown a design (line drawing) for 10 seconds. The design is then removed and the individual is instructed to draw the design from memory. The next design follows, for a total of five different designs. The individual is asked to draw all the designs again after a delay of at least 1 hour. The designs are scored according to their accuracy. The total score is the sum of the scores for all five designs. Immediate and delayed scores are calculated separately, and from these the percent retained score can also be derived. Recognition, copying and matching conditions are also available.

Executive Functions

Test	Description
Control Oral Word Association Test (COWAT)	In this test, the individual must orally generate as many words as possible in one minute that begin with a certain letter. One minute is typically allowed for each of the letters: C, F, and L. This is a phonemic fluency test and is contrasted with semantic fluency tests in which individuals generate the names of things that belong to a semantic category (e.g., animals). The test is sensitive to access to vocabulary knowledge in long-term memory, and also requires executive control over processes such as selective attention, set shifting, and self-monitoring. A very similar test is the FAS test, which uses the letters F, A, and S.
Short Category Test	The short category test is an abbreviation of the full Halstead-Reitan Category Test, and uses the first four subsets of the original scale (108-items). The short form comprises five subtests, and as in the original Category Test, the stimuli are various geometric shapes, lines, colours, and figures. Each subtest is organised around a single principle and always uses the numbers 1, 2, 3 or 4 as responses. For example, in the first subtest, the individual is presented with a series of Roman numerals (I to IV) and selects the number that best represents the figure. As another example, the individual may be presented with four geometrical shapes in a row (three circles and a square) and has to work out which number to press (e.g., the rule may be that the number refers to the position of the odd item in the sequence). Auditory feedback (chime = correct, buzz = wrong) is provided so that the rule is learned within the subtest. The principle used to classify the stimuli changes with each subtest. The usual score is the number of errors made, summed across the subtests. The test is sensitive to non-verbal concept formation and requires reasoning and logical analysis.
Tower of Hanoi Test	The *Tower of Hanoi Test* is a test of problem solving and strategy development. Typically, there are three rods arranged vertically on a wooden platform and a number of disks of various sizes that can fitted on any rod. The problem starts with all the disks arranged in ascending order of size on the first rod; with the smallest disk on top. The task is to move all this entire stack of disks to the third rod according to two rules: (a) only one disk can be moved at a time and (b) no disk can be placed on top of a smaller disk. With three discs the problem can be solved in seven moves, four discs in 15 moves, and five discs in 31 moves. In addition to assessing problem-solving abilities (including memory for previous moves and strategy discovery), the test is affected by visual-motor coordination.

Test	Description
Wisconsin Card Sorting Test	In this test, individuals are presented with a series of stimulus cards and told to match the cards, but they are not told how to match them. After each match they are told whether they are right or wrong. There are usually three stimulus parameters (colour, shape and number). Initially, the classification rule is unknown to the individual and they need to discover this rule based on the feedback (e.g., based on the colour). Following successful rule discovery, the rule is changed (unknown to individual) and the new rule needs to be discovered and, once discovered, a new unannounced rule is used. Four measures of performance are usually obtained: how quickly the initial rule is discovered, how long the previous (now erroneous) rule is used (perseveration), how quickly subsequent rules are discovered, and reversion to a previous rule (set maintenance). The test is sensitive to abstract reasoning abilities and the ability to shift cognitive strategies in response to unannounced rule changes. This is a popular clinical neuropsychological test, is sensitive to frontal lobe functioning and requires normal vision, comprehension of instructions, and the ability to differentiate the various stimulus parameters. There are many popular variations of this test, but all incorporate the ability of individuals to shift their classification rules based on unannounced rule changes on the basis of “correct/incorrect” feedback.

Visual-motor Abilities

Test	Description
California Computerized Assessment (CALCAP): Simple reaction time	In this test, individuals press a key as soon as they detect anything on a screen. The test is sensitive to visual acuity, response speed, and general orientating alertness.
California Computerized Assessment (CALCAP): Choice reaction time	In this test, individuals press a key as soon as a specific number (e.g., 7) is presented on a screen, and otherwise they do nothing. Response time is the main measure, although omissions and intrusions can also be measured. The test is sensitive to visual acuity, response speed, and short-term memory demands. It is similar to the simple reaction time test from the CALCAP, but adds a simple memory element to the reaction time task.
Choice Reaction time	Another variation in the assessment of reaction time entails a cognitive decision to determine which of two or more motor responses should be made. Again, the time between presentation of a stimulus and the making of the appropriate response is measured. In a two-choice reaction time task, the individual views a stimulus

Test	Description
	and has to (a) classify the stimulus into one of two categories and (b) press a response button associated with that category (response selection). For example, individuals may be asked to press one button for red stimuli and another button for green stimuli. The type of classification can vary according to the degree of cognitive processing required. It can include simple stimulus locations in space (e.g., left versus right side of the screen) or be based on the identity of the stimulus (e.g., vowel or consonant, musical instrument or item of clothing). The key element of a choice reaction time task is that the individual has to select the appropriate motor response associated with their classification of the stimulus. The level of choice is determined by the number of responses the individual has to make. Typically, more than two-choice decisions are based on the location of the stimulus in space (e.g., a 4-choice reaction time test means that on each trial one of four different responses needs to be selected, based on, for example, which of the four possible stimulus locations was presented). In addition to being sensitive to factors influencing simple reaction time, the test requires ability to classify the stimulus accurately and select the appropriate response.
Finger Tapping	Individuals place their dominant hand palm down, fingers extended, with the index finger resting on a lever that is attached to a counting device. Individuals are instructed to tap their index finger as quickly as possible for ten seconds, keeping the hand and arm stationary. This trial is repeated five to ten times, until the examiner has collected counts for five consecutive trials that are within five taps of each other. Before starting the test, individuals are given a practice session. They are also given brief rests between each 10-second trial, and one- to two-minute rests after every third trial. This entire procedure is repeated with the non-dominant hand. The test takes approximately ten minutes to complete. The test is sensitive to educational level, intelligence, fatigue, general weakness or lack of coordination, depression, and injuries to the shoulders, arms, or hands.
Groove Pegboard Test	The Grooved Pegboard is a manipulative dexterity test. Individuals are presented with a board consisting of 25 holes with randomly positioned slots into which they must insert the pegs. The pegs have a key along one side and this must be rotated to match the slot in the hole before it can be inserted. This test requires more complex visual-motor coordination than most pegboard tests, and is more sensitive to visual acuity and manual dexterity than the simpler pegboard tests.
Lafayette Pegboard Test	This is another variety of a grooved pegboard test. It comprises a pegboard with holes and a tray containing the pegs. Individuals are told that each peg and hole have the same shaped groove (a round side and a square side). The orientation of the peg needs to match that of the hole to be inserted. The individual is asked to pick a peg from the tray and insert it into hole. After a practice,

Test	Description
	they are asked to put as many pegs as possible into the holes, as quickly as possible. The pegs must be inserted in a specific order (left-to-right) with no gaps between pegs. Most versions have spaces for 25 pegs (5 x 5). The test is undertaken with the dominant and non-dominant hand. The length of time taken to put all the pegs in correctly is the usual measure of performance, although the number of dropped pegs and total number of pegs correctly placed can also be measured. The test is sensitive to manipulative dexterity (e.g., finger and manual dexterity) and speed.
Progressive Ratio	This test is based on a schedule of reinforcement that is characterised by requiring the individual to make progressively more responses in order to obtain some reinforcement. The individual is required to work for reinforcement on a fixed-ratio (FR) schedule that consistently gets longer. The pattern of increment is determined in advance by the experimenter. For example, the schedule may start at FR 2 (two responses must be made), then FR 4, FR 8, FR 16, and so on. At some point, the demand of the schedule will be too high, and the individual will quit responding. This point is called the breaking point, which is defined as the last completed ratio. Thus, if the individual made 64 responses and obtained the reinforcement, but then stopped responding, the breaking point would be 64. The progressive ratio schedule assesses how motivated the individual is to obtain the reinforcement
Purdue Pegboard Test	This is a test of manual dexterity and bimanual coordination. The pegboard consists of a board with two parallel rows with 25 holes into which cylindrical metal pegs are placed. The test involves a total of four trials, usually beginning with a brief practice. The subsets for preferred, non-preferred, and both hands require the individual to place the pins in the holes as quickly as possible, with the score being the number of pins placed in 30 seconds. The test measures two types of activities: one involving gross movement of hands, fingers and arms; and the other involving fingertip dexterity. The test is sensitive to deficits in complex, visually guided, or coordinated movements that are thought to be mediated by the basal ganglia.
Pursuit Aiming Test	The pursuit aiming test requires the individual to use a pencil to place one dot inside each of a series of circles that are arranged sequentially in row, and there are several rows of circles. This task is to be performed as quickly as possible for 60 seconds. Errors occur when the individual puts the dot outside the circle or touching the side of the circle. The test is sensitive to visual acuity, visual-motor coordination (e.g., tremor), and the speed of executing fine visually guided motor movements

Test	Description
Simple Reaction time	There are many varieties of "simple reaction time" tests. In essence these tests require minimal cognitive processing and are mainly concerned with measuring the time taken between the presentation of a stimulus (e.g., a light, sound) and the completion of a movement response to that stimulus. For example, the individual might be asked to press a key or button (e.g., space bar) as quickly as possible when a stimulus (e.g., a light) appears on the screen of a computer. In a discrimination reaction time test, the individual is presented with one of two or more different stimuli (e.g., a red light and a green light) and asked to respond to only one of the stimuli and not the other (e.g., press the button for the red light, but not the green light). The test is sensitive to fluctuations in attention and speed of executing simple motor responses. See also California Computerized Assessment Package: simple reaction time
Santa Ana Dexterity	The Santa Ana dexterity test is another manual dexterity test which requires rapid visual-motor (i.e., eye-hand) coordination. The individual sits in front of a base plate containing 48 square holes arranged in rows, with 12 holes per row. There is an equal number of pegs having a cylindrical upper part and a square lower part. These pegs are placed in the square holes and each peg is half-black and half-white. The individual is instructed to pick each peg out of its hole, rotate it through 180^{o}, and then place it back in the hole. The individual completes as many of these "rotations" as possible in 30 seconds. The two coloured half's of the pegs provide visual feedback on the accuracy of this rotation. The test is normally completed for the dominant, non-dominant, and both hands together. The ability to use the two hands provides an assessment of coordination. In addition to being sensitive to fine skilled motor movements, it also requires good eye-hand coordination. The number of pegs successfully turned is usually recorded as the test score.
WAIS III: Performance IQ	Most subtests used in the construction of the "performance IQ" measure involve effective visual-motor abilities, especially: picture completion, picture arrangement, block design, object assembly, digit symbol, and symbol search.
WAIS: Digit-symbol substitution test	This is a subtest of the Wechsler Adult Intelligence Scale – see description earlier. At the top of a page, the individual is shown a set of digits, each of which is associated with a symbol. Immediately below the "digit-symbol" pairings, there is a random sequence of digits, each with a blank response box below it. The individual must fill the blank response boxes with the paired symbols as quickly as possible over 90 seconds. In some versions, symbols are presented rather than digits, and the individual enters the paired digits in the blank boxes – this is typically referred to as the "symbol-digit" substitution test. Other pairings are also possible (e.g., "letter-letter" or "letter-digit" pairings).

Test	Description
	The test is sensitive to complex timed fine motor abilities (writing the response), visual acuity, visual scanning, processing speed, and memory for the digit-symbol pairings. The usual score recorded is the number of correct items completed during the allowed time.

Self-report Scales

Test	**Description**
General Health Questionnaire (GHQ)	This self-report scale is widely used in the assessment of psychological well-being and is often used for the detection of psychiatric distress related to general medical illness. Individuals respond to a series of questions (e.g., Have you been getting edgy and bad tempered? Have you found everything getting on top of you?) on a four-point scale (i.e., not at all, no more than usual, rather more than usual, and much more than usual). It is typically sub-divided into four scales: somatic symptoms, anxiety and insomnia, social dysfunction, and depression). Usually individuals rate their responses over the last two weeks, but other time frames are possible. The subscales represent dimensions of symptomatology and not distinct diagnoses, and they are not independent of each other. Therefore it is usual to indicate general psychological well-being using the total score, as the subscores do not necessarily indicate specific psychological disorders. There are variations on the scoring method (0, 1, 2 and 3 or 0 and 1) and the number of items included in the scale: 12, 28, 30 and 60. For example, the GHQ-12 uses 12 questions. The most widely used version is the GHQ-28.
Likert Scales	A Likert scale is a scale of agreement. This type of scale asks an individual for an assessment of the applicability of a series of statements, reflecting their evaluation of a particular belief or attitude (e.g., I am good at staying focused). Individuals indicate their reaction to several statements (e.g., I have trouble staying focused on a job, I have a good memory for names) on a response scale ranging from “strongly agree” to “strongly disagree”. Typically, several statements are used to assess an underlying psychological construct (e.g., attention, memory) and these ratings are summed or averaged to provide a score for that construct. It is usual to give a balance of positive and negative statements about the belief to avoid response bias. The response scale typically has five points (strongly agree, slightly agree, neutral, slightly disagree, strongly disagree) although others can be used (e.g., a nine point scale) with little impact on reliability. Normally, the response scale contains a neutral point, but sometimes this is omitted to force the individual to commit. Albers et al (2004c), for example, used a five-point scale to assess beliefs about

	concentration, memory, problem solving, and reading. In that particular study, individuals were asked to compare their ability now with their ability 10 years earlier (e.g., My ability to concentrate now is worse than 10 years ago"). As this is a self-report measure it is necessarily sensitive to conditions that affect confidence about (e.g., anxiety, depression), awareness of (e.g., metacognitive abilities), or insight into (e.g., amnesia, optimism, prejudice) the belief – that is, they can only be indicative of how the individual views that particular ability. It is well known that self-reported abilities and objectively measured abilities are rarely correlated. Likert scales are also commonly used to assess mood states (e.g., arousal, stress, anxiety, depression) because there are currently no credible alternatives for the "objective" assessment of such internal states.
Profile of Mood States (POMS)	The POMS exists in two forms: The long form consists of 65 adjectives (e.g., cheerful, active, alert, relaxed) that are rated by individuals on a five point scale (Not at all, A little, Moderately, Quite a lot, and Extremely) and the short form consists of a subset of 35 of the 65 adjectives. Usually, the individual is asked to rate their feelings "at the moment", or "during the last week", although other time frames can be used (e.g., during the last month, year). Ratings on the POMS are typically used to derive six subscales representing an individual's affective state: tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, vigour-activity, and confusion-bewilderment. Unlike personality traits, these mood states are regarded as transitory and specific to a given situation, and reflect the individual's evaluation of their subjective well-being.
Symptom Checklist-90-Revised	This checklist uses 90 items to assess current psychological symptoms; typically over the last week. Individuals rate various problems and complaints people have (e.g., Feeling hopeless about the future; Soreness of your muscles; Spells of terror or panic) using a five-point scale (0=none through 4=extreme). Nine primary symptom dimensions are obtained (i.e., Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, Psychoticism) and three global indices are derived from these: Global Severity Index (overall psychological distress), Positive Symptom Distress Index (intensity of symptoms), and Positive Symptom Total (number of self-reported symptoms).
Subjective memory questionnaire (SMQ)	This is one of many varieties of questionnaire-based approaches to self-assessment. Examples include: How would you rate your memory overall? (Poor through to Excellent) or How often do you have problems remembering people's names? (Always through to Never). Individuals may be asked about any aspect of their memory (e.g., How often do you have problems remembering people's faces? shopping lists? etc.). In the version of the SMQ by Bennett-Levy & Powell (1980), individuals

	rate 43 questions on a five-point scale ranging from “very bad” to “very good”. Questions include those requesting the individual’s assessment of their memory for people’s names, birthdays, telephone numbers, shopping lists, appointments, song lyrics, jokes, public travel information, previous actions and directions. Formal analysis of this questionnaire indicates a fragmented, multidimensional structure which, except for the first factor, was not possible to interpret psychologically. Typically, such questionnaires reflect beliefs about the individual’s memory capacity, their use of external strategies (e.g., mnemonics) and self-efficacy. It is well known that negative affective states (e.g., depression, anxiety, and stress) lead individuals to evaluate their memory more negatively. Only in a few rare situations do self-reports of memory functioning and objective memory functioning correspond, and they rarely add information of diagnostic significance. The Finnish version of a SMQ has been incorporated in the WHO core battery. This contains 39 items that are responded to as yes/no (e.g., Do you often feel tired after work? Do you have difficulty to concentrate? Do you often have headache?). While no formal analysis of this scale is available, it can be reported according to “pre-existing” categories (i.e., fatigue, sleep disturbances, absent-mindedness, depression, lability, neurovegetative symptoms, gastrointestinal symptoms, peripheral and central neurological symptoms) or as the “total number of symptoms”. This format is not a Likert scale – although it is sometimes referred to as one.