



Committee on Toxicity of Chemicals in Food,
Consumer Products and the Environment

ORGANOPHOSPHATES



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Consumer Products and the Environment

Organophosphates

Chairman
&
Chairman of the Working Group
on Organophosphates:
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I. Executive Summary

1.1 This report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment considers whether single, prolonged or repeated exposure to low doses of organophosphate compounds (OPs) can cause long-term adverse health effects. Low doses were defined as those which do not produce overt acute (short-term) toxicity accompanied by recognised clinical symptoms or signs of acute toxicity. The report was drafted by a specially constituted Working Group of the Committee.

1.2 For practical reasons the Working Group concentrated on effects on human health suspected of being common to OPs in general (i.e. class effects) rather than considering compound-specific effects. In particular, they focused on neurotoxic effects. Most of the relevant scientific evidence concerned possible neurological, psychological or psychiatric effects and these were the types of illness most frequently attributed to OP exposure by those who made submissions to the Working Group. The composition of the Working Group reflected the need for a detailed investigation of this subject and the Working Group sought expert advice on psychiatric issues.

1.3 The Working Group held a total of fourteen meetings between May 1998 and September 1999 and a draft report was submitted to the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment for endorsement in October 1999. Details of the background to the establishment of the Working Group and its methods of working are given in Chapter 2 of the report.

1.4 Chapter 3 describes the nature of OPs, their chemical structures and biological properties. For the purpose of their deliberations, the Working Group defined OPs as organophosphorus compounds that inhibit the enzyme acetylcholinesterase. Their mode of action as inhibitors of this enzyme is described and consideration is then given to the various uses of OPs as pesticides and veterinary medicines, the co-formulants used in such products, and the regulatory process for approval and licensing these products in the United Kingdom.

1.5 Chapter 4 summarises the various ways in which individuals may be exposed to OPs. These include exposure to trace amounts in food and water, through the use of household or garden insecticides, and in the treatment of headlice. Consideration is also given to occupational exposure such as that during orchard spraying and, in particular, sheep dipping. The fate of OPs in the body is described, with sections on their absorption, metabolism and excretion.

1.6 The toxicology of OPs and the mechanisms involved in their acute cholinergic effects and in the induction of delayed polyneuropathy are considered in Chapter 5. The value of the hen test for screening compounds that induce delayed polyneuropathy is discussed. This chapter also examines the scope for potentiation of toxic effects through concomitant exposure to other compounds and individual variations in susceptibility to the effects of OPs.

Organophosphates 1.7 The report then considers the sources of data that were relevant to the Working Group's remit. In Chapter 6 information provided by individuals in personal testimony, and relating to data held by the OP Information Network and the Pesticide Exposure Group of Sufferers, is described. Many individuals reported long-term illness, often severely impairing normal life, which they believed to be caused by exposure to OPs. Data available from adverse reaction schemes (the Health and Safety Executive's Pesticides Incidents Appraisal Panel; the Veterinary Medicines Directorate's Suspected Adverse Reaction Surveillance Scheme; the Medicines Control Agency's Yellow Card Scheme) and data from the National Poisons Information Service were also considered. However, these were found to be of very limited value in relation to the remit of the Working Group. The consequence was that the Working Group were unable to draw on any substantial body of clinical data. The Working Group were thus faced with a major problem. Although many of the individuals who submitted evidence reported very real, distressing illness, often distinguished by unusual combinations of symptoms, few could provide long-term medical observations or supporting clinical data. Many felt that their problems had been inadequately monitored and investigated. Individual case reports were informative but could not be used to make any assessment of cause and effect.

1.8 Chapter 7 consists of a review of the scientific evidence, largely derived from published scientific papers, describing epidemiological studies that were relevant to the deliberations of the Working Group. The Working Group identified 27 reports of such studies as being the most informative with regard to the potential toxicity of low-level exposure to OPs. These are summarised in detail in Appendix 4, with the Working Group's critique of each. Some of them concern the late sequelae of acute poisoning episodes rather than low-level exposure as defined by the Working Group. These were relevant because any chronic health effects that could be shown to result from acute poisoning might also occur with lower exposures and thus would merit special attention. The Working Group also considered the full report of a major study by the Institute of Occupational Medicine published in July 1999. In view of the importance of this study, which investigated an occupational group of particular concern, namely sheep dippers in Britain, it is summarised in detail in Appendix 5 together with a critique by the Working Group.

1.9 The review in Chapter 7 is divided into five sections covering different types of health outcome relating to the nervous system, namely: neuropsychological abnormalities, electroencephalographic abnormalities, peripheral neuropathy and neuromuscular dysfunction, psychiatric illness, and effects on the autonomic nervous system. Within each section consideration is first given to long-term effects following acute OP poisoning. This is followed by consideration of the effects of exposure to OPs in the absence of any recognised acute poisoning episode. It was, in the main, the Working Group's analysis of these studies that underlay the conclusions set out in Chapter 8.

Conclusions

1.10 Chapter 8 gives the Working Group's considered response to the question posed in their remit, namely to advise on whether prolonged or repeated low-level exposure to OPs, or acute exposure at a dose level lower than that causing overt toxicity, can cause chronic ill health. As noted earlier, the Working Group considered not only the evidence relating to

low-dose exposures (i.e. those insufficient to cause overt toxicity) but also studies on the long-term sequelae of recognised acute poisoning episodes. The rationale for this is described in paragraph 1.8.

1.11 Although it has been proposed that dipper's flu is a manifestation of acute OP toxicity, the Working Group concluded that this is unproven. Thus, for the purpose of this report it was not regarded as an indicator of acute OP toxicity.

1.12 In reviewing the scientific evidence the Working Group focused on the five different health outcomes relating to the nervous system that are listed in paragraph 1.9. Of these, the data on EEG abnormalities and effects on the autonomic nervous system were insufficient to allow any firm conclusions to be drawn. The conclusions, which are those of the Committee, regarding the other endpoints are given below.

Long-term sequelae of acute poisoning

Neuropsychological outcomes

1.13 The balance of evidence supports the view that neuropsychological abnormalities can occur as a long-term complication of acute OP poisoning, particularly if the poisoning is severe. Such abnormalities have been most evident in neuropsychological tests involving sustained attention and speeded flexible cognitive processing ("mental agility"). In contrast, current evidence suggests that long-term memory is not affected after acute poisoning.

Peripheral neuropathy

1.14 Peripheral neuropathy, as one feature of OP-induced delayed polyneuropathy, is a well-established complication of poisoning by OPs that inhibit the enzyme neuropathy target esterase. The neuropathy is predominantly motor but possibly also sensory. Compounds that produce more than 70% inhibition of neuropathy target esterase give positive results in the hen test. Compounds evaluated as giving a positive response in the hen test are not used in the United Kingdom and have not been approved or licensed by regulatory agencies (i.e. the Veterinary Medicines Directorate or the Pesticides Safety Directorate).

1.15 The balance of evidence indicates that acute poisoning by other OPs, which do not inhibit neuropathy target esterase, can also lead to persistent peripheral neuropathy detectable by neurophysiological tests. If this occurs, most cases are not at a level that would give rise to symptoms.

Psychiatric illness

1.16 The limited evidence available does not allow any firm conclusions to be drawn regarding the risk of developing psychiatric illness in the long term as a consequence of acute poisoning by OPs.

Organophosphates **Prolonged low-level exposure**

1.17 In comparison with the positive neurological and neuropsychological findings following recognised poisoning incidents, the evidence relating to chronic low-level exposure to OPs, insufficient to cause overt acute toxicity, is less convincing.

Neuropsychological outcomes

1.18 Although some studies suggest impairment in the same tests that are affected after acute poisoning, others do not. The balance of evidence does not support the existence of clinically significant effects on performance in neuropsychological tests from low-level exposures to OPs. If such effects do occur, they must either be relatively uncommon or so small that they are not consistently detectable by standard methods of testing.

Peripheral neuropathy

1.19 The balance of evidence indicates that low-level exposure to OPs does not cause peripheral neuropathy. If effects on peripheral nerve function sufficient to cause severe disability do occur, they must be rare.

Psychiatric illness

1.20 The available data indicate that exposure to OP sheep dips is not a major factor in the excess mortality from suicide among British farmers. However, in general, the evidence relating psychiatric illness to OPs is insufficient to allow useful conclusions.

Acute exposure to OPs at a lower dose than causes frank toxicity

1.21 No studies have examined the long-term effects of a single exposure to OPs insufficient to cause acute toxicity. However, the findings in individuals with prolonged and repeated low-dose exposures, and in those who have suffered recognised acute poisoning, together indicate that any risk of serious health effects from such limited exposure must be small.

Questions posed to the Working Group by the Official Group on OPs

1.22 In addition to addressing the central question stated in the remit of the Working Group, consideration was given to the specific questions (listed in Appendix 2) posed to the Working Group by the Official Group on OPs. These were modified for clarity and as a result of the evolution of the thinking of the Group over time. Answers to these questions, as modified, are given in Appendix 3.

Monitoring of human adverse effects

1.23 It was a matter of particular concern to some members of the Working Group that the present schemes for monitoring human adverse effects had yielded so few relevant data and that little progress had been made in establishing a relevant clinical database.

Outstanding issues

*Executive
Summary*

1.24 In addition to drawing the above conclusions the Working Group identified outstanding issues, which need to be addressed by further research.

1.25 The major gap in current knowledge relates to the possibility that OPs cause disabling neurological or neuropsychiatric disease in a small sub-group of exposed persons. Most research has focused on people who were in work at the time of investigation, and therefore by definition were sufficiently fit for employment. Moreover, the available published studies have generally been designed to look for effects on the mean level of quantitative health indices in the exposed population, rather than exploring the possibility that only a small proportion of subjects may be at increased risk of clinically significant disease. Thus, although the substantial body of evidence that has now accumulated gives little support to the hypothesis that low-level exposure to OPs can cause chronic disease of the nervous system, it does not exclude the possibility that at least some of the illnesses that were described to the Working Group as following such exposure are indeed a manifestation of toxicity.

1.26 Further investigation, using suitably designed studies, is needed to establish whether the risk of more severe neurological or neuropsychiatric disease is increased by low-level exposure to OPs.

1.27 In view of the widespread public concern about OPs, evident from the response to the Working Group's inquiry, there is an urgent need for further research targeted at the issues set out above.

Recommendations for further research

1.28 The Working Group recommended further research to address the outstanding issues. These were grouped around the following questions, the answers to which would help to clarify the remaining uncertainties:

- What are the most common patterns of exposure, clinical presentation and subsequent clinical course among people in the United Kingdom with chronic illnesses that they attribute to OPs?
- How common is dipper's flu, and what causes it?
- Does low-level exposure to OPs cause disabling neurological or psychiatric disease in a small subgroup of exposed persons?
- Do people with chronic disabling illness that is suspected of being related to OPs differ metabolically from the general population?
- Other than acetylcholinesterase inhibition, what mechanisms play an important role in the causation of adverse health effects by OPs?

2. Introduction

2.1 This report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) considers the evidence as to whether exposure to low doses of organophosphate (OP) compounds can cause long-term adverse health effects. The Working Group on Organophosphates (subsequently referred to as the Working Group) set up by the Committee defined low doses for the purpose of their work as doses lower than those causing overt acute toxicity (i.e. symptoms and signs of acute toxicity). It was not possible to give a quantitative definition of “low dose” in view of the differences in potency of different OPs.

Historical perspective

2.2 OPs are a group of chemical compounds used throughout the world. In the United Kingdom (UK) they have been used in agricultural and horticultural pesticides, some veterinary medicines (particularly in sheep dips to prevent and treat sheep scab and other ectoparasitic disorders, also in flea collars), in human medicines (malathion only – as a treatment for head lice), and in various public hygiene products, both for use by professional operators (e.g. for the control of cockroaches and other insect pests in public buildings such as hospitals, schools etc.) and for use by the general public (insecticides for home and garden use). It is upon OPs used for these purposes that this report is focused. In addition, some OPs have been developed as nerve agents (see paragraph 3.6).

2.3 Ministers are advised by several independent expert committees with regard to the safety of OP products. The Advisory Committee on Pesticides (ACP) advises on the approval of pesticide products, including those used in public hygiene. The Veterinary Products Committee (VPC) advises on the authorisation of veterinary medicines; its Medical and Scientific Panel established in 1993 reports to the VPC on human health issues relating to OP products. The Committee on Safety of Medicines advises on the safety of human medicines. These Committees have considered specific OP products on a number of occasions. Their advice has been that scientific evidence supported the continued use of products containing OPs for certain specified purposes provided that they were used in accordance with instructions. In recent years the regulatory authorities have taken steps to increase safety, including more stringent requirements for protective clothing when using products containing OPs. For example, detailed advice on the safe handling of sheep dips was given in the Health and Safety Executive (HSE) leaflet AS 29 in 1991.¹ This was revised in 1994 and again in 1998. Guidance on biological monitoring of workers exposed to OP pesticides was published by HSE in 1980 and updated in 1986.² This is considered in more detail in Chapter 3 and specifically in the chronology of events in Annex 3A.

2.4 It is well established that acute (i.e. occurring within a few days) effects on human health can arise after exposure to sufficiently high levels of OPs; such effects are referred to as the acute cholinergic syndrome. Although rare in the UK, elsewhere in the world there have been large numbers of cases of severe acute OP poisoning.³ It is recognised that

Organophosphates these short-term effects may sometimes be followed by long-term (chronic) neurotoxic effects. More recently data have become available which suggest that long-term adverse effects on human health may result from exposure to low levels of OPs, which in themselves do not produce symptoms of acute toxicity. If correct, this would have important implications for risk assessment and the regulation of OP products. However, to date there has been no scientific consensus on this possibility. The major source of concern relates to possible long-term neurological effects in farmers who have used OP sheep dips but it extends to people exposed to OP products used for other purposes.

2.5 In response to the uncertainty regarding the potential effects of long-term low-level exposure to OPs research has been undertaken, some of it funded by the UK Government. Government-funded Research and Development relevant to OP compounds was listed in the Report to Ministers by the Official Group on OPs in 1998.⁴

2.6 In order to help the consideration of the scientific issues relating to the effects of OPs, the Department of Health (DH) commissioned a review of chronic neurotoxic effects of OPs in humans. This was carried out by Dr D Ray for the Medical Research Council (MRC) Institute for Environment and Health (IEH) and involved a comprehensive review of the then-published scientific literature. The report was published in June 1998.⁵ It found that the existing evidence was not clear cut, with some studies suggesting small long-term effects from low-level exposure whereas others did not. It also raised the possibility that individuals might vary in their susceptibility to the toxic effects of OPs.

2.7 Other reviews have reached different conclusions. Jamal⁶ concluded that the available evidence supported the existence of adverse effects from long-term low-level exposure to OPs but Eyer⁷ and the European Centre for Ecotoxicology and Toxicity of Chemicals⁸ reached similar conclusions to those set out in the IEH review.⁵

2.8 The Chief Medical Officers wrote to all doctors in 1991 and again in 1993 alerting them to the possibility of adverse effects from exposure to pesticides and certain veterinary medicines containing OPs. The Royal College of Physicians of London and the Royal College of Psychiatrists were asked to advise upon the clinical management of patients with chronic symptoms attributed to OP sheep dip exposure and to review any new clinical evidence for such an association. Their report was published in November 1998.⁹ Regarding the question of long-term low-dose exposure producing chronic sequelae, the report noted that this was currently the subject of much research and that the available data had limitations and the question was left open.

2.9 In 1998 the Government set up a committee of senior officials from relevant Departments to examine and to coordinate activity on OP products and to advise Ministers (the Official Group on OPs). Their report to Ministers was published in 1998.⁴ They recommended that the COT should be asked to consider the evidence for long-term effects upon human health from low-dose exposure to OPs. Ministers agreed to this recommendation.

Terms of Reference for the COT Working Group

2.10 Ministers agreed a list of questions recommended by the Official Group on OPs as a starting point for the COT consideration. These are given at Appendix 2 and addressed in Appendix 3.

2.11 At its meeting in February 1998, the COT decided to set up a Working Group to carry out the review. The following terms of reference were agreed subsequently:

“To advise on whether prolonged or repeated low level exposure to OPs, or acute exposure to OPs at a lower dose than causing frank intoxication, can cause chronic ill health effects.”

The Working Group understood “frank intoxication” to mean overt toxicity.

Membership of the Working Group

2.12 The membership of the COT Working Group is given at Appendix 9. This was agreed by Department of Health Ministers who wanted to ensure that, given the complexity of the subject and the degree of public, parliamentary and media interest, members should be appointed with due regard to their expertise and impartiality. It was considered important to avoid duplicating the membership of the current Medical and Scientific Panel of the VPC, and that members of the Working Group should not be involved with pending litigation concerning OPs.

2.13 It was agreed that the Working Group would be chaired by Professor Woods, a clinical pharmacologist and chairman of COT, and that it should include expertise in clinical neurology, clinical neurophysiology, neuropsychology, occupational health, epidemiology and clinical toxicology. The Working Group also included two public interest members. Professor K Hawton, who had recently carried out research on suicides in the farming community, was invited to help the Working Group in considering psychiatric aspects.

Methods of Working

2.14 The OP Working Group met first on 21st May 1998 and subsequently on thirteen further occasions between May 1998 and September 1999. A draft report was submitted to the COT at its meeting on 19th October 1999.

2.15 The Working Group was supported by a Secretariat provided by officials from the Department of Health. The conclusions set out in this report are those of the members of the Working Group and have been endorsed by the COT. The opinions expressed in the report are independent of any other body.

2.16 For practical reasons the Working Group concentrated on effects on human health suspected of being common to OPs in general (i.e. ‘class effects’) rather than considering compound-specific effects. In particular, they focused on human data relating to the

Organophosphates chronic neurotoxicity of OPs; in addition relevant animal data were considered. The major concerns related to this area and the composition of the Working Group reflected the need for detailed investigation of neurotoxic effects. Possible compound-specific effects, including mutagenicity and reproductive toxicity, are being considered in reviews of the individual OPs used in the UK that are currently being carried out by ACP and VPC.

2.17 A comprehensive search of the scientific literature published up to June 1999 was carried out to ensure that all relevant material could be considered by the Working Group; this material formed the main basis for the Working Group's conclusions. However, other possible sources of relevant data were also explored. These included: information from adverse reaction surveillance schemes, principally the Appraisal Panel for Human Suspected Adverse Reactions (SARS) to Veterinary Medicines of the VPC and HSE's Pesticide Incidents Appraisal Panel; information obtained from the centres of the National Poisons Information Service in the UK; information provided in response to an advertisement in the *British Medical Journal*; and submissions received from interested individuals and groups either in response to an invitation from the Chairman or on their own initiative. The extent to which data from these sources cast further light on the questions posed to the Working Group is discussed in Chapter 6.

2.18 The Working Group felt that it was most important to hear at first hand about the experience of those who suffered from illnesses that might be linked with exposure to OPs. Members of the Working Group met Mrs E Sigmund and colleagues involved with the OP Information Network (OPIN) to hear about their experience. They also met Mrs E Chapman and colleagues concerned with the Pesticide Exposure Group of Sufferers (PEGS) register. Lady Mar made a presentation to the Working Group and subsequently members of the Working Group met Mr P Tyler MP and colleagues from his All Party Group on Organophosphates (Dr I Gibson MP, Mr C Gill MP, Mr E Llwyd MP) together with Lady Mar. Dr D Ray attended the first meeting of the Working Group to discuss his review. The Working Group was also very grateful to Dr G Jamal, Dr D Davies, Dr S Hodges, Dr V Howard, Dr P Julu and Professor A Watterson for attending a meeting to make presentations to Working Group members. Mr R Cooke, Mr P Dobson and Mr D McEwan, representatives of NOAH (National Office of Animal Health Ltd), made a presentation to members of the Working Group. In addition, members of the Working Group met Mrs S Bray, Mr G Cleverton, Mr J Coyte, Mr D Hassall and Mrs J Wheatley. Dr R Rawbone and Dr S Smith from HSE attended a Working Group meeting and answered questions. The Working Group was very grateful to Dr A Pilkington, Dr D Buchanan, Mr F Hurley and Dr S Hansen for attending a meeting to discuss the results of their epidemiological study.¹⁰⁻¹² A list of those who had meetings with the Working Group or with group members is given in Appendix 6. Those who made written submissions are listed at Appendix 7.

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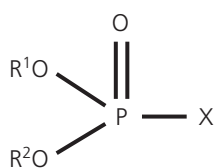
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3. The nature of OPs and their use

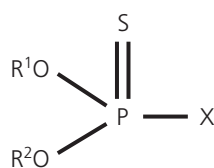
Chemical structures and physicochemical properties

3.1 Organophosphorus compounds include all chemicals which contain both carbon and phosphorus. Many of these do not inhibit the enzyme acetylcholinesterase (see below) and are not used as pesticides. Organophosphate (OP) is a term that can be used to describe all chemical compounds in which a phosphate group, or phosphate derivative, is part of an organic (i.e. carbon-containing) molecule. In practice, and more specifically, it is used to refer to those organophosphorus compounds which inhibit the enzyme acetylcholinesterase. This is the definition used for OP throughout this report and it is these compounds upon which the Working Group focused its attention.

3.2 OPs are usually esters, amides or thiol derivatives of phosphoric acid with the following general formula for (i) phosphates or (ii) phosphorothioates respectively.



(i) Phosphate compound



(ii) Phosphorothioate compound

The P=O containing structure is sometimes referred to as an oxon and the P=S structure as a thion. The R¹ and R² moieties are usually alkyl or aryl groups which may be bonded directly or through oxygen or sulphur atoms (when bonded *via* sulphur the compound is called a phosphorothiolate, see Table 3.1). In phosphoroamidates (see Table 3.1) a carbon atom is linked to the phosphorus atom through an NH group. X represents one of a wide range of substituted or branched aliphatic, aromatic or heterocyclic groups linked to the phosphorus atom through a labile bond. During the process of inhibition of the target enzyme, acetylcholinesterase, the phosphorus atom binds to an amino acid on the enzyme with X being eliminated; the group X is thus often referred to as the 'leaving group'. The lability of the linkage between X and the phosphorus atom is critical with regard to the reactivity of the OP with the enzyme acetylcholinesterase.

3.3 OPs are, as a result of their structure, very reactive chemicals and vary in their biological activities and potencies as acetylcholinesterase inhibitors, depending on the nature of R¹, R², and X. Thions, such as parathion, show lower toxicity in mammals, and usually require metabolism to the corresponding oxygen-containing compound (oxon) in order to inhibit acetylcholinesterase.

3.4 Table 3.1 gives examples of the wide range of structures of OPs that have been used in pesticide products or veterinary medicines. The structure of the nerve agent sarin is also shown.

Organophosphates 3.5 The physicochemical properties of these compounds such as their volatility and lipid-solubility depend on the structures of the substituents R¹, R² and X. OPs which are nerve agent gases, such as sarin, have low molecular weights, with simple R¹ and R² substituent groups and readily displaceable leaving groups (fluoro- and cyano-) which are bound directly to the phosphorus without an intermediate oxygen or sulphur atom. The resulting molecules are both volatile and lipid-soluble. OPs that are used as insecticides are generally far less volatile, are frequently solids at room temperatures, and are more stable in aqueous solution. OPs used as sheep dips have low volatility and high lipid-solubility, and are therefore retained in the lanolin of the fleece.

Biological properties of OPs

Differences between OP pesticides and OP nerve agents

3.6 There are major differences in the biological properties of OP pesticides and OPs used in chemical warfare (nerve agents). OP insecticides are, in general, characterised by their low mammalian toxicity and high acute insect toxicity. This selective toxicity has been designed into the molecule and exploits differences in the metabolism of OPs between mammals and insect pests. Structurally most commercial pesticides are phosphate or phosphorothioates with O,O-dimethyl or O,O-diethyl substituents on the phosphorus atom. To further exploit the metabolic differences between species, some OPs are delivered as 'pro-pesticides' which are, generally, thiophosphate derivatives that are metabolically activated by the target animal species to the proximate organophosphate inhibitors of acetylcholinesterase. The 'phosphorylated' acetylcholinesterase from OP pesticides is, in general, readily reactivated by oxime. Nerve agent OPs have a different chemical structure. In general these are phosphonates, or phosphoramidates (e.g. tabun), in which there is a direct chemical bond between the alkyl substituent and the phosphorus atom. This phosphorus-carbon bond confers a high degree of stability to the OP-inhibited acetylcholinesterase *in vivo* and is in a large part responsible for the toxicity of these compounds, which is orders of magnitude higher than that of the OP pesticides.

Acetylcholinesterase and its inhibition

3.7 Acetylcholine is a neurotransmitter involved in the functioning of neurons within the brain, in the ganglia of the autonomic nervous system, in the parasympathetic nerve endings, and at neuromuscular junctions. It is released in response to nerve stimulation and binds to post-synaptic acetylcholine receptors, thereby transmitting the impulse to the associated neuron or effector organ. The duration of action of acetylcholine is limited by its extremely rapid hydrolysis by the enzyme acetylcholinesterase, which is present in large amounts in the membranes surrounding the synapse or the neuro-effector junction and also in erythrocytes and blood plasma. The hydrolysis of acetylcholine by acetylcholinesterase is shown schematically in Figure 3.1, and involves transfer of the acetyl group from acetylcholine to a serine residue on the esteratic site, followed by rapid hydrolysis to give acetate and the active enzyme.

Table 3.1: Generic structures of some typical OPs

The nature of OPs and their use

Type	Structure	Examples
Phosphate	$\begin{array}{c} \text{O} \\ \\ \text{R}^1\text{O} - \text{P} - \text{OR}^3 \\ \\ \text{OR}^2 \end{array}$	Dichlorvos Chlorfenvinphos
Phosphonate	$\begin{array}{c} \text{O} \\ \\ \text{R}^1\text{O} - \text{P} - \text{OR}^3 \\ \\ \text{R}^2 \end{array}$	Trichlorfon
Phosphorothioate	$\begin{array}{c} \text{S} \\ \\ \text{R}^1\text{O} - \text{P} - \text{OR}^3 \\ \\ \text{OR}^2 \end{array}$	Bromophos Chlorpyrifos Chlorpyrifos methyl Diazinon Parathion
Phosphorothiolate (S-substituted)	$\begin{array}{c} \text{O} \\ \\ \text{R}^1\text{S} - \text{P} - \text{OR}^3 \\ \\ \text{OR}^2 \end{array}$	Demeton-S-methyl Omethoate Profenofos
Phosphorodithioate	$\begin{array}{c} \text{S} \\ \\ \text{R}^1\text{S} - \text{P} - \text{OR}^3 \\ \\ \text{OR}^2 \end{array}$	Dimethoate Disulfoton Malathion Thiometon
Phosphonothioate	$\begin{array}{c} \text{S} \\ \\ \text{R}^1\text{O} - \text{P} - \text{R}^3 \\ \\ \text{OR}^2 \end{array}$	EPN (this is the recognised common name of this pesticide)
Phosphorothioamidate (Phosphoramidothionate)	$\begin{array}{c} \text{S} \\ \\ \text{R}^1\text{O} - \text{P} - \text{N} \begin{array}{l} / \text{R}^3 \\ \backslash \text{R}^4 \end{array} \\ \\ \text{OR}^2 \end{array}$	Isofenphos Propetamphos

Organophosphates **Table 3.1: Generic structures of some typical OPs continued**

Type	Structure	Examples
Phosphorothioamidate (Phosphoramidothiolate)	$ \begin{array}{c} \text{O} \\ \parallel \\ \text{R}^1\text{S} - \text{P} - \text{N} \begin{array}{l} \nearrow \text{R}^3 \\ \searrow \text{R}^4 \end{array} \\ \\ \text{OR}^2 \end{array} $	Methamidophos
Phosphonofluoridate	$ \begin{array}{c} \text{O} \\ \parallel \\ \text{R}^1\text{O} - \text{P} - \text{F} \\ \\ \text{R}^2 \end{array} $	Sarin

Where R¹, R², R³ and R⁴ are chemical groups which may be the same or different. (The reader is referred to reference 4 for more details of the specific structures of these compounds.)

3.8 The process by which acetylcholinesterase can be inhibited by two main classes of compounds, OPs and carbamates*, is outlined in Figure 3.2. In the case of carbamates, the enzyme is carbamylated instead of phosphorylated.

* Carbamates are N-substituted esters of carbamic acid having the following general structure:

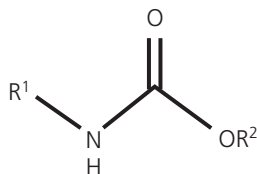
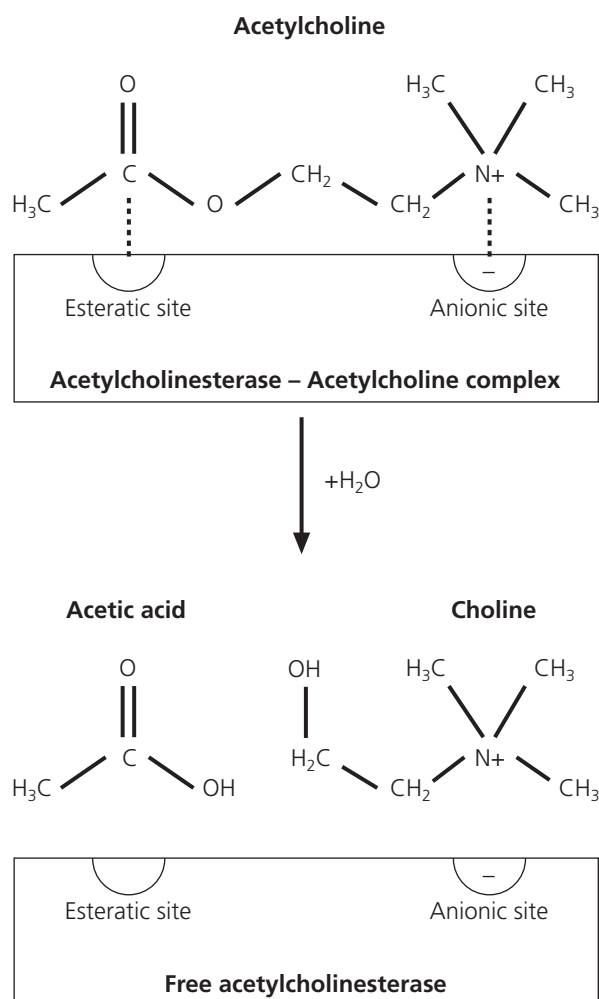


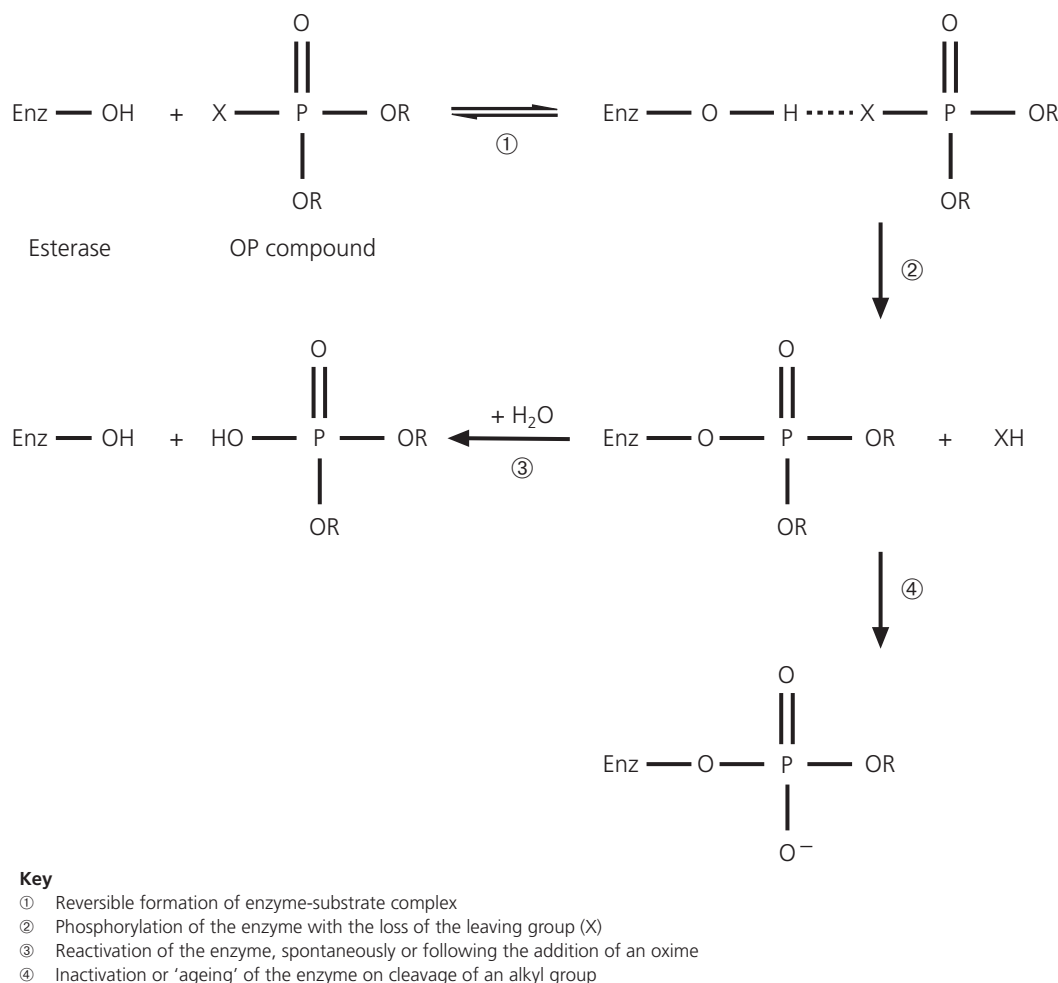
Figure 3.1: Schematic representation of the hydrolysis of acetylcholine by acetylcholinesterase

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3.9 The phosphorylated enzyme may undergo either hydrolysis to yield reactivated acetylcholinesterase, or an irreversible process known as ageing (see Figure 3.2). The relative rate of the two processes depends on the source of the enzyme and the nature of the substituents on the phosphorylated enzyme. The vast majority of insecticidal OPs contain either two methyl R groups or two ethyl R groups so that they produce either a dimethoxyphosphorylated or a diethoxyphosphorylated enzyme. The leaving group does not take part in either the reactivation or the ageing reaction so that the kinetics of reactivation and ageing for each type of phosphorylated enzyme are the same, regardless of the nature of the leaving group. The half-lives for the hydrolysis of dimethoxyphosphorylated and diethoxyphosphorylated human plasma cholinesterase are about 5 and 1400 hours respectively;¹ the corresponding values for the human erythrocyte enzyme are 0.85 and 58 hours respectively.² The presence of secondary or tertiary alkoxy groups increases the stability of the phosphorylated enzyme and further reduces the rate of reactivation. The OPs which have principally been used in sheep dips in the UK produce different forms of phosphorylation product, with chlorfenvinphos and diazinon giving a diethoxyphosphorylated product, and propetamphos an (ethylamino)-methoxyphosphorylated product. As a result, the two principal active agents that are currently used in sheep dips may show differences in the rate of reactivation, in the duration of acute symptoms of toxicity, and in the potential for ageing of the enzyme.

Organophosphates **Figure 3.2: Inactivation ('ageing') of acetylcholinesterase by OP compounds**



3.10 Ageing of the inhibited acetylcholinesterase involves the loss of an alkyl substituent from the phosphorylated enzyme, so that a methoxy (CH₃O⁻) or ethoxy (C₂H₅O⁻) group joined to the phosphorus is converted to an oxygen anion (O⁻), which then stabilises the product and prevents reactivation of the enzyme. The half-life of ageing of human acetylcholinesterase is dependent on the source of the enzyme and the nature of the OP. For example, the half-life for ageing for human erythrocyte acetylcholinesterase is 41 hours for the diethoxyphosphorylated enzyme resulting from interaction with either chlorfenvinphos or diazinon.² The half-life for the product resulting from interaction with propetamphos is not known. The degree of ageing that occurs in practice depends on the balance between ageing and reactivation and it is possible to envisage a situation where repeated exposure could result in a small fraction of the enzyme ageing at each exposure and a large proportion being reactivated. If such repeated exposure were prolonged the amount of reactivated enzyme would decrease while an increasing proportion would become permanently aged. This may be important in the context of repeated occupational exposure.

3.11 OPs are not only inhibitors of acetylcholinesterase but will also bind to other proteins and inhibit a number of other enzymes containing the amino acid serine. In

addition to acetylcholinesterase, known targets include plasma pseudocholinesterase, neuropathy target esterase (NTE), A-esterases (tissue esterases capable of hydrolysing OP esters) and carboxyesterases, as well as other esterases and proteases. The multiplicity of potential binding sites gives rise to a wide range of possible sites and modes of action. For OPs used in pesticides and veterinary medicines, acetylcholinesterase is one of the most sensitive targets and currently its inhibition is used as the critical effect in risk assessment. It has been proposed that covalent binding to other proteins such as enzymes in the central nervous system (CNS) might contribute to OP toxicity.³ Before the potential contributions of these targets to the spectrum of OP-related toxicity can be evaluated dose-response relationships need to be established for their binding in comparison with that for acetylcholinesterase inhibition. This has been carried out for NTE but data for other putative targets are either very limited or non-existent. It is also possible that covalent binding to sites other than acetylcholinesterase could in some circumstances protect against toxicity by lowering the amount of active OP available to bind to acetylcholinesterase.⁴

The use of OPs

History

3.12 The early developmental work on OPs was carried out in the 1930s by Schrader and co-workers at IG Farben in Germany (see review by Marrs⁵). The anticholinesterase properties of the compounds were recognised and they were investigated as potential insecticides. However, because of the very high mammalian toxicity of the compounds investigated, they were of little use for this purpose. Major research on OPs was carried out in the period immediately preceding the Second World War and continued in Germany during the war with the development of compounds which showed high volatility, rapid skin penetration and potent effects on the central nervous system. These nerve agents, which are volatile liquids, include tabun, sarin and soman. Other agents developed during and after the Second World War included the so-called V agents such as VX; these are less volatile but more toxic than the earlier nerve agents.

3.13 Some OP compounds were developed for uses not dependent on their inhibition of acetylcholinesterase, for example as defoliants and fire retardants (see review by Marrs⁶). Most of these compounds do not have any significant anticholinesterase activity, for example triorthocresyl phosphate (TOCP) which has been used as an additive in aviation fuel. Therefore they are not included in the general definition of OPs used in this report (see paragraph 3.1) and are not covered in this review.

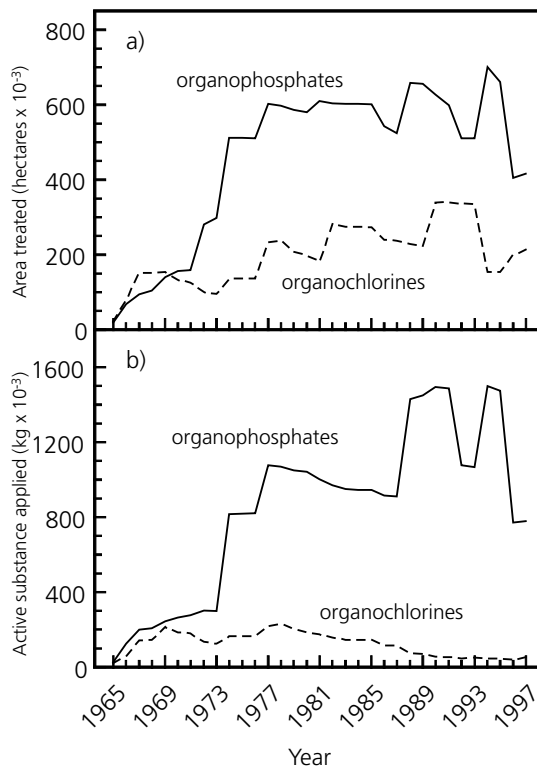
OPs use in pesticide products

3.14 During the 1950s and 1960s OPs with selective toxicity to insects and relatively low toxicity to mammals were developed and their use increased rapidly in the 1970s. Levels of use then plateaued and declined with the increasing use of pyrethroid insecticides. Figure 3.3 illustrates graphically the amounts of OP and organochlorine pesticides (in kilograms) applied to crops and the areas treated (in hectares) in Great Britain during the period 1965-1997. For OPs there is a clear relationship between the area treated and weight of active

Organophosphates substances applied. For organochlorine pesticides the discrepancy between area treated and weight of active substance applied may be related to the use of lindane as a seed treatment (now withdrawn) which involved the application of small amounts to seed which was then sown over a wide area.

3.15 In 1998 there were 24 OPs approved by the Pesticides Safety Directorate (PSD) as active ingredients in insecticide products. These were: azamethiphos, chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, demeton-S-methyl, diazinon, dichlorvos, dimethoate, disulfoton, ethoprophos, etrimfos, fenitrothion, fonofos, heptenophos, iodofenphos, malathion, mephosfolan, phorate, phosalone, pirimiphos-methyl, quinalphos, thiometon, triazophos, trichlorfon. However, three of these were subject to revocation procedures later in 1998: these were demeton-S-methyl, fonofos and triazophos. In addition, as a result of a review of anticholinesterase compounds announced by the PSD in 1998, with a requirement for notification of support for products and submission of comprehensive data on safety, a further seven of these compounds were not supported by industry for commercial reasons. These were diazinon, heptenophos, mephosfolan, phosalone, quinalphos, thiometon and trichlorfon. Approval of products containing these active ingredients has now been revoked.

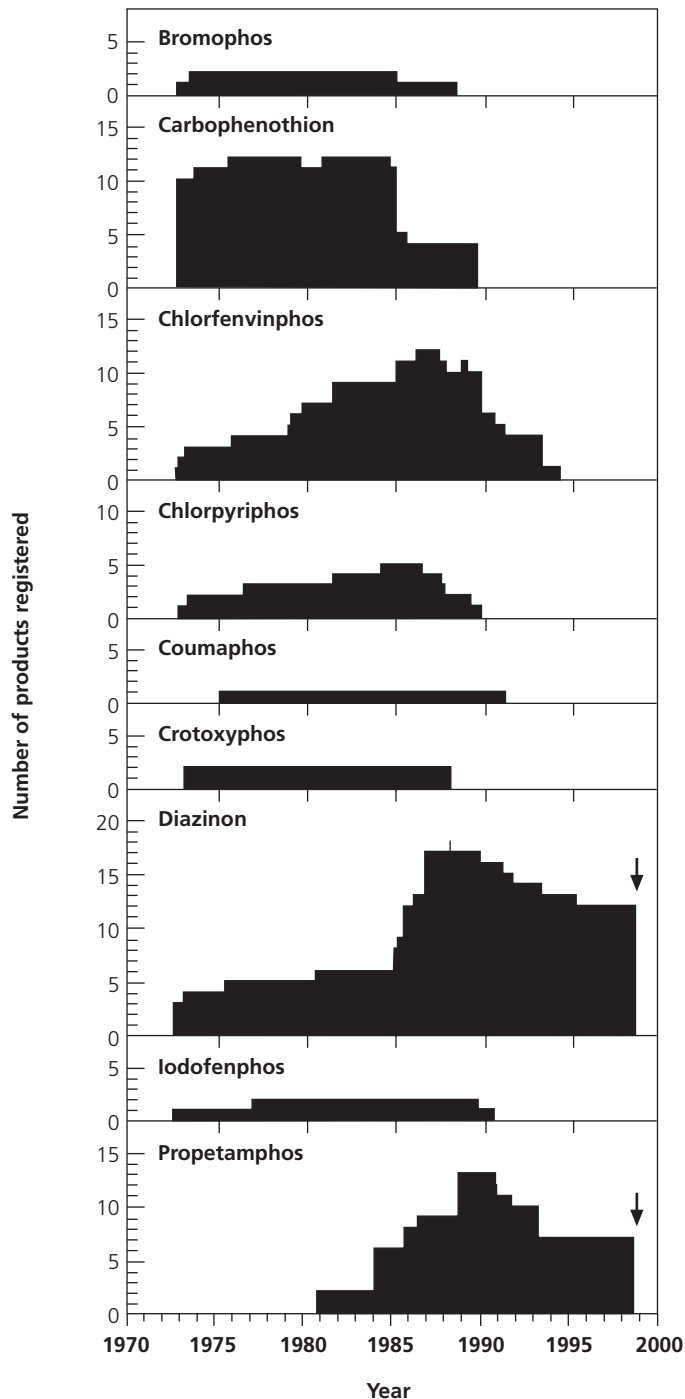
Figure 3.3: The usage of OP and organochlorine pesticides in Great Britain during the period 1965 to 1997, plotted as a) the areas treated (in thousands of hectares), and b) the amounts applied to crops (in thousands of kilograms).



(Data supplied by the Pesticides Safety Directorate)

Figure 3.4: Number of sheep dip products registered at any one date which contained the OP active ingredient indicated, some products contained more than one OP active ingredient. The period covered is September 1972 to September 1998. The arrows indicate those products still registered at the end of this period.

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(Data supplied by the Veterinary Medicines Directorate)

3.16 In 1998 pesticides containing the following OPs were approved by HSE for use in non-agricultural pesticide products: azamethophos, chlorpyrifos, chlorpyrifos-methyl, diazinon, dichlorvos, fenitrothion, iodofenphos and trichlorfon. Following the decision to review all anticholinesterase compounds announced by HSE's pesticides registration section

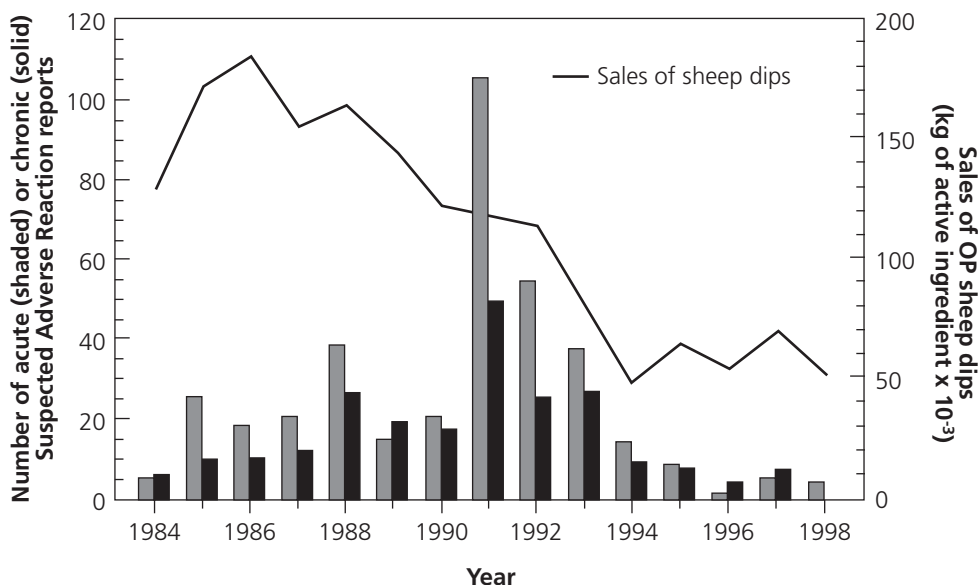
Organophosphates in 1998, a number of active ingredients were not supported by industry for commercial reasons; these were chlorpyrifos-methyl, diazinon, iodofenphos and trichlorfon. Approval of all products containing these active ingredients has now been revoked.

OP use in sheep dips and other veterinary medicines

3.17 OPs are used as veterinary medicines to control ectoparasites in animals, and especially in sheep, where they are active against sheep scab, ticks and blow fly strike. The only OP compounds currently licensed in the UK for use in sheep dips are diazinon, which is a phosphorothioate, and propetamphos, which is a phosphorothioamidate (see Table 3.1). Other active OP ingredients in sheep dips licensed since 1972 have included carbophenothion (licences expired 1979 to 1989), chlorfenvinphos (licences expired 1987 to 1994), chlorpyrifos (licences expired 1986 to 1989), coumaphos (licence expired 1991) and crotoxyphos (licences expired 1988). The pattern of usage in relation to date is shown in Figure 3.4.

3.18 Total sales of OP sheep dip products in the UK increased from the early 1970s until 1986 and have steadily declined since then, as is shown in Figure 3.5. It was compulsory to dip sheep twice a year during the period 1984 to 1988 and once a year during 1988 to 1991. Compulsory dipping was discontinued as from July 1st 1991.

Figure 3.5: Trend in annual sales of the active ingredients of OP sheep dips, in thousands of kg, right hand scale and solid line. Annual numbers of human Suspected Adverse Reactions to sheep dips by year of onset of the adverse reaction (if provided), left hand scale; acute adverse reactions are shown by shaded bars and chronic adverse reactions by solid bars.



(Adapted from reference 8. Data supplied by the Veterinary Medicines Directorate)

3.19 A number of OPs are also used in veterinary medicines other than sheep dips. Diazinon, naled and tetrachlorvinphos are used in flea powders and collars for dogs and cats, dichlorvos and fenitrothion are used in the treatment of dogs and cats, azamethiphos

and dichlorvos are used in the treatment of salmon, coumaphos is used in wound dressings for horses and phosmet pour-ons are used for pigs and cattle.

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Human medicines

3.20 There is one approved use of an OP compound in human medicines in the UK, that is for the treatment of head lice with malathion. Products are available over-the-counter for this purpose.

Approval of OPs used in the UK

3.21 Human and veterinary medicines and pesticide products can only be put on the market after they have been through a system of scientific scrutiny, on the basis of which they are approved by Ministers and licensed. The criteria for approval are safety, quality and efficacy. The advisory process is outlined in paragraph 2.3.

3.22 A major component of the evaluation of pesticides approved for use in the production of human food items is the determination of a safe level of exposure for humans to a specific active compound, known as the Acceptable Daily Intake (ADI). The ADI is defined as “the amount of a chemical which can be consumed every day of an individual’s entire lifetime in the practical certainty, on the basis of all known facts, that no harm will result”.⁷ The COT use the following definition of ADI in the context of food additives: ‘An estimate of the amount of food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risks’. ADI values are determined by consideration of all adverse effects reported in studies in animals and humans, and are calculated as the No-Observed-Adverse-Effect-Level (NOAEL – the maximum observed daily dose which does not produce the effects detected at higher doses) for the most sensitive, relevant effect divided by an uncertainty (safety) factor. Normally uncertainty factors of 100-fold and 10-fold are applied to NOAEL values from studies in animals and humans respectively. Thus the ADI value is an estimate which includes a safety margin for any reported adverse effects. Although the ADI is usually determined largely from a single critical effect, protection from this effect should also give adequate protection from other effects produced at higher dosages.

3.23 In addition, the approval schemes in the UK consider the safety of workers applying pesticides. Acceptable Operator Exposure Levels (AOELs) are established for worker exposure to agricultural pesticides using general principles analogous to those used in deriving an ADI, but noting that the major routes of exposure are dermal and by inhalation.

3.24 Similar considerations apply to the licensing of veterinary medicines including sheep dips. OP-containing sheep dips have been considered by the VPC on a number of occasions over the last decade and steps have been taken particularly from 1991 to increase awareness of the potential hazards involved and the need for the use of appropriate protective clothing. A chronology of events in relationship to the licensing of sheep dips containing OPs is given in Annex 3A.

Organophosphates **References**

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Annex 3A

Chronology of events with regard to licensing of sheep dips containing OPs and recommendations for their safe use

Information available for the period prior to 1991

Only limited information was available to the Working Group concerning the advice given prior to 1991 (since this predates the establishment of the VMD).

The toxicity of OPs used in agriculture was recognised as a cause for concern in the early 1950s. (Ministry of Agriculture and Fisheries. *Toxic Chemicals in Agriculture*. Report to the Minister of Agriculture and Fisheries of the Working Party on precautionary measures against toxic chemicals used in agriculture, 1951. London: His Majesty's Stationery Office.)

The Agricultural Development and Advisory Service (ADAS) of MAFF published a leaflet on Sheep Dipping and Spraying in 1979 (ADAS 1979: short term leaflet 161). This noted that sprays were not acceptable for sheep scab but may be useful for blowfly control. There is a section on safety where it was pointed out that many of the poisonous compounds incorporated in sheep dip preparations can be readily absorbed through the skin, nose, eyes and mouth and can cause toxic effects. Some were covered by the Health and Safety (Agriculture) (Poisonous substances) Regulations 1975, and in certain circumstances protective clothing must be worn. When handling the substances in their concentrated form overalls, rubber gloves and a face shield must be worn. Although not covered by the regulations it was stated that operators should always wear protective clothing whilst dipping or spraying sheep or handling freshly treated sheep.

The need to wear overalls, rubber gloves and a face shield when handling the concentrate was emphasised in a MAFF leaflet on sheep scab, dipping procedures published in 1985, and also in a further ADAS leaflet published in 1986 (ADAS leaflet P593 Sheep Scab 1986).

HSE first published their Guidance Note MS17 on biological monitoring of workers exposed to OP pesticides in 1981, and updated this in 1987. These were in their medical series intended for the Employment Medical Advisory Service occupational health physicians. The latter document included a section on symptoms and signs and noted that regular monitoring should be considered for anything more than occasional exposure to OPs such as garden use.

These HSE guidance notes were not sent to GPs or hospitals but the DH produced a booklet entitled Pesticides Poisoning: notes for the guidance of medical practitioners in 1985, a second edition being produced in 1996. This contained a section on the acute and chronic toxic effects of OP pesticides and was sent to all GPs, and hospital Accident and Emergency Departments.

Organophosphates More detailed information provided by VMD is available from 1991, this is given below.

Information for the period 1991-1999

1991

- April** Letter sent to all doctors from the Chief Medical Officer (CMO, England) on reporting of pesticide incidents.
- August** Advice on safe handling and disposal of sheep dips. A4 wall chart PB 0645 and leaflet “Safe handling and disposal of sheep dip” issued.
- September** HSE leaflet AS29 issued on ‘Sheep Dipping: Protect Your Health’.

1992

- January** VPC reviewed sheep dip products. They emphasised the need to read and follow label instructions and wear the protective clothing recommended in HSE leaflet AS29. They recommended the following:
- Studies on operators including blood tests and collection of information about clothing worn and which areas of the body were exposed.
 - Further studies on the persistence of residues in fleece.
 - Further data be obtained from companies and the Suspected Adverse Reaction Scheme (SARS) for consideration at the end of 1992.
- May** National Office of Animal Health (NOAH) article “Safe Dipping” appeared in the journal “Sheep Farmer”.
- June** Revised edition of “The safe handling and disposal of sheep dips. Advisory note to farmers” produced by VMD.
- November** NOAH leaflet on OP sheep dips published.

1993

- February** Meeting of Minister and parliamentary delegation resulting in a VPC meeting to consider a moratorium on OP dips until product review complete.
- March** VPC meeting to consider the above. Found no case for a moratorium but noted poor use of personal protective equipment (PPE) and that further research into the effects of OPs on human health was underway. The following recommendations were made.
- Further re-emphasise the hazard of products.
 - Hazard warnings to be standardised and to go on the labels.
 - Clear advice should be available on the types of PPE to use.
- As a follow up to the above the following steps were taken:
- May** Joint NOAH/WHO leaflet and poster sent to all sheep farmers. 500,000 stick-on labels with the toxic symbol plus reference to the need to read the new advisory leaflet prepared for placement on all containers of sheep dip products.

July	Joint letter from the CMO (England) and the VMD Director to all GPs drawing their attention to the recent publicity material and to ask them to be aware of the possibility of OP poisoning.	<i>Chronology of events with regard to licensing of sheep dips containing OPs and recommendations for their safe use</i>
October	Further VPC meeting to consider research work being generated. Again VPC did not recommend a ban but made the following recommendations: <ul style="list-style-type: none">● In view of the non-use of protective clothing there should be a certificate of competence scheme introduced such that only dippers holding this could purchase OP dips.● The establishment of a Medical and Scientific Panel to coordinate research into the long-term health effects arising from exposure to OPs.● A review to ensure sheep dips are disposed of safely.	

1994

March	The Statutory Instrument enacting a Certificate of Competence Scheme (SI 599/1994) for sheep dippers, The Medicines (Pharmacy and Merchants List) (Amendment) Order, was laid before Parliament. A letter was sent to all sheep farmers urging them to enrol for the Certificate.
April	All those wishing to purchase OP sheep dips required to show they were registered for the Certificate of Competence Scheme run by the National Proficiency Test Council (NPTC).
May	A revised version of HSE's AS29 leaflet entitled "Sheep dipping" produced and sent to all sheep farmers.
July	Letter in <i>Veterinary Record</i> from the presidents of the Royal College of Veterinary Surgeons (RCVS) and the British Veterinary Association (BVA) and the Director of the VMD reminding veterinary surgeons of the position on the prescribing of OP containing sheep dips.
September	New advisory leaflet on sheep scab issued and backed by posters urging farmers to treat their sheep.
October	Reminder letter sent to all sheep farmers about the need for the Certificate of Competence if they plan to buy OP dips.

1995

March	Revised edition of HSE's AS29 "Sheep Dipping" produced.
April	From this time only holders of the Certificate of Competence could purchase OP dips.
May	Report from the Institute of Occupational Health (IOH) in Birmingham published with summary in the <i>Lancet</i> . Report showed again that many of those involved in sheep dipping are not using PPE and indicated possible

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small differences in some neurophysiological tests between dippers and a control group.

July Following a VPC meeting which considered the IOH report, the Minister announced acceptance of its advice that whilst the Report contributed to the body of knowledge it was not a definitive study and did not find sufficient evidence to support a ban on OPs, but that a contract for further studies should be placed as a matter of urgency. The VPC also stressed the need for farmers to follow the advice in AS29 and the Control of Substances Hazardous to Health (COSHH) regulations.

October The Institute of Occupational Medicine (IOM) in Edinburgh was awarded a contract for research into the possible long-term health effects of OP dips valued at £500,000.

A review of the current rules governing OP sales was also to be undertaken with further advice expected early in 1996.

December Announcement of consultation of interested parties on the effectiveness of the restrictions on the purchase of OP sheep dips.

1997

February After a consultation announced at the end of December 1995, VPC advised that sheep dips are safe when used according to the manufacturers' instructions. The following conclusions were drawn by VPC:

- The marketing of OP sheep dips should continue
- The Certificate of Competence should be extended to include users of OP dips
- OP sheep dips should only be made available to clients holding a Certificate of Competence
- OP sheep dips should retain their Pharmacy and Merchants List (PML) classification
- Labelling of OP dips should be simplified
- There should be a mechanism for informing water regulators on the use of sheep dips and consulting them on the means of disposal
- Further basic research needed
- There should be a review of non-OP dips within a year

1998

January Announcement that synthetic pyrethroid sheep dips should be included in the Certificate of Competence scheme for purchasing dips.

March Publication of new AS29 (rev 2) booklet. New advice warned of the dangers of not following the correct procedures when using sheep dips.

May	Announcement by MAFF Minister of State that the Certificate of Competence required for purchases of OP sheep dips was being extended to cover all types of sheep dip.	<i>Chronology of events with regard to licensing of sheep dips containing OPs and recommendations for their safe use</i>
December	Announcement that Certificate of Competence was required for purchases of all sheep dips.	
1999		
July	Results of IOM research into the possible long-term health effects of OPs published. MAFF Minister of State asked companies to come up with proposals for improved packaging of concentrated OP sheep dips within 3 months.	

4. Exposure to OPs

Sources of exposure

4.1 Exposure of humans to OPs can occur:

- from trace OP contaminants present in food and drinking water;
- through their use as household or garden insecticides, or in the treatment of headlice;
- through exposure during their manufacture, formulation or use.

4.2 Data on OP intakes from food are available from the annual reports of the Working Party on Pesticide Residues (WPPR). These reports provide the results from the extensive monitoring programmes undertaken by MAFF. They include comparisons of any residue levels and estimates of intake with the corresponding ADI values. Recent analyses¹ indicate that dietary intakes of OPs from food, as determined in total diet samples, are usually well below the ADI values for each specific pesticide analysed, but there have been exceptions, with exposures up to twice the ADI being estimated for propetamphos and phosalone in infants (see Table 4.1). Pesticides were chosen for analysis in this survey on the basis that they had been regularly found during previous monitoring, or could be expected to occur as residues. It was noted by the Working Party on Pesticide Residues that propetamphos has not been found regularly in recent monitoring.¹ Total dietary surveys are carried out at approximately five-yearly intervals, and data from two earlier surveys (1984/1985 and 1989/1990) are given in Table 4.2. These data are more limited than for 1995/1996.

Table 4.1: Dietary intakes of OPs as determined in total diet samples (1995/1996) reported as a percentage of the ADI for the individual pesticides

Pesticide	ADI (mg/kg b.w. per day)	Dietary intake as a %age of the ADI*		
		Adults	Schoolchildren	Infants
Chlorpropham †	0.1	3	5	7
Chlorpyrifos-methyl	0.01	0.6	0.8	1
Dimethoate	0.0008	13	15	45
Etrimfos	0.003	2	3	3
Parathion ‡	0.005	0.8	1	3
Phosalone ‡	0.001	53	61	176
Phosphamidon	0.0005	12	16	20
Pirimiphos-methyl	0.03	0.7	1	1
Propetamphos †	0.0001	140	180	200
Triazophos	0.001	1	2	4

Adapted and updated from the Annual Report of the Working Party on Pesticide Residues: 1996, see reference 1

* Bold text indicates where the ADI was exceeded

† These are temporary ADIs, that for propetamphos is currently under discussion

‡ The ADIs for parathion and phosalone have since been revised to 0.004 and 0.02 mg/kg b.w. per day respectively, see references 30 and 31

Organophosphates **Table 4.2: Dietary intakes of OPs as determined in total diet samples 1984/1985 and 1989/1990 (data only available for adults)**

Pesticide	ADI (mg/kg b.w. per day)	Dietary intake as a %age of the ADI	
		1984/1985	1989/1990
Chlorpropham †	0.1	No residues detected	0.1%
Chlorpyrifos-methyl	0.01	No residues detected	0.1%
Etrimfos	0.003	No residues detected	0.11%
Malathion	0.02	0.01%	Less than 0.01%
Pirimiphos-methyl	0.03	0.1%	0.17%

Adapted from Report of the Working Party on Pesticide Residues 1988-1990, see reference 32

† This is a temporary ADI

4.3 In theory OPs could enter drinking water *via* industrial effluent, seepage from toxic waste sites, from the washing of fleeces of treated sheep, and from run-off water after agricultural use.² In the UK, run-off water from agricultural use is likely to be the most important route by which water supplies are contaminated. Exposure to OPs *via* drinking water is limited by their hydrolysis and degradation through the effects of light and pH (acidity and alkalinity), and through metabolism by micro-organisms. Data collected between 1993 and 1996 show that 5 to 10% of fresh water samples and 1 to 3% of ground water samples in England and Wales contained detectable concentrations of diazinon and propetamphos.³ Higher concentrations (>100 ng/l) were present in 2 to 3% and 0 to 1% of fresh water and ground water samples respectively. Recent results from a monitoring programme by the Environment Agency, Wales, indicated that the presence of diazinon in river water was widespread, detectable amounts being found in 75% of the 107 river sites monitored.⁴ In 29 cases, levels were above the maximum allowable concentration in the Environmental Quality Standard (100 ng/l). Ingestion of two litres of water containing 100 ng/l by an adult would give an oral intake of about 3 ng/kg per day which is less than 0.2% of the ADI for diazinon and less than 4% of the ADI for propetamphos.

4.4 No data are available on the extent of exposure arising from household uses of OPs or from secondary exposure to occupational sources (e.g. in farming families) in the UK.

4.5 In the UK the highest exposure of humans to OPs is from two agricultural procedures, namely crop spraying (including that in glass houses) and sheep dipping. Most reports of adverse health effects relate to these procedures, and they provide the basis for most of the epidemiological studies discussed in Chapter 7. Exposures in these applications differ from those arising from consumption of contaminated food and water in the dose and route of exposure and also in the scope for concomitant exposures to co-formulants.

4.6 Co-formulants are added in order to increase the stability of the OP during storage, and/or to increase contact between the OP and the target organism, or to reduce inactivation in the target species. It has been suggested that co-formulants contribute significantly to the production of adverse effects in exposed individuals. Theoretically, co-formulants could increase OP toxicity through:

- chemical reactions within the formulation,
- the enhancement of OP uptake across the skin,
- synergism or potentiation in the body – in which the response to the combination is much greater than predicted by simple addition, the latter being the default assumption.

4.7 Chemical changes within formulations during storage under hot conditions (38°C) have resulted in enhanced toxicity of malathion in experimental animals (decrease in LD₅₀* from 2.7 g/kg body weight to 0.6 g/kg body weight) but not in the toxicity of some other OPs.² Data on the stability of formulations are examined as part of the approval processes for OPs in the UK.

4.8 Transdermal absorption of chemicals depends on both the nature of the chemical and the formulation in which it occurs. Enhanced absorption and toxicity have been reported for some OP formulations in organic solvents as compared with the pure compound or an aqueous preparation.²

4.9 The metabolism of OPs could be inhibited *in vivo* if a co-formulant were a substrate for the same metabolising enzyme. For example, piperonyl butoxide is used in some formulations because it inhibits the inactivation of the OP in the target (pest) species thereby producing a synergistic effect. This compound has the potential to inhibit metabolism *via* cytochrome P-450 in humans, but because these enzymes are involved in the bioactivation process (converting thions to oxons) the result may be a decrease in toxicity. However, in some cases the cytochrome P-450 system also contributes to detoxification (dealkylation); the net effect observed will vary according to the OP.

4.10 Each of these possible effects of co-formulants would be more likely to occur following exposure to the concentrate rather than to a dilute formulation, e.g. the sheep dip or spray in use, because dilution with water to create the sheep dip or spray would minimise any effect of the co-formulant. A possible synergistic interaction with a co-formulant directly enhancing the toxicity would be of concern. The Working Group was provided with a list of the co-formulants currently used in the UK (see Table 4.3), and these were not thought likely to cause significant neurotoxic interactions with OPs through known mechanisms of toxicity. The presence of co-formulants was considered by the Working Group as likely to be a minor determinant of differences in toxicity compared to other sources of variability such as work practices, and differences in exposure levels together with interindividual variability in transdermal penetration and interindividual differences in susceptibility.

*LD₅₀: estimated dose that would result in the death of 50% of the exposed animals

Organophosphates **Table 4.3: Co-formulants that have been used in the UK in pesticide products or sheep dip products containing OPs**

Pesticide products
<p>Solvents</p> <p>Aromatic hydrocarbon solvent*, aliphatic hydrocarbon solvent*, <i>n</i>-butanol, cyclohexane*, cyclohexanone*, dichloromethane*, dimethylformamide*, isopropanol, isoparaffin*, kerosene*, <i>n</i>-nonanol, paraffin oil, petroleum naphtha*, trichloroethane*, xylene*.</p>
<p>Surfactants/wetting agents/emulsifiers</p> <p>Alkyl aryl sulphonate*, alkyl lauryl sulphonate*, alkyl phenoxy polyethoxyethanol, alkyl phenol ethoxylate, calcium alkyl aryl sulphonate, calcium dodecyl phenyl sulphonate*, cetyl octyl alcohol ethoxylate, castor bean ethoxylate, castor oil ethoxylate*, cetyl oleyl alcohol ethylene oxide condensate, cyclic alkylamine alkyl aryl sulphonate, epoxidised soya bean oil*, epoxidised linseed oil*, linear fatty acid ethoxylate*, octaphenoxy polyethoxy ethanol*, nonyl phenol ethoxylate*, nonyl phenol ethylene oxide condensate*, sodium lauryl sulphate*, polyoxyethylene sorbitol monoesterate, poly(oxy-1,2-ethylenediyl)-<i>a</i>-(1-oxo-9-octadecaenyl) <i>n</i>-hydroxy monoethylene glycol, diethylene glycol, butoxyethanol*, polypropylene glycol*.</p>
<p>Other compounds (stabilisers, preservatives etc)</p> <p>Acetic acid, acetic anhydride, 2,6 di-<i>t</i>-butyl cresol*, butyl dioxitol, dibutylphthalate*, dioctylphthalate, formaldehyde, vinylpyrrolidone styrene copolymer, paradichlorobenzene, piperonyl butoxide and sorbic acid (synergist), dyestuffs.</p>
Sheep dips
<p>Solvents</p> <p>Aliphatic hydrocarbon solvent*, aromatic hydrocarbon solvents*, kerosene, xylene, anthracene oil*, mineral oil, 'natural' oil, spindle oil*, tall oil.</p>
<p>Surfactants/wetting agents/emulsifiers</p> <p>Calcium dodecylbenzene sulphonate, castor oil soap, 'ionic and non-ionic' emulsifiers*, linear alkyl sulphonate, nonylphenol ethylene oxide condensate*, phenylsulphonate, polyalkylene glycol ether; soap emulsifier, tributyl phenol ethylene oxide condensate.</p>
<p>Cresols/Phenols/Tar oils</p> <p>Coal tar phenol, cresylic acid (cresols)*, cresylic creosote*, phenols.</p>
<p>Miscellaneous</p> <p>Dichlorometaxlenol, epichlorohydrin, epoxidised soya bean oil*, parachlorometaxlenol, propylene oxide*</p> <p>* currently used (Information provided by PSD and VMD)</p>

4.11 Workers involved in the manufacture of OPs may also be exposed, although this exposure should be minimal if appropriate workplace practices are adopted.²

Routes of exposure

4.12 Ingestion is the main route of exposure for the general public and arises from contamination of food and water with trace amounts of pesticide. This may be the source of the low levels of OP metabolites sometimes found in the urine of apparently unexposed subjects. Occupational exposure may be dermal, by inhalation or by the oral route. In the

case of medical treatments with malathion products to control head lice dermal exposure predominates.

*Exposure
to OPs*

Dermal exposure

4.13 Dermal exposure may arise during handling of the concentrate and application of the diluted OP during spraying or sheep dipping. In addition, handling of treated sheep may result in exposure due to residues in the fleece. OP adsorbed onto clothing can be a source of continued absorption, especially if the clothing is wet. *In vitro* studies on the absorption of malathion across cadaveric human skin have shown extensive absorption following direct application of a solution, or of a cotton material containing the solution, but limited absorption if the solution or the cotton material was allowed to dry. Extensive absorption occurred if the contaminated dried cotton fabric was wetted again (as could occur if a worker wore previously contaminated clothing).⁵ The dermal route is also the principal route of exposure during spray application of pesticides (see paragraph 4.14).

Inhalational exposure

4.14 OPs inhaled into the respiratory tract (as vapour, droplets or dusts) would be absorbed more rapidly than when they contaminate the skin. Despite this, studies in workers involved in the formulation of OPs, or spraying orchards with OP-containing products, have indicated that transdermal absorption rather than inhalation represents the main route of exposure even after allowance has been made for the difference in uptake between these routes. The doses arising from skin contamination in workers at an OP formulation plant and in workers involved in spraying OPs were about 100-fold higher than those *via* inhalation.² Results from a study of orchard sprayers using chlorpyrifos in England during the period 1996 to 1998 indicated that exposures were very much higher (about 400-fold) by the dermal route than by inhalation.⁶ Median daily exposures were 6.13 mg chlorpyrifos by the dermal route compared with 0.016 mg by inhalation.

Oral exposure

4.15 Although oral exposure should not arise from occupational uses, in practice observations of agricultural workers, sheep dippers and others show significant potential for exposure *via* this route, for example through smoking or eating without having first washed their hands.⁷

The fate of OPs in the body

Absorption following different routes of exposure

Dermal absorption

4.16 The skin acts as a barrier and dermal absorption tends to be slow although lipid-soluble compounds may produce a depot in the skin from which there can be slow release into the circulation. The extent of transdermal absorption depends on a number of factors including the chemical nature of the formulation and those factors influencing skin permeability, such as temperature and the degree of hydration of the skin. Organic solvents in the concentrate would enhance transdermal absorption.² Only limited data exist

Organophosphates on the extent of metabolism within the skin before entry into the general circulation. The results of an *in vitro* study using parathion and porcine skin indicated that the absorbed material was present largely as metabolites (paraoxon and 4-nitrophenol) with less than 20% (of the absorbed material) being unchanged parathion.⁸ The skin acts as a very efficient barrier to absorption compared with other routes. Studies in experimental animals (rats, guinea pigs, dogs) have shown that ¹⁴C-diazinon is eliminated rapidly after oral or intravenous dosage with half-lives of 24 hours or less. Slower elimination was reported after dermal administration to rats, consistent with slow absorption across the skin.⁹ Exposure of the hand and forearm of a volunteer to 48% parathion (v/v) as an emulsifiable concentrate for two hours did not alter erythrocyte acetylcholinesterase activity (a biomarker of effect, see paragraph 4.29), and negligible changes in acetylcholinesterase activity were found in a volunteer completely covered in 2% parathion dust and then enclosed in a rubber suit for seven hours.²

4.17 *In vivo* experimental studies in human volunteers on the absorption of topically applied OPs have also indicated low levels of transdermal uptake with small amounts of metabolites excreted in the urine, although most have had limitations with regard to the quantitative measurement of total excreted material. Transdermal absorption of ¹⁴C-labelled malathion has been reported to be less than 5% of the applied dose over seven days.¹⁰ Although the greatest urinary excretion of absorbed radiolabel occurred during the first 24 hours, there was a continued low-level excretion throughout the seven days of the study, with little decrease in the excretion rate between days three and seven which amounted to about 0.25% of the applied dose per day. The site of application was washed after 24 hours, to prevent further uptake, and the prolonged and relatively consistent, low-level excretion suggests that a depot of absorbed material may have formed in the skin. Alternatively, the half-life could have been very long because of a slow release from adipose tissue and, in consequence, the calculated extent of absorption may have considerably underestimated the true uptake. A similar argument applies to data for ¹⁴C-diazinon reported by Wester *et al.*,¹¹ in spite of their attempts to correct for incomplete urinary recovery. The uncorrected data showed 2% excretion of the dosed ¹⁴C-radiolabel in the urine but not all of the remainder was recovered from the application site by skin stripping (i.e. removing surface layers of skin) and washing 24 hours after application. The sites of application were not occluded for the 24 hours of exposure prior to washing and thus loss onto clothing may have occurred. This low absorption calculated from an *in vivo* study contrasts with *in vitro* data, which showed about 14% absorption across human skin over a period of 24 hours. Extensive *in vivo* absorption combined with very slow elimination over a period of days or weeks cannot be excluded.¹¹ (See also the discussion of urinary metabolites in paragraphs 4.25 to 4.28.)

4.18 A small amount of the OP may be metabolised in the skin during dermal absorption, but this would be less than that metabolised in the lumen and wall of the gastrointestinal tract during oral absorption. Generally, for OPs there is a wide range of tissues and body fluids in which metabolism may occur.

Inhalation

4.19 Most OPs used as insecticides are liquids at room temperature and have a low vapour pressure so that only small amounts of the compounds will be present in the air in

gaseous form. The airborne concentration will depend on the volatility of the OP and also its concentration and partial pressure in the formulation. Organic solvents would reduce the vapour pressure of OPs but the concentration in the air above the sheep dip concentrate would be higher than in the air above diluted dip because of the higher concentration in the liquid phase. Inhalation of vapour is of greater relevance in enclosed spaces than outdoors. Because of the low volatility of pesticide OPs, inhalation of aerosols such as sprays and dusts is generally more important than inhalation of vapour. Absorption of inhaled OPs deposited in the lungs is rapid and almost complete with limited metabolism before they reach the general circulation and thus the main determinant of absorbed dose by this route is the generation of an inhalable dosage form.

Ingestion

4.20 The gastrointestinal tract has a large surface area and rapid and complete absorption would be expected for lipid-soluble compounds such as the OPs used in sheep dips. The OP may be metabolised in the gut lumen and gut wall, with greater amounts being metabolised in the liver before reaching the general circulation.

Metabolism

4.21 Mammals can metabolise OPs through various pathways that involve a range of enzyme systems. The consequences of metabolism depend upon the biological activities and physico-chemical characteristics of the parent compound and its metabolite(s). The pathways involved differ from compound to compound and the general summary below provides an overview and concentrates upon those reactions which could give rise to interindividual variability in bioactivation and/or detoxification.

4.22 The most common pathway for the metabolism of OPs is *via* hydrolysis, which results in removal of the more labile leaving group. The products of such hydrolysis include dimethylphosphate (DMP), diethylphosphate (DEP), dimethylphosphorothioate (DMPT) and diethylphosphorothioate (DEPT) which are excreted in urine. Because insecticidal OPs differ mostly in the structure of the leaving group, the rates of hydrolysis will differ from compound to compound, despite similarity in some of the resulting urinary metabolites. The enzymes involved in OP hydrolysis and inactivation are present in many tissues, with high activities in liver, intestine and plasma. The principal enzymes are microsomal enzymes and the A-esterases, a group of enzymes capable of hydrolysing a broad range of substrates, including OPs.² The activity of A-esterases can be quantified in relation to specific OPs used as substrate and there is a comprehensive literature on "paraoxonase" (A-esterase activity using paraoxon as the substrate). Paraoxonase activity has been measured in the serum of a large number of human subjects and shows very wide (up to 10-fold) differences within Caucasians¹² and in other ethnic groups.¹³ The enzyme exhibits genetic polymorphism in humans with a sub-group (40-50%) of the population in the UK and United States of America (USA) having low activity.^{12,14} The possible consequences of this polymorphism are complicated because of the compound-specific nature of the variation in enzyme activity, the low activity form for paraoxon having a relatively high activity for diazinon as a substrate. In contrast, erythrocyte acetylcholinesterase activity shows less variation between individuals.¹² It follows that

Organophosphates variability in hydrolytic inactivation may be a major source of variability in the response of individuals to OPs in addition to variability in exposure, although recent data suggests that variability in oxidative metabolism (see paragraph 4.24) is more important at toxicologically relevant concentrations.¹⁵ Serum cholinesterase and lymphocyte neuropathy target esterase (NTE) activities in humans also show wide inter-individual variability. The low activity of OP hydrolysis in insects contributes to the organism's susceptibility to OPs and the development of resistance in insects can be due to their possessing high esterase activity.

4.23 The leaving substituents of OPs (X in the general formulae given in paragraph 3.2) may contain esterified carboxylic acid side chains, e.g. in malathion, and these may be hydrolysed by carboxylesterases producing more water-soluble and less toxic metabolites.²

4.24 OPs can also undergo metabolic oxidation resulting in:

- desulphuration (e.g. conversion of parathion to paraoxon),
- N-dealkylation of substituted amide side chains,
- O-dealkylation (e.g. replacement of R¹ and R² in the general formulae of paragraph 3.2 by a hydrogen atom when these are methyl or ethyl groups),
- side chain oxidation of alkyl-, aryl- and thioether substituents.

These oxidation reactions are catalysed by cytochrome P-450 enzymes predominantly in the liver. The cytochrome P-450 enzymes are a family of enzymes involved in the oxidation of lipid-soluble molecules such as drugs, sterols and many environmental chemicals. The specific cytochrome P-450 isoenzyme responsible for the oxidative desulphuration of parathion is CYP3A4,¹⁶ which is present in human liver. It can be induced or inhibited by certain therapeutic drugs, and is responsible for the oxidative metabolism of many drugs in common use. CYP3A4 activity shows very wide interindividual differences (up to 15-fold), both *in vitro* in liver samples¹⁶ and *in vivo* when drug substrates are used as probes.¹⁷ CYP3A4 activity is high in the intestine but not in the skin or lungs, and therefore there may be differences in the quantitative balance between parent compound and oxidised metabolites entering the general circulation dependent upon the route of exposure. Although CYP3A4, together with CYP2C8, is able to oxidise chlorpyrifos, *in vitro* studies indicate that the action of A-esterases in the liver is the most important route of metabolism for this OP. These enzymes are involved in both the activation and detoxification of thion OPs. Changes in their activity have complex effects, the overall change in toxicity depending upon the balance between the two processes.

Methods for assessment of exposure: biomarkers

Urinary excretion of OP metabolites

4.25 Urinary excretion of metabolites can be used as a biomarker of exposure to some OPs, but the time-course of excretion may vary with the dose.² In consequence, the most reliable data are derived from the analysis of 24-hour urine collections made prior to and

after the period of exposure. Sampling immediately prior to exposure is necessary because of the low, but variable, levels of these metabolites excreted in the absence of known recent exposure of the individuals. However, caution is needed with this approach when there may have been exposure to several OPs, because of differences in the extent to which different OPs produce the same metabolite (e.g. parathion-methyl and fenitrothion). In addition, any presystemic metabolism (metabolism at the site of entry into the body before uptake into the general circulation), especially hydrolysis, would result in an over-estimation of the systemic dose when it is based upon the urinary excretion of inactive metabolites. Thus, urinary metabolites should only be regarded as biomarkers for exposure and uptake and not for toxic effects.

4.26 There are limited data on the rates of elimination of OP metabolites in urine. It was concluded in the IEH review¹⁸ that “with transient exposure complete excretion can take as long as two days, especially after dermal exposure”. However, some data are consistent with very slow elimination over many days or possibly weeks. Very slow elimination of earlier doses might explain the pre-exposure levels found in many subjects in controlled studies (e.g. of sheep dipping^{7,19}) and those found in kibbutz workers before the spraying season.²⁰ In the latter study, urinary metabolites were not detectable in laboratory workers at any time. The possibility of a very prolonged half-life appears, at first sight, to be incompatible with the half-lives of diazinon in experimental animals (paragraph 4.16) and with the finding that in rhesus monkeys about 40% of an intravenous dose of ¹⁴C-diazinon is eliminated within the first 24 hours.¹¹ However, this study reported a subsequent very slow and almost constant elimination of about 5% of the dose per day between days 3 and 7, and a similar time profile of excretion has been reported in humans following dermal application.¹¹ The apparent discrepancy could arise from a combination of efficient metabolism and high lipid-solubility. Diazinon is highly lipid-soluble, so that it would be readily taken up by adipose tissue and very slowly released back into the general circulation. The rapid initial excretion of metabolites after intravenous dosage in monkeys could arise from metabolism of the higher circulating concentrations of the compound which occur during the distribution phase, following which the rate of metabolite formation would be largely determined by release from adipose tissue. Propetamphos has a similar lipid-solubility to diazinon and probably shows a similar *in vivo* rate of elimination. However, this suggestion of retention of part of the dose in adipose tissue, and its subsequent slow release and metabolism appears incompatible with the absence of any evidence of accumulation, as measured by excretion of metabolites by the sheep handlers in a trial which involved the repeated handling of dipped sheep over a period of 10 weeks.²¹

4.27 About 70% of an oral dose of 0.5 mg/kg of chlorpyrifos given to humans was recovered as metabolites in the urine within 6 to 7 days of dosing and this was accompanied by significant lowering of erythrocyte acetylcholinesterase activity.²² Dermal application of a similar dose resulted in between 1 to 2% appearing in the urine as metabolites and was accompanied by no inhibition of acetylcholinesterase, which indicated limited uptake. The elimination half-life was about 24 hours following the oral dose, but urinary measurements following dermal application indicated considerably slower elimination over the first 5 to 8 days. The site of dermal application was not occluded and the subjects washed the area 12 to 20 hours after application. Thus the metabolic fate of the remaining 98% of the dose was not known. The possibility of slower urinary

Organophosphates elimination after dermal compared with oral dosage suggests that the value of 1 to 2% uptake noted above may be an underestimate.

4.28 Interpretation of urinary metabolite data is complicated by uncertainty about the source of low levels of such metabolites in the absence of known recent exposure to OPs and whether these represent unrecognised exposures or late elimination from exposures that occurred many days previously. It is possible that these background levels may be derived in part from food and water (see paragraphs 4.2 and 4.3). The use of urinary metabolite data as a biomarker of exposure should be regarded with caution pending definition of the time-relationships between OP exposure and elimination of metabolites, as determined by an adequately performed radiolabel balance study. The results of a recent study²³ show a clear temporal relationship between handling sheep dip concentrate and the excretion of urinary metabolites, but a less consistent and weaker relationship with dermal exposure to the diluted dip itself.

Blood cholinesterase activities

4.29 In addition to being found in nerve synapses, cholinesterase activity is present in erythrocytes, lymphocytes and serum or plasma. Erythrocyte acetylcholinesterase, which is similar to the synaptic enzyme, shows different substrate and inhibitor specificity from that demonstrated by the serum (plasma) enzyme, which is also called pseudocholinesterase. In consequence, erythrocyte acetylcholinesterase inhibition may be used as a biomarker for the cholinergic effects of OPs, whereas inhibition of pseudocholinesterase is not related closely to this effect and is solely a marker of exposure and uptake. Some studies have used whole blood cholinesterase activity (which would include both enzymes), and this should also be viewed as a biomarker of exposure. An esterase occurring in lymphocytes is similar to NTE and inhibition of this enzyme is linked to delayed neuropathy in experimental animals (see paragraphs 5.9 and 5.10): this esterase has been measured in some studies, especially those of cases of severe acute poisoning.²

4.30 When average acetylcholinesterase activities are derived for groups of people, small but statistically significant differences in activity can be a measure of exposure or effect. However interpretation of acetylcholinesterase activity in an individual is difficult in the absence of baseline (pre-exposure) data: without baseline measurement exposure can only be inferred with confidence if inhibition exceeds the normal range of values found in unexposed subjects. A further problem in the interpretation of acetylcholinesterase activities is that the inhibited enzyme can undergo reactivation on storage of a blood sample.

4.31 The correlation between inhibition of erythrocyte acetylcholinesterase and clinical symptoms and signs is strong for short-term high-dose exposures. However, the relationship is weaker for prolonged exposures, possibly due to the development of tolerance, in which the clinical features become less apparent with the passage of time despite continuing inhibition of acetylcholinesterase.²

4.32 The use of erythrocyte acetylcholinesterase activity to compare the effects of different OPs on the nervous system assumes that there is equal access from the circulation to the enzyme for the different compounds. In relation to possible adverse effects within

the brain, differences in permeability of the blood: brain barrier to different OPs could result in different concentrations in the brain for a given concentration in blood, and therefore different biomarker:effect relationships. It has been suggested that the stress associated with farming may have contributed to enhanced central nervous system effects in farmers exposed to OPs as a consequence of sheep dipping and that this could occur in the absence of lowered erythrocyte acetylcholinesterase activity.²³ Animal experiments have shown that physical stress can increase the permeability of the blood:brain barrier for the polar drug pyridostigmine (not an OP), giving rise to increased drug concentrations and consequent inhibition of acetylcholinesterase within the brain.²⁴ Stress did not affect entry into the brain, or activity within the brain, of the lipid-soluble drug physostigmine. The Working Group concluded that stress-associated changes in brain uptake would not be relevant to the two principal OPs used in sheep dips (diazinon and propetamphos) because, like physostigmine, these are very lipid-soluble and would readily cross the blood:brain barrier under normal conditions.

The extent of occupational exposure

Sheep dipping

4.33 Sheep dipping involves handling of the concentrated formulation when preparing the dip together with the processes of handling and submerging the sheep in the diluted dip. During the former there is a greater potential for exposure and uptake because of the use of concentrated formulation, which is likely to enhance dermal absorption and which, because of its greater concentration, would give a higher vapour pressure of OP than the diluted dipwash. The dip bath is topped-up with concentrate at intervals during the day and handling the concentrate is therefore not a single event occurring at the initial bath preparation but is repeated on average eight times during a day's dipping depending on the size of the flock and the product used.²³ However the process of preparing the dipwash is of much shorter duration than dipping itself which may take up to eight hours. The recent IOM study showed that the majority of uptake was due to handling the concentrate, rather than being a result of being splashed by the dipwash itself.²³

4.34 Before 1991 users of sheep-dips received limited advice about potential adverse effects, and there was a general view that OPs were less harmful than the organochlorine agents they replaced. In consequence, exposures at this time are likely to have been higher than those measured more recently.

4.35 A study of sheep dippers in 1993 found that airborne levels of diazinon during dipping were below the limit of detection (<0.01 mg/m³), indicating that inhalation would be of negligible importance.⁷

4.36 In another study of exposure during sheep dipping there was no correlation between urinary OP metabolites and estimated exposure as judged by "splashing score" (adjusted for protective clothing).⁷ In contrast, there was a significantly higher excretion of metabolites by those who handled the sheep dip concentrate ($P<0.01$). A similar conclusion was reached in the recent IOM study.²³ The exposure index calculated in Phase I of that study was determined principally by the handling of the concentrate. This

Organophosphates conclusion is consistent with data on the urinary OP metabolites excreted by different occupational groups²⁵ (see Table 4.4). These findings suggest that exposure to the higher concentrations of OPs present in the concentrated formulations (range 8 to 60%) as compared with the diluted dip (0.03 to 0.04%) outweighs the longer duration of exposure to the dip.

4.37 In a further study on sheep dippers there was no statistically significant decrease in erythrocyte and plasma cholinesterase activities following a single sheep dipping session using normal clothing, or using protective clothing.^{7,19} Protective clothing reduced the contamination of normal clothing (worn inside the protective clothing) by up to 100-fold. However, urinary excretion of OP metabolites was similar in studies of sheep dipping in which protective clothing was worn¹⁹ and when fewer precautions were taken.⁷ The similarity of the urinary metabolite levels in these two studies remains unexplained. A possible explanation for the absence of an effect of protective clothing on the excretion of urinary metabolites could be the presence of significant non-dermal exposure in both situations. This is unlikely however, in view of the undetectable amounts of OP in the air and the nature of the supervision of the workers throughout the studies. The finding illustrates the difficulty of interpreting the measurements of biomarkers at low levels of exposure, under circumstances where erythrocyte acetylcholinesterase activity is unchanged, together with the problems of the presence of measurable pre-exposure concentrations of metabolites in some subjects.

4.38 OPs persist in the fleece for a time after dipping, with a half-life for loss from the fleece of 12 to 53 days, depending on the nature of the OP and site of measurement.²⁶ The potential for exposure of workers from handling sheep in the weeks following dipping would be limited, compared with that during dipping, because of the small surface area of skin exposed (primarily the hands) and the vehicle (wool grease which would not wet large areas of the skin and would not enhance absorption). In one study the handling each week of dipped sheep during 1 to 10 weeks after dipping with a diazinon formulation resulted in the deposition of 1-2 mg diazinon on the hands (which was removed by washing) and slightly less on the boiler suit worn during handling.²¹ The weekly urinary excretion of diazinon metabolites, diethylphosphate (DEP) and diethylphosphorothioate (DEPT), was usually less than 10 nmole/m mole creatinine and varied from being below the limit of detection to 26 nmole/m mole creatinine. These values are lower than those reported following sheep dipping which caused a mean increase of 23 nmole/m mole creatinine, with a range from below the limit of detection to 151 nmole/m mole creatinine²³ (see Table 4.4).

Spray workers

4.39 Results from a study of orchard sprayers using chlorpyrifos have recently been reported.⁶ Urinary excretion of metabolites was measured before, during and after the spraying session. Elevated levels of DEP were found in pre-exposure urine from 11 out of 63 individuals studied, with the highest amounts in those who had been exposed to OPs in the week prior to the investigation. The excretion data are summarised in Table 4.4.

Table 4.4: Urinary excretion of OP metabolites

Exposed group	Metabolite measured	Pre-exposure	Post-exposure	Reference
Agriculture Spray workers	DMP	0-21 nmoles per mmole creatinine	0-76 nmoles per mmole creatinine	Nutley and Cocker (1993) ²⁵
	DEP	0-4 nmoles per mmole creatinine	0-43 nmoles per mmole creatinine	
	DEPT	0-4 nmoles per mmole creatinine	0-116 nmoles per mmole creatinine	
	DMPT	0-22 nmoles per mmole creatinine	0-474 nmoles per mmole creatinine	
Sheep Dippers	DMP	0-8 nmoles per mmole creatinine	0-51 nmoles per mmole creatinine	
	DEP	0-7 nmoles per mmole creatinine	0-63 nmoles per mmole creatinine	
Formulation Workers	DMP	nd	0-352 nmoles per mmole creatinine	
	DEP	nd	0-386 nmoles per mmole creatinine	
	DEPT	nd	1-93 nmoles per mmole creatinine	
	DMPT	nd	1-320 nmoles per mmole creatinine	
Pesticide (Diazinon) applicators	DMPT ^(a)	0-40 µg per gram creatinine	0-31 µg per gram creatinine	Maizlish <i>et al.</i> (1987) ¹⁷
	DEPT	0-44 µg per gram creatinine	0-356 µg per gram creatinine	
	DEPT	0-34 µg per gram creatinine	0-84 µg per gram creatinine	
Sheep Dippers	DEP + DEPT	–	45 (27-63) nmoles per mmole creatinine (mean and 95% CI)	Stephens <i>et al.</i> (1996) ²⁸
Quarry Workers (controls)	DEP + DEPT	–	5 (1-8) nmoles per mmole creatinine (mean and 95% CI)	
Pesticide applicators (These were stratified into different number of hours sprayed in the preceding 4 days)	DEPT ^(b)	–	2-396 ppm urine	Stokes <i>et al.</i> (1995) ²⁹
0.5 – 8 hours	DEPT ^(b)	–	2-143 ppm urine	
9–18 hours	DEPT ^(b)	–	4-274 ppm urine	
19–61 hours	DEPT ^(b)	–	8-396 ppm urine	

Organophosphates **Table 4.4: Urinary excretion of OP metabolites** continued

Exposed group	Metabolite measured	Pre-exposure	Post-exposure	Reference
Spray workers (chlorpyrifos)	DMP	0-48 nmoles per mmole creatinine	0-135 nmoles per mmole creatinine	HSE (1998) ⁶
	DMPT	0-41 nmoles per mmole creatinine	0-120 nmoles per mmole creatinine	
	DEP	0-42 nmoles per mmole creatinine	0-48 nmoles per mmole creatinine	
	DEPT	0-13 nmoles per mmole creatinine	0-20 nmoles per mmole creatinine	
Sheep Dippers	DMP ^(a)	0-38 nmoles per mmole creatinine	0-84 nmoles per mmole creatinine	Niven <i>et al.</i> (1993) ⁷
	DEP	0-39 nmoles per mmole creatinine	0-84 nmoles per mmole creatinine	
	DMPT	0-24 nmoles per mmole creatinine	0-107 nmoles per mmole creatinine	
	DEPT	0-26 nmoles per mmole creatinine	0-102 nmoles per mmole creatinine	
The sheep dippers were stratified by job description as follows:				
Paddler	DEP+DEPT	0-14 nmoles per mmole creatinine	0-48 nmoles per mmole creatinine	
Chucker	DEP+DEPT ^(c)	0-39 nmoles per mmole creatinine	4-154 nmoles per mmole creatinine	
Helper	DEP+DEPT	0-65 nmoles per mmole creatinine	0-75 nmoles per mmole creatinine	
Contractor (n= 3 only)	DEP+DEPT	0-7 nmoles per mmole creatinine	17-42 nmoles per mmole creatinine	
Sheep handlers (overall)	DEP	0-13 ^(d) nmoles per mmole creatinine	0-18 nmoles per mmole creatinine	CVL (1993) ²¹
	DEPT	0-5 nmoles per mmole creatinine	0-10 nmoles per mmole creatinine	
After 1 week handling sheep	DEP	0-9 nmoles per mmole creatinine	0-17 nmoles per mmole creatinine	
	DEPT	0-4 nmoles per mmole creatinine	4-9 nmoles per mmole creatinine	
After 10 weeks handling sheep	DEP	0-8 nmoles per mmole creatinine	0-9 nmoles per mmole creatinine	
	DEPT	0-5 nmoles per mmole creatinine	0-4 nmoles per mmole creatinine	
Sheep dippers	DEP+DEPT+DEPDT	0-54 nmoles per mmole creatinine	0-227 nmoles per mmole creatinine	Niven <i>et al.</i> (1994) ¹⁹

Table 4.4: Urinary excretion of OP metabolites continued

Exposed group	Metabolite measured	Pre-exposure	Post-exposure	Reference
These were stratified by job description:				
Paddler	DEP+DEPT+ DEPDT	0-26 nmoles per mmole creatinine	16-138 ^(d) nmoles per mmole creatinine	
Chucker	DEP+DEPT+ DEPDT	0-6 nmoles per mmole creatinine	5-222 ^(e) nmoles per mmole creatinine	
Helper	DEP+DEPT+ DEPDT	0-54 nmole creatinine	0-70 nmoles per mmole creatinine	As above in both cases
Sheep dippers	DEP	0-28 nmoles per mmole creatinine	0-85 nmoles per mmole creatinine	Sewell <i>et al.</i> (1999) ²³
	DEPT	0-47 nmoles per mmole creatinine	0-348 nmoles per mmole creatinine	

(a) Would not be expected to be a metabolite of diazinon.

(b) A metabolite of azinphos-methyl which was used by 80% of pesticide applicators in this study.

(c) Both subjects with values >100 nmoles per mmole creatinine handled the concentrate.

(d) One spurious value excluded.

(e) The two subjects with values >100 nmoles per mmole creatinine worked a long session on the same farm and the concentrate was spilled on the feet of the subject with a value of 138 nmoles per mmole creatinine.

Key

DMP	=	dimethylphosphate
DEP	=	diethylphosphate
DMPT	=	dimethylphosphorothioate
DEPT	=	diethylphosphorothioate
DEPDT	=	diethylphosphorodithioate
nd	=	not determined

4.40 The presence of aerosols or particulates probably accounts for the high airborne concentrations of parathion detected in the cockpits of aircraft used for crop spraying and in the aircraft washing area compared with that detected in the loading area.²⁰

4.41 Comparisons of whole blood, erythrocyte and plasma cholinesterase activities in workers who were spraying OPs, and local kibbutz residents who were either exposed or not exposed to spray drift found no significant differences between groups or changes between baseline and in-season measurements.²⁰ In contrast, there was a significant difference in the urinary excretion of metabolites between kibbutz workers and kibbutz residents, indicating that despite its limitations (see paragraph 4.28) the excretion of urinary metabolites was a more sensitive biomarker of exposure than cholinesterase inhibition in these circumstances.

Organophosphates 4.42 The degradation products of OPs which arise as a consequence of their exposure to light and air may produce adverse effects in humans more rapidly than the original OP, and this may be particularly relevant when workers re-enter sprayed fields if an inadequate time is allowed between spraying and re-entry.

Workers involved in formulation of OP products

4.43 Among a group of formulation workers a high proportion of individuals were found to excrete DEP and DEPT (62% and 86% respectively) in their urine after exposure, with a wide range in the urinary levels of these metabolites.²⁵ This was higher than the proportion of individuals excreting these metabolites after exposure through agricultural spraying (58% and 30%) and dipping sheep (34% and 47%). The ranges of DEPT excretion were similar in all three groups, but the maximum excretion of DEP (and dimethyl-metabolites) was much higher in formulation workers (see Table 4.4 for details).

4.44 The combined urinary metabolite excretion data during a one year period in one formulation worker paralleled changes in plasma pseudocholinesterase activity and provided a sensitive biomarker with the maximum excretion (about 500 nmoles of DEP + DEPT per mmole of creatinine) coinciding with a 30 to 40% reduction in plasma pseudocholinesterase activity; in contrast, erythrocyte acetylcholinesterase activity was not reduced significantly at any stage.²⁵

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5. Toxicology of OPs and the mechanisms involved

Toxicology: class effects of OPs

5.1 As noted in the introduction, the Working Group concentrated their attention on toxic effects that are known or suspected to be common to OPs in general (i.e. class effects) rather than compound-specific effects.

5.2 The accepted general “class effects” of OPs fall into three main groups:

- acute (short-term) effects of acetylcholinesterase inhibition (the acute syndrome);
- delayed effects following inhibition of acetylcholinesterase (the intermediate syndrome);
- delayed polyneuropathy.

5.3 The characteristic effect of OPs is inhibition of acetylcholinesterases and inhibition of erythrocyte acetylcholinesterase activity in humans exposed to OPs has been considered to be the most sensitive indicator of possible adverse effects,¹ although measurement of urinary metabolites is now considered to be the most sensitive indicator of uptake.²

The acute syndrome – symptoms and signs

5.4 The mechanism underlying the characteristic acute toxic effects produced by OP pesticides is well established.¹ It is based upon the reaction of the OP with the enzyme acetylcholinesterase resulting in the production of organophosphorylated derivatives of the enzyme which are inactive (see paragraphs 3.7 to 3.9 and Figure 3.1). Acetylcholinesterase is responsible for metabolising acetylcholine, an important chemical transmitter at neural and neuromuscular synapses (junctions). Inhibition of the enzyme results in accumulation of acetylcholine at receptors in the brain and spinal cord, at neuromuscular junctions, at ganglia of the autonomic nervous system and at parasympathetic (muscarinic) nerve endings. The resulting excess cholinergic drive and continued high level of receptor activation leads to the characteristic symptoms and signs of acute toxicity of OPs, which are summarised in Table 5.1.

Organophosphates **Table 5.1: Acute cholinergic effects of OPs, see reference 9**

Receptor	Site	Symptoms and signs
Mixed	Central nervous system	Giddiness, anxiety, restlessness, headache, confusion, failure to concentrate, respiratory depression
Muscarinic	Glands	Excessive secretion, e.g. saliva, mucus, sweat, tears (parasympathetic nervous system)
	Smooth muscle	Effects on gastrointestinal tract (diarrhoea), pupil (miosis and failure to focus), bladder (involuntary micturition) and heart (bradycardia)
Nicotinic (N1)	Autonomic	Increased sympathetic drive, e.g. hypertension, tachycardia
Nicotinic (N2)	Neuromuscular junction	Fasciculation of muscle followed by weakness and paralysis

5.5 All OPs used in pesticide products or veterinary medicines are capable of producing the effects shown in Table 5.1. Their severity and magnitude depends upon the reactivity of the OP (see paragraph 3.2), the route and extent of exposure, the relative extent of bioactivation and inactivation of the OP, and on other factors such as its deposition in fat. Symptoms and signs of toxicity that follow a single exposure are closely related to inhibition of acetylcholinesterase. Life-threatening effects mediated through muscarinic sites can be prevented by administration of antagonists such as atropine. Phosphorylated acetylcholinesterase can be reactivated by the administration of oxime derivatives, for example, pralidoxime,³ but this is unsuccessful once complete ageing of the phosphorylated product has occurred (see Figure 3.2).

5.6 With repeated exposure to OPs, the induction of tolerance may result in the loss of cholinergic symptoms and signs despite continued inhibition of acetylcholinesterase. The mechanism for this tolerance is unknown but may involve an altered regulation of muscarinic (acetylcholine) receptors.

The intermediate syndrome

5.7 This syndrome was not recognised until recently, despite the many cases of acute OP poisoning in humans reported throughout the world.⁴ The syndrome is characterised by muscle weakness involving the limb, neck and respiratory muscles which starts 1 to 4 days after a poisoning incident. The effects last from 5 to 18 days and may result, in part, from muscle necrosis.⁵ The established syndrome does not respond to treatment with atropine or oximes and can result in respiratory failure. Experimental animal studies indicate that muscle necrosis following OP poisoning can be reduced or prevented by treatment with acetylcholinesterase reactivators⁶ or acetylcholine antagonists.⁷ It has been suggested² that early administration of acetylcholinesterase reactivators to poisoned humans would reduce the severity of the intermediate syndrome but there are no reported clinical studies demonstrating this.

Delayed polyneuropathy

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5.8 Organophosphate-induced delayed polyneuropathy (OPIDPN) is a sensorimotor polyneuropathy, predominantly affecting the lower limbs, combined with varying degrees of ataxia. Symptoms and signs develop over a period 1 to 4 weeks after intoxication.^{8,9} Degeneration of the distal ends of longer axons of some distal and spinal nerves is followed by myelin breakdown, Schwann cell proliferation and macrophage accumulation.^{10,11} The condition does not respond to treatment with drugs having an anticholinergic action or oximes, and recovery is slow and often incomplete; the CNS component does not recover.¹² For OPs capable of producing OPIDPN the successful treatment of the acute intoxication does not prevent the occurrence of the delayed neuropathy.

5.9 Not all OPs cause OPIDPN and testing for this property is a crucial component of the screening tests for approval of OPs. The mechanism is believed to be associated with the phosphorylation and ageing of a particular enzyme, neuropathy target esterase (NTE, previously known as neurotoxic esterase) within neurons by processes analogous to those described for acetylcholinesterase.^{13,14} The generation of a negative charge at the phosphorylated site underlies the ageing process and appears to be an essential step in the generation of OPIDPN. Certain 'inhibitory' phosphinates and carbamates have chemical structures that allow them to bind covalently to NTE but preclude the possibility of an ageing reaction. Such compounds do not produce OPIDPN and their binding to NTE prevents certain other OPs producing OPIDPN.

5.10 NTE is found in all mammalian species including humans, and OPIDPN occurs in all species tested. However, it does not always occur to the same extent, and clinical signs are difficult to produce in rodents. The hen is more sensitive than many other species to the development of an OPIDPN, and develops clinical signs similar to those seen in humans.¹⁵ It is therefore used as the test species when screening compounds for the potential to cause this syndrome. The current test includes a requirement for both a measure of the inhibition of NTE and for monitoring of clinical and morphological signs of OPIDPN.^{16,17} Inhibition of NTE to the extent of 70% or more, together with the observation of clinical effects and pathological changes, defines a positive result. Both humans and hens develop an OPIDPN at similar degrees of NTE inhibition and the hen test is therefore a good predictor of neurotoxic potential. The absolute sensitivity of humans to this type of OP toxicity depends on the OP and species-specific pharmacokinetic factors, which are less well predicted by the hen test. Some caution in interpretation of the hen test is required since there is the possibility that the metabolism of OPs in the hen may differ from that in humans. The hen does have a lower ability than humans to detoxify many OPs and it is regarded as a sensitive model for OPIDPN. However, this relates principally to quantitative (effective dose) considerations rather than to qualitative differences since the only proximal toxins produced in either species are esterase inhibitors and the relative sensitivity of hen and human enzymes (acetylcholinesterase and NTE) have been shown to be similar.

5.11 A hen test is included as a requirement of the regulatory approval process for OPs in both pesticides and veterinary medicines. All OP pesticides approved in the UK have been evaluated to be negative in the test, in some cases using the current guideline. Furthermore, as part of the approval process consideration is also given to chronic toxicity studies (which may include electrophysiological measures) in rodents; data from metabolic

Organophosphates studies in human tissues may also be available. These data provide additional useful information when interpreting hen tests. Given the far lower sensitivity of NTE than acetylcholinesterase to all registered OP pesticides and veterinary medicines, classical OPIDPN would not now be expected to occur in the UK in the absence of acetylcholinesterase inhibition and signs of acute toxicity.

5.12 Pesticides known to produce OPIDPN in the hen at very high doses which can only be survived if antidotal treatment is given are: chlorpyrifos, coumaphos, cyanofenphos, DEF (*S,S,S*-tributylphosphorotrithioate), dioxabenzophos, EPN, dichlorvos, haloxon, isofenphos, leptophos, merphos, methamidophos, mipafox and trichlorfon.¹ The first two of these in the past have been used in licensed sheep dip products in the UK but the others have not; licences of chlorpyrifos products expired in 1989 and those of coumaphos products in 1991.

Other putative mechanisms of toxicity

5.13 As noted earlier, mechanisms underlying the acute syndrome, and the initial toxicity seen in the intermediate syndrome, relate to inhibition of acetylcholinesterase. The mechanism involved in the induction of OPIDPN relates to phosphorylation and ageing of the enzyme NTE. Consideration is given in the following sections to other mechanisms that might be involved in producing longer-term effects either following episodes of acute toxicity or following low-level exposure to OPs that was not associated with any of the characteristic symptoms or signs of acute OP toxicity.

Longer term effects after acute poisoning

5.14 There are several possible mechanisms by which longer-term toxic effects might follow acute poisoning by OPs. These are summarised in Table 5.2. Not all of these relate directly to acetylcholinesterase inhibition.

5.15 In parallel with the binding of OPs to acetylcholinesterase, OPs may also bind to serine residues in proteins such as proteases, esterases and sites of protein kinase action in cell signalling. For example, pirimiphos-methyl has been shown to inhibit proteases in the liver at doses that have little effect on acetylcholinesterases.¹⁸ Such phosphorylation of proteases could lead to general degenerative effects such as cell death through apoptosis of neurons or glial cells in the peripheral and central nervous systems. Alternatively, there could be disturbance of cell signalling pathways through binding of OPs to tyrosine or serine residues in proteins.

Table 5.2: Summary of putative mechanisms of OP toxicity other than inhibition of acetylcholinesterase or neuropathy target esterase

Phosphorylation of proteases, esterases or proteins involved in cell signalling
Interaction with cytoskeletal proteins
Excessive calcium influx in cells at nerve endings
Prolonged receptor stimulation at nerve endings leading to muscle fasciculation and necrosis
Hypoxic brain damage
Psychological stress from an acute episode producing post-traumatic stress disorder

5.16 OPs can react with cytoskeletal proteins in nerve cells leading to histological damage and resultant functional impairment within the nervous system. For example, neurotoxicity is associated with increased phosphorylation of the enzyme calmodulin kinase II together with enhanced phosphorylation of cytoskeletal proteins.^{19,20} These effects are not dependent on acetylcholinesterase inhibition.

5.17 Short-term exposure to toxic doses of potent OPs can produce effects on the “jitter” of muscle impulses with increased variation in the delay between stimulation of motor nerves and the subsequent production of action potentials in the muscle. Excessive calcium influx (which can produce local muscle fibre damage) at the motor end-plate is a putative mechanism for the long-term effects seen on jitter in skeletal muscles following exposure to the potent OP sarin (a nerve agent). In a study of sarin, effects on jitter measured by single fibre EMG (SFEMG) lasted for 30 months after a single exposure, considerably beyond the time when the acetylcholinesterase activity would have returned to normal.²¹

5.18 The excessive accumulation of acetylcholine at neuromuscular junctions and the prolonged transmitter-receptor interaction produced by OPs can lead to contractions of the innervated muscle fibres and fasciculation (visible flickering of muscles). In some muscle fibres this is associated with cell death. It is thought that the necrosis results from excessive entry of calcium ions into muscle cells.²²⁻²⁴ This mechanism is responsible for the intermediate syndrome, which may occur after severe acute poisoning (see paragraph 5.7), and might also give rise to longer-term effects.

5.19 Another possible cause of long-term toxicological sequelae following moderate to severe acute toxicity is anoxia or hypoxia resulting from convulsions or respiratory impairment. This may lead to death of nerve cells and irreversible neurological dysfunction through hypoxic brain damage.

5.20 In addition, it is possible that psychological stress following an acute poisoning episode could trigger psychiatric illness such as post-traumatic stress disorder.

5.21 In summary, there are various putative mechanisms whereby long-term toxic effects could follow acute cholinergic episodes. These mechanisms may explain why adverse effects may be seen a long time after acetylcholinesterase levels have returned to normal. Mechanisms that could lead to irreversible damage to nerves and muscle fibres include organophosphorylation of proteases and other esterases, muscle fasciculation, necrosis and anoxia.

Longer-term effects following low-level exposure not associated with overt acute toxicity

5.22 In theory some of the mechanisms outlined above could also produce long-term adverse effects from exposures to OPs insufficient to cause overt toxicity through inhibition of acetylcholinesterase. Thus, inhibition of other enzymes, specifically proteases or esterases, might produce general degenerative effects in nerve cells at exposures producing no significant effects on acetylcholinesterase levels (see paragraph 5.15). Similarly chemical reactions with cytoskeletal proteins in nerve cells could result in impaired function independent of any effects on acetylcholinesterase (see paragraph 5.16).

Organophosphates 5.23 When considering possible long-term effects of OPs it is pertinent to note that the approval process for pesticides and veterinary medicines includes a requirement for chronic toxicity studies in animals as part of the database to be assessed before approval. These are used to reveal adverse effects produced after prolonged high-dose exposure and to define an exposure that does not produce adverse effects in animals.

Potentialiation

Interactions between OPs

5.24 Interactions between OPs could occur at the site of action (toxicodynamics) or by interference with detoxification and elimination (toxicokinetics). Toxicodynamic interactions arising from exposure to a number of OPs would be expected to give additive effects rather than potentialiation. Potentialiation of toxins may occur when the enzymatic detoxification of one compound is inhibited by the presence of a second compound, thus resulting in an increase in toxicity. Such effects are rare but may be significant when the second compound is a potent inhibitor of the detoxification process. The ability of OPs to interact in this way has been studied in detail and the results have been reviewed comprehensively.²⁵ Most combinations produced additive effects rather than potentialiation. Limited potentialiation has been observed between malathion and EPN but only when the dosage of each OP was high and close to that which would saturate the detoxification enzymes.²⁶ If the doses of the two compounds were sufficiently small and there was enough enzyme present to detoxify both compounds, potentialiation would not occur. There has been one documented instance of substantial potentialiation (resulting in an 88- to 134-fold increase in activity) between malathion and TOCP which is a potent inhibitor of malathion detoxification.²⁷ It may also induce the cytochrome P-450 that activates malathion to its oxon (P. Blain, personal communication). Although TOCP is an organophosphate it is not a pesticide and thus concurrent exposure to both compounds is unlikely.

5.25 In order for potentialiation to occur, the interacting compounds need to be present simultaneously, or almost simultaneously, at concentrations that are near to those producing toxic effects. It has been noted that there is unlikely to be any danger of potentialiating effects arising from OP residues in food provided that the tolerances for the individual pesticides are not exceeded.²⁸ A rare instance of OP poisoning in an occupational context resulting from potentialiation involved a malathion formulation that had deteriorated on storage in the tropics. The enhanced toxicity resulted from an interaction with isomalathion.²⁹

Interaction with other compounds

5.26 Interactions between OPs and other chemicals could involve effects on either kinetics or dynamics. Toxicokinetic effects would occur when there is exposure to, and uptake of, two or more compounds that share the same metabolic pathways of activation and detoxification. In general such effects are only important at relatively high exposure levels, since with low-level exposure there is usually sufficient metabolic capacity to

metabolise the OPs and the other compounds concurrently. There are numerous potential interactions of this type, the vast majority of which have not been investigated. In the case of OPs the most important enzymes in their detoxification and elimination are esterases and cytochrome P-450 3A (also known as CYP3A4), or other cytochrome P-450 enzymes. These cytochromes may be involved in the metabolism of a wide range of drugs (e.g. erythromycin, terfenadine, ketoconazole, nifedipine and also some used in the treatment of AIDS), and thus there is a theoretical potential for interaction in people exposed to OPs who are undergoing drug therapy. Again, however, saturation of the enzyme by the combined substrates would be necessary for interaction. In addition certain drugs such as the anti-ulcer drug cimetidine, and dietary components such as bergamottin, and naringenin (which occurs in grapefruit juice) and quercetin (occurring in some vegetables) are potent CYP3A inhibitors.

5.27 Toxicodynamic interactions are less well documented but an example is the increase in the severity of OPIDPN as a result of subsequent exposure to “promoters”, i.e. substances that are not neurotoxic in themselves but can enhance the neuropathy caused by another agent in the hen and rodent models. For example, phenylmethanesulphonyl fluoride (PMSF) has been shown to increase the severity of OPIDPN lesions by three-fold in the hen, possibly by inhibiting repair mechanisms.³⁰ Human exposure to such promoters is rare since they are chemicals used principally in research.

5.28 The possibility of interaction of OPs with anaesthetics has been highlighted in the report of the Royal Colleges of Physicians and Psychiatrists.³¹ Potential mechanisms could be through interaction of effects (i.e. toxicodynamics) or interaction in the metabolism of the compounds (i.e. toxicokinetics). It was considered by the Working Group that OPs would be more likely to prolong recovery from anaesthesia than to cause post-operative mortality, and the former would be difficult to investigate because data are not routinely collected on such effects. Currently there is no evidence to support an interaction of this type.

5.29 Possible interactions of OPs with co-formulants used in pesticide or veterinary medicine products have been considered earlier (see paragraph 4.10).

Variation in individual susceptibility to the toxic effects of OPs

5.30 The severity of the toxic effects of OPs is to a large extent dependent on rates of metabolic activation and detoxification. Many OPs widely used as insecticides, e.g. parathion, chlorpyrifos, propetamphos and diazinon, are thion compounds and undergo oxidative metabolism to biologically active oxon forms. Hydrolysis then yields inactive metabolites. It is known that there is considerable individual variability (up to about 15-fold) in the activity of the key enzymes involved, e.g. cytochrome CYP3A4, responsible for oxidative desulphuration of parathion, and paraoxonase, an esterase in blood responsible for the hydrolysis of many oxons. This is considered in more detail in paragraphs 4.21 to 4.24. In addition there is likely to be variability between individuals in the reactivation and ageing of acetylcholinesterase (see paragraphs 3.9 and 3.10). There is thus the potential for considerable variation in an individual's response to OPs with regard to adverse effects observed. It should be noted, however, that this is similar to the situation with many other toxic chemicals.

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6. Chronic toxicity of OPs: the basis for concern

6.1 As has been explained in the Chapter 2, the Working Group was anxious to take account of all possible data that might be relevant to their remit. In addition to information available from the published literature, which is examined in depth in the next chapter, evidence was sought from various other sources. The nature of these sources and the information obtained from them is summarised in this chapter.

Data from submissions made by individuals and groups

6.2 Several individuals gave personal testimony of the illnesses they had suffered following exposure to OPs, and the Working Group were informed that data are held by two organisations, the OP Information Network (OPIN) and Pesticide Exposure Group of Sufferers (PEGS) which indicated that many other people exposed to OPs suffered from similar symptoms. Many such individuals claimed long-term illness as a result of exposure to OPs during sheep dipping or other agricultural activities. A list of symptoms and signs reported by individuals who believed that they had suffered from exposure to OPs is given in Table 6.1. In many cases these severely impaired important aspects of normal life.

6.3 In addition, there were frequent references to dipper's flu being a concomitant of dipping. It was clear from enquiries made by the Working Group that there is no generally agreed definition of what constitutes dipper's flu. It is a term that has been used in common parlance in the farming community since the early 1990s. It is used to describe "flu-like" symptoms, including runny nose, headache, aching limbs and malaise occurring shortly after the time of dipping and persisting for up to 48 hours. The cause of dipper's flu is not known. It may be related to the anticholinesterase properties of OPs. However, there is an alternative hypothesis that it arises from exposure to endotoxins that accumulate in sheep dip. The Working Group concluded that the hypothesis that dipper's flu is a manifestation of OP toxicity is not proven. Research is needed both to characterise the nature of dipper's flu more fully and to identify the mechanism involved in its causation.

6.4 The nature of exposure to OPs recalled by individuals varied, some describing particular incidents of direct contact through handling the concentrated formulation, or through being splashed and soaked by the dipwash. Others referred simply to repeated exposure during regular cycles of dipping, notably when that process was compulsory. Some individuals pointed out that sheep continued to be handled from time to time during the months after dipping. There were varying accounts given in the submissions of the care taken with regard to the use of protective clothing and other protective measures (e.g. changing and washing of soaked clothing and washing of exposed skin) during the period when official guidance was first being promulgated and then later strengthened. It was difficult to establish any norm for the degree of exposure encountered, or evidence of excessive exposure by those who later developed more severe symptoms. It was notable that, despite the large quantities of OPs used in arable farming, relatively few cases were

Organophosphates brought to the attention of the Working Group of long-term adverse effects from the use of OPs in that sector of farming, or in horticulture.

Table 6.1: Symptoms/signs mentioned most frequently by individuals who believe that they have suffered long-term ill health from exposure to OPs*

Anxiety
Confusion
Depression (including suicidal thoughts in severe cases)
Headache
Fatigue
Impaired concentration
Incoordination
Increased sensitivity to repeated exposure to OPs
Intolerance to alcohol and other chemicals
Irritability
Memory loss
Muscular pains
Muscular spasms
Nausea
Nightmares
Numbness of the extremities
Other psychiatric disorder
Respiratory disease
Sleep disorders

* These are listed alphabetically and not in any order of priority

Data from Adverse Reaction Schemes

6.5 The Working Group also sought any relevant data that were available from the schemes for reporting adverse reactions to pesticides and veterinary medicines. These are the HSE's Pesticides Incidents Appraisal Panel (PIAP) and the VMD's Human Suspected Adverse Reaction Surveillance Scheme (SARSS).

6.6 In the case of PIAP, data from HSE's Pesticide Incidents Reports were available referring to the period from 1989/1990 until 1996/1997. These included information on the number of incidents assessed by PIAP when at least one of the active ingredients in the pesticide product was an OP. It was noted that PIAP only assessed cases that had been brought to the attention of HSE, and had subsequently been investigated by HSE or by a local authority. Only minimal data were available on symptoms and signs. There were a total of 69 confirmed cases over the study period, but all related to acute effects and there were no cases of chronic toxicity recorded in this process. Therefore analysis of these data was not particularly helpful to the Working Group.

6.7 In the case of veterinary medicines, data from the reports of the Appraisal Panel for SARS were also considered. Six hundred and fifty one reports of suspected adverse

reactions due to OP sheep dips have been received since 1985. Prior to 1991 the reports received each year were relatively few (ranging from 5 to 19 each year) with the greatest numbers being in 1991, 1992 and 1993 (127, 129 and 180 reports respectively) and then falling to 17 reports in 1998.¹ The number of cases by reported year of onset of the adverse reaction, which may differ from the year in which the reaction was reported, are shown above in Figure 3.5 and compared with annual sales of OP sheep dips over the same time period. In contrast to reported pesticide incidents, a substantial proportion of reported reactions to OP sheep dips involved persistent symptoms. Symptoms noted in the chronic cases were headache, fatigue, tiredness and, in a number of instances, numbness or tingling of the extremities.

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6.8 Following the recommendations from a review of the procedures for monitoring and investigating human suspected adverse reactions to veterinary medicines,² the Appraisal Panel no longer classifies individual cases according to likely causation. In the years up to 1997, after which this change in practice took place, the Appraisal Panel classified none of the chronic cases as showing strong evidence for an association with exposure to the cited dip product (the classification system used before 1998 is shown in Table 6.2).

Table 6.2: Classification system used by VMD's Appraisal Panel for Human Suspected Adverse Reactions until 1997

Class	Criteria
1	Clinical signs and symptoms typical of exposure to the cited formulated veterinary medicine combined with corroborating medical evidence, e.g. for OP sheep dip reports, cholinesterase depression.
2	The balance of evidence based on current knowledge, circumstances, clinical symptoms and signs, or biochemical evidence, where appropriate is consistent with ill health due to exposure to the cited formulated veterinary medicine.
3	There is strong evidence including medical reports that the symptoms are <u>not</u> related to the use of the cited formulated veterinary medicine.
4a	The reported ill health is not consistent with the known potential ill health effects of the cited formulated veterinary medicines given the reported exposure circumstances, but the implied association cannot be entirely discounted in the light of current knowledge.
4b	The evidence may be consistent with exposure to the cited veterinary medicine being the cause of the reported ill health but alternative explanations/confounding factors were involved e.g. pre-existing neurological, respiratory, or cardiac problems, or cases where concurrent infections could not be discounted.
5	Insufficient data were available to make a conclusion on the case. These may include those which are historical reports (often passed to the VMD by a third party), where further information is unavailable/unobtainable, or current reports where follow-up data is unavailable/not provided.

Data from the National Poisons Information Service (NPIS)

6.9 In addition to considering information from the specific adverse reaction schemes relating to pesticides and veterinary medicines, the Working Group sought data from all the NPIS centres in the UK. The extent and format of the data varied from centre to centre. The emphasis of the Working Group was on incidents due to inhalation or dermal exposure rather than deliberate ingestion, because the main concern of the Working Group was with

Organophosphates low level exposure. Although there were a large number of reports of such incidents, in nearly all cases the individuals were asymptomatic or suffered only mild, transient symptoms. However, it was recognised that the system was not designed to follow up poisoning incidents and that follow-up data were available in only a few cases.

Data from the Medicines Control Agency (MCA)

6.10 It was noted that within the data held by NPIS there were a number of reports of incidents relating to use of malathion in preparations to treat headlice, although in most cases these were due to accidental ingestion (100% of cases reported from one NPIS Centre involved ingestion); in all cases individuals were asymptomatic or suffered transient effects. These data were consistent with those provided by MCA from their yellow card adverse reaction reporting scheme for human medicines. The data related to the period January 1990 to September 1998. Only a small number of cases had been reported and there was no consistent pattern. The Working Group noted, however, that any delayed effects would be unlikely to be detected by this system, particularly for over-the-counter products such as shampoos for the treatment of headlice.

Conclusions

6.11 The substantial number of individuals with disabling illness that has been reported as following exposure to OPs is a major cause for concern. As a means of assessing the extent of the problem, the data considered from the various reporting schemes (PIAP, SARSS, MCA) and from the NPIS were of limited value to the Working Group. All of the reporting systems focused principally on the acute effects and were not designed to detect long-term consequences of prolonged or repeated low-level exposure, particularly if the illness produced was not specific to OPs. It was not possible therefore to draw any conclusions from these schemes regarding the frequency of possible delayed (or chronic) effects.

6.12 It also became clear from individual submissions that there were barriers to full reporting, such as consequences for employment, the large numbers in self-employment, and a culture of stoicism among agricultural workers. These would all reduce the number of cases reported. In addition, there was no means of gauging the overlap between the official reporting data, the case reports collected by OPIN, and the case reports collected by PEGS.

6.13 The Working Group was thus faced with a major problem regarding the data available. The sufferers reported very real, and for both them and their families, distressing illness, often distinguished by unusual combinations of symptoms. For these people, the illness is palpable and because their symptoms have developed since exposure they believe the cause to be exposure to OPs. But few had long-term medical observations or results of tests to present with their accounts. Many individuals felt that their problems had been poorly recognised, inadequately monitored and investigated, and exacerbated by lack of appropriate medical advice. It was claimed that only one or two individual practitioners recognised and addressed their problems. It is to be hoped that the situation will be

improved following the report of the Royal College of Physicians of London and the Royal College of Psychiatrists.³ This report, and the recommendations it contains on diagnosis and management of patients, were drawn to the attention of doctors by an article in CMO's Update, a quarterly publication sent to all doctors in England and CMOs in Wales, Scotland and Northern Ireland.⁴

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6.14 The consequence was that the Working Group was unable to draw on any substantial body of clinical data. The inquiry of the Working Group would have been helped greatly by a systematic description of the clinical features of a large case series, such as might have been provided by the clinical database proposed by the British Medical Association.⁵ This is considered further in Chapter 9. The individual case reports were informative, but were inadequate to define the syndrome, if it existed. Furthermore, they could not be used to make any assessment of cause and effect. In order to draw definite conclusions in this regard data from appropriately designed epidemiological studies are needed. This is considered in the next chapter.

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7. Chronic toxicity of OPs: the scientific evidence

Introduction

7.1 As has been described in Chapter 6, various types of chronic illness have been reported in individuals who have been exposed to OPs, and these illnesses are suspected to occur as a result of a toxic effect of such exposure. However, the fact that in some individuals an illness develops following exposure to OPs does not in itself establish that OPs have caused the condition. Before it can be concluded that there is a causal link there is a need for the following:

- reliable evidence that the illness is more common in people who have been exposed to OPs and that this excess is unlikely to be explained by other known causes of the illness;
- a plausible toxic mechanism through which OPs could give rise to the illness.

7.2 The mechanisms whereby OPs might cause adverse health effects in the long term have been discussed in Chapter 5. This chapter reviews the scientific evidence relating exposure to OPs to the frequency of different types of illness, and sets out the Working Group's interpretation of the findings.

7.3 The evidence considered comes largely from published scientific papers. Some of these were identified by the individuals and organisations who submitted information to our inquiry. In addition, the Working Group carried out a systematic search of the scientific literature up to June 1999. The papers examined are listed in Appendix 8. Among them are 27 reports that the Working Group considered to be the most informative with regard to the potential toxicity of low-level exposure to OPs. These reports are summarised in Appendix 4 with a discussion of their individual strengths and limitations. Some of them concern the late sequelae of acute poisoning episodes, rather than low-level exposure as defined by the Working Group. However, they are relevant either because they identify long-term health effects that might also be associated with lower exposures, or because they indicate that the frequency of certain health outcomes does not appear to be elevated even after episodes of acute toxicity. The absence of any increased incidence of an illness in subjects with exposure to OPs sufficient to cause overt acute toxicity makes it less likely that such effects would occur from low-level exposure, although there remains the possibility of cumulative effects occurring after prolonged low-level exposure. An outline of the 27 studies, giving information on the type of investigation and the exposed population is in Table 7.1.

Organophosphates **Table 7.1: Key epidemiological studies**

Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Ames <i>et al.</i> (1995) ¹³ [A2, C2, D2]	Follow-up study of workers who had been removed from exposure to OPs because of low acetylcholinesterase activities, to assess whether there were chronic neurological sequelae	45 male pesticide workers (Californian) removed from exposure to OPs because of low acetylcholinesterase activities. They had not shown symptoms or signs of overt acute OP toxicity	No data on the specific OPs involved
Amr <i>et al.</i> (1997) ⁴¹ [D2]	Cross-sectional study of pesticide workers to assess psychiatric morbidity	380 workers exposed during formulation (208) or application (172) of pesticides in Egypt	No details on specific pesticides or exposure levels
Cole <i>et al.</i> (1997) ¹⁶ [A2, D2]	Cross-sectional study of individuals from a rural population to investigate neuropsychological function	144 individuals from a farming community in Ecuador sub-divided into pesticide applicators (123), field workers 'generally' exposed to pesticides (28) and those only exposed by consumption of local potatoes	No details of specific pesticides, erythrocyte acetylcholinesterase activities of all groups slightly lower (11-15%, p<0.001) than in a 'non-farm' population
Cole <i>et al.</i> (1998) ³⁸ [C2]	Cross-sectional study of individuals from a rural population to investigate effects on the peripheral nervous system	As above	As above
Daniell <i>et al.</i> (1992) ¹² [A2, C2]	Prospective longitudinal study of pesticide applicators to assess neurological performance	57 applicators involved in orchard spraying in Washington State, USA	Azinphos-methyl was the main pesticide used. No quantitative data on exposure

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Table 7.1: Key epidemiological studies continued

Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Davies <i>et al.</i> (1999) ⁶ [D2]	Postal surveys (2) to assess neuropsychiatric symptoms in individuals exposed to OPs	175 randomly selected farmers from Cornwall and west Devon in first survey of whom 45 were not exposed to OPs. The second survey involved 179 sheep dippers and 32 non-sheep dippers, all of whom had been exposed to OPs	Estimated from questionnaire but no details of specific OPs nor any quantitative data
Duffy <i>et al.</i> (1979) ¹⁴ [B1]	Study to investigate EEG abnormalities at least one year after an episode of acute OP poisoning by sarin	77 male workers who had one or more episodes of acute toxicity due to sarin exposure at least one year previously	All had experienced symptoms and signs of acute sarin toxicity and a reduction in erythrocyte acetylcholinesterase activity of at least 25%
Engel <i>et al.</i> (1998) ³⁹ [C2]	Cross-sectional study to assess peripheral neurophysiology in apple-thinners	67 Hispanic farm workers in Washington State USA who were indirectly exposed to OPs via foliar residues	Azinphos-methyl was the main pesticide used. No quantitative data on exposure. No depression of erythrocyte acetylcholinesterase activity seen at time of examination
Fiedler <i>et al.</i> (1997) ¹⁵ [A2, C2, D2]	Cross-sectional study of tree fruit farmers to assess neuropsychological and psychiatric variables.	57 fruit farmers who were licensed pesticide applicators in New Jersey, USA	No data on pesticides used or exposure levels. No history of acute poisoning and erythrocyte acetylcholinesterase activity normal at time of study
Gomes <i>et al.</i> (1998) ¹⁷ [A2, C2]	Morbidity study, including neurological function assessment of farm workers	226 expatriate workers (mainly ethnic Asians) employed on farms in United Arab Emirates	No information on specific pesticides used. Erythrocyte acetylcholinesterase activity significantly lower than controls (about 15%, p<0.01)

Organophosphates **Table 7.1: Key epidemiological studies continued**

Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Hawton <i>et al.</i> (1998) ⁴³ [D2]	Investigation of suicide and stress in farmers involving psychological autopsy study and study of geographic distribution of suicides	The autopsy study involved 84 cases (71 suicides and 13 open verdicts). The geographic study considered 719 deaths reported in farmers over the period 1981-1993 with suicide or open verdicts	No information on exposure to specific pesticides
Jager <i>et al.</i> (1970) ³³ [C2]	EMG study on workers involved in manufacture or formulation of pesticides	66 workers in a Dutch factory: 36 exposed to both OPs and organochlorine pesticides, 24 to organochlorine only and 6 following acute exposure only	No data on specific OPs or on exposure levels. Erythrocyte acetylcholinesterase activity normal
London <i>et al.</i> (1997) ¹⁸ [A2, C2]	Cross-sectional study to investigate neuropsychological effects in pesticide applicators	163 male pesticide applicators in orchards in South Africa	No data on specific OPs or on exposure levels. Plasma cholinesterase activity normal
London <i>et al.</i> (1998) ⁴⁴ [C2, D2]	Cross-sectional study to investigate neurological function in pesticide applicators	164 male pesticide applicators in orchards in South Africa	No details of specific OPs. Plasma cholinesterase activity normal
Maizlish <i>et al.</i> (1987) ¹¹ [A2, C2]	Comparison of neuropsychological tests pre- and post-shift in pesticide applicators	46 pesticide (diazinon) applicators involved in Japanese beetle control in California	Slight increase in urinary levels of diazinon metabolite DEPT at end of shift
McConnell <i>et al.</i> (1994) ³¹ [C1]	Investigation of indices of peripheral neuropathy following acute poisoning with OPs	38 individuals with medical records of work-related OP poisoning incident who had been admitted to hospital	21/36 cases of acute poisoning due to methamidophos. Identity of the other OPs not stated

Table 7.1: Key epidemiological studies continued

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Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Misra <i>et al.</i> (1988) ³² [C1]	Study to investigate neuromuscular function in pesticide applicators	24 pesticide sprayers using the OP fenthion. No protective clothing worn	No data on fenthion levels. Serum cholinesterase activity significantly ($p < 0.01$) lowered by about 30% immediately after spraying
Otto <i>et al.</i> (1990) ³⁶ [A2, C2]	Cross-sectional study on workers at a pesticide factory to assess neuropsychological and neurophysiological function	229 workers at a pesticide plant in Egypt involved in formulation of several different OPs	No data on specific OPs. Serum cholinesterase activity lower by about 15% in the pesticide workers
Pickett <i>et al.</i> (1998) ⁴² [D2]	Case-control study to investigate link between suicide and exposure to pesticides	Cases comprised 1457 suicides in Canadian farmers over period 1971-1987. Exposure to pesticides based on questionnaire relating to acres sprayed with herbicide, or insecticide and costs of agrochemicals bought	No data on specific pesticides or exposure levels
Pilkington <i>et al.</i> (1999a) ² [C2, E2]	Cross-sectional study to investigate (in the field) indices of peripheral neuropathy in sheep dippers exposed to OPs	612 sheep farmers involved in the use of OP sheep dips	Cumulative exposure estimated from model of OP uptake developed in earlier phase of study
Pilkington <i>et al.</i> (1999b) ³ [A2, C2, E2]	Nested clinical study on a sub-group of the sheep dippers described above to investigate indices of peripheral neuropathy and associated neuropsychological abnormalities	76 sheep farmers (a subset of the cohort described above)	As above

Organophosphates **Table 7.1: Key epidemiological studies continued**

Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Reidy <i>et al.</i> (1992) ⁹ [A1, C1, D1]	Investigation of long-term sequelae following episodes of acute OP poisoning in farmers	21 Hispanic workers who had experienced two episodes of hospital treatment following pesticide poisoning by a combination of mevinphos, methomyl and maneb	Individuals stated to be subject to chronic low-level pesticide exposure but no details given
Rosenstock <i>et al.</i> (1991) ⁸ [A1, C1]	Retrospective cohort study to investigate neurological sequelae following acute OP poisoning in agricultural workers	38 men discharged from hospital over period 1/7/86 to 31/7/88 after acute OP poisoning (occupational) in Nicaragua	No data reported in this paper but see also McConnell <i>et al.</i> (1994) ³¹
Savage <i>et al.</i> (1988) ⁷ [A1, B1, C1, D1]	Study to investigate chronic neurological sequelae following acute OP poisoning	100 individuals with physician's diagnosis of OP poisoning in Texas USA. Various OPs involved	No data on exposure levels
Steenland <i>et al.</i> (1994) ¹⁰ [A1, C1, D1]	Study to investigate chronic neurological sequelae following acute OP pesticide poisoning	128 male cases of accidental exposure to OPs in California. A wide range of OPs involved	No data on exposure levels
Stephens <i>et al.</i> (1995) ¹⁴ [A2, D2]	Cross-sectional study in sheep dippers to assess neuropsychological effects	146 sheep farmers involved in use of OP sheep dips	No quantitative data on actual exposure. Estimates made from questionnaire

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Table 7.1: Key epidemiological studies continued

Reference, [Indication of the outcomes under which considered *]	Type of study	Study population	Exposure
Stephens <i>et al.</i> (1996) ⁴⁵ [A2, D2]	Investigation of relationship between chronic neuropsychological abnormalities and effects of acute exposure in sheep dippers	77 sheep farmers involved in use of OP containing sheep dips (diazinon, chlorfenvinphos or propetamphos)	Data on urinary excretion of metabolites of diazinon and chlorfenvinphos provided (stated to cover 43 of the 77 subjects). Mean values in morning following exposure for DEP/DEPT were 44.9 nmole/mole creatinine compared with 4.7 nmole/mole creatinine in the controls
Stokes <i>et al.</i> (1995) ³⁷ [C2]	Investigation of peripheral neuropathy in pesticide applicators	90 pesticide applicators in New York State. Principal compound used was the OP azinphos-methyl but at least 10 other OPs were used, plus other pesticides	Exposure to azinphos-methyl confirmed by urinary excretion of metabolite. No quantitative data on exposure levels
Stoller <i>et al.</i> (1965) ⁴⁰ [D2]	Geographic study comparing incidence of mental illness in areas of high and low OP usage	All male admissions to mental health institutions of Victorian Mental Health Authority considered. This included rural fruit growing regions of Victoria, Australia	No direct measures of exposure but total sales of OP within a given area used as a surrogate

* The codes in brackets, explained below, indicate the endpoints under which the studies were considered and, if in bold text, where mention of them can be found in this chapter. Summaries and critiques of all the studies can be found in Appendices 4 and 5.

- A1 Neuropsychological abnormalities in subjects with a history of acute OP poisoning, paragraphs 7.12 to 7.18
- A2 Neuropsychological abnormalities in subjects with no history of acute OP poisoning, paragraphs 7.19 to 7.26
- B1 Electroencephalographic abnormalities in subjects with a history of acute OP poisoning, paragraphs 7.31 to 7.33
- C1 Peripheral neuropathy and neuromuscular dysfunction in subjects with a history of acute OP poisoning, paragraphs 7.41 to 7.46
- C2 Peripheral neuropathy and neuromuscular dysfunction in subjects with no history of acute OP poisoning, paragraphs 7.47 to 7.60
- D1 Psychiatric illness in subjects with a history of acute OP poisoning, paragraphs 7.64 and 7.65
- D2 Psychiatric illness in subjects with no history of acute OP poisoning, paragraphs 7.62 and 7.66 to 7.70
- E2 Autonomic nervous system effects in subjects with no history of acute OP poisoning, paragraphs 7.71 to 7.73

Organophosphates 7.4 The Working Group also considered in detail the full report of a major study by the Institute of Occupational Medicine (IOM) published in July 1999 (included in Table 7.1).¹⁻³ This focused on the relationship between exposure to OP sheep dips and indices of peripheral neuropathy and neurophysiological abnormalities in sheep farmers and dippers. It was divided into three phases. Phase 1 involved development and validation of an OP uptake model for sheep dippers.¹ Phase 2 was a cross-sectional field study of peripheral neuropathy in sheep dippers and controls.² Phase 3 comprised a clinical neurological, neurophysiological and neuropsychological study in a sample of sheep dippers with evidence of peripheral neuropathy in phase 2.³ In view of the importance of this study, which investigated an occupational group of particular concern, sheep dippers in Britain, it is summarised in detail in Appendix 5 with a consideration of its strengths and weaknesses.

7.5 In addition to published studies, consideration was given to unpublished scientific material provided as submissions to the Working Group. In general, these data were less detailed than those obtained from published reports and sometimes had not been collected through rigorously designed studies. They therefore cannot be given the same weight. Nevertheless, they provide useful information for comparison with data in published studies and suggest further lines of research. An individual who was well prior to exposure and subsequently became unwell after exposure to OPs may well believe that there is a cause and effect relationship between exposure and illness. However, the possibility of a chance association must be considered and this cannot be excluded without properly designed and conducted studies.

7.6 In evaluating the evidence, consideration must be given to any deficiencies in the design or execution of individual studies that would tend to bias their results, and also to the potential for confounding of associations with exposure to OPs by other factors that independently influence the health of the population under investigation. A further matter that must be taken into account is that most of the studies that have been carried out are small so that the confidence intervals associated with their results are wide. It must also be remembered that observations can be misleading through the play of chance. The issues of bias, confounding and chance are discussed in the answer to question 1 posed to the Working Group by the Official Group on OPs (see Appendix 3).

7.7 Also of importance to interpretation is the consistency of observations from one study to another. A finding becomes more credible if it is demonstrated consistently in several independent investigations with different designs and differing potential for bias.

7.8 This review is divided into five sections focusing on different 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.

7.9 Within each section consideration is given first to long-term effects of exposure to OPs following acute OP poisoning. This is followed by consideration of the effects of exposure to OPs in the absence of any recognised acute poisoning episode.

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7.10 The Working Group was aware of concerns about other toxic endpoints that might occur as class effects of OPs, e.g. effects on the cardiovascular system, respiratory system and on bone density. However, for the reasons noted earlier (paragraph 2.16) the Working Group focused on neurotoxic effects.

Neuropsychological abnormalities

7.11 Many of the illnesses that were reported to the Working Group as following exposure to OPs featured abnormalities of higher neurological function such as difficulties with memory, speech, concentration and cognition. Jamal⁴ and Davies^{5,6} have postulated a syndrome of chronic OP-induced neuropsychiatric disorders (COPIND) that includes these features. The severity of such disorders in patients presenting to clinicians has been sufficient to cause disability. As a proportion of all people with exposure to OPs these patients are relatively few in number. However, if the risk of such illness were increased by exposure to OPs, it is possible that similar but more minor abnormalities would occur more commonly in exposed populations, and with sufficient frequency to be detectable in epidemiological surveys. A number of studies have investigated this issue, comparing the results of neuropsychological tests in people exposed to OPs and unexposed controls.

Studies in individuals with a history of acute OP poisoning

7.12 Some of these studies have focused on subjects who have previously suffered one or more episodes of recognised acute OP poisoning. The most informative investigations of this type are those by Savage *et al.*,⁷ Rosenstock *et al.*,⁸ Reidy *et al.*,⁹ and Steenland *et al.*¹⁰

7.13 In the study reported by Savage *et al.*⁷ previous poisoning was associated with significantly reduced scores in a wide variety of tests and the pattern of impaired function was striking. It included poor performance on tests stressing speeded, flexible information processing (e.g. digit-symbol substitution, digit span, Wisconsin card sort) that would be expected to be vulnerable to generalised brain damage. However, the poisoned group also had lower scores in tests that are held to be relatively insensitive to brain damage, such as the Wechsler Adult Intelligence Scale (WAIS) vocabulary subtest, and measures of single word reading and spelling. Also notable is the fact that despite the number of tests in which impairment was observed, few significant effects were found in tests relating to non-verbal function. The single test of long-term memory that was administered did not detect differences between the poisoned and control groups. This pattern of abnormalities suggests that the exposed subjects suffered predominantly from impaired verbal function, perhaps reflecting cerebral dysfunction lateralised to the left hemisphere (although the results of a clinical test of aphasia were reported not to differentiate between the poisoned and control groups). Alternatively the pattern could in part reflect a failure to match the groups adequately for verbal intelligence in the design of the study.

Organophosphates 7.14 Rosenstock *et al.*⁸ studied patients who had required admission to hospital for acute poisoning, and employed an extensive battery of tests. Unlike Savage *et al.*⁷ they found no differences between the poisoned and control groups in the vocabulary subtest of the WAIS. However, differences were observed in a wide range of other tests, including digit span, digit-symbol substitution, visual, but not verbal, memory, and block design (a test of non-verbal reasoning). These findings suggest that the results of Savage *et al.*⁷ are not entirely explained by poor matching of poisoned and control subjects for verbal intelligence.

7.15 The results of these two studies contrast with those of Reidy *et al.*⁹ who employed a similarly extensive test battery to study patients who had been treated in hospital for acute OP poisoning. They found differences in measures of motor function and mood but little evidence of cognitive impairment.

7.16 Similar results were obtained by Steenland *et al.*¹⁰ who studied cases of pesticide poisoning reported to physicians. Again an extensive battery of tests was employed. They found no evidence of poorer performance by their poisoned group in three out of the four cognitive tests employed (including digit-symbol substitution). The exception was a measure of sustained attention (a continuous performance task).

7.17 Each of these studies has methodological limitations, the more important of which are summarised in Appendix 4. In particular, the poisoned and control subjects may have differed not only in their exposure to OPs, but also in other ways that could have influenced their performance in the neuropsychological tests, and which were not adequately taken into account in the statistical analysis. Among the most important of these potential confounding variables are general intelligence (usually estimated by measures thought to be relatively insensitive to neurological damage, such as vocabulary range and reading ability) and age. It is also possible that people who perform poorly on neuropsychological tests are more prone to accidental poisoning when they use pesticides, but the Working Group thought it unlikely that any such tendency would be sufficient to explain the associations that the studies found.

7.18 Despite their methodological limitations the findings in studies of poisoned subjects are unlikely to be wholly attributable to confounding effects or to chance. The patterns of abnormality reported are not entirely consistent, but this may in part reflect differences in the severity of the poisoning which subjects had suffered. On balance, the Working Group interpreted the studies as providing reasonable, although not conclusive, evidence that OP poisoning of sufficient severity to require hospital admission can lead to persistent cognitive impairment. This effect is most evident in neuropsychological tests involving sustained attention and speeded, flexible cognitive processing, for example, the digit-symbol substitution test. The studies reviewed provide no evidence that long-term memory is affected by acute OP poisoning apart from impaired visual memory in one investigation. Thus, if low-level exposure to OPs causes neuropsychological abnormalities these are most likely to be shown on tests of cognitive function other than memory.

Studies in individuals with no past history of acute OP poisoning

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7.19 Other epidemiological studies have examined the relation of long-term OP exposure to neuropsychological function in people who have not suffered from acute poisoning. The most informative are those by Maizlish *et al.*,¹¹ Daniell *et al.*,¹² Ames *et al.*,¹³ Stephens *et al.*,¹⁴ Fiedler *et al.*,¹⁵ Cole *et al.*,¹⁶ Gomes *et al.*,¹⁷ and London *et al.*¹⁸

7.20 Two of these investigations, by Daniell *et al.* and Ames *et al.*,^{12,13} 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.*¹² studied 57 pesticide applicators involved in orchard spraying. That of Ames *et al.*¹³ was a follow-up study of 45 pesticide workers who had been removed from exposure to OPs because of low acetylcholinesterase activities, but who had not shown symptoms or signs of acute OP toxicity.

7.21 Maizlish *et al.*¹¹ 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.

7.22 Stephens *et al.*¹⁴ investigated 146 sheep farmers involved in the use of OP 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.

7.23 Fiedler *et al.*¹⁵ 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.*¹⁴ They did not include either the 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.

7.24 Gomes *et al.*¹⁷ studied expatriate farm workers exposed to OPs 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 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 OPs. The interpretation of this finding is made difficult, however, because possible differences in

Organophosphates 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.

7.25 Cole *et al.*¹⁶ compared three groups of individuals who had been exposed to pesticides (including OPs) 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 OPs and other agents.

7.26 London *et al.*¹⁸ 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 OP exposure in other studies. Two measures of motor function showed modest ($p < 0.05$) relationships with estimated cumulative OP 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 OP exposure and cognitive function.

7.27 Each of the published studies that we have reviewed in this section has its own particular limitations, and these are summarised in Appendix 4. They include:

- the possibility of differences between exposed and control subjects, other than their contact with OPs, which might spuriously influence their performance on neuropsychological tests, and which may not always have been taken into account adequately in the statistical analysis of the results;
- small study size, so that effects that are large enough to be important medically may not have been distinguishable from random variation;
- possible biases due to an association between subjects' willingness to participate in the research and their state of health. For example, in some studies subjects who suspected that they had been made ill by OPs may have been more inclined to take part;
- the inclusion in some studies of subjects with an unrecognised past history of acute poisoning. This could have resulted in some spurious abnormalities on neuropsychological testing;
- restriction to workers currently or recently exposed to OPs. This means that individuals too ill to remain in employment will have been excluded.

7.28 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 OPs 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 OP 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 OPs (see paragraphs 7.13 to 7.14).

7.29 Most of the individuals in the exposed groups studied were in active employment at the time they were investigated. Therefore it is not surprising that where decrements in performance have been found, the extent of the deficit has generally been small and not at a level that would normally result in their being unable to work. The research provides little evidence that low-level exposure to OPs is a common cause of neuropsychological abnormalities but, because of the size and design of the studies, the possibility that it leads to serious neuropsychological disorders in a small sub-group of individuals cannot be excluded.

Electroencephalographic (EEG) abnormalities

7.30 One of the limitations of studies assessing neuropsychological outcomes is the possibility that the performance of subjects in tests is influenced by their knowledge that they have been exposed to or poisoned by OPs. A more objective index of central nervous system function is provided by the EEG, although the clinical relevance of abnormal patterns on EEGs is not always clear. In general, prominent EEG changes are usually confined to the acute stages of toxic encephalopathies with minor and essentially non-specific abnormalities or normal EEG findings in the chronic stages.

Following acute poisoning

7.31 Two studies have looked for EEG abnormalities following acute poisoning by OPs. Duffy *et al.*¹⁹ studied 77 workers who had been poisoned by the nerve agent, sarin. They were investigated at least one year after an accident in which they had been poisoned by the compound and, in addition to routine visual analysis of the EEG, computerised spectral analysis was used. In comparison with 39 unexposed controls employed at the same industrial plant, computerised analysis demonstrated significant abnormalities of both waking and sleeping EEGs.

7.32 In contrast, follow-up of 100 patients with previously documented acute OP poisoning, due to agricultural pesticides, in Colorado and Texas revealed no significant abnormalities on EEG by conventional visual inspection when they were compared to an unexposed control group.⁷

Organophosphates 7.33 These studies suggest that, if sufficiently sensitive techniques are used, long-term changes can be detected in brain electrical activity following acute OP poisoning. However, the implications of these EEG changes for neuropsychological function are unclear.

No past history of acute poisoning

7.34 An extensive literature search did not reveal any studies that have investigated the possible effects of OPs on EEG activity in the absence of acute poisoning.

Peripheral neuropathy and neuromuscular dysfunction

Organophosphate-induced delayed polyneuropathy: OPs that inhibit NTE

7.35 As described in Chapter 4, exposure to certain OPs can cause severe peripheral polyneuropathy. Organophosphate-induced delayed polyneuropathy (OPIDPN) is a well-recognised complication of acute poisoning by OPs that inhibit the enzyme NTE.²⁰ For example, peripheral neuropathy occurred in an episode of TOCP poisoning in the United States of America arising from contamination of a tonic known as Jamaica ginger²¹ and in an episode in Morocco caused by contaminated cooking oil.²²

7.36 The onset of OPIDPN usually occurs 7 to 21 days after an episode of poisoning, so that there is often a period after the resolution of cholinergic symptoms and before the onset of neuropathy when the patient is relatively well. Moreover, not all patients with cholinergic symptoms necessarily go on to develop OPIDPN.²³ The disorder usually takes the form of a predominantly motor neuropathy affecting the lower limbs more than the upper limbs.²³ The onset is characterised by cramps, paraesthesiae of the extremities and distal weakness.²⁴ The symptoms then worsen over the course of about two weeks. At the time when the illness is at its worst there is severe distal wasting and weakness together with mild proximal weakness, ataxia and loss of the ankle reflexes.²⁵ This clinical picture is similar to that of Guillain-Barré syndrome, the commonest cause of acute neuropathy throughout the world. Indeed if the history of OP poisoning were absent the clinical manifestation of OPIDPN would fulfil the internationally accepted diagnostic criteria for Guillain-Barré syndrome. Some patients poisoned by OPs have been regarded as having this condition by the authors of case reports, for example Fisher²⁶ and Adlakha *et al.*²⁷ However, the diagnostic criteria for Guillain-Barré syndrome specifically exclude disease that follows exposure to toxins²⁸ and the pathogenesis of OPIDPN appears to differ from that of Guillain-Barré syndrome (see Chapter 5).

7.37 One report has claimed that neuropathy after poisoning by OP compounds that are NTE inhibitors can sometimes be predominantly sensory in nature. Thus Kaplan *et al.*²⁹ described eight cases of this type following exposure to chlorpyrifos spray within an enclosed space: chlorpyrifos is only a weak, non-cumulative inhibitor of brain NTE.³⁰ Moretto and Lotti²³ have questioned whether OP poisoning was responsible for the cases described by Kaplan *et al.*²⁹ but, in the absence of a satisfactory alternative explanation for the occurrence of neuropathy in these cases, a predominantly sensory neuropathy appears a possible, albeit unusual, form of OPIDPN.

7.38 The weakness that occurs in OPIDPN usually persists for many months and although, in most cases, substantial improvement eventually occurs, some individuals are left with permanent footdrop and resultant disability. During recovery lower limb spasticity can be detected, indicating that damage has occurred not only to peripheral nerves but also to central nervous system pathways including the corticospinal tracts.²²

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OPs that do not inhibit NTE

7.39 It has been postulated that peripheral neuropathy and long-term abnormalities of neuromuscular function can result from exposure to OPs that are not NTE inhibitors. Few of the symptoms reported to the Working Group by individuals and patient groups as being associated with OP exposure suggest the presence of peripheral neuropathy. However, Jamal has listed the disorder as one of the features of the syndrome of COPIND.⁴ In addition, the cases of sensory neuropathy reported by Kaplan and colleagues²⁹ (see paragraph 7.37) may be relevant since the OP incriminated, chlorpyrifos, is only a weak inhibitor of NTE.

7.40 Several epidemiological studies provide data on peripheral nerve function, either in patients who have been acutely poisoned by OPs or in individuals exposed to OPs who do not have a history of recognised poisoning episodes. These studies are summarised in paragraphs 7.41 to 7.46 and 7.47 to 7.60 respectively.

Following acute OP poisoning

7.41 A cross-sectional comparison of 100 individuals who had a documented history of acute OP poisoning with matched controls included a conventional neurological examination and various neuropsychological tests.⁷ There were no differences between the groups in the neurological examination of the cranial nerves, motor system, sensory system, or tests of coordination, balance and gait. Significantly impaired performances were found in some neuropsychological tests which could be affected by abnormalities in the peripheral nervous system (e.g. the finger oscillation test and peg board test) but not in others (e.g. tactile performance location, tactile form recognition, hand dynamometer, and whole-body steadiness).

7.42 Rosenstock *et al.*⁸ and McConnell *et al.*³¹ studied 52 patients who had previously been admitted to hospital in Nicaragua with acute OP poisoning, and compared them with age- and sex-matched siblings or friends who had not been treated for OP poisoning. The poisoned group had significantly impaired performance on neuropsychological tests, including two which would be affected by peripheral neuropathy (a pursuit aiming task and a manual dexterity task). In addition, vibration sensory thresholds were significantly increased in the fingers and toes of the poisoned subjects. Some of the subjects in this study had been exposed to methamidophos, an inhibitor of NTE, but abnormalities were also apparent in those who had been poisoned by other OP compounds (the identities of the latter compounds were not given in the published report).

7.43 Reidy *et al.*⁹ studied 21 Hispanic field workers who had experienced two documented episodes of acute toxic exposure to OPs. Following the second exposure five

Organophosphates subjects were diagnosed as having peripheral neuropathy. A neuropsychological test battery showed impairments compared to controls in finger tapping and peg board tests, both of which might be due to peripheral neuropathy, although they could also have been the result of damage to central nervous system pathways. Because of the way in which subjects were selected, less weight can be given to this study (see Appendix 4).

7.44 Steenland *et al.*¹⁰ investigated 128 patients who had suffered from probable or definite acute OP poisoning, together with a control group who were friends of the poisoned subjects. The study incorporated a clinical neurological examination, vibration threshold tests, nerve conduction measurements, and neuropsychological tests. When all the poisoned subjects were considered, there were no significant differences, compared to the control subjects, in vibration thresholds in the fingers or toes; median, ulnar or sural sensory nerve conduction velocities and amplitudes; median, ulnar or peroneal motor nerve conduction velocities; or compound muscle action potential amplitudes (cMAPs). Nor was there any significant difference in the neuropsychological tests that might have been affected by peripheral nerve function, such as pursuit aiming, dexterity, and postural sway. However, in the 83 subjects who had had definite poisoning, and in the 36 subjects who had been admitted to hospital because of the poisoning, mean vibration thresholds were abnormal in the fingers and toes, suggesting the presence of a subclinical sensory neuropathy in those with the most severe poisoning.

7.45 In India, Misra *et al.*³² studied 24 workers who regularly sprayed the OP fenthion and whose mean duration of exposure was 8.5 years. The subjects complained of headache, giddiness, paraesthesiae, and ocular symptoms on the day after spraying. Standard nerve conduction measurements at this time were normal and similar to those in a control group apart from repetitive cMAPs (similar to those seen in myasthenic patients overtreated with carbamate anticholinesterases) in 29% of sprayers compared to none in the controls. On repeat neurophysiological examination three weeks after exposure (by which time their serum cholinesterase activities had risen by 23%), the repetitive cMAPs were no longer seen. Also, there were minor but significant improvements in motor nerve conduction indices for the group, though no intra-individual comparisons were made.

7.46 Some of the health effects assessed in the investigations described in this section provide more direct indices of peripheral neuropathy than others. For example, nerve conduction velocities and amplitudes are specific measures of peripheral nerve function, whereas performance on a pursuit aiming test can be influenced by pathology in the central, as well as the peripheral, nervous system. When viewed together, the findings suggest that severe acute poisoning with OPs that do not inhibit NTE can sometimes lead to persistent peripheral neuropathy, although in most cases the effects are not at a level that would give rise to symptoms.

No past history of acute OP poisoning

7.47 In addition to the studies that have followed up patients after acute poisoning by OPs, a few investigations have suggested that OPs can also cause peripheral neuropathy or abnormal neuromuscular function in the absence of overt acute poisoning.

7.48 Jager *et al.*³³ studied neuromuscular transmission in factory workers exposed to OP and organochlorine pesticides. On nerve stimulation 16 of 36 workers exposed to both OPs and organochlorine pesticides had repetitive compound muscle action potentials in their hand muscles. In addition 14 of the 36 showed a decrease in the first cMAP evoked after a period of voluntary muscle contraction compared to the pre-exercise response. In all, 17 were regarded as abnormal, as opposed to one of 24 workers exposed to organochlorines alone and to none of 28 unexposed control workers. The findings, which did not correlate with blood acetylcholinesterase activities, were interpreted as providing evidence for abnormal neuromuscular transmission. However, subsequent studies by other investigators have cast doubt on the reliability of cMAP amplitude when used in this way as a measure of neuromuscular dysfunction and have failed to find any difference between exposed and unexposed workers.^{34,35} Furthermore, morphological changes in cMAPs indistinguishable from repetitive responses can readily occur for a variety of technical reasons. Thus, this study provides only weak evidence for persistent abnormal neuromuscular transmission in workers exposed to OPs.

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7.49 Otto *et al.*³⁶ compared a sample of male production workers exposed to OPs (including some that were NTE inhibitors) at a pesticide formulation plant in Egypt and a comparison group from a fertiliser plant and a textile factory. The pesticide workers had a higher prevalence of several symptoms that can occur in peripheral neuropathy and of abnormal vibration sensation.

7.50 In the United States, Stokes *et al.*³⁷ compared 68 pesticide applicators and an equal number of controls matched for age, sex and county of residence. Vibration sensory thresholds were significantly increased in the index fingers of the applicators. However, the weight that can be given to this finding is reduced by the absence of a similar abnormality of sensation in the toes. A toxic peripheral neuropathy would normally be expected to affect sensation in the toes before the fingers.

7.51 In a cross-sectional survey in the United Arab Emirates, Gomes *et al.*¹⁷ found that symptoms of muscle pain and weakness were much more common in farm workers exposed to OPs than in unexposed control individuals. In addition, there was a significant impairment on an aiming task in the exposed group. However, erythrocyte acetylcholinesterase activities were reduced significantly in the exposed group as compared with the controls, raising the possibility that some or all of the observed differences were attributable to short-term effects of recent exposure.

7.52 In a cross-sectional study of three groups of farm workers in Ecuador, Cole *et al.*³⁸ found a significant increase in symptoms suggestive of peripheral neuropathy together with signs of poor coordination and abnormal tendon reflexes in pesticide applicators compared with non-exposed workers. There was a non-significant trend towards an increase in the threshold for detection of vibration sensation in the big toe in pesticide applicators, which was significant in the case of those individuals who reported symptoms of previous pesticide poisoning. There was also a significant increase in symptoms suggestive of peripheral neuropathy in exposed subjects who had not been applying pesticides.

Organophosphates 7.53 Most recently a British survey found a higher prevalence of symptoms suggestive of peripheral neuropathy in 612 sheep farmers than in a control group of 107 ceramic workers, although not in comparison with a second control group comprising 53 farmers who had not dipped sheep (see Appendix 5).²⁻³ In addition, thresholds for cold sensation were higher in the sheep farmers, but no consistent difference was observed in thresholds for sensation of heat or vibration. Within the sheep farmers, symptoms of neuropathy were associated with higher estimated cumulative exposure to OPs, but no corresponding relationship was observed with more objective measures from sensory testing. Symptoms and vibration sensory thresholds were both related to intensity of exposure.

7.54 In contrast to the studies summarised above, several other epidemiological studies that have collected information relevant to peripheral nerve or neuromuscular function have found no evidence of an association with long-term exposure to OPs.

7.55 Maizlish *et al.*¹¹ investigated 46 pest control workers before and after short-term low-level exposure to diazinon. The test battery included hand-eye coordination and finger tapping. The results of these tests might have demonstrated impairment if moderate or severe peripheral neuropathy had been present. However, after allowance for possible confounding factors, no significant differences were found for these measures in comparison with unexposed controls. In addition, the study incorporated a neurological examination and, although the results are not reported in detail, no mention is made of neurological abnormalities in the subjects exposed to OPs. Furthermore, no significant excess of weakness in the hands, weakness in the legs, tingling in the toes, tingling in the fingers, muscle twitching or loss of balance was reported in the exposed group; these are all possible symptoms of peripheral neuropathy.

7.56 In a prospective longitudinal study, Daniell *et al.*¹² found no differences in finger tapping or hand-eye coordination between 49 pesticide applicators with exposure mainly to azinphosmethyl and 40 control subjects. Also, Fiedler *et al.*,¹⁵ as part of a cross-sectional assessment of neuropsychological performance, showed that hand-eye coordination and performance in grooved pegboard tasks were unimpaired in OP-exposed tree fruit farmers as compared with unexposed controls. Although these tests are not designed as tests of peripheral nerve function they would become impaired if there were moderate or severe peripheral neuropathy.

7.57 In a cross-sectional study Ames *et al.*¹³ compared 46 workers with a history of moderate OP exposure causing asymptomatic depression of acetylcholinesterase activities and 90 unexposed controls. There was no significant difference in median, ulnar or peroneal nerve conduction velocities, muscle action potentials, sensory action potentials, or vibration thresholds in the fingers or toes.

7.58 Engel *et al.*³⁹ examined sensory and motor nerve conduction and neuromuscular transmission in 69 workers exposed to low levels of OPs while apple thinning, the exposed group having worked for 80 hours or more in the current season. Nerve conduction was normal and similar to that in control non-exposed workers. No significant dose-response relationship was observed between hours spent thinning and any

neurophysiological measure, and repetitive cMAPs to nerve stimulation were present in a smaller proportion (15%) of exposed than unexposed (22%) subjects.

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7.59 In a cross-sectional survey of 164 pesticide applicators and 83 control subjects in Western Cape Province, South Africa, neurological symptoms were more common with higher exposure to OPs, but the association was not statistically significant, and a similar relation was also found with other “dummy” symptoms.⁴⁴ In addition, there was no significant association between long-term exposure to OPs and vibration sense threshold or tremor intensity in the dominant hand, although tremor was more common in workers with recent exposure.

7.60 When the above studies are viewed together, and their individual strengths and weaknesses are taken into account, there is no clear evidence that peripheral neuropathy can be caused by low level exposure to OPs that do not inhibit NTE. If clinically important neuropathy does result from such exposure then it must be a rare effect.

Psychiatric illness

7.61 Many of the health problems that have been described to us in people who have been exposed to OPs are psychiatric in nature (see Chapter 6). They have ranged from mild mood changes to severe depression and suicide and it was suggested in submissions to the Working Group that OP toxicity may be part of the explanation for the high rates of suicide among British farmers.

7.62 Dr Davies in his presentation to the Working Group explained how he had identified a characteristic pattern of symptoms in patients with psychiatric problems in the context of long-term OP exposure. These are listed in Table 7.2. A patient showing at least seven of these symptoms would be diagnosed as having COPIND. This is supported by the results of two postal surveys. The first found a significantly ($p < 0.001$) increased prevalence of COPIND symptoms, including personality changes and impulsive and suicidal thoughts in farmers exposed to OPs. In the second study questionnaires were sent to individuals concerned about their health after OP exposure, their names having been obtained from the OP Information Network (OPIN) database. A similar pattern of symptoms was noted in those exposed to OPs from sheep dipping and those exposed to OPs in other ways and the authors suggest that the only identifiable, uniting factor was exposure to OPs. These studies have been published subsequently.⁶

Organophosphates **Table 7.2: Symptoms of Chronic Organophosphate Induced Neuropsychiatric Disorder (COPIND) as described by Dr D.R. Davies (see also reference 6)**

Exacerbation of dipper's flu
Personality changes
Impulsive suicidal thoughts
Cognitive impairment
Language disorder
Alcohol intolerance
Heightened sense of smell
Handwriting deterioration
Sensitivity to OPs
Decreased exercise tolerance

7.63 The Working Group therefore examined the scientific evidence from other published epidemiological studies linking OPs with psychiatric illness.

Following acute OP poisoning

7.64 A small number of studies have sought evidence of increased psychiatric morbidity following acute poisoning by OPs. Thus Reidy *et al.*⁹ found higher levels of anxiety and depression in a sample of farm workers who had suffered acute toxicity from mevinphos in combination with a carbamate and a dithiocarbamate as compared with unexposed controls; Steenland *et al.*¹⁰ reported increased tension and confusion on follow-up of reported cases of poisoning in California. Also, Savage *et al.*⁷ described an excess of depression in a similar study in Colorado and Texas.

7.65 It appears from the available published information that the abnormalities of mood recorded in these studies were not of sufficient severity to require treatment. Also, apart from their history of poisoning, there may have been other differences between the exposed subjects and controls, which differentially influenced their mental health. For example, the subjects investigated by Reidy *et al.*⁹ were involved in litigation, and this in itself may have affected their reporting of symptoms. It is therefore difficult to draw firm conclusions regarding the risk of significant psychiatric illness following acute OP poisoning.

No past history of acute OP poisoning

7.66 An early study by Stoller *et al.*⁴⁰ explored the geographical correlation between hospital admission for psychiatric illness and usage of OPs in Victoria, Australia. There was an increased incidence of admission for schizophrenia in one fruit growing area where OPs were most used. However, none of the 18 patients from this area with schizophrenia had been exposed to OPs.

7.67 More recently, the prevalence of psychiatric symptoms has been assessed in several cross-sectional surveys of people who have worked with OPs. Stephens *et al.*¹⁴ reported an increased vulnerability to psychiatric disorders in sheep farmers exposed to OPs as

compared with controls who worked as quarrymen; London *et al.*⁴⁴ found an excess of dizziness, sleepiness and headache in South African pesticide applicators exposed to OPs; and Amr *et al.*⁴¹ described an increased prevalence of depression, irritability and erectile dysfunction in Egyptian pesticide applicators and formulators working with a range of products including OPs. However, Ames *et al.*¹³ found no significant abnormality of mood in subjects with a past history of asymptomatic inhibition of acetylcholinesterase; and Fiedler *et al.*¹⁵ found no evidence of disordered emotion or personality in a sample of fruit farmers with a history of long-term exposure to OPs.

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7.68 In addition, two studies have addressed the relation of pesticide exposure to the risk of suicide in farmers. The first⁴² compared 1457 Canadian farmers who had died by suicide with 11,656 living controls. No information was available on exposure to OPs specifically, but after statistical adjustment for potential confounding factors there was no evidence of any association with the use of pesticides in general as reported at an earlier census.

7.69 The second study explored determinants of suicide in British farmers and was discussed in detail by the Working Group with Professor Hawton.⁴³ The rate of suicide among farmers was significantly elevated in the county of Devon where exposure to OPs has been common among sheep farmers, but overall there was no clear geographical correlation with particular types of farming. In comparison with a sample of living controls, cases of suicide were more likely to have been pig farmers, but the proportion of sheep farmers did not differ significantly between suicide cases and living controls. Among sheep farmers who committed suicide, use of sheep dip was more common than in sheep farming controls but the overall proportion of farmers with a history of symptoms attributed to OPs was similar in the suicide and control groups.

7.70 These findings indicate that OP exposure is not a major factor in the excess of suicide among British farmers. Among the epidemiological studies of low-level exposure to OPs that have been carried out to date, the psychiatric symptoms that have been reported to occur in excess have generally been minor, and the findings have not been consistent from one study to another. It is unlikely, however, that individuals with more severe psychiatric illness would have been recruited into these investigations, and different study designs would be needed to detect a causal effect of OPs on the occurrence of serious psychiatric disease. This gap in knowledge is considered further in our recommendations for future research (Chapter 9).

Effects on the autonomic nervous system

7.71 Dr Julu reported to the Working Group on possible specific effects of OP exposure on autonomic function in the skin. He had examined 15 male patients referred because of the suspected presence of autonomic lesions. All had been exposed to OPs in sheep dip. Almost all (13 out of 15) showed evidence of a selective thermoregulatory vasoconstrictive failure in the skin of the dominant hand and foot, with increased blood flow. Some abnormality in skin-related measures was seen in all subjects. There was also evidence of damage to the walls of the large blood vessels shown by abnormalities of peripheral baroreceptors. These findings, with the skin being the primary target followed by the blood vessels and then the heart, were markedly different from the abnormalities seen in diabetes.

Organophosphates 7.72 In a recent British survey the prevalence of symptoms suggestive of abnormalities in the autonomic nervous system was higher in 612 sheep farmers than in two control groups comprising 53 farmers who had not dipped sheep and 107 ceramic workers.²⁻³

7.73 The Working Group considered that these observations suggesting autonomic dysfunction would need further investigation by more rigorously designed studies in larger samples of subjects before any firm conclusions could be drawn.

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8. Conclusions

8.1 The remit of the Working Group was to advise on whether prolonged or repeated low-level exposure to OPs, or acute exposure at a dose lower than causes overt toxicity, can cause chronic ill health. For practical reasons the Working Group restricted its attention from the outset to class effects of OPs.

8.2 In the course of the work it became apparent that the large majority of the relevant scientific evidence concerned neurological, psychological or psychiatric health effects. In addition, these were the types of illness most frequently attributed to OP exposure by those who made written or oral submissions to the Working Group. Although the evidence on other possible class effects of OPs was sparse, to address it satisfactorily would have required additional expertise that was not represented in the Working Group and additional time which would have delayed significantly the completion of this report. A decision was made, therefore, to concentrate upon health effects in the nervous system.

8.3 In addressing the question posed in paragraph 8.1, the Working Group considered not only the evidence relating to low dose exposures (i.e. those insufficient to cause overt acute toxicity), but also studies on the long-term sequelae of recognised acute poisoning episodes. Any chronic health effects that could be shown to result from acute poisoning might also occur with lower exposures, and thus would merit special attention. On the other hand, if a particular health outcome did not appear to be a problem following recognised poisoning incidents, it was unlikely to result from a single acute exposure insufficient to cause overt toxicity.

8.4 Although it has been proposed that dipper's flu is a manifestation of acute OP toxicity, the Working Group concluded that this is unproven. Thus, for the purpose of this report it was not regarded as an indicator of acute OP toxicity.

8.5 In reviewing the scientific evidence the Working Group focused on five different health outcomes relating to the nervous system. These were: neuropsychological abnormalities, EEG abnormalities, peripheral neuropathy and neuromuscular dysfunction, psychiatric illness and effects on the autonomic nervous system. Of these, the data on EEG abnormalities and effects on the autonomic nervous system were insufficient to allow any firm conclusions to be drawn. Conclusions regarding the other endpoints are given below.

Long-term sequelae of acute poisoning

Neuropsychological outcomes

8.6 The balance of evidence supports the view that neuropsychological abnormalities can occur as a long-term complication of acute OP poisoning, particularly if the poisoning is severe. Such abnormalities have been most evident in neuropsychological tests involving sustained attention and speeded flexible cognitive processing ("mental agility"). In contrast, current evidence suggests that long-term memory is not affected after acute poisoning.

Organophosphates Peripheral neuropathy

8.7 Peripheral neuropathy, as one feature of OP-induced delayed polyneuropathy, is a well-established complication of poisoning by OPs that inhibit the enzyme NTE. The neuropathy is predominantly motor but possibly also sensory. Compounds that produce more than 70% inhibition of NTE give positive results in the hen test. Compounds evaluated as giving a positive response in the hen test are not used in the UK and have not been approved or licensed by regulatory agencies (i.e. the Veterinary Medicines Directorate or the Pesticides Safety Directorate).

8.8 The balance of evidence indicates that acute poisoning by other OPs, which do not inhibit NTE, can also lead to persistent peripheral neuropathy detectable by neurophysiological tests. If this occurs, most cases are not at a level that would give rise to symptoms.

Psychiatric illness

8.9 The limited evidence available does not allow any firm conclusions to be drawn regarding the risk of developing psychiatric illness in the long term as a consequence of acute poisoning by OPs

Prolonged low-level exposure

8.10 In comparison with the positive neurological and neuropsychological findings following recognised poisoning incidents, the evidence relating to chronic low-level exposure to OPs, insufficient to cause overt acute toxicity, is less convincing.

Neuropsychological outcomes

8.11 Although some studies suggest impairment in the same tests that are affected after acute poisoning, others do not. The balance of evidence does not support the existence of clinically significant effects on performance in neuropsychological tests from low-level exposures to OPs. If such effects do occur, they must either be relatively uncommon or so small that they are not consistently detectable by standard methods of testing.

Peripheral neuropathy

8.12 The balance of evidence indicates that low-level exposure to OPs does not cause peripheral neuropathy. If effects on peripheral nerve function sufficient to cause severe disability do occur, they must be rare.

Psychiatric illness

8.13 The available data indicate that exposure to OP sheep dips is not a major factor in the excess mortality from suicide among British farmers. However, in general, the evidence relating psychiatric illness to OPs is insufficient to allow useful conclusions.

Acute exposure to OPs at a lower dose than causes frank toxicity

Conclusions

8.14 No studies have examined the long-term effects of a single exposure to OPs insufficient to cause acute toxicity. However, the findings in individuals with prolonged and repeated low-dose exposures, and in those who have suffered recognised acute poisoning, together indicate that any risk of serious health effects from such limited exposure must be small.

Questions posed to the Working Group by the Official Group on OPs

8.15 In addition to addressing the central question stated in the remit of the Working Group (see paragraph 8.1), consideration was given to the specific questions (listed in Appendix 2) posed to the Working Group by the Official Group on OPs. These were modified for clarity and as a result of the evolution of the thinking of the Group over time. Answers to these questions, as modified, are given in Appendix 3.

Monitoring of human adverse effects

8.16 It was a matter of particular concern to some members of the Working Group that the present schemes for monitoring human adverse effects had yielded so few relevant data and that little progress had been made in establishing a relevant clinical database.

Outstanding issues

8.17 The major gap in current knowledge relates to the possibility that OPs cause disabling neurological or neuropsychiatric disease in a small sub-group of exposed persons. Most research has focused on people who were in work at the time of investigation, and therefore by definition were sufficiently fit for employment. Moreover, the available published studies have generally been designed to look for effects on the mean level of quantitative health indices in the exposed population, rather than exploring the possibility that only a small proportion of subjects may be at increased risk of clinically significant disease. Thus, although the substantial body of evidence that has now accumulated gives little support to the hypothesis that low-level exposure to OPs can cause chronic disease of the nervous system, it does not exclude the possibility that at least some of the illnesses that were described to the Working Group as following such exposure are indeed a manifestation of toxicity.

8.18 Further investigation, utilising an appropriate study design, is needed to establish whether the risk of neurological or neuropsychiatric disease is increased by low-level exposure to OPs in a sub-group of individuals. If there were an excess risk it would be necessary to establish how far it is determined by direct toxicity and how far by psychological or other mechanisms. It is important to consider such possibilities because it is well established that psychosocial circumstances can have a profound influence on the incidence and severity of many types of disease. This does not mean that such diseases are not real or are imagined nor does it detract from the severe disability that they may cause.

Organophosphates 8.19 The widespread public concern about OPs, which was evident from the response to the Working Group's inquiry, underlines the urgency of the need for further research targeted at the outstanding issues. The next chapter describes the specific avenues of investigation that the Working Group considered to be most useful.

9. Recommendations for further research

9.1 A substantial body of scientific evidence has now accumulated on the long-term toxicity of OPs. As described in the previous chapter, this allows certain conclusions to be drawn, but there are important issues that remain unresolved. This chapter sets out questions that are amenable to research, answers to which would clarify some of the remaining uncertainties. The order in which the questions are listed is not intended to indicate their relative priority.

9.2 What are the most common patterns of exposure, clinical presentation and subsequent clinical course among people in the UK with chronic illnesses that they attribute to OPs?

The case series that have been reported to date investigated only a small number of individuals, and a systematic description of a much larger sample of cases, such as from the OPIN and PEGS databases, would be valuable. Particularly useful would be information on: the types of OP product implicated; the duration, frequency, circumstances and extent of exposure before the onset of illness; the clinical features of the illness and its subsequent course (including any effects of further exposure to OPs). This would help in the planning of further research to test hypotheses about specific syndromes. The Working Group are aware that a proposal for a systematic descriptive study of this type has been submitted to MAFF.

9.3 How common is dipper's flu, and what causes it?

Many of those who submitted evidence to the Working Group referred to dipper's flu as being a common problem in sheep farmers. It is unclear whether the phenomenon is an acute toxic effect of one or more OPs, or whether it results from other pathological mechanisms. If it could be established that it were a toxic effect of OPs, it might provide a useful index of exposure. Moreover, the finding might shed light on potential mechanisms of chronic toxicity. It would be valuable to establish how frequently dipper's flu occurs, whether it occurs with the use of non-OP sheep dips, whether similar symptoms occur in relation to other uses of OPs, and how it relates to changes in erythrocyte acetylcholinesterase activity.

9.4 Does low-level exposure to OPs cause disabling neurological or psychiatric disease in a small sub-group of exposed persons?

To address this question, studies should allow for the fact that people with disabling disease may have long ceased any significant exposure to OPs, and may not be currently employed or may be in a different occupation. Various designs might be used, including cross-sectional surveys in the general population of selected areas, case-control investigations, and retrospective cohort studies of industrial populations previously exposed during the manufacture or formulation of OPs.

Organophosphates 9.5 **Do people with chronic disabling illness that is suspected of being related to OPs differ metabolically from the general population?**

Current evidence indicates that most people with low-level exposure to OPs suffer no detectable adverse effects on the function of the nervous system. It follows that if the same exposures can cause serious illness through a toxic mechanism in a small sub-group of individuals, those individuals must be unusually predisposed to the toxicity, either through genetically determined differences in their metabolism, or their target organ sensitivity, or as a consequence of other components of their environment such as diet or medication. The possible existence of sub-groups in the population that are particularly susceptible to the effects of OPs has been noted by others.^{1,2-4} There may be scope to look for evidence of such susceptibility in people with illnesses suspected of being caused by OPs. For example, case-control methods could be used to test for genetic differences in potentially relevant aspects of metabolism. Also, with suitable precautions and appropriate clinical support, it might be possible to explore the effects of challenge with small doses of OPs in a group of cases. However, the Working Group recognised that to carry out such studies may present great difficulties

9.6 **Other than acetylcholinesterase inhibition, what mechanisms play an important role in the causation of adverse health effects by OPs?**

A number of putative mechanisms of toxicity were put forward in Chapter 5. These include phosphorylation of proteases or esterases, interaction with cytoskeletal proteins and prolonged receptor stimulation at nerve endings leading to muscle fasciculation and necrosis. Scientific data to support the existence of such mechanisms is lacking. Research is needed to elucidate these mechanisms and to explore their relevance to human disease.

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