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Organophosphorus Pesticides and Long-term Effects on the Nervous System

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European Centre for Ecotoxicology and Toxicology of Chemicals

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SUMMARY AND CONCLUSIONS 1

1. INTRODUCTION AND TERMS OF REFERENCE 5

2. DEFINITIONS 8

3. REVIEW OF CHRONIC TOXICITY 10

3.1 CHRONIC EFFECTS OF ACUTE OR REPEATED, SYMPTOMATIC EXPOSURE 11
3.2 EFFECTS OF CHRONIC, LOW-LEVEL, APPARENTLY ASYMPTOMATIC EXPOSURE 17
3.3 BEHAVIOURAL EXPERIMENTS IN ANIMALS RELATED TO CHRONIC EFFECTS 30
3.4 POTENTIAL MECHANISMS OF CHRONIC EFFECTS 33

4. REVIEW OF TESTING PROTOCOLS WITH RESPECT TO THEIR CAPABILITY TO IDENTIFY CHRONIC EFFECTS 37

4.1 APPROACH TAKEN 37
4.2 PREDICTABILITY OF ACUTE EFFECTS (ACUTE AND REPEATED EXPOSURE) 41
4.3 PREDICTABILITY OF DELAYED NEUROPATHIC EFFECTS (OPIDN; ACUTE AND REPEATED EXPOSURE) 44
4.4 PREDICTABILITY OF CHRONIC EFFECTS INDUCED BY SHORT-TERM, HIGH-LEVEL EXPOSURE 46
4.5 PREDICTABILITY OF CHRONIC EFFECTS OF CHRONIC, LOW-LEVEL EXPOSURE 48

5. CONCLUSIONS 50

BIBLIOGRAPHY 52

APPENDIX A: REVIEW OF ACUTE AND DELAYED TOXICITY 73

A.1 ACUTE EFFECTS 73
A.1.1 Pharmacology of organophosphorus compounds and kinetics of effects 73
A.1.2 Acute clinical effects in humans 77
A.1.3 Pathology produced by acute intoxication 82
A.1.4 Concluding remarks on acute effects 84

A.2 INTERMEDIATE SYNDROME 84
A.2.1 Features and mechanisms of the intermediate syndrome 84
A.2.2 Concluding remarks on the intermediate syndrome 86
A.3 DELAYED NEUROPATHY 86
A.3.1 What is OPIDN ? 86
A.3.2 Are OPIDN Type I and Type II two different types of neurotoxicity ? 88
A.3.3 Molecular mechanisms of OPIDN 89
A.3.4 OPIDN in man 91
A.3.5 OPIDN from prolonged, low-level exposure 95
A.3.6 Concluding remarks on OPIDN 97
LIST OF ABBREVIATIONS 98
LIST OF OPs 100
MEMBERS OF THE TASK FORCE 109
MEMBERS OF THE SCIENTIFIC COMMITTEE 110

SUMMARY AND CONCLUSIONS

Organophosphorus pesticides (OPps) have been used for decades. In view of their potent biological effects, these agents are subject to toxicological scrutiny aimed at the recognition of potential hazards and their risk management. In addition to the "classical" hazards of acute toxicity and delayed neuropathy, termed organophosphorus-induced delayed neuropathy (OPIDN), other specific types of organophosphorus neurotoxicity, such as "intermediate syndrome", ocular "Saku disease" and more recently "chronic syndrome" have been allegedly associated with OPp exposure. It has been claimed that even low-level, apparently asymptomatic chronic exposure could cause long-term adverse effects on the nervous system. This prompted ECETOC to review the existing epidemiological data and toxicological testing protocols for the development and registration of OPps, with respect to their sensitivity and reliability for detecting such effects.

The present report reviews studies relating to long-term effects in humans, discriminating between chronic effects of acute or repeated exposure strong enough to produce clinical signs and symptoms (symptomatic exposure) and the effects of chronic, low-level, apparently asymptomatic exposure. These two fundamentally different situations are often confused in the literature. In addition, chronic, low-level exposure may have been associated in some studies with undocumented episodes of acute intoxication and the signs, symptoms and sequelae of acute exposure mistakenly interpreted as effects of chronic exposure. Some epidemiological studies demonstrate the absence of adverse effects in cohorts exposed but protected (by protective clothing) and thus confirm the possibility of using OPps safely. Others report adverse effects that vary but are predominantly either psychological, neurological or ophthalmological. Unfortunately, both the "positive" and the "negative" epidemiological studies often lack sufficient details of the conditions, levels of exposure and compounds involved and data are frequently confounded by the spontaneous occurrence of changes related to aging and underlying diseases. Moreover, most studies are retrospective and methods of examination have not been standardised, are frequently subjective and rarely appropriately controlled. Therefore, at present, the evidence for the alleged chronic effects arising from low-level exposure appears insufficient. In most studies, there are no specific complaints about oculotoxicity resulting from chronic exposure. This is of interest in that oculotoxicity was previously suspected and special animal tests for this effect were required.

This report also reviews experimental and mechanistic studies focused on chronic OPp effects. It concludes that animal experiments, aimed at further characterising neurotoxicity induced by specific OPps, confirm acute and protracted effects on cognitive functions but have not demonstrated the alleged effects of prolonged, low-level exposure. Kinetic data demonstrate that inhibition of

cholinesterase (ChE) by multiple low doses increases only during the initial phase of exposure and thereafter reaches a steady state, so that there is no long-range cumulative inhibition. No convincing explanation of how chronic, low-level exposure could possibly culminate in chronic dysfunction has been provided. Several mechanisms have been proposed, such as changes in receptors or neurotransmission, potential non-cholinergic effects of ChE or of OPps, or inhibition of other proteins/enzymes. However, in the absence of a demonstration that such effects really do occur at doses too small to cause acute or sub-acute effects, these hypotheses cannot be used as a foundation for the understanding of OPp neurotoxicity.

The testing protocols used in animal safety studies have been reviewed with respect to their ability to identify OPp effects. The effects known or alleged to occur in humans have been compared to the toxicological “end-points” observed in animals. This comparison has demonstrated good predictability of the acute and delayed neuropathic effects. Although some chronic effects can be predicted from animal studies, there are restrictions caused by the short natural lifespan of experimental animals and by the restricted capability of animal experiments to test for exclusively-human mental performance.

For the sake of completeness and to facilitate the comprehensive understanding of OPp toxicology, an Appendix to the report has been provided, with reviews of experimental and clinical data related to acute effects, intermediate syndrome, and OPIDN.

The Task Force has arrived at the following conclusions:

Acute and intermediate effects

The primary neurotoxic effects of OPps on humans and animals are well understood. Such effects are generally reversible. Nevertheless, acute poisoning due to severe overdose can produce persistent changes. The acute toxicity of OPps is appropriately assessed in regulatory animal safety studies, well documented in humans, and clinically manageable. The intermediate syndrome is always associated with prior acute effects and the number of reported cases is limited. Risk from such hazards can be managed by minimising exposure.

Neuropathic effects (OPIDN)

The potential to induce OPIDN can be reliably detected by monitoring neuropathy target esterase (NTE) inhibition and by adequate neuropathological examination in delayed neurotoxicity studies in hens. It is unlikely that chronic, low-level inhibition of NTE could cause neuropathy, because OPIDN is a threshold event. In any case such effects would have been observed in sub-chronic and chronic

safety studies in rats and dogs had they occurred. Most cases of OPIDN have been caused by non-pesticidal organophosphorus compounds such as triorthocresyl phosphate (TOCP).

Long-term effects

There is no pharmacokinetic evidence for cumulative effects of chronic exposures to OPps at levels which are not acutely toxic. With prolonged substantial exposure the sensitivity to OPps decreases owing to the development of tolerance, and the effects are reversible after cessation of exposure. Based on well-established pharmacological and toxicokinetic principles, irreversible sequelae of low-level exposure are considered unlikely. Tolerability studies in human volunteers conducted with a number of specific OPps indicate the absence of sequelae from daily, low-level asymptomatic exposure lasting for several weeks.

There is insufficient evidence in the epidemiological literature that there is a “chronic syndrome” resulting form chronic, apparently asymptomatic exposure. To resolve the question of whether or not such a syndrome does exist, surveillance of the exposed populations is considered more appropriate than additional animal studies. The described features of “chronic syndrome” resemble the complaints and changes sometimes seen in the general population and known to be linked to other societal and socio-economic factors. Interpretation of epidemiological observations should take such confounding factors into consideration.

Some of the toxic effects claimed to constitute the alleged “chronic syndrome” can be detected in general regulatory safety studies and in special neurotoxicity studies within the constraints of inter-species extrapolation. There are human mental capabilities which are not testable in animals. Therefore, and in the absence of hard evidence of the key features of ‘chronic syndrome’ in humans with low-level, asymptomatic exposure, it is not feasible to propose modifications of present regulatory animal studies.

Oculotoxicity

Review of reported complaints indicates that oculotoxicity does not appear to be a hazard of chronic OPp exposure. Persistent ocular effects are not observed in most epidemiological studies. In animal studies, ophthalmoscopy and biomicroscopy, supported by adequate pathology examination, provide sufficient sensitivity for detecting adverse effects in standard animal regulatory safety studies.

Recommendations

Based on the current state of the science, the Task Force has concluded that the evidence for “chronic syndrome” is insufficient, but recommends that the epidemiology studies currently planned are followed up and the issue re-visited when results are available.

1. INTRODUCTION AND TERMS OF REFERENCE

Organophosphorus pesticides (OPps) by virtue of their biocidal properties form a sub-set of the large family of (>50,000) organophosphorus compounds (OPs). OPps comprise insecticides, acaricides, nematicides, rodenticides, fungicides, herbicides and defoliants. Some are utilised as pharmaceutical compounds in veterinary and human medicine, particularly as antiparasitics (Rollo, 1980). Many OPs are used as industrial chemicals (e.g. flame retardants, fluids, lubricants, plasticisers, UV-stabilisers). Although the latter are generally less toxic than OPps, some also have unexpected and specific effects on the nervous system.

The introduction of OPps about 50 years ago represented great progress both in terms of efficacy and of environmental and toxicological properties, when compared to the older-fashioned, drastic agents such as arsenicals, that were widely used before the OPps became available. More than 200 OPps with insecticide activity have been introduced on to the market, representing about 37% of all insecticdes. Since OPps cannot currently be completely replaced by other compounds, they are probably going to remain important long after the year 2000.

Owing to the potency of their biological effects, many OPps must be handled using protective measures to minimise human exposure. Despite such precautions, cases of unintentional or intentional intoxication do occur. Since the market introduction of OPps, various adverse effects in man have been reported. The effects of acute neurotoxicity (accidental or by suicidal abuse) can be well predicted by toxicological studies, the risk managed, and the victims treated with antidotes. Also organophosphorus-induced delayed neuropathy (OPIDN) can be predicted and its risk managed. Currently, in addition to these classical manifestations of organophosphorus neurotoxicity, a variety of adverse effects in man are attributed to prolonged, low-level exposure, which, at least initially, is asymptomatic (Allsopp *et al,* 1995; Davies, 1995; NFU Conference Report, 1995).

In the light of these latter reports, the ECETOC Task Force on oculotoxicity which had been commissioned to analyse the potential toxic effects of OPps on the visual system, recommended in its report (ECETOC, 1994), that a new Task Force be appointed to review the evidence for neurotoxicity arising from chronic, low-level OPp exposure.

ECETOC responded by commissioning the Task Force “Organophosphorus pesticides and long-term health effects” with the following terms of reference:

- Review briefly the characteristics of the classical neurotoxicity (acetylcholinesterase inhibition and OPIDN) that may be associated with organophosphorus pesticides;

- review the clinical and patho(physio)logical characteristics of the “chronic neurotoxic syndrome” recently alleged to occur in man, and the exposure situations in which it has been described. Evaluate the evidence that this is an organic syndrome;

- review the commonly-used (and available) testing (general and neurotoxicological) protocols in respect to their sensitivity to the characteristics of this “syndrome”. Evaluate the extent to which existing experimental data can contribute to an assessment of the organic nature of this “syndrome”;

- make suggestions for an experimental approach for the investigation of the “syndrome” and/or the prediction of a potential to cause it.

In accordance with the above Terms of Reference, this document focuses on the compounds that are OPps having one or more of the following activities: cholinesterase (ChE) inhibition, with or without the "intermediate syndrome", induction of OPIDN and inhibition of neuropathy target esterase (NTE), alleged chronic effects from levels of exposure which are insufficient to cause symptoms typical of cholinesterase inhibition.

In addition to proved or alleged neurotoxic effects, a variety of other adverse effects, most frequently carcinogenicity, immunotoxicity and developmental toxicity, have been attributed to OPps (Allsopp *et al,* 1995) some resulting from secondary damage to organs and tissues during acute cholinergic crisis, others attributed to direct action. Such effects are often compound-specific and are not the subject of this report. In addition, the controversial issue of the so-called "Gulf War Syndrome" has not been discussed.

Whereas the analysis of potential adverse effects of OPps is best carried out by their classification as OPIDN, acute, intermediate and chronic effects, the real or alleged health impairment of the affected individuals can represent a combination of these various effects. This is to be expected in situations where there will be repeated exposure to a variety or mixture of agents and with various conditions and levels of exposure. It is recognised that the choice of diagnostic methods should appropriately match the spectrum of possible effects, both in the experimental and in the medical diagnostic field.

This report deals essentially with the chronic effects of OPps, particularly those included in the appended “List of OPs”. Reviews of OPIDN, acute effects and intermediate syndrome, together with information which may be needed for an adequate understanding and interpretation of the chronic effects, have been located in Appendix A.

2. DEFINITIONS

In the references quoted in this report the terms “acute” and “chronic” are sometimes used with different implications. This can lead to confusion and difficulties in comparing the different scenarios. Also, it is important to distinguish chronic exposure from chronic health effects which may have been induced by previous short-term or even acute exposure.

The following definitions are proposed to facilitate the understanding of the report.

Duration of exposure

- Acute exposure: exposure during a short period of time, as a rule less than a day.
- Repeated exposure: any exposure with the same compound(s) which is repeated at least once after an interval of at least 24 hours. Repeated exposure can continue over weeks, months or years.
- Chronic exposure: exposure of long duration mostly over months and years.

In animal experiments the duration is more precisely defined:

- Acute exposure: one exposure on one day, or divided exposures over one day.
- Sub-acute exposure: daily exposure for 14-28 days.
- Sub-chronic exposure: daily exposure for more than 1 and less than 6 months (usually 3 months).
- Chronic exposure: daily exposure for at least 6 months. In animal tests, chronic exposure may cover the greater part of the species-specific life span.

Types of exposure

- Symptomatic exposure: exposure of any duration resulting in clinical signs or symptoms, whether functional or behavioural.
- Asymptomatic exposure: exposure of any duration resulting in no clinical signs or symptoms.

Duration of effects

- Acute effect: an effect which lasts only for a short time, at the most a few hours or days.
- Protracted effect: an effect which can last up to several months.
- Chronic effect: an effect which lasts for months or years.
- Persistent effect: an effect induced by any type of exposure (acute, repeated, chronic) which still occurs and is not observed to be reversible despite the cessation of exposure.
- Reversible effect: an effect, which declines in severity after the termination of exposure. If the effect eventually disappears, it is completely reversible.

Types of effects

- Cholinergic crisis: a severe poisoning manifested by abrupt occurrence of signs and symptoms characteristic of cholinergic stimulation. Some reports use the term “acute syndrome” in this context.
- Intermediate syndrome: paralysis and/or respiratory depression occurring 24 hours to several days after severe acute AChE inhibition.
- “Chronic syndrome”: commonly used to cover not only, 1) the chronic effects of acute or repeated, high-level, symptomatic exposure to OPps but also 2) the alleged effects of long-term, low-level, apparently asymptomatic exposure. This report discriminates between these two different situations.
- Organophosphorus induced delayed neuropathy (OPIDN): damage to peripheral nerves and long spinal cord tracts which is unrelated to cholinergic effects and occurs 2-3 weeks after exposure.

3. REVIEW OF CHRONIC TOXICITY

In the assessment of the chronic toxicity of OPps it is important to discriminate between ”chronic” effects of acute or repeated, *symptomatic exposure*, and the effects allegedly resulting from exposure which does not produce any of the symptoms classically associated with acute exposure (*asymptomatic exposure*). The chronic effects of symptomatic exposure are avoidable by reducing exposure. The occurrence of chronic effects after apparently asymptomatic exposure could cast doubt on the safety of exposure levels currently considered to be acceptable.

The scientific value of human epidemiological studies investigating effects of OPp exposure are often limited by uncertainties in relation to the compounds involved, their quantity and exposure duration. For retrospective cohort studies it is obviously difficult, if not impossible, to identify these parameters. Results obtained in studies with poorly-documented exposure conditions can only be used to generate hypotheses. Moreover, case reports describing effects observed in a few subjects would have to be very well-defined before they could be generalised. Suspected effects based on such observations need to be confirmed in prospective studies or supported by animal studies. Unfortunately, few prospective studies have been conducted with OPps and, of these, several do not clearly indicate exposure conditions and exposure levels. The following overview of the literature includes only those studies which evaluate the effects of exposure to OPps specifically. Studies dealing with “pesticides” in general, or where subjects had been exposed primarily to pesticides other than OPps, are of little use in establishing causal relationships and have not been included. The reports are assessed based on their estimation of exposure levels and duration and occurrence of symptoms or neurobehavioural effects. Evaluation of neurobehavioural effects by objective test methods is considered superior to the use of questionnaires or the evaluation of subjective complaints.

Spencer and Schaumburg (1985) suggested that for the assessment of neurotoxicity in general, effects should be considered as substance-related when the following criteria are fulfilled:

- The substance induces a consistent pattern of neurological or neurobehavioural dysfunction(s);

- the effects (or an equivalent in the view of this Task Force) seen in man can also be induced in animals under comparable exposure conditions; and

- reproducible lesions or functional changes in the nervous system of exposed humans and/or animals account for the neurobehavioural dysfunction.

The reproducibility of findings is a central paradigm of science; it also underlies the above criteria.

3.1 CHRONIC EFFECTS OF ACUTE OR REPEATED, SYMPTOMATIC EXPOSURE

Clinical observations

The question of chronic sequelae was first addressed 30 years ago when there were allegations that a substantial proportion of those who suffered acute OPp poisoning continued to suffer ill effects for years afterwards. These could take the form of asthenovegetative syndrome (Faerman, 1967) or of hypertension, gastrointestinal (GI) disturbances, or a variety of other complaints (Watanabe, 1972). Other investigators, however, who considered a larger series of cases (235 individuals occupationally exposed in California) found, three years after poisoning, no consistent pattern of long-term adverse effects in most subjects, but a variety of complaints in a small group, falling into the following categories: optic, gastrointestinal, cardiorespiratory, neuropsychiatric. Intolerance to the odour of pesticide preparations, mentioned by 20 individuals was considered to be psychogenic or a conditioned reflex (Tabershaw and Cooper, 1966).

Metcalf and Holmes (1969) described the results of multidisciplinary investigation of industrial and agricultural workers acutely exposed to a variety of pesticides, and focused on workers manufacturing OPps. The initial investigation was carried out in 1952 at the University of Colorado Medical Center. Psychological testing done within 3 days of a symptomatic exposure generally showed erratic and slowed functioning, indicating the presence of clinical delirium. However, analysis of data from a battery of psychological tests carried out at a later stage, revealed no formal evidence of organic brain damage in psychological test terms. Neurological examinations revealed multiple minor signs, such as generalised weakness and confusion shortly after exposure, but no clear neurological signs were evident following clinical recovery. EEG (electroencephalography) showed slow-wave bursts after activation by hyperventilation. This was originally considered reversible, but upon re-evaluation persistent abnormal features were detected. Psychiatric interviews were conducted on 56 men with histories of exposure and on 22 controls. There were a variety of chronic complaints in the exposure group with increased frequency as compared with controls. A subsequent examination was carried out in 1965 on a group of men from the same industrial population, many of whom had been continuously employed since 1950. The examination included psychological testing, psychiatric interviews, neurological examinations, EEG, visual and auditory evoked responses, physical examinations and a special all-night sleep (EEG) study. Table 1 summarises the results (A and B) together with those of 3 other cohorts (C, D and E).

Table 1: Chronic complaints and observations in subjects with previous symptomatic exposure

Domain	Complaints/observations	A*	B*	C*	D*	E*
Language	linguistic disturbance			+	-	
Attention	lethargy/difficulty in maintaining alertness/vigilance and focusing of attention/concentration	+	+	+	+	+
Memory	difficulty/slowness in thinking/slowing of information processing and psychomotor speed	+	+		+	+
	forgetfulness/disturbed memory/memory deficit	+	+	+	+	+
Skill/reflexes	disturbed dexterity/co-ordination		+	+	+	-
Mood	anxiety				†	
	depression			+		-
	irritability/impatience	+	+			
Symptoms	muscular aches and pains	+	+			
	general fatigue/fatigability	+	+			-
	visual difficulty/oculomotor imbalance	+	+			
	headaches	+	-			
	increased perspiration	+	-			
	trouble sleeping/increased dreaming	-	+			
	libido increased or decreased	+	+			
Other	EEG changes/changes in sleep patterns		+	-		
	visual/auditory evoked responses - trends to lower amplitudes and longer latencies		+			
	sensory functions			-		+
	decreased nerve conduction velocity/amplitude					+

A* Metcalf and Holmes, 1969 (1952 examinations)
B* Metcalf and Holmes, 1969 (1965 examinations)
C* Savage *et al,* 1988
D* Rosenstock *et al,* 1991
E* Steenland *et al,* 1994

\+ reported
\- reported absent
† "Scandinavian questionnaire" significantly positive

The conclusion from the observations of Metcalf and Holmes (1969) was that long-term exposure to OPps could possibly induce irreversible or only slowly-reversible brain dysfunction. However, this conclusion may not be justified based on the available data. Many if not all individuals examined had obviously had a previous acute symptomatic exposure which could be responsible for the observed findings; in addition, the "control group" was not clearly identified. The changes in EEG mirrored to a lesser degree the more severe disturbances seen after acute exposure. Work history and exposure data were lacking and it was not reported whether the workers had also been exposed to chlorinated hydrocarbons. Furthermore, it was not clear from the EEG studies whether the persistent EEG changes were matched by changes in psychological or behavioural parameters.

Levin and Rodnitzky (1976) reviewed and compared reports about observations in individuals with clinical symptoms and in apparently asymptomatic workers repeatedly using OPps. They concluded that the investigators generally agreed on the presence of several behavioural sequelae of OPp poisoning. They also concluded that the few available studies of apparently asymptomatic workers at risk from repeated exposure to OPps produced only equivocal findings in relation to the presence of behavioural abnormalities.

Duffy *et al* (1979) examined the EEG of 77 workers who had a documented history of one or more accidental exposures to toxic levels of sarin; no exposure had occurred in the year preceding the examination. The control group was composed of 38 industrial workers from the same plant who had had no work exposure to OPs. The criteria of exposure included the characteristic clinical signs and reduction of erythrocyte (RBC) ChE (≤ 75% normal value). Statistically-significant group differences included increased Beta activity, increased Delta and Theta slowing, decreased Alpha activity, in the EEG, and also increased amounts of rapid eye movement (REM) sleep in the exposed group. These findings indicated the persistence of functional brain changes resulting from short-term exposure to toxic levels of sarin. It should be noted, however, that there is some controversy concerning the value of computerised analysis of brain-wave topography and caution was expressed about its uses. In a commentary on these studies, the authors themselves raise the question of the toxicological significance of the findings (Duffy and Burchfiel, 1980).

Savage *et al* (1988) evaluated a heterogeneous group of individuals with a history of occupationally-related OPp poisoning that had occurred on average 9 years prior to the study, and found abnormalities in a wide range of neuropsychological variables, including visuomotor, attention and language function. Persistent abnormalities in affective behaviour, especially anxiety, were also found. Although the authors stated that the poisoning documentation was screened for completeness, it is not clear on what criteria it was based. In particular the clinical severity of poisoning, the toxicological evidence of poisoning and the nature of other intercurrent diseases were not reported. For instance,

one exclusion criterion was that of head trauma with periods of unconsciousness totalling more than 15 minutes. Cases with periods of unconsciousness of less than 15 minutes were thus included and therefore the changes in the cognitive functions might represent a consequence of brain hypoxia or other intercurrent factors, given the large variability in the time elapsed from poisoning to assessment. Generally most observed differences between the groups were within normal variability. Certainly other factors, such as education might account for differences, for instance, in comprehension, arithmetic and vocabulary. Moreover, toxicological analysis showed that the blood level of organochlorine pesticides in the study group was about twice that of controls. Since statistical analysis failed to show any association between blood levels and the neuropsychological tests, the authors ruled out organochlorines as the causative agents of such impairment. However, from the toxicological standpoint, organochlorine exposure might have been more relevant given the enormous pharmacokinetic differences between these pesticides and OPps.

Rosenstock *et al* (1991) examined 36 male farm workers in Nicaragua about 2 years after an accidental poisoning episode with OPps. The age-matched control group consisted of 36 men in the same community who had never been treated for pesticide poisoning. The study was carried out before the onset of the spraying season to reduce to a minimum the potential influence of acute pesticide exposure. According to these data, a proportion of both groups had had some recent exposure. The performance of the poisoned group was significantly worse than that of the control group in a battery of neuropsychological tests. The authors concluded that even a single episode of clinically-significant OPp intoxication could be associated with a persistent decline in neuropsychological functioning. The type of OPps involved and the severity of poisoning were not reported and the design and statistical significance of the study were criticised (Schuman and Wagner, 1991). Since the control group had been exposed to OPps and given the end-points used (e.g. visuomotor performance) and the lack of follow-up studies, it cannot be excluded that pre-existing neuropsychological deficits could have biased the study results.

Steenland *et al* (1994) examined 128 men in California poisoned by OPps between 1982 and 1990. The control group consisted of 90 “non-poisoned friends of the subjects not currently working with pesticides”. The tests included a neurological physical examination, 5 nerve conduction tests, 2 vibrotactile sensitivity tests, 10 neurobehavioural tests, and a postural sway test. In tests for sustained visual attention and mood scales, the performance of the poisoned group was significantly worse than the control group. The pesticides to which the patients had been exposed included chlorpyrifos (CPF), diazinon, dimethoate, methyl demeton and methyl oxydemeton, mevinphos, parathion, phosalone and others. A pesticide-specific analysis disclosed significant deficits in nerve conduction among men exposed to CPF and phosalone, and reduced vibrotactile sensitivity among those exposed to methyl demeton. These findings indicated the possible occurrence of a neuropathy affecting both the upper and the lower limbs; this, in turn, could have biased the results of other tests. However the clinical and

toxicological assessment of poisoning was imprecise and the involvement of the upper limbs is not expected in mild OPIDN. Moreover since actual electrophysiological and vibration sensitivity data were not reported, it is difficult to assess the biological significance of these changes. The mean age of the non-poisoned control group was significantly lower than that of the poisoned subjects.

Keifer *et al* (1996) explored the effects of pesticide drift on residentially-exposed populations in Nicaragua. They compared a group of 100 residents randomly-selected from a community surrounded by actively-sprayed cotton fields with a similar community far from agricultural spraying. Residents from the exposed community had significantly lower RBC AChE activity and a significantly higher proportion of subjects complained of acute or chronic symptoms. The results indicated “a strong association between exposure to aerial pesticides and symptoms”. This study was carried out in 1990, at the end of the cotton pesticide-application season (August-December). There are no data relating to reversibility of complaints. Apart from a comparison of pre- and post-application findings, it would be difficult to assess reversibility in situations such as this where pesticides are applied regularly.

Wesseling (1997) reported a cross-sectional study of 211 banana plantation workers in Costa Rica, testing the hypothesis that chronic neurobehavioural deficits occur as a consequence of previous intoxication with ChE-inhibiting pesticides. The performance of 81 previously-poisoned workers on a battery of neurobehavioural tests was compared with that of 130 randomly-selected non-poisoned plantation workers. It was estimated that 1.5% agricultural workers/year suffered a work-related pesticide poisoning requiring medical attention. The examination was carried out between May and September 1994. As it was not feasible to conduct the study outside a spraying season, a substantial proportion of the study population had been recently in contact with ChE inhibitors, although none complained of overt poisoning symptoms. There was no difference in RBC AChE activity between sub-sets with/without recent exposure, or between the poisoned/ non-poisoned groups. However, the mean plasma ChE level of the workers with recent exposure, was lower than for the workers without recent exposure and within this sub-set with recent exposure, the previously-poisoned workers had a lower mean value than the non-poisoned controls. The previously-poisoned subjects performed less well than non-poisoned controls on most psychometric tests; more-severely poisoned subjects had markedly-increased neuropsychiatric symptoms. The study, like other similar studies, provided no evidence of adverse effects in "non-poisoned" subjects exposed to OPp levels considered to be acceptably low.

At a conference organised in 1997 by the Scottish Agricultural College, Julu *et al* (1997) reported significant abnormalities of the peripheral nervous system (PNS) in sheep-dipping farmers. The finding was more pronounced in subjects with previous episodes of poisoning, but was also present in those without previous symptoms. Another group of patients who experienced previous acute OPp

poisoning had autonomic failure, mainly of the sympathetic nervous system. The authors maintained that the findings were different from OPIDN and supported the following conclusions: 1) long-term sequelae follow acute OPp intoxication and 2) chronic effects may follow repetitive low-level exposure to OPps. These findings have yet to be substantiated by controlled studies on randomly-selected samples from the exposed population. It is not possible to infer causation from the data so far presented.

At the same conference Davies (1997) described "*chronic organophosphorus-induced neuropsychiatric syndrome*" in "*victims of chronic organophosphorus exposure*", particularly sheep-dipping farmers. He indicated that his findings would be published and that the reports were in press. He contended that there was a *"spectrum of symptoms with no convincing psychiatric explanation"* (= a new disease). According to a transcript of a participant's notes the symptoms as presented by Davies are: *Exacerbation of "dippers' flu" (*said to resemble increased sensitivity to infections as observed in Gulf War veterans); *personality change* (not depression but mood destabilisation, irritability, rage); *intensive suicidal ideation and suicidal behaviour*; *memory and concentration impairment* (no quantitative results available, but universal complaint of inability to finish a task; and having to begin again); *language disorder* (difficulty in finding the correct word, 2 victims were talking "*absolute rubbish"); decreased alcohol tolerance* (half a pint of lager and they were lying on the floor*); heightened sense of smell* (to perfumes and fragrances); *heightened sensitivity to organophosphorus compounds* (feeling ill in the presence of OPps); *handwriting deterioration* (can write no more than a paragraph); *impaired exercise tolerance* (chronic fatigue; not chronic fatigue syndrome but something else). These orally-presented findings cannot as yet be critically evaluated or compared with the research published by other authors in internationally-accepted, peer-reviewed journals.

Concluding remarks on the chronic effects of symptomatic exposure

Most of the above reports describe chronic, persistent effects of symptomatic exposure related to prior episodes of acute exposure at relatively-high exposure levels.

The characteristic complaints and findings include poorer performance or significant changes in attention or vigilance, memory functions (speed of thinking, information processing) and velocity of psychomotor reactions. Less frequently disturbances in language functions, visual functions, dexterity, mood (irritability, depression) and sleep have been reported. As further demonstrated in this report (Section 4), many of these features represent characteristic human functions, for which no reasonable animal end-points can be defined in toxicity tests.

Most of these complaints and symptoms also appear to be characteristic of changes expected with advancing age. Therefore, a challenging question in the interpretation of epidemiological studies is the

discrimination of normal age-related changes, from abnormal performance not attributable to age. This is of particular importance, when reversibility of effects is to be assessed in elderly subjects after years of exposure. In consequence, the use of age-matched controls in epidemiological studies is of utmost importance.

Few human epidemiology studies have been carried out to date and those that are available have methodological shortcomings. The individual levels of exposure and extent of ChE inhibition are generally poorly documented. The studies and findings are mostly related to “OPps” as a group and little attention has been paid to substance-specific findings. The spectrum of tests applied in examinations is different in various studies, and few of the studies reported a complete examination of all possibly-affected organs and functions. In most studies the control groups consisted of individuals potentially chronically-exposed to low levels, but considered to be “healthy”.

Chronic sequelae of clinically-manifest severe OPIDN are different from the other chronic effects of symptomatic exposure, as discussed above. Late signs of severe OPIDN consist mainly of spasticity affecting the lower limbs. This observation is consistent with the known pathology of OPIDN.

3.2 EFFECTS OF CHRONIC, LOW-LEVEL, APPARENTLY ASYMPTOMATIC EXPOSURE

For a report of persistent effects of prolonged, asymptomatic exposure to OPps to be scientifically acceptable, examination of subjects should be conducted at a sufficiently long interval after the last exposure (ideally before the next spraying season) to allow recovery of ChE. Furthermore, there should be available a reasonable estimate of exposure conditions and subjects should not have been previously treated or hospitalised for OPp intoxication. Although several studies claim to have investigated the persistent effects of prolonged asymptomatic OPp exposure, in reality only a few fulfil the criteria listed above.

Allegations about the occurrence of chronic effects

Schizophrenic and depressive reactions, with severe impairment of memory and difficulty in concentration, were reported in 16 workers after variable exposures to OPps (Gershon and Shaw, 1961). However in conclusion the authors themselves state: "It may be objected that our results are due purely to chance and that exposure and mental change are not correlated. It is true that our results have not been analysed statistically and that they are drawn from the community at large". This report was severely criticised because of serious flaws, including the lack of evidence for

exposure, the detailed clinical description of only a few cases and the inconsistency with previous larger studies (Barnes, 1961).

Durham *et al* (1965) examined 50-70 exposed farm workers and up to 25 controls using vigilance tests and determining Complex Reaction Time. During exposure, which was neither estimated nor controlled by determining ChE inhibition, exposed farm workers performed slightly more poorly in the vigilance tests and responded more slowly to the visual stimuli. Performance returned to normal in the post-exposure sessions.

In an epidemiological survey Stoller *et al* (1965) compared several Australian areas to determine whether areas of "high" OPp usage had a higher proportion of admission for psychiatric disorders than "low"-usage areas. Sales of OPps were highest in the Goulburn valley, an area with one of the highest psychiatric admission rates. However, by a follow up of the patients' records, the authors found that none of the patients "had worked on a fruit farm and none had been exposed to organophosphorus insecticides".

In another study, 24 workers (13 commercial applicators and 11 farmers) with unspecified exposure to OPps were compared, by means of a personality test, a structured interview and ChE levels, with a control group of 24 farmers uninvolved in application (Levin *et al,* 1976). Commercial sprayers, but not farmers, showed elevated levels of anxiety and lower plasma ChE levels than controls. Assessment of other behavioural manifestations and RBC ChE failed to disclose other group differences. The authors concluded that these findings were tentative until confirmed by additional studies.

In Hawaii, Korsak and Sato (1977) examined 32 individuals with chronic occupational exposure to pesticides (mainly OPps but also carbamates and organochlorines) using neuropsychological tests and EEG. Participants were segregated according to frequency of exposure into 2 groups of “low” versus “high” exposure. No unexposed control individuals were included in the study and the methods used for group composition and data analysis were poor. Plasma ChE levels were the same in both "low" and "high" exposure groups. Actual exposure data were not reported and the EEG changes were different from those reported by Metcalf and Holmes (1969). Both neuropsychological and EEG data indicated a "selective' effect on the left frontal hemisphere. This is an unusual toxic effect, in that the blood supply to the brain is equal for both hemispheres and there is no reason why the left hemisphere should be more susceptible than the contralateral one.

Davies (1995) examined more than 20 individuals with “significant exposure to organophosphorus pesticides”. He noticed a frequent history of personality changes with destabilisation of mood, depression and irritability being the most prominent features. The patients reported extreme and intense “suicidal ideation”. The author reviewed animal data, clinical and epidemiological studies and

concluded that there was a relationship between chronic exposure to OPps and the development of affective disorders and suicidal behaviour, to which mood destabilisation, directly induced by exposure to OPps, could contribute.

Stephens *et al* (1995) compared the neuropsychological performance of 146 sheep farmers exposed to OPps in the course of sheep dipping with 143 non-exposed quarry workers. The authors concluded that "the farmers performed significantly worse than controls in tests of sustained attention and speed of information processing" with no changes in short-term memory or learning. In addition, "farmers also showed greater vulnerability to psychiatric disorders as assessed by a questionnaire". This large and generally well-conducted study provided clear evidence of several small but significant differences between the two populations in neuropsychological performance, one of which could be related to flock size. However, since no direct measure of chronic exposure was available, it is difficult to decide whether the slightly worse performance of the farmers was due to OPp use or to some other variable. No gross or subjectively apparent differences were seen in this study.

Stephens *et al* (1996) analysed the relationship between acute exposure and chronic neuropsychological effects in 77 UK sheep dippers who were a sub-group of the above-described study by Stephens *et al* (1995). Acute effects were assessed using a purpose-constructed symptom questionnaire. A low level of exposure to diazinon and chlorfenvinphos was confirmed by urine analysis in 43 farmers; the remaining farmers were exposed to propetamphos. Chronic effects were assessed at least 2 months after exposure using computerised neuropsychological tests and questionnaires regarding general health and memory. Mathematical analysis (simple correlation and multiple linear regression) provided no evidence for an association between acute and chronic effects. The authors concluded that individuals might experience chronic effects without the "earlier warning" signs of acute exposure. If these findings as reported are correct, they cannot be explained by the known biochemical and toxic effects of OPps and their dose-response relationships. The selection of workers belonging to the experimental group was different from that of the controls, because of the different response rates. While there were statistically-significant differences as compared with controls, several factors might have been involved in the determination of such differences. Long-term exposure data were assessed by means of a retrospective-exposure questionnaire. A dose-response relationship was found for only one test (syntactic reasoning). No explanation for these inconsistencies is offered by the authors. Assessment of exposure based only on memory is virtually impossible (Taubes, 1995). There might be a recall bias ("recent worries about the possible long-term effects of pesticides used in sheep dips") in the selection of workers to participate in the study and their responses.

Parrón *et al* (1996) retrospectively investigated 251 suicides which occurred in 3 different areas in the province of Almeria in 1976-1987 and compared this with the suicide rate in Spain as a whole. The eastern area of Almeria has the highest density of greenhouses in the world. The authors showed that the suicide rate in Almeria was higher than that of Spain and was highest in the eastern area of the province. The authors concluded that "a strong positive association between suicide and exposure to pesticides is therefore possible". The interpretation of this finding, however, would require the suicide rates to be broken down by occupational groups to identify whether previous acute poisoning might have been a pre-disposing factor.

Fiedler *et al* (1997) compared 57 fruit-tree farmers (exposed) to 42 age-matched (unexposed) cranberry/blueberry growers and hardware store owners and found significantly slower reaction time (dominant hand in the “high-exposure” subgroup) in exposed subjects. This was largely attributable to age and did not correlate with pesticide exposure. There were no significant differences in tests of concentration, visuomotor skills, memory, or in expressive language and mood.

Reports of chronic exposure without adverse effects

Rodnitzky *et al* (1975) performed a study on 12 apparently asymptomatic, chronically-exposed farm workers and 11 pesticide applicators in Iowa, USA. The control group consisted of 23 farmers who were tested prior to the spraying season or were not personally handling pesticides. Subjects were tested for abnormalities in memory, signal processing, vigilance, language and proprioceptive feed-back performance. The performance of the exposed group was not deficient as compared with the controls and plasma and RBC ChE levels were in the normal range. The mean plasma ChE was significantly lower in the exposed group, because of lower values in the sub-group of pesticide applicators. There was no significant difference in mean RBC ChE activity. The total duration of exposure was not evident from the report, the OPps used were not specified and in addition various non-OPps were used. Exposure was in general considered to be minimal although no quantitative data were available; exposure of the control group could not be totally excluded. The authors commented that their results showed no measurable abnormalities, but did not necessarily prove that brain chemistry and physiology were unaffected in such workers. The study only indicated that minimal exposure was “unlikely to result in widespread neurobehavioral dysfunction of practical importance”.

Rodnitzky *et al* (1978) carried out a prospective study in volunteers who ingested small doses of ethyl and methyl parathion and were subjected to a battery of neurobehavioural tests including memory, vigilance, information processing, language, proprioceptive feedback performance, anxiety level and depression. Two male volunteers, 53 and 62 years of age, respectively, were tested pre-exposure to obtain control values. Each completed four 5-day periods of parathion ingestion separated by a period

of 1-8 weeks. The total amounts ingested were 30 and 12 mg methyl and ethyl parathion respectively. Plasma and RBC ChE levels were measured before and at the end of each ingestion period. There was no significant depression of ChE activity and no significant effects on cognitive and psychomotor performance or emotive functions. The authors concluded that their results did not support the existence of latent or sub-clinical neurobehavioural effects from “minimal chronic exposure” (low-level, repeated). In this study the subjects’ own pre-exposure performances were used as controls, but with only two individuals involved, statistical evaluation is not feasible. Repetition of the same tests can result in a "practice/effect" biasing the results. The subjects’ performance improved in some cognitive and psychomotor tasks. If this trend was real and unrelated to practice/effect, then it shows that some neurobehavioral effects, albeit “beneficial”, did occur.

Maizlish *et al* (1987) studied neurobehavioural effects of low-level exposure to diazinon in pest control workers in California. Mean duration of application was 39 days and 46 applicators were compared to 53 non-applicators, using a computer-assisted neurobehavioural test battery. The study showed a small but significant deterioration in neurobehavioural function over a working shift, but no persistent effects. However, because of limitations in the study design, concern over adverse behavioural effects of long-term, low-level diazinon exposure could not be completely excluded. Moreover, the applicators were on the average younger, had fewer medical problems, less recent home use of pesticides and less temporary worries. These factors could potentially contribute negatively and confound the study results. Cholinesterase inhibition was not monitored and the investigation was not repeated at a later time-point.

In the State of Washington, USA, Daniell *et al* (1992) carried out a prospective study examining effects of chronic occupational exposure to OPps on the neuropsychiatric performance of 49 apple orchard pesticide applicators, using a comparison cohort of 40 beef slaughterhouse workers. The nature and the extent of pesticide exposure were carefully assessed. The major OPp involved was guthion (azinphosmethyl). There were no statistically-significant differences in results from the computerised neurobehavioral test battery. The authors concluded that there was no clear evidence in applicators of clinically-significant decrements in neuropsychological performance following low, intermittent and well-controlled OPp exposure over 6 months. Monitoring of RBC ChE in these cohorts demonstrated no changes in the slaughterhouse workers and a mild post-seasonal inhibition (< 15%) in the applicators (Karr *et al*, 1992). Potential confounding factors in this study were that some control subjects reported greater amounts of alcohol consumed than other sub-groups and a tendency towards a greater prevalence of previous head injury among the controls than the applicators. Furthermore, a proportion (27%) of the controls reported having worked with pesticides in the past. The findings were representative only of relatively low agricultural exposures with ground vehicle-based methods of spray application and regular use of personal protective equipment.

Ames *et al* (1995) studied 45 professional pesticide applicators who were using a variety of OPps and had at least one documented episode of ChE inhibition but no symptoms of frank poisoning (RBC ChE inhibition ≤ 70%, plasma ChE inhibition ≤ 60%). No central nervous system (CNS) or PNS effects were observed when compared with 90 controls. The authors concluded that there is some evidence that preventing acute OPp poisoning also prevented neurologic sequelae. A possible criticism of this study is that selecting subjects, based on a lack of symptoms of poisoning despite ChE inhibition could have led to a selection of individuals with especially robust nervous system, refractory to excessive cholinergic stimulation. The OPps involved were not identified and some workers were also exposed to carbamates. The individual time period between the observed ChE inhibition and the examination is not evident, but was probably 1 year or more, since the workers were examined in 1990 based on medical records of 1985, 1988 and 1989.

Reviews

Karczmar (1984) reviewed the "acute and long-lasting central actions of organophosphorus agents." He hypothesised that the psychological and EEG effects could not depend only on ChE inhibition and ACh accumulation, as these effects might outlast the biochemical changes and persist for days, months, and even years. He quoted the features of "chronic organophosphorus syndrome" as "excessive dreaming, loss of libido and memory, irritability, concentration deficit…" and suggested that a cholinergic alert non-mobile behaviour (an effect of ChE inhibitors) could be disquieting or stressful and evoke schizoid or paranoid response.

D'Mello (1993) reviewed available information describing the behavioural changes induced by ChE inhibitors. He concluded that with respect to the changes reported in humans (such as loss of appetite, difficulty in concentration, impairment of memory, ataxia, muscular weakness, decreased reaction, emotional lability, confusion and anxiety) the data were not sufficiently reliable and that too little was known about the behavioural changes induced by ChE inhibitors. On the other hand the experimental animal data, usually more reliable, were considered rather superficial, especially with respect to the specificity of the observed effects.

Ecobichon (1994) compiled numerous reports of chronic effects in humans and correctly discriminated "neurological" from "psychological" effects. The "neurological" effects were generally sequelae associated with symptomatic exposure ("poisoning") and were occasionally complicated by OPIDN. The descriptions of "psychological" effects also included examples of acute effects and their sequelae. Potential chronic effects associated with professional activity were considered including Wehrmacht personnel engaged in the manufacture and handling of chemical warfare agents during World War II, and agricultural pilots involved in accidents during spraying operations. Although the author acknowledges that easily-recognisable serious or permanent symptomatology has not been observed

frequently enough to establish a recognisable pattern, he contends that a variety of suspected or real biological effects occur in a sizable proportion of cases. In his opinion, it is worthwhile examining the data, since sufficient anecdotal information can be found to indicate that there might be persistent and serious complaints, lasting from 6 months to several years and possibly a lifetime. On the other hand, he also clearly states that the incidents reported in the published literature are of limited use as they tend to be anecdotal, bizarre single cases, resulting from accidental high-level exposure or suicidal intent and poorly documented in medical terms. Therefore, his concerns would seem to be intuitive rather than based on substantiated records. The attempt to relate the neurological, psychological, and neuromuscular effects to the electrophysiological mechanisms results in his concluding that "the importance of the cholinergic system in the central nervous system is poorly understood".

Mearns *et al* (1994) reviewed the literature and concluded that "long-term psychological effects of low-level exposure have not been determined satisfactorily", and "not all studies found ill effects". It was suggested that enhanced psychological observation of exposed individuals in longitudinal studies was needed.

The Greenpeace report "The Effects of Organophosphorus Pesticides on Human Health" (Allsopp *et al,* 1995) pays particular attention to "effects on the central nervous system following repeated exposure to small quantities of organophosphorus compounds". The report quotes the multinational epidemiology study on long-term health effects from low-level exposure published in 1993 by the World Health Organization (WHO). The work was carried out between 1983 and 1986 on a total of 752 individuals from Belgium, Czechoslovakia, Hungary, Israel, Poland, Turkey and Yugoslavia. Agricultural, manufacturing and packing workers were exposed to one or more of the following agents: methyl azinphos, chlorfenvinphos, dimethoate, CPF, fenitrothion, malathion, methidathion, methyl parathion, mevinphos, parathion, phorate, phosalone, pirimiphos, quinalphos, tetrachlorvinphos, thiometon, trichlorfon and vamidothion. The workers were considered to be "healthy" and none had suffered acute poisoning during the previous 2-3 years. The assessment of long-term health effects was carried out using "WHO Neurobehavioural Core Test Battery". The results were somewhat heterogeneous, but in general indicated that many exposed individuals did not feel well and had mood problems. The study concluded that low-level exposures could produce symptoms and signs warranting preventive action to reduce exposures. However, considering the work pattern of this cohort, the low-level exposures could also reasonably be expected to have included episodes of substantial over-exposure and certainly included a number of subjects with alcohol-related illnesses.

In the UK in 1991, the National Farmers' Union (NFU) carried out a study on farmers in response to growing publicity about ill health caused by sheep dips. About a third of the respondents reported complaints such as fatigue, aching limbs, loss of concentration, depression, and acute symptoms such

as headache, dizziness and nausea. It was concluded there was concern that these effects might represent cumulative toxicity of OPps (Allsopp *et al,* 1995; NFU Conference Report, 1995).

Jamal (1995) reviewed the data related to chronic effects on the CNS and PNS. He expressed concern that "it is possible that chronic intoxication as a result of repeated exposure to very small doses could result in cumulative poisoning which may produce sub-clinical effects initially but may render the individual susceptible to further toxic insults thus producing progressive effects on the nervous system". He also admitted that potential effects induced by low doses of OPps were still controversial and needed further substantiation. He concluded that the question of possible long-term OPp effects urgently needed further investigation and that future studies must be appropriately designed, controlled and use modern sensitive and comprehensive techniques.

Eyer (1995) reviewed the available literature dealing with neuropsychopathological changes after exposure to OPps. He concluded that the available data did not indicate that apparently asymptomatic exposure was connected with an increased risk of delayed or permanent neuropsychopathological effects.

More recently, Steenland (1996) commented on a variety of findings and complaints reported in human epidemiology studies with a warning that "sub-clinical damage does occur, but longer follow-up studies are needed", and so confirmed the shortcomings of data presently available.

Tolerability studies in human volunteers

In human volunteers experience from tolerability studies of various duration (up to several weeks of daily dosing) indicates an absence of any sequelae of repeated "low-dose" exposure. "Low-dose" in these studies is typically a multiple of the dietary ADI (acceptable daily intake) for the general population. Such studies have been and still are being performed, particularly towards protecting the general population from any adverse effects from pesticide residues in food.

In studies on methyl parathion reviewed in the International Programme on Chemical Safety (IPCS) Environmental Health Criteria (WHO, 1993) there was no evidence of toxic effects when 4.5-7 mg/day methyl parathion (~ 3-5 x ADI) was given daily to groups of 5 male volunteers for 118 days or when volunteers received increasing daily doses of up to 19 mg/day for 30 days. The lowest dose that produced some inhibition of plasma and RBC ChE activities was 24 mg/day (20 × ADI).

IPCS (WHO, 1989) reviewed a large number of human volunteer studies conducted with dichlorvos, via the oral and particularly the inhalation routes of exposure. Repeated oral doses of up to 16mg/kg/day dichlorvos for up to 60 days and various inhalation regimes were employed. No effects

were observed, apart from plasma ChE inhibition and irritation of respiratory pathways at high atmospheric concentrations (~ 50 mg/m^3 air). As aircraft personnel were involved, EEG and ECG (electrocardiography) were among the clinical parameters included in a study on 26 men and 6 women exposed in a chamber to dichlorvos concentrations of 1 mg/m^3 air for up to 7.5 hours. It was concluded that "the use of dichlorvos as an insecticide in the form of sprays or slow-release strips (at recommended levels) does not constitute a short- or long-term hazard for the general population".

In male and female volunteers, oral doses of up to 0.2 mg/kg/day dimethoate for up to 39 days were tolerated without any changes; 0.43 mg/kg for 57 days reduced whole blood ChE activity by 24% on day 20. Dose levels of 0.59 and 1 mg/kg (45 and 14d, respectively) produced only inhibition of whole blood ChE activity (Edson *et al*, 1967).

Many other compounds, some widely used as therapeutics, for example malathion, metrifonate and tetraethyl pyrophosphate, have been studied in controlled trials in humans. Dose levels eliciting no or only slight ChE inhibition consistently failed to produce any other effects. The studies were reviewed by Gallo and Lawryk (quoted in Hayes and Laws, 1991).

Exposure in the human volunteer studies was well characterised, but in general no psychological tests were included in the study protocol. In the absence of such sensitive measurements, a relationship between OPp exposure and subtle functional changes as reported in the epidemiological studies can neither be substantiated nor contradicted.

Electrophysiological tests of neuromuscular functions in humans

In several studies peripheral nerve function was assessed by means of electrophysiological tests. Such tests are delicate and are performed more reliably with needle than with surface electrodes. All studies with needle electromyography were negative and most effects were trivial and within normal variability. Moreover several studies assessed toxic neuropathy by measurements made in the upper limbs, thus failing to appreciate that distally-accentuated neurotoxicity is more likely to occur first in the longer axons in the legs.

Neuromuscular function was assessed with surface electrodes on the upper limbs of workers exposed to OPps and organochlorine pesticides (Jager *et al,* 1970). A higher incidence of electromyographical changes (repetitive activity and reduced amplitude) was detected in 36 workers exposed to both chemical classes as compared to 24 exposed to organochlorine only and 28 controls. The biological significance of these small changes is unclear and no data on exposure levels were provided. Changes were thought to be related to synaptic dysfunction because they were similar to those found

in myasthenic patients over-treated with anti-ChE drugs. However, these changes were associated in the patients with substantial inhibition of AChE, whereas changes in workers were not associated with whole blood AChE depression.

Fifty-three workers exposed to both OPps and organochlorines were examined shortly after the start and towards the end of the spraying season (Drenth *et al,* 1972). The surface electrode electromyography records of 12 men changed from normal to abnormal, while those of 13 men changed from abnormal to normal. No differences in blood ChE were detected. No data on exposure levels were given. It is therefore difficult to support even the cautious conclusion of the authors that electromyography abnormalities represent only an indication of the need for more protection of workers but not the evidence of immediate health problems.

Minimal electromyographic changes were detected by surface electrodes in 102 workers exposed to OPps when mean values were compared to an unmatched control group of 75 subjects (Roberts, 1976). Fifty six workers were examined before and after a holiday period. Subjects displaying these changes improved somewhat after holidays, whereas some unspecified variability was observed in exposed subjects with normal electromyography. No exposure data were available.

In a study on 6 workers occupationally exposed to OPps over a 7-9 month period, surface electrode electromyography indicated that voltages varied in a manner reflecting a vague assessment of the pattern of exposure (Roberts, 1977). It is difficult to assess these results given the lack of actual exposure data.

In a study on volunteers, mevinphos (25 µg) was administered orally to 8 subjects daily for 28 days; a placebo was given to 8 controls (Verberk and Sallé, 1977). RBC AChE activity was depressed by 19% but no correlation was found with the neurological changes detected. A 7% decrease was found in slow fibre motor nerve conduction velocity and a 38% increase (as % of pre-exposure values) in Achilles tendon reflex force at the end of exposure. No effect on neuromuscular transmission was detected. The authors concluded that the significance of the detected effects with regard to health was not clear.

Neuromuscular function was assessed with surface electrodes in a group of 11 spraymen during a period of exposure to OPps and after 1-4 weeks of non-exposure (Stälberg *et al,* 1978). Plasma ChE activity was significantly reduced after work, whereas RBC AChE was not. A slight reduction in sensory conduction velocity and increased fibre density was detected in some workers. However, there was no correlation between electrophysiological effects and plasma enzyme inhibition.

Exposure to OPps was approximately assessed in a study on 4 groups of workers comprising “highly” exposed (42/group), seasonal workers exposed and re-examined after exposure (14), agricultural workers with “low” exposure (129) and agricultural workers not exposed (26) (Jusic *et al,* 1980). The authors concluded that synapse testing with needle electromyography and clinical examination did not detect any latent OPp interference with peripheral neurological/muscular response.

A study was conducted in workers exposed to the defoliant S,S,S-tributyl phosphorothioate (DEF), where needle electromyography and biochemical examination (lymphocytic NTE and blood ChE) were performed before and after the spraying season (Lotti *et al*, 1983). Air and dermal exposure were measured on a typical working day. No electrophysiological changes were detected, although there was ~ 60% inhibition of lymphocytic NTE. A consideration of the pharmacokinetics of DEF might explain why lymphocytic NTE inhibition was not associated in this study with electrophysiological changes indicative of PNS damage. DEF requires metabolic activation, mainly in the liver, to be an esterase inhibitor. The active metabolite formed is highly reactive and is unlikely to reach the nervous system unless very large amounts are formed. Some active metabolite reaches the blood, however, where it inhibits lymphocytic NTE. This might explain why, in this case, the inhibition of lymphocytic NTE is not associated with electrophysiological changes indicative of peripheral nerve damage.

Oculotoxicity from chronic, low-level exposure

Prolonged exposure to OPps has been suspected to have a causal relationship to the entity known in Japan as “Saku Disease” (Ishikawa 1971, 1973; Ishikawa and Miyata, 1980; Dementi, 1994). The major ocular signs of this disease are reduced vision, a narrowing of the peripheral visual field and/or central scotoma and abnormal refraction or myopic tendency with or without vertical corneal astigmatism. The epidemiological feature of the disease is characterised by its restriction to a fraction of the Japanese population and to certain geographical regions. The difficulty in defining “Saku Disease” led the Japanese Ministry of Public Welfare to list criteria for which at least 5 of 8 must be present for diagnosing the disease (Plestina and Piukovic-Plestina, 1978). The major OPps used in Japan up to the early 1970s included malathion, O-ethyl-O-4-nitrophenyl phenylphosphonothioate (EPN) and ethyl and methyl parathion. They were replaced by fenthion, dipterex, fenitrothion and diazinon as the latter group was considered to be less toxic to humans. All have been implicated in the development of “Saku Disease” (Ishikawa and Miyata, 1980).

Plestina and Piukovic-Plestina (1978) carried out an extensive review of ophthalmological findings in humans in Yugoslavia who were exposed to pesticides and workers who had been formulating pesticides for longer than 5 years; the control comprised workers with “no closer contact with pesticides than the general population”. Visual impairment and eye abnormalities were found more

frequently among exposed than among control subjects, although the causal relationship to pesticides was not fully established. They concluded, however, that "findings in workers heavily exposed to organophosphorus insecticides for a very long time were not consistent with the suggestion that this group of chemicals might be a cause of severe eye troubles in persons whose only exposure is environmental" i.e. although some changes have been reported in heavily-exposed subjects, no ocular problems were likely to be produced by chronic asymptomatic exposure.

In India, Misra *et al* (1985) carried out a detailed neuro-ophthalmological evaluation of 79 workers chronically exposed to fenthion for 1-18 years; the control group consisted of 100 hospital workers. The exposed workers had mean serum ChE levels lower than controls (~ 26% with butyryl thiocholine and ~ 30% with acetyl thiocholine as substrate). The symptoms in the exposed workers included eye irritation, diminution of vision, blurring of vision, discomfort from bright light, black dots and flashing lights. Fifteen of 79 subjects (exposure 7-9 years) had macular changes characterised by perifoveal irregularity of pigmentation and areas of hypopigmentation. The macular changes were attributed to OPp exposure. A physical examination, including a detailed neurological assessment, was performed. As no results were reported, it is uncertain whether the affected subjects had other neurologic symptoms attributable to exposure.

Concluding remarks on the chronic, apparently asymptomatic exposure

It is still a matter of controversy whether the "chronic syndrome" from low-level, apparently asymptomatic exposure really does exist, and if so, what its specific features are. In his review, D'Mello (1993) concluded that the available data on behavioural toxicity of OPps were unreliable. The main points of criticism are listed in Table 2.

Table 2: Shortcomings of reports of effects in man *

- Exact circumstances of exposure are often unknown
- Agent-specific effects and possible interactions between different agents are not considered
- Some effects can be attributed to therapy with medicaments (other than OPps)
- Methods used to collect and integrate information are primarily anecdotal
- Lack of appropriate controls
- Number of subjects tested is usually small

***** D'Mello 1993

To overcome these methodological shortcomings there is an obvious need for standardisation of epidemiological examinations and this has been initiated under the auspices of the WHO (Becking 1992).

The requirements for adequate epidemiological analysis of behavioural effects of OPp pesticides in man, as formulated 20 years ago by Levin and Rodnitzky (1976), are fulfilled in few of the available reports, viz. "*Longitudinal, double-blind studies utilising behavioural and toxicological measures are required to more adequately evaluate the impact of organophosphorus pesticides on occupational health and safety. Variables such as pre-exposure personality, age, nutritional status and occupational stress, may modulate the behavioural expression of organophosphorus exposure and must be taken into account in any such study. Epidemiological studies must take account of the likelihood that seasonal use of pesticides may produce slight, transient disturbance in workers and that affected workers may tolerate such symptoms without seeking treatment. With a better understanding of these potential pitfalls, future studies are quite likely to result in a more complete and accurate cataloguing of the behavioural effects of organophosphorus pesticides ".*

A specific problem in the analysis of the potential chronic effects of low-level OPp exposure is the apparent discrepancy in the effects observed. For example, in instances of "Saku Disease" the ocular effects are the most important symptoms reported, while in reports of the "chronic syndrome", ocular effects, apart from "blurred vision" shortly after exposure, are not mentioned. Unless these differences are due to substance-specific effects or to diagnostic shortcomings, the pictures of "Saku Disease" and of chronic syndrome are quite different and may have different aetiology. Factors such as a specific predisposition, possibly related to an underlying natural disease and unrelated to OPps may have been involved in at least some cases. Imai (1986) re-examined 9 patients originally diagnosed as "Saku Disease" and concluded that these patients did not have any systemic problems and their ocular abnormalities could be fully explained by common diseases.

Another particular problem in retrospective epidemiological studies is the difficulty, if not impossibility, of identifying the spectrum of pesticides involved and their specific levels of exposure. Furthermore, the effects considered as permanent or chronic may in fact represent acute effects of other OPps or pesticides of other chemical classes. The chronic effects attributed to low-level, asymptomatic exposure may also be associated with previous symptomatic but unreported exposure.

Human volunteer tolerability studies with known specific compounds reported no significant chronic effects, although such studies normally involve oral exposure and are not focused on subtle psychological effects. Electrophysiological studies of neuromuscular function in humans generally detected only trivial findings within normal variability and provided no evidence of latent toxic effects.

There is no known mechanism by which chronic, low-level OPp exposure could culminate in episodes of manifest acute or intermediate syndrome. Weak ChE inhibition by OPps is not known to be cumulative beyond the increase seen during the first few days of exposure with ChE inhibition reaching a plateau. It has been suggested that the pre- and post-synaptic molecular changes in neurotransmission could be related to those involved in the intermediate syndrome (De Bleecker, 1995) and could be instrumental in the chronic effects (Bushnell *et al*, 1994). However tolerance, i.e. desensitisation of cholinergic receptors resulting from prolonged and substantial increase of ACh, only develops when the inhibition of AChE is substantial; this is manifested by at least mild signs and symptoms, and hence no such effects would result from asymptomatic exposure.

3.3 BEHAVIOURAL EXPERIMENTS IN ANIMALS RELATED TO CHRONIC EFFECTS

Various experimental studies using EEG yielded findings partly similar to changes reported in humans. Long-lasting changes in the frequency spectrum of spontaneous EEG have been reported in primates following a single symptomatic exposure or a series of sub-convulsive exposures to sarin (Burchfiel *et al, 1976).* Results of statistical analysis of EEG power spectra showed increases in relative amounts of β-voltage (15-50 Hz) which persisted for one year and were most prominent in the states of awakeness during darkness or in drowsiness. These data are difficult to assess because of the limited number of animals employed, the complex statistical treatment of the electrophysiological parameters and the fact that it is not clear whether the variability of these EEG parameters was assessed in the controls over time. The toxicological significance of these changes thus remains to be explained. Parallel experiments with dieldrin, an organochlorine insecticide, produced similar alterations in the EEG spectrum. Hence the interpretation is further complicated by the fact that the same effects are produced by compounds with markedly different chemical structures and mechanisms of neurotoxicity.

Synchronisation in the 8 Hz range was observed in rats after acute or repeated administration of diisopropyl fluorophosphate (DFP) or soman in doses that "caused no overt symptoms" (Wolthuis *et al,* 1991*).* In the same animals, shuttle-box performance was decreased by repeated administration. While tolerance to the performance decrement developed in DFP-treated animals, tolerance did not develop after repeated injections of soman.

Behavioural studies in animals have demonstrated that selected ChE inhibitors, with predominantly CNS effects, improve performance in rats in a number of cognitive or neuromotor tests at doses far below those that cause overt clinical signs (Wolthuis and Vanwersch, 1984) and in marmosets (Wolthuis *et al,* 1995*).* These effects could well be due to sub-threshold AChE inhibition in the nervous tissue.

Russell *et al* (1986) studied the effects of repeated exposures to asymptomatic levels of soman administered subcutaneously (s.c.) to rats daily for 3 days, or 3 times a week for 22 days at doses of 0.035 mg/kg. In most animals this dose reduced body temperature and pain sensitivity, and impaired temporal perception, general activity and cognitive functions but did not induce overt signs. Most of these initial effects disappeared when tolerance developed, although some, such as decreased pain sensitivity, persisted. Assay of brain AChE showed that its activity was reduced to 38% after the third injection and remained at a mean of 40% at 3 hours after the subsequent treatments.

Pope *et al* (1992) examined the prolonged effects of a single dose of CPF which did not produce acute cholinergic crisis. Rats received a single s.c. injection of 279 mg/kg and were examined 2, 4, 6 and 12 weeks later. Cholinesterase in the brain cortex and striatum was inhibited by 94-96%, 82-83% and 58-60% at 2, 4, and 6 weeks respectively. The muscarine-binding sites for muscarinic receptors were also reduced, in the cortex by 34, 33, and 18% and in the striatum by 48, 40 and 23% at 2, 4 and 6 weeks respectively. Twelve weeks after treatment both ChE activity and receptor density had returned to control values. Behavioural observations indicated reduced locomotor activity for the first 2 days after treatment, with a return to control values afterwards. When, however, the animals were challenged with scopolamine, an anti-muscarinic agent known to induce hyperactivity, the CPF-treated rats had a higher locomotor activity than the controls at 2, 4, 6, 8, and 12 weeks. These results were considered to indicate persistence of changes in the cholinergic system. However the possibility of decreased inactivation of scopolamine, a reaction catalysed by esterases/amidases, must be taken into consideration in the interpretation of the results.

Llorens *et al* (1993) studied behavioural effects of repeated exposure to disulfoton, administered to rats in doses of 0.5, 1 or 2 mg/kg/day intraperitoneally (i.p.) for 30 days. The animals had decreased brain AChE activity (40-90%) and down-regulated muscarinic receptors (25% in top-dose group). They developed a tolerance only to clinical cholinergic signs and not to decreased motor activity. Retention of a passive avoidance response and acquisition in a Morris Water Maze test were not impaired by the exposure.

Ehrich *et al* (1993) used a “functional observational battery” and neuropathology according to US EPA guidelines to screen for neurotoxic effects 1, 2, and 3 weeks after a single administration of various ChE inhibitors. Seven compounds: triorthotolyl phosphate (TOTP), DFP, phenyl saligenin phosphate (PSP), mipafox, malathion, dichlorvos and carbaryl were administered in low, intermediate and high doses. The compounds were selected to represent a variety of potential effects i.e. some were NTE inhibitors, some were cholinergic stimulators and some caused both cholinergic poisoning and NTE inhibition. Taken together the high doses of selected compounds were shown to induce acute cholinergic poisoning or to profoundly inhibit brain NTE. Determination of enzyme activities in sub-

groups of animals demonstrated that the high doses inhibited AChE and NTE in the brain by at least 37 and 64% at 4 and 48 hours respectively after treatment. All 7 compounds induced changes in parameters indicative of CNS excitability. In addition, dose-related alterations in response to approach were induced with DFP, malathion, dichlorvos and carbaryl. Neuropathic changes were found in the rostral dorsal spinal columns of animals treated with TOTP, DFP, PSP and mipafox. Lesions occurred at all dose levels and, with the exception of PSP, their grading increased in a dose-related manner.

Bushnell *et al* (1993) compared performance in a delayed matching-to-position test following a single administration of CPF or DFP given for 4 weeks. Both treatments induced a comparable ChE inhibition in whole blood and brain and down-regulation of muscarinic receptors that lasted for 6-8 weeks. Performance improved after a few days with CPF; with DFP-induced impaired performance, recovery occurred only at the end of treatment. This demonstrated that functional impairments could not be fully explained by inhibition of ChE or muscarinic receptor binding.

Bushnell *et al* (1994) studied the effects of repeatedly administered CPF to rats. Weekly-administered s.c. injections of 15, 30 or 60 mg/kg inhibited ChE activity in the whole blood by 60-90% after 5 weeks; the highest dose also induced tremor, working memory impairment and motor slowing in daily delayed matching-to-position/visual discrimination tests. Reducing the frequency of administration to every other week reduced the inhibition of whole blood ChE activity to 50-75% of control and ameliorated all behavioural deficits. Reinstatement of weekly administrations, but at levels of 15, 30 and 45 mg/kg for 10 weeks inhibited whole blood ChE activity by 75-90%. Tremor was not observed during this period; however motor slowing and working memory impairment persisted throughout the dosing period in all treated groups. Unfortunately the authors did not assess the persistence of these effects. Possible critical points of this study included the massive extent of ChE inhibition which obviously precluded the establishment of a clear dose-effect relationship, and difficulty in the interpretation of findings which at the high dose levels could have been secondary to general toxicity.

Padilla (1995) reviewed epidemiological studies and suggested that short-term exposure to some ChE-inhibiting insecticides might precipitate long-term adverse effects. She added that recent experimental studies (Ehrich *et al,* 1993; Pope *et al,* 1992; Tandon *et al, 1994)* “demonstrated that a single or short-term exposure to some organophosphorus compounds may produce behavioural or neurochemical changes lasting for days or months, presumably outlasting the ChE inhibition.”

Sheets *et al* (1997) tested 6 OPps for neurotoxic potential in sub-chronic neurotoxicity screening studies according to US EPA (FIFRA) requirements. The tested compounds sulprofos, disulfoton, azinphos-methyl, methamidophos, trichlorfon and tebupirimphos were administered in diet for 13 weeks to separate groups of rats. Neurobehavioural findings due to cholinergic effects occurred only at dietary levels producing more than 20% inhibition of AChE. The AChE activity was the most

sensitive index of exposure: in general, RBC activity was inhibited to a greater extent than plasma activity, and brain activity was the least sensitive. The greatest effects in the neurobehavioural tests were observed during weeks 4 and 8; there was no evidence of additional cumulative toxicity after 8 weeks of treatment.

Prendergast *et al* (1997) observed an impairment in performance of the spatial test of working memory in rats for up to 21 days after cessation of 14 days s.c. administration of 250 μg/kg DFP. AChE activity was suppressed to ~ 43% of controls in the frontal cortex and to ~ 50% in the hippocampus 3 days after cessation of treatment and recovered to normal levels by 21 days. The rate of recovery was lower in the hippocampus than the cortex and this was tentatively associated with the observed impairment of working memory. Repeated doses of 50μg/kg did not affect maze performance but slightly increased locomotor activity. Unfortunately the authors did not measure AChE activity in this group.

Ivens *et al* (1997) tested behaviour, motor activity, learning and memory in male and female rats ingesting 0, 0.5, 2 and 8 ppm parathion for up to 15 weeks. Tests were repeated during weeks 1-4 and 10-14, as well as 30-34 weeks after the end of treatment. Parathion caused no clinical signs and no, or only borderline, inhibition of brain AChE. Notably no cumulative or adverse effects were detected on learning and memory. Indeed, there was even an improvement of learning in the reference memory type of the Morris Water Maze.

These animal experiments demonstrate that prolonged exposure may, in some instances, result in effects associated with down-regulation of receptors and development of tolerance, while other effects appear to persist. The tolerance would result from the prolonged substantial inhibition of AChE and substantially increased presence of ACh. The observed behavioural effects may be related to substantial AChE inhibition; they provide no evidence that neurobehavioural changes occur without AChE inhibition. It follows that no novel neurochemical screening procedure specific for the alleged features of "chronic syndrome" can be recommended.

3.4 POTENTIAL MECHANISMS OF CHRONIC EFFECTS

Chronic inhibition of AChE resulting from exposure to OPps is thought to result in higher than normal levels of ACh within synapses, ultimately leading to a down-regulation of post-synaptic receptors. Consequently, a given concentration of ACh at these synapses gives less of a response since fewer post-synaptic receptors are available. Following continuous exposure to OPps several studies have demonstrated decreased numbers of post-synaptic muscarinic and nicotinic receptors in various tissues, including different regions of the brain. In addition, down-regulation of pre-synaptic muscarinic

receptors has been observed, indicating that pre-synaptic receptor-mediated inhibition of ACh release is reduced during continued exposure, accounting for an increase in the release of ACh from the pre-synaptic terminals in the exposed individuals. Numerous studies have demonstrated that repeated exposure to OPps renders mammals less susceptible to the toxic effects of these ChE inhibitors, even though ChE activities are below normal. This can be interpreted as exposure-induced tolerance (Sultatos, 1994).

A prolonged perturbation in the cholinergic second messenger system has been demonstrated in the retina of rats administered a single dose of fenthion (Tandon *et al,* 1994). In the retina and frontal cortex of treated rats a transient reduction in ChE activity and muscarinic receptor density was observed. In contrast to the transient reduction in ChE and receptor density, there was a persistent decrease of carbachol-stimulated release of inositolphosphate (an intracellular second messenger of some muscarinic receptors) in the retina of treated animals for up to 56 days after dosing. This effect was not found in the brain cortex, suggesting a persistent effect on signal transduction in the retina at the time when the density of muscarinic receptors had already returned to control levels. Although the decrease in carbachol-stimulated inositolphosphate release can be causally related to the initial ChE inhibition and receptor down-regulation, the prolonged duration of this effect could be responsible for late functional effects.

Williams *et al* (1997) demonstrated in male albino mice, additive AChE-inhibitory effects of multiple low doses of the compounds of the direct acting OPps, ecothiopate, paraoxon and mipafox and also additive inhibitory NTE effects of mipafox. The “multiple” doses consisted of 5 consecutive s.c. administrations/day; an additional group was given 8 administrations of mipafox. The total s.c.-administered dose was equal to twice the single dose which had reduced AChE inhibition to 60% of the control. During this period the inhibitory effects were additive, and, according to the data presented, leveled off afterwards, indicating that a ”steady state” was attained. These data, therefore, do not appear to prove that prolonged exposure to low levels of OPps could lead to a massive enzyme inhibition resulting form an insidious “cumulative” effect. They show that what is generally accepted for pharmaceuticals is also true for OPps, namely that a low initial enzyme inhibition increases when the exposure is repeated for several days. This increase is not linear, but asymptotically approaches a steady-state level. Therefore the term “cumulative” is misleading when used for long-term ChE inhibition by OPps as it suggests an effect continuously increasing during chronic exposure.

In a companion report Kelly *et al* (1997) demonstrated increases in “prejunctional and postjunctional jitter” (variability of latencies of evoked potentials) in the diaphragm of treated mice. They started from a previous experiment in which only mipafox, a neuropathic OPp, increased jitter and tested the hypothesis that increased jitter was related to inhibition of NTE. They observed that increased prejunctional jitter also occurred in paraoxon-treated mice and thus attributed this effect to “long-term

AChE inhibition" and not to NTE inhibition (again, the duration of treatment was 5 or 8 days). They speculated that the observed electrophysiological effects "may have health implications for exposure of humans to organophosphorus compounds". Although this demonstration of induced jitter is of interest, it is premature to designate this finding as a sign of toxicity before its biological mechanism and meaning have been elucidated.

Davies (1995) in his analysis of a relationship between OPp actions and affective disorders and suicide, pointed to the monoamine theory of depression and the importance of serotonin and other monoamines in the pathogenesis of numerous psychic disorders. He reviewed several animal studies indicating OPp effects on serotonin metabolism and brain levels. He also speculated that by influencing phosphorylation reactions in the nervous system, OPps could possibly influence neuronal systems and receptors functioning with other transmitters, such as glutamate.

There is evidence from psychopharmacological research, that increase in cholinergic activity is correlated with depression. Janowsky *et al* (1972,1973) reported that the centrally-active ChE inhibitor physostigmine caused dramatic but brief (20-90 min.) reduction in manic symptoms in bipolar patients or precipitated or aggravated depressive symptoms (Risch *et al*, 1981). In parallel with depression, increases in REM sleep duration and REM sleep density have been reported (Berkowitz *et al,* 1990) as well as activation of the hypothalamo-pituitary-adrenal axis as indicated by increased secretion of adenocorticotropic hormone, cortisol or prolactin (Janowsky and Risch, 1984) and increases in serum epinephrine levels (Janowsky *et al*, 1988, Janowsky and Overstreet, 1995). These effects are not a compound-specific effect of OPs, but are related to their ChE-inhibiting effect and the related stimulation of cholinergic activity. This central cholinergic control of mood is further supported by similar changes in mood induced by parasympathicomimetics (cholinomimetics) such as physostigmine, arecoline, oxotremorine, or ACh precursors such as deanol, choline or lecithin as well as by increases in REM sleep duration and REM sleep density induced by these compounds (Berkowitz *et al*, 1990; 1982, Janowsky *et al*, 1974). Similarly changes in mood can be antagonised either by centrally-active cholinolytics such as scopolamine or by catecholaminergic agonists such as methyl phenidate (Janowsky *et al*, 1973) indicating that a balance in central cholinergic and catecholaminergic activity plays a pivotal role in the regulation of mood. Changes in mood, REM sleep and hormonal reactions induced by cholinergic compounds or OPs have been reported only at doses which induced cholinergic signs or markedly inhibited ChE activity (Bowers *et al*, 1964; Janowsky *et al*, 1973; Rowntree *et al*, 1950). Therefore test subjects were often pre-treated with atropine to suppress cholinergic signs (Janowsky *et al,* 1972). Changes in mood were rather short-lived and disappeared together with the normalisation of the central cholinergic-adrenergic balance (Bowers *et al*, 1964; Janowsky *et al*, 1973 and Rowntree *et al*, 1950).

In addition to an effect on mood, involvement of cholinergic systems in cognitive functions such as attention, memory and learning is generally accepted and supported by a plethora of investigations in man and animals (Deutsch, 1971; Squire, 1987). In animals acquisition and recall have been shown to depend on an optimal level of central cholinergic activity with impairments seen at higher and lower cholinergic activities (Wilson and Cook, 1994). The optimal cholinergic activity level also depends on the type of task and the level of training (Lydon and Nakajima, 1992). In man, improvement of cognitive functions in general is seen at low asymptomatic or slightly symptomatic dose levels (Bierer *et al*, 1993) with impairments occurring at higher, symptomatic doses. Although cognitive effects intimately related to the activity of the cholinergic systems have been demonstrated in animals (Section 3.3), the persistence of such changes has not been demonstrated in animals exposed to low levels of OPps.

Contrary to the seemingly prolonged duration of the symptoms in the “chronic syndrome”, the changes in mood, REM sleep and hormonal reactions induced by cholinergic compounds are rather short-lived and seem to disappear with the normalisation of the central cholinergic-adrenergic balance

There is also evidence that chronic administration of nicotine has neuroprotective effects and protects neurons from damage (Socci and Arendas, 1996). This effect could be due to stimulation of cholinergic nicotinic receptors. In view of such data, the effect of ChE inhibition and cholinergic stimulation on the CNS system cannot be qualified as only beneficial or only deleterious; either judgement would oversimplify the true situation.

4. REVIEW OF TESTING PROTOCOLS WITH RESPECT TO THEIR CAPABILITY TO IDENTIFY CHRONIC EFFECTS

The more we know about the similarities of structure and function of higher organisms at the molecular level, the more we are convinced that mechanisms of chemical toxicity are, to a large extent, identical in animals and man. Gerhard Zbinden, 1991.

4.1 APPROACH TAKEN

The science of experimental toxicology focuses on the identification of potential toxicological hazards to human health using surrogate animal models in laboratory experiments (Johannsen, 1990). The relevance of findings from such animal experiments is founded on the established biological basis for the cross-species extrapolation, resulting from the phylogenetic continuity of organisms (Schmidt-Nielsen, 1975; Krasovskij, 1976; Russell, 1991; Winneke, 1991). This hazard identification is based on two main principles that underlie all animal toxicity testing. The first is that any effects produced by a chemical agent in laboratory animals may be applicable to man. The second is that exposure of experimental animals to toxic agents in high, toxic doses is a useful and valid method of discovering possible hazard to man.

The use of high, toxic doses to discover hazard is based on the quantal dose-response concept that the incidence of an effect in a population is greater as the dose or exposure increases. This concept at the same time implies that the severity of a toxic effect seen in an individual has to increase with increasing dose or with prolonged duration of exposure. With decreasing doses or shorter exposure, the incidence and severity of toxic effects diminish until a point is reached when they are no longer detectable. Any toxic effect is the net outcome of 2 processes i.e. the primary action of the toxic agent and the repair or the regenerative capacity of the system. Only if the capacity of the repair system is exceeded will a net toxic effect be seen. This balance tends to generate a threshold below which no net adverse effect is visible. A threshold may also result from pharmacokinetic factors,such as when the capacity to metabolise an agent vastly exceeds its capacity to penetrate a tissue. The sub-threshold dose is called the no-observed-effect level (NOEL) and can be the basis for the calculation of a maximum permissible exposure level for man. Depending on the biomarker or end-point used, different NOELs may be obtained (Classen *et al,* 1996). With the development of increasing insight into toxic mechanisms, more appropriate biomarkers have been selected, resulting in a steady increase in sensitivity of toxicity tests. The selection of appropriate biomarkers for neurotoxicity end-points is a critical step that can greatly influence risk assessment. The accuracy with which such end-points can be used to predict toxic effects in man depends on several criteria. An ‘ideal’ biomarker for

neurotoxicity should be a) valid, b) specific, c) sensitive and d) reliable (Vorhees, 1987). For a test to be *valid* it should measure a toxic end-point that is relevant for the human situation, should accurately predict the occurrence of this effect and should be interpretable. For a test to be *specific* it should be reliable in identifying compounds inducing this effect and recognising those not inducing it. For a test to be *sensitive* it should detect a particular type of impairment and should be able to detect small effects. For a test to be *reliable* it should produce a consistent pattern of results, within the same laboratory, concurrently and over time, as well as across different laboratories.

When assessing the capability of animal studies to predict OPp-related neurotoxic effects, several limitations have to be taken into account. Prediction of human toxicity based on animal data implies that the agent, subject, and exposure conditions should be as close to the human situation as possible. Therefore, evaluation of animal tests required for the registration of OPps will be grouped according to potential human exposure. Since most of the effects induced by OPps are related to ChE inhibition, this biomarker represents a good basis for extrapolation and will thus be used whenever possible. For the registration of many of the first-developed OPs only few safety tests had to be conducted. Some of these first OPs, for example TOCP and leptophos, were responsible for neurotoxic effects in man and/or animals and were responsible for triggering further tests which are now conducted routinely. Thus, when considering the toxicity of OPps in general, the different safety standards in operation when the compounds were first marketed have to be borne in mind.

In standard toxicity studies, the end-points used to identify potential neurotoxicity are either lesions detected in the nervous system during routine histological examination and/or changes in behaviour seen when observing the animals throughout the in-life phase of a study. In special neurotoxicity studies, which are usually conducted in rodents, refined histological techniques are used in addition to some reflex tests and measurement of motor activity. Plasma or RBC ChE activity is usually measured when testing OPps but these end-points reflect exposure rather than neurotoxicity. For OPs a specific study in hens is conducted to identify the compound's potential to induce OPIDN. In such studies the end-points include behaviour, locomotion or neuromotor performance (ataxia), histopathology of peripheral nerves and parts of the CNS, and in addition NTE activity, the substantial inhibition of which may be associated with the induction of delayed neuropathy.

Epidemiological studies investigating subjects exposed to OPps often report effects on motor and/or autonomic functions, cognition, sleep, and mood. Therefore, signs and symptoms observed in man are grouped according to the functional domains listed in Table 3. Inter-species comparison will be structured according to these functional domains. In Tables 5 to 9 effects reported in man are compared with end-points observed in toxicity and neurotoxicity animal studies. The end-points required by test guidelines are shown in shading in the Tables. End-points in italics are related to the

corresponding signs or symptoms seen in man, but prediction of effects in man is limited due to the less specific character of the findings in animals.

Table 3: Signs/symptoms in man grouped by functional domains

Functional domain	Function affected
Cognition	attention/vigilance memory speed of thinking (information processing)
Sensorimotor	speed of psychomotor reactions dexterity slurred speech
Mood	anxiety irritability depression
Sleep	fatigue/drowsiness sleep disturbances
Other	cholinergic signs visual dysfunction

In Table 4 standard and special tests are listed that are required for the registration of agrochemicals in the USA or in other OECD countries.

The recently-proposed protocols for the determination of potential ocular effects of OPps (Hamernik, 1994) have been commented on in ECETOC Monograph No. 22 (1994) and will only be addressed in the conclusions of this report.

Table 4: Toxicology data requirements for registration of agrochemicals (mammalian toxicity)

USA (40 CFR 158)	OECD Guideline	Study type
		Acute toxicity
§81-1	OECD 401/(420)	acute oral (rat, fixed dose method)
§81-2	OECD 402	acute dermal
§81-3	OECD 403	acute inhalation (rat)
		Irritation studies
§81-5	OECD 404	acute (primary) dermal irritation/corrosion
§81-4	OECD 405	acute (primary) eye irritation/corrosion
§81-6	OECD 406	skin (dermal) sensitisation
		Sub-acute and sub-chronic toxicity
	OECD 407	repeated dose oral (rodent, 28d)
§82-1	OECD 408	sub-chronic oral (rodent, 90d)
§82-1	OECD 409	sub-chronic oral (non-rodent, 90d)
§82-2 *or* §82-3	OECD 410	repeated dose dermal, (21,28 or 90d)
§82-4	OECD 411 [a)]	repeated dose inhalation (21,28 or 90d)
		Reproduction toxicity
§83-3	OECD 414	teratogenicity (2 species)
§83-4	OECD 416	2-generation
		Special studies
§85-1	OECD 417	toxicokinetics (metabolism)
§85-2		dermal penetration
		Chronic toxicity and oncogenicity
§83-2	OECD 451	oncogenicity (2 species)
§83-1	OECD 452	chronic (2 species)
	OECD 453 [a)]	chronic and oncogenicity (rat)
		Neurotoxicity
§81-8		neurotoxicity screening battery (acute)
	OECD 407 [a)]	repeated dose oral (rodent, 28d)
§81-7	OECD 418	delayed neurotoxicity (hen, acute exposure)
§82-6	OECD 419 [a)]	delayed neurotoxicity (hen, 28d)
§82-7	OECD draft [b)]	neurotoxicity screening battery (90d*)* neurotoxicity test guideline
§83-8	OECD draft [b)]	developmental neurotoxicity test guideline

a) test not generally required
b) guideline in development

4.2 PREDICTABILITY OF ACUTE EFFECTS (ACUTE AND REPEATED EXPOSURE)

In man, exposure to OPs induces signs and symptoms that vary depending on the exposure level. These are due to a direct effect of OPs on the nervous system, appearing minutes to hours after exposure and lasting for days, weeks, or in some cases months, depending on the OP involved. In Tables 5 and 6 the signs and symptoms observed after symptomatic exposure in man are compared with clinical signs observed in animals after a single exposure. Where possible, inhibition of RBC ChE activity is used as a crude basis for comparing sensitivity of different species.

Comparison of signs and symptoms observed in humans with end-points measured in acute toxicity and/or neurotoxicity studies demonstrates that most of these OP-induced effects can be detected in animal studies. Symptoms reported in humans, such as dizziness, jitteriness, increased tension, headache, disturbance of speech, consciousness or cognition, excessive dreaming, insomnia or nightmares, do not, however, have corresponding or detectable specific end-points in animals. Depending on the OP, different cholinergic signs may be observed in animals after acute (Ehrich *et al*, 1993) or after sub-chronic exposure (Sheets *et al*, 1997). Independent of the duration of exposure, these signs appear only at doses which inhibit RBC ChE activity by more than about 40%. These symptoms are reported to occur in humans only at doses which substantially inhibit AChE and are therefore not of specific concern in risk assessment (Grob *et al*, 1947a,b,c; Harvey *et al*, 1947; Bowers *et al,* 1964); an inhibition of RBC ChE of $\leq$ 20% is regarded as a NOEL in humans (WHO, 1990). This is clearly below an exposure level shown to induce signs or symptoms in animals or man.

Table 5: Comparison of acute signs/symptoms in man and animals

Functional domain	Signs/symptoms in man	Corresponding end-point	
		toxicity study	neurotoxicity study
Cognition	attention/vigilance	-	operant behaviour
	memory	-	working memory
	speed of thinking (information processing)	-	working memory operant behaviour
	speed of psychomotor reactions	-	operant behaviour
Sensorimotor	dexterity	*ataxia* histopathology	*ataxia* neuropathology sensorimotor tests
	language function	-	-
	visual functions	pathology of eye	ERG, operant behaviour
Mood	anxiety	ease of removal/handling	ease of removal/handling plus-maze
	irritability	ease of removal/handling	reaction to sensory stimuli ease of removal/handling
	depression	-	*behavioural despair*
Sleep	sleep	-	24-hr activity, sleep EEG
	fatigue	-	*motor activity*
Other	sexual habits	*delayed insemination*	-
	cholinergic signs [a)]	cholinergic signs	cholinergic signs

[a)] see Table 6

italics: end-point not strictly related to corresponding symptom
shading: end-point explicitly required by test guidelines

Table 6: Comparison of acute cholinergic signs/symptoms in man and animals

Signs/symptoms in man [a)]	Corresponding end-point in animals	
	toxicity	neurotoxicity
Minimal exposure (< 40% inhibition of RBC AChE) no signs or symptoms	no clinical signs	no effects
Mild to moderate exposure (40-80% inhibition of RBC AChE)		
Weakness		reduced grip strength
Dizziness		
Headache		
Sweating		
Nausea and/or vomiting	*increased vomiting (dogs)*	
Salivation	salivation lacrimation, rhinorrhoea	salivation lacrimation, rhinorrhoea
Blurred vision		
Miosis	miosis	miosis
Dyspnea	dyspnea	dyspnea
Abdominal pain and/or diarrhoea	diarrhoea	diarrhoea
Incontinence	urination	urination
Excessive dreaming		
Insomnia		
Jitteriness, restlessness, increased tension, emotional lability	ease of removal/handling	*ease of removal/handling*
Nightmares		
Moderate to severe exposure (> 80% inhibition of RBC AChE)		
In addition to above signs/symptoms:		
Difficulty in walking	abnormal gait	abnormal gait
Muscular fasciculations, tremor	fasciculations, tremor	fasciculations, tremor
Convulsions	convulsions	convulsions
Disturbance in speech		
Disturbance in consciousness		
Fever		increased rectal temperature
Bronchopharyngeal secretion	rales	rales
Changes in blood pressure		
Loss of pupillary reflex		pupillary reflex absent
Dyspnea or cyanosis	dyspnea, cyanosis	dyspnea, cyanosis

[a)] Namba *et al*,1971

italics: end-point not strictly related to corresponding symptom

shading: end-point explicitly required by test guidelines

4.3 PREDICTABILITY OF DELAYED NEUROPATHIC EFFECTS (OPIDN; ACUTE AND REPEATED EXPOSURE)

Some OPs can induce a delayed neuropathy. The cases occurring in man triggered the development of the delayed neurotoxicity study in the adult hens, which is now compulsory for the registration of OPps. The OPIDN has been characterised as a distal degeneration of long sensory and motor neurons. Although some functional recovery is possible, delayed neuropathy causes persistent damage and therefore ideally the reliability of a predictive test has to be absolute. The reliability of the 'acute hen test' to identify OPps that have a neuropathic potential was evaluated by Capodicasa *et al* (1991). The authors concluded that "all but two organophosphorus pesticides (methamidophos and CPF) which caused OPIDN in man were positive in the hen test." Methamidophos, which produced a false negative result, had a higher cholinergic toxicity in hens than in man, while with the CPF, OPIDN was observed in man only after massive suicidal intoxication. For these two OPps, delayed neuropathy was later demonstrated in the hen test at very high doses (Bertolazzi *et al*, 1991; Capodicasa *et al*, 1991). As demonstrated with CPF, the sensitivity of the hen test is not increased by repeated administration of an OPp (Richardson *et al*, 1993).

In the early 1990s, both the OECD and EPA test guidelines for the hen studies were revised and NTE-inhibition measurement added; inhibition of this enzyme is considered a pre-requisite of OPIDN. Furthermore, the OECD guideline now provides a detailed description of how to examine the cerebellum, the part of the nervous system most affected in OPIDN. Classen *et al* (1996), showed that inhibition of NTE is the most sensitive end-point for OPIDN. It tells much about a sub-threshold potential to produce neuropathy. Axonal degeneration in the cerebellum is the most reliable end-point because it shows if OPIDN actually occurred. Studies conducted according to the new test guidelines are thus optimally sensitive and can even identify OPps with a low potential to induce OPIDN.

In addition to this specific hen test, a complete set of toxicity studies in rodents and non-rodents has to be conducted for the registration of an OPp. Although, in general, other species are less sensitive than hens (Soliman, 1983) OPIDN has been induced in different species, namely, dogs, cats, rats, rabbits, sheep, horse, cattle, and monkeys (Davis and Richardson, 1980; Barrett and Oehme, 1985). Following the acute or repeated administration of specific OPs, the induction of behavioural and/or histological evidence of delayed neuropathy has been described, particularly in the standard species used in toxicity studies with pesticides. In dogs, administration of trimethyl phosphate for up to 4 months induced ataxia, impaired sensorimotor responses and reflexes, and neuropathology in spinal cord and peripheral nerves (Schaeppi *et al*, 1984). In mice, TOCP administered as a single dose (Veronesi *et al*, 1991) or for 9 months (Lapadula *et al*, 1985) was shown to induce weakness, ataxia and progressive hind-limb paralysis and/or neuronal lesions in spinal cord and peripheral nerves. Similarly in rats, acute (Moretto *et al*, 1992; Inui *et al*, 1993; Veronesi *et al*, 1986; Veronesi and Padilla,

1992) or repeated (Lehotzky and Ungvary, 1976; Veronesi, 1984) administration of specific OPs induced clinical or neuropathological evidence of OPIDN. These studies indicate that axonal lesions are induced in standard species under the test conditions used in regulatory toxicity studies. Similarly with other toxic end-points, dose-effect studies indicate that independent of exposure duration, OP-induced neuropathy is a non-linear phenomenon with a clear no-effect level (Abou-Donia and Preissig, 1976a; Prentice and Majeed, 1983). As histopathological examination of brain, spinal cord, and peripheral nerves is conducted routinely in regulatory toxicity studies, delayed neuropathy induced by repeat-dose, low-level exposure would be detected in sub-chronic and chronic toxicity studies. These studies routinely include a maximum tolerated dose and thus allow an estimate of the risk of OPIDN induced by neuropathic OPps administered over a prolonged period. Negative results in such studies indicate that OPIDN will not occur at low exposure levels even with extended duration of exposure.

Table 7: Comparison of effects indicative for OPIDN in man and animals

Effects in man	Corresponding end-point in animals	
	toxicity study	neurotoxicity study
Numbness and tingling in feet	-	*response to tactile stimulus*
Elevated vibration threshold	-	-
Weakness of lower limbs	-	reduced grip strength
Loss of reflexes	-	patellar reflex (dogs)
Inability to keep balance	gait disturbance	gait disturbance
Paresis, paralysis of lower limbs	hind-limb paresis/paralysis	hind-limb paresis/paralysis
Nerve conduction velocity reduced	-	neurophysiology
Axonal degeneration	histopathology	neuropathology

italics: end-point not strictly related to corresponding symptom
shading: end-point explicitly required by test guidelines

4.4 PREDICTABILITY OF CHRONIC EFFECTS INDUCED BY SHORT-TERM, HIGH-LEVEL EXPOSURE

In man, chronic, persistent effects other than OPIDN, induced by short-term, high-level exposure have been reported. Since the recovery period in animal safety tests is limited to 2-4 weeks, the design of these studies does not allow the persistence of any chronic effects to be demonstrated (Ehrich *et al*, 1993). In addition, since acute neurotoxicty studies as well as sub-chronic toxicity and neurotoxicity studies are aimed at determining a NOEL, the top dose often does not induce severe, acute intoxication, comparable to that in man (Sheets *et al*, 1997).

Although deficits in learning or performance have been reported in special animal studies after high doses of OPs (George *et al*, 1992; Hymowitz *et al,* 1990; McDonough *et al,* 1986), testing was done at a time when the animals had not fully recovered from acute effects. Single toxic doses of OPs can induce local damage to the CNS which is correlated to cognitive deficits (Hymowitz *et al*, 1990; McDonough *et al*, 1986). It is not clear if irreversible chronic effects may also result from functional changes induced in the absence of any morphologic lesions. As long as chronic effects are exclusively associated with acute, symptomatic exposure they can be avoided by adequate protective measures. As maximum tolerated exposure levels will be based on the most sensitive parameter, namely, AChE inhibition, such effects will not occur if exposure is adequately controlled.

Table 8: Comparison of chronic sequelae of symptomatic exposure in man and animals

Functional domain	Signs/symptoms in man [a)]	Corresponding end-point	
		toxicity study	neurotoxicity study
Cognition	difficulty/slowness in thinking/ slowing of information processing and psychomotor speed	-	working memory operant behaviour
	forgetfulness/disturbed memory/ memory deficit	-	working memory (Morris or tunnel maze)
	lethargy/difficulty in maintaining alertness, vigilance and focusing of attention, concentration	*reduced activity*	operant behaviour: performance, motor activity
Sensorimotor	muscular weakness	histopathology of muscles (muscle wasting) and nervous system	grip strength, histopathology of muscles, neuropathology
	visual difficulty/oculomotor imbalance	-	operant behaviour
	disturbed dexterity/co-ordination	*ataxia*	*ataxia*, operant behaviour neuropathology
	linguistic disturbance/slurring of speech	-	-
	muscular aches and pains	*reduced activity*	-
	visual/auditory evoked responses trends to lower amplitudes and longer latencies	-	EVPs
Mood	anxiety	*ease of removal/handling*	plus-maze
	depression	*reduced activity*	behavioural despair [b)]
	irritability/impatience	*touch response/ease of removal*	startle response/operant behaviour
Sleep	general fatigue/fatigability	*reduced activity*	*motor activity, 24-h activity*
	trouble sleeping/increased dreaming	-	*24-h activity* sleep EEG
	EEG changes/changes in sleep patterns	-	EEG
Other	increased perspiration	-	*temperature regulation*
	headaches	*reduced activity*	-
	changes in libido	*delayed insemination*	*evaluation of sexual behaviour*

[a)] Metcalf and Holmes, 1969 (1952, 1965 examinations); Levin and Rodnitzky, 1976; Rosenstock *et al*, 1991;
[b)] predictability of this test system for this application not generally accepted
italics: end-point not strictly related to corresponding symptom

4.5 PREDICTABILITY OF CHRONIC EFFECTS OF CHRONIC , LOW-LEVEL EXPOSURE

In chronic toxicity and carcinogenicity studies the duration of exposure is long enough to allow chronic effects to develop. Most of the initial neuro-active effects of OPs are expected to disappear gradually due to the development of tolerance. On the other hand, late effects are expected to take weeks to develop. As is the case in human epidemiological studies, age-related changes can occur in old animals and may confound study results. Furthermore, in animal studies it is difficult to determine the persistence or reversibility of chronic effects, because the duration of the recovery period is limited by practical as well as by biological constraints, such as the natural life-span of selected animal species.

Animal studies using a range of dose levels indicated that for all behavioural changes a NOEL could be established (Ivens *et al*, 1997; Mattsson *et al*, 1996; Sheets *et al,* 1997; Wolthuis *et al,* 1995). Invariably the NOEL for behavioural changes lies above that for AChE inhibition. The existence of a NOEL also fits presently accepted theories in toxicology and is of particular importance in relation to the alleged chronic effects induced by prolonged, asymptomatic exposure. Owing to the paucity of relevant data published so far, the evaluation is limited to identifying end-points in toxicity studies that correspond to signs and symptoms reported in man. Such a comparison indicates that in toxicity studies at least some of these symptoms can be identified. Although Table 9 may give the impression that most of the symptoms observed in man can be assessed in animals, the limitations in this extrapolation to man should be emphasised. Furthermore, many of these tests are used only by individual laboratories and their validity is not generally accepted. In addition such tests need prolonged experience and/or training of animals and are thus not well-suited as tests in regulatory studies.

Regarding proposals for further work, well-conducted epidemiology studies including a good estimate of dose and employing sufficiently sensitive and relevant effect indices are considered key to the understanding of the potential long-term effects of OPps. Two major studies are on-going or planned, one in the UK on sheep dippers and another in the USA on residents exposed to “off label” use of methyl parathion. This Task Force does not therefore at the present time recommend conducting any specific animal study but proposes instead to await the results of the on-going epidemiology investigations. A follow-up of the situation in due time by ECETOC is envisaged.

Table 9: Comparison of alleged sequelae of repeated, asymptomatic exposure in man and corresponding end-points in animals

Functional domain	Signs/symptoms in man	Corresponding end-point	
		toxicity study	neurotoxicity study
Cognition	reduced attention/vigilance	-	operant behaviour
	memory disturbance	-	working memory
	reduced speed of thinking (information processing)	-	working memory, operant behaviour
Sensorimotor	reduced speed of psychomotor reactions	-	operant behaviour
	impaired dexterity	*ataxia*	e.g. staircase test
	slowness of language function	-	-
	disturbed visual functions	pathology of eye	ERG, operant behaviour
Mood	anxiety	*ease of removal/handling*	*ease of removal/handling*
			plus-maze
	irritability	*ease of removal/handling*	*reaction to sensory stimuli* *ease of removal/handling*
	depression	-	*behavioural despair* [a)]
Sleep	sleep disturbances	-	*24-h activity*, sleep EEG
	general fatigue	-	motor activity
Other	altered sexual habits	*delayed inseminational*	-

[a)] predictability of this test system not generally accepted

italics: end-point not strictly related to corresponding symptom

shading: end-point explicitly required by test guidelines

5. CONCLUSIONS

- The primary neurotoxic effects of OPps on humans and animals are well understood. Such effects are generally reversible. Nevertheless, acute poisoning due to severe over-dose can produce persistent changes. The acute toxicity of OPps is appropriately assessed in regulatory animal safety studies, well documented in humans, and clinically manageable. The intermediate syndrome is always associated with prior acute effects and the number of reported cases is limited. Risk from such hazards can be managed by minimising exposure.

- The potential to induce OPIDN can be reliably detected by monitoring NTE inhibition and by adequate neuropathological examination in delayed neurotoxicity studies in hens. It is unlikely that chronic, low-level inhibition of NTE could cause neuropathy, because OPIDN is a threshold event. In any case such effects would have been observed in sub-chronic and chronic safety studies in rats and dogs had they occurred. Most cases of OPIDN have been caused by non-pesticidal OPs such as TOCP.

- There is no pharmacokinetic evidence for cumulative effects of chronic exposures to OPps at levels which are not acutely toxic. With prolonged substantial exposure the sensitivity to OPps decreases owing to the development of tolerance, and the effects are reversible after cessation of the exposure. Based on established pharmacological and toxicokinetic principles, irreversible sequelae of low-level exposure are considered unlikely. Tolerability studies in human volunteers conducted with a number of specific OPps indicate the absence of sequelae from daily, low-level asymptomatic exposure lasting for several weeks.

- There is insufficient evidence in the epidemiological literature that there is a “chronic syndrome” resulting from chronic, apparently asymptomatic exposure. To conduct appropriate surveillance of the exposed populations is considered superior to mounting additional animal studies to resolve the question of whether or not such a syndrome does exist. The described features of “chronic syndrome” resemble the complaints and changes sometimes seen in the general population and known to be linked to other societal and socio-economic factors. It is essential to correct the epidemiological observations for such confounding factors.

- Some of the toxic effects claimed to constitute the alleged “chronic syndrome” could be detected in general regulatory safety studies and in special neurotoxicity studies within the constraints of inter-species extrapolation. There are human mental capabilities which are not testable in animals. Therefore, and in the absence of hard evidence of the key features of ‘chronic syndrome’ in humans with low-level, asymptomatic exposure, it is not feasible to propose modifications of present regulatory animal studies.

- Review of reported complaints indicates that oculotoxicity does not appear to be a hazard of chronic OPp exposure. This is of interest in that oculotoxicity was previously suspected to result from OP exposure and special animal tests for this effect were required. In most epidemiological studies of OPp exposure, persistent ocular effects are not observed. In animal studies, ophthalmoscopy and biomicroscopy, supported by adequate pathology examination, provide sufficient sensitivity for detecting adverse effects in standard animal regulatory safety studies

- Based on the current state of the science, the Task Force concludes that the evidence for a "chronic syndrome" is insufficient. It is recommended that the epidemiology studies currently planned are followed up and the issue re-visited when the results are available.

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APPENDIX A: REVIEW OF ACUTE AND DELAYED TOXICITY

A.1 ACUTE EFFECTS

A.1.1 Pharmacology of organophosphorus compounds and kinetics of effects

The acute toxic effects of OPs are generally attributed to their anti-ChE activity and in particular to inhibition of the enzyme AChE which catalyses the hydrolysis of the neurotransmitter ACh. The inactivation of AChE results in the accumulation of ACh at all sites of cholinergic transmission. At muscarinic receptor sites this causes prolongation of the characteristic response, either excitatory (e.g. bronchoconstriction) or inhibitory (e.g. vasodilatation). At nicotinic receptors (e.g. motor end plates) the effect is first excitatory (muscular fasciculation) followed by inhibition (muscular paralysis) on the same synapse due to desensitisation of receptors and to muscle fibre exhaustion. Activation of nicotinic receptors causes a rapid response typical for synaptic transmission and includes the opening of the receptor ionic channel. Activation of muscarinic receptors leads to activation of a second messenger system. Prolonged periods are thus required for the onset and termination of responses, suggesting that ACh acts through muscarinic receptors as a modulator in the CNS, rather than as a transmitter (Koelle, 1994).

Exposure to OPs inhibiting AChE results in effects equivalent to activation of cholinergic systems. In order to induce pharmacological effects, the degree of AChE inhibition must surpass the physiological “reserve capacity”. For instance a 50% inhibition of erythrocyte AChE or 60% inhibition of plasma AChE was associated with signs of poisoning in field workers (Ames *et al,* 1989; Lotti, 1995) and in animals, as little as 26% acute inhibition of brain AChE was associated with neurobehavioural changes (Tilson *et al,* 1992).

The effect of different OPs on inhibition of ChEs other than AChE seems to be rather variable. Some agents preferentially inhibit butyryl cholineresterase (BChE) while others preferentially inhibit AChE (Hayes, 1982), the structure-activity requirement for inhibition of the two proteins being very different. Although there are data indicating the substance-specific extent of inhibition of AChE caused by different OPs in particular brain regions (Santhoskumar *et al,* 1996), such selective inhibition in brain areas has not yet been firmly established. Development of tolerance to the behavioural effects of ChE inhibition on prolonged exposure is known to prevent clinical symptoms even at 90% ChE inhibition (activity as low as 10% of normal) (Hayes, 1982).

Monitoring of ChE activity can be carried out on CNS or PNS (in animal studies), blood plasma, or RBC. Brain AChE inhibition is considered to indicate adverse effects on the nervous system and may be used to define a critical effect. Blood ChE inhibition provides direct evidence of exposure but only indirect evidence of neurotoxicity or adverse effects. In humans, examination is restricted to blood, since, for obvious reasons, no samples of the nervous tissue can be taken. RBC ChE is AChE both in man and in most animal species, whereas plasma ChE is often regarded as mainly unspecific BChE. However, in rat plasma the ChE activity represents mostly the neuronal form of AChE and it has been suggested that it could well represent the brain AChE activity (Sette 1997). The view of the "Acute Cholinesterase Risk Assessment Work Group" (Draft Report 1997) was that plasma ChE inhibition was not an adverse effect and therefore should not be utilised in risk assessments; similarly RBC AChE inhibition was not *per se* an adverse effect and the data on brain AChE inhibition should take precedence for determining a NOEL in animal studies. This group suggests a default NOEL threshold of ≤ 20% activity difference both for RBC and for brain AChE. (Some authors give "serum" values of activity/inhibition. Serum is plasma minus the proteins involved in the clotting process).

The inhibition of AChE occurs by covalent binding of the OP. AChE activity is restored by slow *de novo* synthesis of fresh enzyme and also to some extent as a result of spontaneous dephosphorylation of the inhibited enzyme. The rate and the degree of this spontaneous reactivation depends on the exact structure of the covalently-bound OP residue and can, in some cases, be accelerated by treatment with therapeutic oximes (Karalliede and Henry, 1993).

During the initial phase of exposure to low doses of OPps, AChE activity successively decreases until the steady-state level of inhibition is reached corresponding to the level of exposure. The level of inhibition represents the balance between continuous constant inhibition of enzyme and restoration of its activity by re-synthesis or dephosphorylation/reactivation. This steady state level is then maintained without further cumulative effects, even though the exposure level remains constant. At the same time tolerance develops to many of the functional effects of the AChE inhibition (Swamy *et al*, 1992 a, b).

There are data indicating that AChE also has effects not mediated through cholinergic mechanisms. For example, AChE is present in non-cholinergic neurons and non-neural tissues, it occurs transiently in developing neurons, and it can occur in catalytically inactive forms. There is a soluble form of AChE which undergoes secretion, often independent of cholinergic transmission, in response to physiological stimuli. There is evidence that AChE produces changes in neuronal activity, unrelated to hydrolysis of ACh, by opening K+ channels, inactivating Na+ channels and enhancing excitatory synaptic events in selected brain areas (Appleyard, 1994). AChE may have other catalytic functions, unrelated to neurotransmission and non-catalytic functions involved in structural interactions, for instance cell adhesion (Massoulië *et al,* 1993). If AChE has major physiological functions unrelated to cholinergic neurotransmission, it can be speculated that inhibition of AChE by exposure to OPs could also

influence such functions. However the fact that the catalytic centre of Ache is blocked does not imply that functions besides catalytic activity are altered. Such proposed mechanisms are at present of theoretical experimental interest only, since as yet there is no evidence for their practical relevance in potential toxic effects.

In addition to the known effects of OPs on ChE activity, there is experimental evidence indicating differential, agent-specific, and possibly direct effects on the post-synaptic receptors. For instance, following maximum tolerated doses, parathion and CPF show similar levels of ChE inhibition, but marked differences in toxicity. In the brain cortex and striatum, both suppress ligand binding to muscarinic receptors, whereas in the cerebellum, parathion suppresses but CPF increases, this binding. Following a pre-incubation with paraoxon, the density of muscarinic receptors in all 3 areas of the brain was decreased by parathion but increased by CPF (Chaudhuri *et al,* 1993). Paraoxon is the biologically-active metabolite of parathion. In spite of this, parathion has the greater potency to block nicotinic response in mouse neuroblastoma cells. Both parathion and paraoxon interact with muscarinic receptors in Chinese Hamster ovary cells and induce ion channel activation by a second messenger pathway other than ACh. These results indicate possible direct effects of parathion and paraoxon on nicotinic and muscarinic receptors (van den Beukel *et al,* 1996). However, it is unclear whether such effects observed at high concentrations *in vitro* will be relevant in practice. Beside these actions on cholinergic neurotransmission some OP-acid triesters have been reported to inhibit kynurenine formamidase, an enzyme in the biosynthesis pathway of tryptophan (Seifert and Pewnim, 1992). As a result of this enzyme inhibition it was speculated that serotonin (5-HT) biosynthesis might be reduced. The significant changes shown in the catecholamine levels in the brain and spinal cord of rats (Ali *et al,* 1979) may have been secondary to the cholinergic hyperactivity associated with toxicity and it is not clear if they would be seen at low doses. This again indicates that experimental evidence has to be provided before relevance for OPp toxicity can be accepted. Despite some speculations on other mechanisms of toxicity, it is still generally accepted that the primary mode of action of OPs and their toxic sequelae in mammals are related to AChE inhibition. The experiments indicating other mechanisms have still to be validated, and the effects tested at reasonably low exposure levels. Nevertheless, there may be potential mechanisms for effects not obviously related to ChE inhibition. However, there is no justification for the effects to be attributed to OPs as a chemical class, since there is no evidence that any such chronic effects occur with all OPs.

The same considerations apply to "novel protein targets" of OPs recently reported to occur in the brain. In particular, a protein identical to N-acetylpeptide hydrolase, an enzyme of unknown biological function but with significant esterase activity, was found to be sensitive to OP inhibition (Richards *et al,* 1998). As is well established for plasma ChE, interaction with protein does not necessarily indicate an

adverse effect and further studies are needed to elucidate if such reactions have any fuctional consequences.

In medicine OPs have been used for several “indications” as, for example, to reduce elevated intraocular pressure in glaucoma, to improve cholinergic innervation in myasthenia gravis or to treat atony of the GI tract and urinary bladder (Massouliè *et al,* 1993; Koelle, 1994). Ophthalmic use of some OPps was associated with formation of lenticular opacities and in the other “indications” no therapeutic advantage was evident over other pharmaceuticals. Currently, OPs and other ChE inhibitors are being tested for the symptomatic treatment of senile dementia of Alzheimer’s type (Hinz *et al*, 1996a, b). Both the undesirable and the desirable effects of OPs have been directly attributed to their influence on the cholinergic neurotransmission; the desirable effects include therapeutic applications.

The role of genetic polymorphism in differential susceptibility to OPp effects

There are data indicating that genetic predisposition can influence the individual reaction to OPp exposure. For instance the extent of AChE inhibition depends on the extent of inhibition of BChE. BChE is considered to function as a “scavenger” of ChE inhibitors, thus protecting the AChE. People with unusual phenotypes of BChE can have a higher proportion of AChE inhibition when the proportion of the inhibitor binding to the BChE is diminished. This situation was demonstrated by Fontoura da Silva and Chautard (1996) who showed that farmers, exposed to pesticides and classified as "mildly poisoned" based on RBC AChE inhibition, had a higher frequency of unusual phenotypes of BChE (13.1%) as compared to exposed farmers who had normal AChE activity (1.7%). Atypical BChE was found in an Israeli soldier who suffered severe symptoms following pyridostigmine (carbamate cholinesterase inhibitor) prophylaxis during the Gulf War. His serum BChE was less sensitive than normal BChE to inhibition by pyridostigmine and several other carbamate agents (Loewenstein-Lichtenstein *et al* 1995). Masson *et al* (1997) investigated several mutants of BChE which affect the aspartate-70 site (Asp-70), the defining amino acid in the peripheral anionic site of human BChE. Asp-70 was found to be important for binding positively charged OPs (e.g. ecothiopate), but not for neutral OPs (e.g. paraoxon, isoOMPA, DFP). Similarly, binding of the positively charged pralidoxime (2-PAM) was influenced while irreversible aging was reduced. It was predicted that people with the atypical variant of BChE will be equally sensitive to paraoxon and DFP but more sensitive to the toxic effects of ecothiopate. In addition they will be resistant to re-activation of BChE by 2-PAM. This will be offset by the lower rate of irreversible aging of inhibited BChE, thus allowing some regeneration by spontaneous hydrolysis.

Furthermore, there are genetic differences in the enzymes metabolising the OPs. The enzyme paraoxonase (PON1) contributes significantly to the detoxication of several OPs. The PON1 gene

contains 2 polymorphic sites at amino acid positions 55 and 192, which result in several alloenzymes of PON1 in human serum. The 192 as well as the 55 polymorphism significantly influences PON1 activity (Furlong *et al,* 1993; Li *et al*, 1993; Mackness *et al,* 1997). Multiple regression analysis on data from 279 healthy human subjects indicated that the 192 polymorphism, 55 polymorphism and serum PON1 concentration were responsible for 46, 16 and 13% of the variation in PON1 activity, respectively (Mackness *et al*, 1997). As discussed by Li *et al* (1993), there is evidence suggesting that serum paraoxonase protects against poisoning by OPs. Birds have very low levels of paraoxon hydrolysing activity in their sera and are very susceptible to parathion poisoning. Rabbits, with a sevenfold higher enzyme level compared with rats, have a fourfold higher resistance than rats to paraoxon poisoning. Rabbit paraoxonase hydrolyses CPF-oxon with a much higher turnover rate than rat paraoxonase, resulting in a very high resistance of rabbits to CPF toxicity. Direct tests of paraoxonase protection have been carried out by injecting purified rabbit enzyme into rats. The protection achieved was higher for CPF-oxon than for paraoxon, probably due to the high hydrolytic activity of the rabbit enzyme for CPF-oxon. In humans, a substrate-dependent polymorphism of serum paraoxonase is observed, where one isoform of paraoxonase has a high and the other a low turnover for paraoxon. Both isoforms appear to hydrolyse CPF-oxon and phenylacetate at the same rate. Arginine at position 192 determines high paraoxonase activity, and glutamine at this position, low paraoxonase activity. In contrast, relative isoenzyme activity is reversed for the hydrolysis of diazoxon, soman and especially sarin with 192Glu determining high enzyme activity (Davies *et al,* 1996). It is interesting to note that the prevalence of the different isoforms can vary in people of different races. For example in the Japanese population the Arg192 allele is more common than in people of other races (Yamasaki *et al*, 1997).

A.1.2 Acute clinical effects in humans

The route of exposure (e.g. oral, dermal, inhalation) may markedly affect distribution and metabolism or may localise effects in the absence of significant systemic absorption (Gage, 1967; Hayes, 1982). Effects specific for the site of entry, however, are characteristic for those substances which act immediately and do not require metabolic activation. The exposure of an unprotected person to *vapour or aerosol* affects predominantly the eyes, nose and airways. The symptoms include miosis, dim or blurred vision, pain around the eye, nausea, headache, rhinorrhoea (running nose), and a feeling of tightness in the throat and the chest. *Dermal* exposure will cause local sweating, with occasional blanching and local muscular fasciculations at the site. The first systemic effects are usually GI, namely, nausea, vomiting and possibly diarrhoea, and occasionally generalised sweating and a feeling of uneasiness or weakness. The initial effects from *ingestion* of a sub-lethal amount of a ChE-inhibiting substance are nausea and vomiting and possibly diarrhoea. After a large, potentially lethal exposure by any route, the usual symptoms are loss of consciousness, seizure activity, apnoea,

flaccidity, copious secretion from the nose and mouth, and occasional involuntary micturition and defecation (Sidell, 1994). In general, the extent of exposure and AChE inhibition are correlated with the severity of signs and symptoms. Examples of various degrees of severity of effects are shown in Table 10:

Table 10: Examples of degrees of severity of OP poisoning [*]

Severity	Signs and Symptoms
Latent	no clinical manifestations; serum ChE activity 50-90% of normal
Mild	fatigue, headache, dizziness, numbness of extremities, nausea and vomiting, increased salivation and sweating, chest tightness, abdominal cramps or diarrhoea; serum ChE activity 20-50% of normal
Moderate	unable to walk, generalised weakness, difficulty in talking, fasciculations, and miosis in addition to above symptoms; serum ChE activity 10-20% of normal
Severe	unconsciousness, miosis and loss of pupillary light reflex, fasciculation, flaccid paralysis, secretions from the mouth and nose, moist rales, respiratory difficulty and cyanosis; serum ChE activity < 10% of normal

[*] Namba *et al,* 1971, mainly from experience with methyl parathion; note that serum ChE activity is an indicator of exposure but is not identical with AChE values in RBC or the nervous tissue.

These signs and symptoms can be attributed to AChE inhibition in the CNS or PNS or in peripheral organs and tissues. The various target tissues and the signs and symptoms related to local cholinergic stimulation as compiled by Sultatos (1994) are listed below in Table 11:

Table 11: Symptoms of ACh inhibition [**]

Location	Receptor	Target	Signs and symptoms
CNS	muscarinic (and nicotinic)	brain	hypothermia, lethargy, tremors, depression, convulsions, coma
PNS	nicotinic	heart and blood vessels	tachycardia, blood pressure increase
		skeletal muscle	fasciculations, ataxia, weakness, paralysis
	muscarinic	heart and blood vessels	bradycardia, blood pressure decrease
		lungs	excess secretions, bronchial constrictions
		bladder	incontinence
		GI tract	vomiting, diarrhoea
		exocrine glands	salivation, sweating, lacrimation
		eye	miosis, blurred vision

[**] Sultatos, 1994

The effects on the respiratory system are complex, involving increased secretions of the respiratory tract, constriction of airways, and muscle weakness. The most common cause of death in cases of acute OP poisoning is respiratory paralysis and consequent oxygen deficiency (Allsopp *et al,* 1995). Acute toxic effects, also called "cholinergic crisis" may lead to death within 24 hours in untreated cases. If respiratory paralysis due to cholinergic block is treated and the patient is protected from excessive secretion by atropine, complete recovery from such acute intoxication is the rule. Pralidoxime hydrochloride, if given promptly, may reverse the binding of the OP to the ester site, and convulsions will cease and consciousness return soon after its administration (Hopkins, 1975).

In addition to these acute cholinergic effects, inhibition of ChE may interfere with CNS functions such as attention, cognition, mood or sleep. Effects on mood as well as on cognitive functions have been investigated more thoroughly in animals and humans using cholinergic agonists and antagonists. Uniformly, these studies showed involvement of cholinergic mechanisms in the regulation of both mood and cognitive functions (Gitelman and Prohovnik, 1992; Hasselmo *et al*, 1992; Warburton and Rusted, 1993) (See also Section 3.4).

Grob *et al* (1947a,b,c) carried out studies on the effects of the potent anti-ChE compound DFP. DFP was administered daily intramuscularly (i.m.) to normal humans. The treatment regimens were about 1.5 mg for 3 to 5 days resulting in a decrease of ChE in plasma by 3-12 % and in RBC by about 30%. The authors observed EEG changes indicating an increased neural activity. The changes appeared following the daily administration of DFP and persisted for 8-42 days. The changes consisted of an increase in the amplitude, frequency and irregularity of rhythm and the intermittent appearance of abnormal waves "similar to those seen in patients with grand mal epilepsy". The symptoms, referable to the CNS, disappeared within 1-4 days, i.e. much faster than the EEG changes (Table 12).

Table 12: CNS symptoms following deliberate exposure to DFP *

Sleep	drowsiness, insomnia, excessive dreaming, nightmares
Psychology	headache, giddiness, mental confusion, emotional lability, increased tension, subjective tremulousness, visual hallucinations, increased libido
Neurology	jitteriness and restlessness, paresthesias, tremor, leg pains of sciatic distribution

* Grob et al, 1947

Effects on cognitive functions and EEG are also confirmed by more recent pharmacological studies. Holsboer-Trachsler *et al* (1993) tested a novel brain-selective AChE inhibitor Exelon (SDZ ENA-713),

a carbamate drug under development for the treatment of dementia of the Alzheimer type, and found significant increase in the density of REM sleep in young male volunteers. The authors also quoted a number of other studies showing that cholinergic agents facilitated REM sleep in man, possibly acting via muscarinic receptors of the M2 type. In view of this pharmacological evidence the possibility that anti-ChE effects of pesticides could have some, albeit reversible influence on the sleep in humans appears quite plausible. Anand *et al* (1996) published a review of human studies with Exelon, including two placebo-controlled studies in 516 patients and a safety/tolerability study in 50 patients. Results suggested that 6-12 mg/day were likely to prove efficacious (for treatment of Alzheimer's symptoms) and were well tolerated.

Epidemiological Studies

Whorton and Obrinsky (1983) described a 4-month follow-up of farm workers acutely poisoned by a combination of mevinphos (Phosdrin) and phosphamidon (Dimecron) in California. The workers had mild-moderate acute symptoms and suppressed ChE activity. Plasma ChE and erythrocyte activity ranged respectively from 10-60% and from 40-85% of normal. Two to 3 months were required for recovery from major symptoms and return to normal ChE levels. In the majority of patients eye complaints such as blurred vision, discomfort while reading and photophobia still persisted at 4 months. Similar slow recovery of visual function was reported by Rengstorff (1994) who described the cases of two men accidentally exposed to sarin vapour. One presented with ChE activity in RBC of 19% and the other of 84% of the normal value. Both men had pronounced miosis which recovered only after 30-45 days; their recovery to normal ChE activity was gradual over a 90-day period. These reports demonstrate protracted effects of acute exposure.

Richter *et al* (1992) summarised their findings on effects of OPps in selected occupational and community groups in Israel. The worker groups were pilots, ground-crews and field workers; the exposed non-workers were adults and children living in kibbutzim with drift exposures and household residents in houses treated by exterminators. In all groups, there was evidence of exposure-associated illness even though "persons with acute poisoning were not seen." It is not clear what the authors intended to convey in this statement, since symptoms characteristic of acute exposure were reported, namely, headache, dizziness, fatigue, nausea, breathing problems, abdominal cramps, and tingling in extremities. These were associated with "within normal" depressions in ChE activity (maximum 13% in whole blood of kibbutz field workers and residents close to sprayed fields) although this could be an under-estimate if spraying and ChE measurements were not synchronised. Tests for vigilance, sensorimotor functions, memory span and cognitive performance conducted in 90 kibbutz workers and residents during and after the spraying season revealed a slight impairment during exposure. Similarly, assessment of "Profile of Mood Status" indicated increased feelings of anger, depression and fatigue during exposure.

Ciesielski *et al* (1994) carried out a study on ChE inhibition and symptoms in North Carolina migrant farm workers exposed to ChE-inhibiting pesticides. The control group consisted of “non-farm workers.” About half of the workers were acutely exposed to pesticide spray while working in fields. In exposed farm workers RBC ChE levels were significantly lower than in the control group. Symptoms, representing predominantly acute effects of exposure, included diarrhoea, nausea, rash, irritated eyes, fever, increased sweating, increased anxiety, dizziness, headache, blurred vision, muscular symptoms, chest pain, sialorrhoea, difficulty breathing, ataxia, and memory loss.

In a terrorist attack in 1995 on a Tokyo subway, a toxic gaseous substance, later identified as a diluted form of the chemical warfare agent sarin, resulted in the death of 11 commuters and in more than 5,000 persons requiring emergency medical evaluation. Okumura *et al* (1996) described 640 of the victims who were evaluated or treated at St. Luke's International Hospital. The patient's condition, based on signs and symptoms, was classified as mild, moderate or severe. The 528 "mild" cases had only ocular sign or symptoms such as miosis, eye pain, dim vision or decreased visual acuity. One hundred and seven cases were characterised as "moderate" by systemic signs and symptoms such as weakness, difficulty in breathing, fasciculations and convulsions but did not require specifically mechanical ventilation. Among the 5 "severe" cases one died before admission and the remaining 4 were intubated and ventilated. Of the latter, one sustained severe hypoxic brain damage and died in hospital 28 days later. The "severe" cases had prominent inhibition of RBC ChE with values < 20 IU/l (international units/ litre) compared to a lower limit of normal values in the examining laboratory of 100 IU/l. This enzyme inhibition persisted for 51, 59 and 72 days respectively in the 3 surviving "severe" cases. In 37 patients acute stress disorder was diagnosed and an anti-depressant prescribed following admission. During a 3-month follow-up period, post-traumatic stress disorder developed in 4 patients. At the time of publication the 638 surviving patients were classified as "full recoveries". However the authors indicated that patients of the "severe" and "moderate" categories would require surveillance, possible for years, to determine the presence or extent of possible adverse, long-term health effects.

Baker and Sedgwick (1996) studied electromyographic changes in 8 healthy volunteers deliberately exposed to low levels of sarin. Following exposure the subjects displayed miosis and some of them photophobia and dyspnea; all symptoms resolved within 48 hours. The activity of RBC AChE was about 58% at 3 hours and 61% at 3 days after the exposure. There were no neuromuscular signs or symptoms on clinical examination. However, single fibre electromyography detected abnormal findings beginning at 3 hours and persisting for 4-15 months.

A.1.3 Pathology produced by acute intoxication

Acute toxic effects of OPs result from an inhibition of AChE. This inhibition is reversible, so that even the victims of sub-lethal or lethal exposure are expected to recover fully when treatment is timely. It is therefore surprising to observe that in experimental work, partly sponsored in relation to chemical weapons, it was claimed that potent ChE inhibitors caused persistent brain lesions and damage to heart, skeletal muscles and some exocrine glands. Because of the similarity of mechanism of action of these compounds to that of OPps, these results cannot be ignored.

De Groot (1988) observed brain damage in rats intoxicated with a near LD_{50} dose of soman. The lesions were largely the result of soman-induced convulsions, but some electron microscopic findings were considered to reflect a direct soman effect. Goldman *et al* (1993) exposed rats to convulsant and sub-convulsant levels of soman and produced evidence for marked neuropathology even in the absence of electrographic seizures, clinically-manifest convulsions or elevated blood pressure. Although the degree of pathology was greater when convulsive doses of soman were administered, seizures were not necessary for significant soman-induced pathology. Baze (1993) reviewed available published and unpublished technical reports on soman-induced morphological changes in the non-human primates. The acute lesions were characterised by neuronal degeneration and necrosis and oedema of neuropil (i.e. swelling of interneuronal areas). They were usually present in the frontal cortex, entorhinal cortex, amygdaloid complex, caudate nucleus, thalamus and hippocampus, and resembled in appearance and location the lesions produced by hypoxic-ischemic injury or by seizures. Nerve agent therapy (pre-treatment with pyridostygmine and treatment with pralidoxine chloride and atropine) supplemented with anticonvulsant reduced or prevented soman-induced acute neural lesions. Acute changes in non-neural tissues were limited to the heart and skeletal muscles and were mainly necrotic in character. Heart lesions were similar to those in OP-intoxicated people. Seizures appeared to be the primary cause of soman-induced lesions. Tryphonas and Clement (1995) studied soman-induced lesions in the brain and heart of rats and suggested that brain lesions were associated with protracted seizure activity and the heart damage could be neurogenic. Clement and Broxup (1993) demonstrated that soman-induced convulsions and necrotic brain lesions in rats could be prevented by administration of diazepam given 10 minutes before soman.

Kadar *et al* (1995) administered sarin, a highly toxic ChE inhibitor, at a near LD_{50} dose to rats and, in most of the surviving animals observed brain lesions similar to those produced by soman, but with a slightly different distribution. The authors suggested that the development of lesions was related to persistent seizures triggered by the elevation of ACh, and the differences in lesion distribution between soman and sarin could be due to different functional effects of these agents, possibly differentially affecting AChE.

Occurrence of necrotic changes in the brains of individuals afflicted by severe respiratory or cardiovascular dysfunction represents a treatment-related albeit secondary event, but does not provide evidence that functionally-toxic chemicals are neuropathic. Krinke *et al* (1978) demonstrated development of necrotic brain lesions in animals treated with non-neuropathic chemicals. Convulsions were induced in mice by administration of amphetamine and necrotic brain lesions were found in a mouse that died after 5 days. In the same study, repeated intravenous administration in dogs of atropine and histamine resulted in only moderate convulsive episodes but profound respiratory distress and necrotic brain lesions. In the context of a possible association between acute lesions and seizures, it is of interest to note that a coincidence of necrotic lesions of the brain and heart was observed many years ago in people affected by epileptic convulsions. It was considered to reflect a disproportion between the metabolic demand of the tissue and the metabolic supply available (Scholz, 1951). Other studies have described a "brain-heart syndrome" and have shown that head trauma, CNS infections, degenerative diseases and convulsions are often associated with heart lesions (Baze, 1993).

Molecular mechanisms explaining how acute functional effects and accumulation of ACh could result in tissue damage are not fully understood. De Groot *et al* (1990) suggested that accumulated ACh potentiates glutamate-induced neuron degeneration, most likely by lowering the threshold for glutamate excitation at the n-methyl-d-aspartate (NMDA)-receptor site. The activation of the NMDA-ionic channels would then lead to massive Ca^{2+} fluxes into the post-synaptic cell, causing cell degeneration. Naarala *et al* (1995) demonstrated production of reactive oxygen metabolites and oxidative stress in neural cells stimulated by the muscarinic receptor agonist carbachol. Although these findings indicate the possibility of a direct effect of the accumulated ACh on the tissues in which lesions are found, the simple and obvious medical experience, that tissues with high physiological metabolic demand will undergo necrosis when this demand cannot be satisfied owing to circulatory and respiratory dysfunction, should not be overlooked. Direct functional effects of OPs on the circulation and respiration are well known.

Exaggerated cholinergic stimulation can produce structural changes in effector tissues such as the muscle fibres or the glands. Comment on skeletal muscle necrosis is included in Section A.2 Intermediate Syndrome. Among the exocrine glands, the pancreas appears to be especially susceptible. For instance, changes interpreted as "acute oedematous pancreatitis" were observed in experimental animals as early as 2 hours after treatment with a sub-lethal dose of diazinon. They were characterised by vacuolation of the acinar cells, interstitial oedema and vasculitis (Frick *et al*, 1987). Vacuolation of secretory cells is a non-specific functional response related to increased secretory activity; it has been observed in exocrine glands such as the lacrimal gland, the salivary gland or the exocrine pancreas following stimulation (Krinke *et al,* 1996).

A.1.4 Concluding remarks on acute effects

Acute toxic effects of OPs are attributed to their anti-ChE activity leading to accumulation of released ACh and exaggerated functional response ("cholinergic crisis"). Pathological changes may occur in the brain, heart, skeletal muscles and the exocrine glands. These probably represent indirect sequelae of functional toxicity, although other mechanisms have been proposed, such as: 1) accumulated ACh may initiate excitotoxic or oxidative tissue damage, 2) inhibition of AChE can disturb functions unrelated to cholinergic neurotransmission and 3) selected OPs may act directly on the post-synaptic receptors. Even if pathology produced by acute exposure is indirect and non-specific, it can persist causing sub-acute and chronic symptoms, since, in general, damage to CNS is irreversible.

OPps are potentially acutely toxic if not used as intended and according to the manufacturers' safety instructions. Regrettably these warnings are not always heeded; the majority of occupational poisonings result from failure to follow recommended precautions.

A.2 INTERMEDIATE SYNDROME

A.2.1 Features and mechanisms of the intermediate syndrome

The term "intermediate syndrome" was introduced by Senanayake and Karalliedde (1987) who observed paralysis and/or respiratory depression in their patients 24-96 hours after severe poisoning with OPs. "Intermediate" pertains to the time period of onset which is after the first 24 hours of exposure, but earlier than OPIDN. A similar condition, characterised by onset of paralysis and/or respiratory depression 24 hours after exposure, was previously referred to by Wadia *et al* (1974) as "Type II or late onset paralysis".

The syndrome is invariably preceded by acute cholinergic symptoms, and manifested by respiratory paresis, weakness of facial, palatal, external ocular, nuchal and proximal limb muscles, and depressed tendon reflexes. There is severe ChE inhibition and electromyography indicates an impairment in neuromuscular transmission. Muscarinic symptoms are not a feature of the intermediate syndrome. Clinical recovery occurs after several days or weeks.

According to Karalliede and Henry (1993), the initial neuromuscular effect of the OPs during the acute cholinergic phase consists of a prolonged depolarisation of the motor end plate, the so-called depolarisation block. This effect is reversible following dispersal of the OP and paralysis tends to last for minutes rather than hours or days. A similar paralysis of short duration is encountered with reversible inhibitors of AChE, such as the carbamates, neostigmine and physostigmine. The excessive ACh at the motor nerve endings stimulates nicotinic receptors and evokes antidromic action

potentials resulting in fasciculation, i.e. synchronous contraction of muscle fibres within a motor unit. The effects observed in the intermediate syndrome have been associated with muscle necrosis due to a prolonged transmitter-receptor interaction. Since muscle necrosis can be prevented in animal experiments by tubocurarine or α-bungarotoxin, a direct necrotising effect of OPs or ACh on muscle fibres is improbable. De Bleecker (1995) demonstrated that muscle necrosis could be induced in rats both with paraoxon, which does not induce intermediate syndrome, and with fenthion, which does. These observations, supported by results of electromyography, indicated that a combined pre- and post-synaptic impairment of neuromuscular transmission was the cause of the intermediate syndrome, rather than muscle necrosis. Moreover, Senanayake and Sanmuganathan (1995) added a new dimension to intermediate syndrome by identifying signs of extrapyramidal dysfunction. They observed tremor, cog-wheel rigidity, chorea and dystonia in 6 patients intoxicated with fenthion. These extrapyramidal signs were preceded by cholinergic crisis followed by other signs of intermediate syndrome; they disappeared spontaneously in about 1-4 weeks. If such extrapyramidal signs represent a feature of the intermediate syndrome, then the syndrome must be attributable to impaired neurotransmission rather than to muscle necrosis. On the other hand, the importance of ChE inhibition and abnormal neurotransmission in the pathogenesis of muscle necrosis is beyond doubt. For instance, a single administration of paraoxon to rats causes prolonged muscle fasciculations after 1-2 hours and produces a grouped skeletal muscle fibre necrosis 24 hours later. Wecker and Dettbarn (1977) demonstrated that muscle necrosis in rats experimentally produced with paraoxon could be prevented by denervation prior to paraoxon administration. Cholinesterase present in the denervated muscle was less susceptible to inhibition by paraoxon and its recovery was improved by prolonged denervation. Subsequently, Wecker *et al* (1978) demonstrated that muscle necrosis is initiated by AChE inhibition, the extent of necrosis depending on duration and degree of inhibition. They also produced muscle necrosis by multiple injections of reversible ChE inhibitors, physostigmine and neostigmine.

More recently Sedgwick and Senanayake (1997) suggested that down-regulation of ACh receptors could explain the intermediate syndrome and neurophysiological findings. They hypothesised that the heavily-activated receptors could become desensitised and be rapidly removed, to become restored after 5-18 days.

Originally the intermediate syndrome was associated with exposure to dimethoate, methamidophos, malathion, monocrotophos or fenthion. Subsequently it was also observed with parathion combined with methyl parathion. Although it is most likely to occur with these OPs, it is not confined to them and may be expected to occur in any instance of prolonged extensive AChE inhibition. The risk of being affected by the intermediate syndrome is increased by exposure to OPs with high lipid solubility and by

impairment of vital systemic functions (cardiovascular, hepatic, renal) that may prolong metabolism and elimination of OPs (De Bleecker, 1995; De Bleecker *et al,* 1992a,b).

A.2.2 Concluding remarks on the intermediate syndrome

Although the intermediate syndrome is more likely to occur with certain OPs than with others, it is not directly agent-specific. It is a dysfunction in neurotransmission associated with prolonged extensive AChE inhibition. The occurrence of intermediate syndrome has always been preceded by cholinergic crisis. There is no evidence that apparently asymptomatic, chronic low-level exposure could lead to a development of the intermediate syndrome. Although animal experiments into the pathogenesis of the intermediate syndrome have a high scientific value, there is no obvious need for special animal safety studies for the detection of a chemical's potential to induce such a syndrome. Experience with the intermediate syndrome is limited, but it appears possible that, in the case of new compounds, the potential can be predicted from the standard set of data normally available for substances of these classes, such as lipid solubility and the dose-related extent and duration of AChE inhibition.

A.3 DELAYED NEUROPATHY

A.3.1 What is OPIDN ?

Some, but not all, OPs can induce a neurodegenerative disorder known as "Organophosphorus-compound induced delayed neuropathy" (OPIDN), which is unrelated to cholinergic effects and occurs about 2-3 weeks after the exposure to a neuropathic dose. Long sensory and motor fibres in the peripheral nerves and spinal cord undergo degeneration similar to that occurring after an interruption of the axon. The clinical signs include tingling, weakness and ataxia in the lower limbs which can progress to paralysis and in severe cases may involve the upper limbs as well. Since multiple nerves may be affected, the term "polyneuropathy" (OPIDP) is sometimes used to make a distinction from conditions affecting single nerves and causing a "mononeuropathy". In this report the term OPIDN is used.

Early experience with OPIDN was obtained with TOCP, a compound of low acute toxicity originally used as a plasticiser and flame retardant. Through adulteration of a beverage it was consumed by thousands of human victims who developed so called "ginger paralysis". The original studies of TOCP neuropathy in man and animals were focused on the evidence of a causal relationship between exposure and occurrence of the lesions (Smith and Lillie, 1931). Little attention was paid to differential susceptibility of various areas or structural elements of the nervous system. Such original reports gave rise to the erroneous belief that TOCP neuropathy was essentially myelin damage affecting the motor neurons. The first comprehensive characterisation of the pathologic features of this neuropathy

in animal models was provided by Cavanagh (1954, 1963) and Beresford and Glees (1963). Their findings demonstrated that myelin damage was a sequel to initial axonal damage and the ascending sensory long spinal tracts were affected more severely and earlier than the descending motor spinal tracts. These studies established TOCP as the “positive control” for evidence of sensitivity of animal experiments. The data compiled by Davis and Richardson (1980) and referred to in Table 13 demonstrate the levels and routes of single administration of TOCP leading to the induction or failure to induce OPIDN in various animal species. The data indicate that the hen is more susceptible than other species, the adult hen more susceptible than the young chick, and parenteral administration more effective than oral administration. Thus oral administration to animals other than hens may yield false negative results.

Table 13: Species sensitivity to TOCP following a single exposure *

Species	OPIDN		Route of administration
	induced	not induced	
Chick (10 - 50 d)		1 g/kg	oral
Chick (72, 100 d)	1 g/kg		oral
Hen	0.5-1 ml/kg		oral
Rat		2-10 g/kg	oral, s.c.
Rabbit		0.05-0.1 g/kg	oral
Guinea pig		0.1 g/kg	oral
Cat	0.5-0.75 ml/kg		i.m.
Dog		0.4-4 ml/kg	oral
Dog	0.4-1.6 ml/kg		s.c.
Calf	0.2 g/kg		oral, i.m.
Rhesus monkey	0.5-1 ml/kg		s.c.
Rhesus monkey		3-15 ml/kg	oral

* Davis and Richardson, 1980

Glees and White (1961) demonstrated that OPIDN can be induced in hens by painting 0.1-0.2 ml/kg TOCP on their comb skin. Initial lesions were observed after a single dose of 0.05 ml/kg suggesting

that repeated exposure to such low levels might result in toxicity. This model was adapted by Abou-Donia and Graham (1978) for testing delayed neurotoxicity from long-term, low-level topical exposure of hens to leptophos. Percutaneous administration of 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 or 20 mg/kg for 183-323 days resulted in neuropathy in all hens given 0.5-20 mg/kg/day. The "latent period" before onset of neuropathy, and its severity were dose-related. In contrast, a single oral dose of 200-800 mg/kg was needed to induce OPIDN in hens (Abou-Donia and Preissig, 1976b). Abou-Donia *et al* (1980) also produced delayed neuropathy in hens by sub-chronic oral treatment with 10 mg/kg/day TOCP, a daily dose much lower than that required to induce OPIDN by a single exposure. Thus repeated moderate-level exposure to neuropathic OPs results in a neuropathy similar to that which can be induced with a single, high-level exposure. Long-term exposure is capable of inducing neuropathy even in species, such as the rat, which is resistant to single administration. Veronesi (1984) observed neuropathy in rats given TOCP in oral doses of either 1160 mg/kg intermittently every 2 weeks, or 116 mg/kg for 5 days/week for 24 weeks. Remarkably, despite the presence of microscopic lesions in rats exposed to the lower dose of 116 mg/kg, the functional abnormalities at this dose level were not very prominent.

Neuropathic effects different from OPIDN have been reported, but these probably represent secondary changes similar to those discussed, with pathology produced by acute intoxication. Veronesi *et al* (1990) treated young rats (2 months) and adult rats (12 months initially) dermally with 25 mg/kg fenthion 3 times/week for up to 10 months. Gliosis, neuronal necrosis and loss in the hippocampus, were observed after 2 months' exposure. Aged animals showed more extensive histopathology. However, no data were presented on the areas susceptible to OPIDN. A similar, probably ischemic nerve cell injury in the hippocampus and some other brain areas, was induced in rats by convulsive doses of malaoxon. Aged rats were more susceptible, a dose of 8.7 mg/kg i.p. causing convulsions, whereas in young rats a higher dose of 39.2 mg/kg i.p. was needed to induce such an effect (Hirvonen *et al,* 1993).

A.3.2 Are OPIDN Type I and Type II two different types of neurotoxicity ?

Several compounds have been found which induce neurotoxicity similar to that of TOCP, for instance, DFP, mipafox, or leptophos. Other compounds, triaryl phosphites, such as triphenyl phosphite (TPP) and tri-ortho, -meta, or -paracresyl phosphite have been reported to induce a neuropathy somewhat different from the TOCP-induced lesion. The terms OPIDN "Type I" and "Type II" were introduced for TOCP- and TPP-like effects respectively to discriminate between these two apparently different conditions. Investigations into the pattern of distribution of TPP-induced axonal degeneration in various animal species indicate the involvement not only of the peripheral nerves and spinal cord tracts, but also of multiple mid- and fore-brain areas including the cerebral cortex in those species where it is present (Tanaka *et al,* 1992; Lehning *et al,* 1996). Although obtained with the capricious and artefact-prone Fink-Heimer silver impregnation technique, these findings give rise to concerns

about the possibility of producing similar lesions in humans. Following the reports that neurotoxicity induced in cats and rats with TPP differed from Type I OPIDN, Carrington *et al* (1988) studied the effects of TPP in hens. Dual effects were observed, namely axonal damage characteristic of Type I OPIDN and additional neuronal damage characterised by neuronal chromatolysis and necrosis in the spinal cord. More recently, Fioroni *et al* (1995) revisited this question and concluded that TPP neuropathy in the hen was essentially the same as other OPIDNs, and that the differences in histopathology might simply represent more advanced lesions. Other authors, however, consider TPP neuropathy as a separate, novel category of OP-induced neurotoxicity, manifested by characteristic symptoms and lesions (Lehning *et al,* 1996). With respect to reliability of safety studies in hens it is reassuring to know that the most sensitive areas of the nervous system, the cerebellar lobules IV and Va, are susceptible to agents inducing both types of OPIDN and the lesions affecting any other area of the nervous system appear to coincide with the cerebellar lesions (Krinke *et al,* 1997). Therefore, when appropriately conducted, the established animal models for OPIDN are capable of predicting with sufficient probability all the neuropathic potential of the OPs.

A.3.3 Molecular mechanisms of OPIDN

Molecular mechanisms instrumental in the development of OP-induced delayed neurotoxicity have not been completely elucidated. The propensity to induce OPIDN is associated with the inhibition of an enzyme with an unknown physiological role, named NTE. According to the investigations carried out by Johnson (for review see Johnson, 1982 and Sultatos, 1994) NTE, to induce OPIDN, must be phosphorylated on a hydroxyl group and then an alkyl side-chain removed from the phosphate moiety completely in the process of “ageing”. It has also been suggested that the removed alkyl group binds covalently to an adjacent “Z” site, and that this alkylation of the Z site might lead to OPIDN. Inhibition and ageing of at least 70-80% NTE activity is predictive of the development of OPIDN. It has been suggested that although all NTE inhibitors are potentially neuropathic, some are potent while others are weak. Moreover, it has been shown that the non-ageing, non-neuropathic (i.e. substance itself is unable to induce a neuropathy) NTE inhibitors, namely phenylmethanesulfonyl fluoride (PMSF), phenyl N-methyl N-benzyl carbamate and phenyl di-n-pentyl phosphinate, protect against OPIDN when administered before, but promote OPIDN when administered after, other neuropathic (capable of inducing a neuropathy) OPs. While protection may be attributed to an interaction of these agents with NTE, promotion probably results from the interaction of promoters with a molecular target other than NTE. This view is compatible with the finding that O-(2-chloro-2,3,3-trifluorocyclobutyl) O-ethyl S-propyl phosphothioate (KBR-2822) administered to hens in doses which do not inhibit NTE, promoted the OPIDN induced with neuropathic agents (Moretto *et al,* 1994; Sultatos, 1994; Peraica *et al,* 1995).
The early neuropathic changes occur in the axons of the affected neurons and consist of the aggregations of phosphorylated neurofilaments, indicating that the aberrant phosphorylation of

cytoskeletal elements is instrumental in the production of OPIDN (Jensen *et al,* 1992). Studies in hens treated with TOCP or with DFP provide evidence of an increased phosphorylation of cytoskeletal protein such as neurofilament proteins, tubulin, microtubule associated protein-2 (MAP-2), myelin basic protein, and autophosphorylation of calcium/calmodulin-dependent protein kinase II (CaM-kinase II) in the brain, spinal cord and sciatic nerve. The aberrant phosphorylation of axonal neurofilaments appears to be associated with an increased CaM-kinase II activity (Gupta and Abou-Donia, 1995). It is unclear whether inhibition of NTE is causally related to aberrant cytoskeletal phosphorylation, and if so, by which mechanisms this occurs. The normal physiological function of NTE is still subject to investigation. Recent advances in this field include: partial purification and sequencing; availability of a monoclonal antibody to chicken brain sub-unit and of polyclonal antibody to synthetic peptides; and screening of cDNA libraries with synthetic oligonucleotides, polymerase chain reaction-developed primers and with antibodies, to obtain cloned NTE which could be applicable to study the function of normal and OP-modified NTE in cultured neural cells (Johnson and Glynn, 1995).

A large number of OPs were tested at single doses in the hen for their potential to induce OPIDN, and many were positive (Lotti, 1992). Although it has been shown that the neuropathic propensity of such OPs is related to their potential to react with NTE rather than their potential to inhibit AChE, acute and sub-acute cholinergic effects of variable degree are also features of intoxication with these agents. Therefore, dual or manifold types of neurotoxicity are attributed to particular agents. When massive ChE inhibition is expected in animal experiments, protection against acute cholinergic effects by agents such as atropine and oxime reactivators is necessary to assure survival after treatment at high levels. With insecticides, the relationship between AChE and NTE inhibition has been called the “therapeutic index” (Kaplan *et al*, 1993), but “esterase inhibition index” is considered more appropriate. For example, this index is low for TOCP, a weak AChE inhibitor, while the index is high for CPF, a potent AChE inhibitor. Currently-used OPps have a high esterase inhibition index and, therefore, it is most unlikely that the threshold of NTE inhibition could be exceeded and OPIDN produced without preceding massive cholinergic effects; however, cholinergic desensitisation resulting from prolonged ChE inhibition reduces the extent of clinical signs, which would then be less prominent.

With respect to the alleged difference between Type I and Type II OPIDN it is of interest that the inhibition of NTE occurs in both situations. In hens both types of neuropathy are associated with an inhibition of about 70% (65-70% in Type I, 70% in Type II). In comparison to hens, rats are less sensitive to Type I than to Type II; NTE inhibition associated with neuropathy amounts to 65-70% with Type I agents but only to 39% with Type II agents. In most species the onset of clinical signs is earlier with Type II than Type I agents (Abou-Donia, 1992), indicating that Type II OPs may indeed be more potent than Type I.

Research into the neurotoxic mechanisms of OPs must include a consideration of the effects of their metabolites. For example, TOCP is a protoxicant, and its metabolically-activated neurotoxic form is phenyl saligenin phosphate (PSP) (Jortner and Ehrich, 1987). Those OPs that contain a sulphur attached to the phosphorus atom by a double bond (thiophosphoryl group) have little or no capacity to inhibit AChE; they undergo metabolic activation to their corresponding oxygen analogues, or oxons, which are potent ChE inhibitors (Sultatos, 1994). Abou-Donia *et al* (1979) reported that DEF administered orally to hens was metabolised to n-butyl mercaptan which apparently caused "late acute toxic effects" 4 days after administration. In contrast, topically-administered DEF, which was not subjected to GI tract hydrolysis, caused delayed neurotoxicity but did not produce late acute effects. Using the example of leptophos Abou-Donia *et al* (1980) demonstrated significant differences in the toxicity of the "pure" as compared to the "technical grade" chemical and its "degradation" products.

A.3.4 OPIDN in man

Most human cases of OPIDN have been due to the inadvertent, accidental ingestion of neuropathic OPs or to suicide attempts with such agents. The symptoms described represent a combination of severe acute, probably cholinergic, signs and subsequent delayed neuropathy. Shortly after ingestion of the poison, there may be some GI distress with nausea, vomiting, and diarrhoea, lasting a few hours to a few days. A latent period of 8-18 days generally follows, depending on the level and duration of exposure. Most patients then complain about sharp, cramp-like pain in the calves, and numbness and tingling in the feet and perhaps the hands. This is followed by increasing weakness of the lower limbs, loss of reflexes and inability to balance. One or 2 weeks after the onset of paralysis in the lower limbs, the patient may experience weakness in the hands (Davis and Richardson 1980).

In most victims of the "ginger paralysis," which occurred in the USA in 1930, symptoms progressed to quadripareses and often frank quadriplegia with foot and wrist drop. Examination usually showed muscular weakness and wasting, with flaccidity and hypoactive reflexes most noticeable in the feet and legs. In most cases, no sensory deficit was manifest. In a few patients there was impairment of sphincter control and some men were sexually impotent for a time after the illness. During subsequent years, the flaccidity and muscle weakness were replaced by hypertonicity, hyper-reflexia, clonus, and abnormal reflexes, reflecting apparent persisting damage to pyramidal tracts and a permanent upper motor neuron syndrome. Spasticity replaced flaccidity, and scissoring replaced or accompanied steppage. Eleven patients observed 47 years after the episode still showed spasticity and abnormal reflexes of an upper motor neuron syndrome. Although this chemically-induced neuropathy appeared to be restricted to the peripheral nerves and spinal cord, 47 years after exposure when the observed 11 victims were 64-81 years old, there was some evidence of frontal release signs and mild dementia. This was attributed to advanced age and cerebrovascular sclerosis, although the

possibility of TOCP effects on cerebral function was not dismissed entirely (Morgan and Penovich, 1978).

In Romania, Vasilescu and Florescu (1980) examined 12 patients with neuropathy following accidental ingestion of alcohol polluted by TOCP. Depending on the amount of TOCP ingested, patients developed flaccid paralysis, predominantly of the lower limbs 15-30 days later. Two to three months after ingestion the symptoms of peripheral neuropathy were complicated by pyramidal signs (pathological reflexes indicating damage of corticospinal nerve fibres). After 1-2 years there was recovery of peripheral neuropathy but progression of the pyramidal signs. Thermal, tactile and painful hypoaesthesia was present distally in the hands and particularly the feet of patients with predominantly peripheral neuropathy, while in patients with predominantly pyramidal signs the sensibility was not disturbed. Re-examination of 2 patients after 13 years showed tendinous retraction of toes, fingers and knees and difficult and spastic gait indicating persistent upper motor neuron lesions. Electrophysiology of the peripheral nerves showed an improvement of motor conduction velocity, however, with values still below the normal control values.

In Sri Lanka, Senanayake (1981) reported clinical and neurophysiological findings in 20 Tamil girls who had received contaminated gingili (sesame) oil. The toxic contaminant was an isomeric mixture of tricresyl phosphate containing uncertain amount of TOCP. The patients repeatedly ingested small doses over a 2-week period; the total amount of the toxic contaminant was estimated as 2.8-5.6 g. In the following 14-30 days a neuropathy developed, beginning with an intense pain in the calf muscles, followed by limb weakness, first affecting the lower and then the upper limbs. The patients had difficulty in walking and using their hands. Flaccid weakness of the distal limb muscles was associated with loss of ankle jerks in most patients and exaggerated knee jerks in some. Electrophysiology including electromyography and nerve conduction monitoring indicated abnormal motor function with an improvement after a 3-year period. Sensory changes occurred only in a few patients and sensory conduction studies after 3 years produced normal values. In the more advanced stage the pyramidal tract signs became more apparent. These were a mild spasticity of lower limbs and an exaggeration of knee jerks. None of the patients complained of GI symptoms, sphincter disturbances or visual abnormalities.

Tosi *et al* (1994) examined 7 of 41 people, who 50 years previously had been affected by a strange epidemic paralysis while working or living on a farm near Verona, Italy. The most severe cases had a spastic paraplegia and lower leg muscle atrophy without sensory impairment, a clinical syndrome characteristic of late-stage OPIDN. The epidemic paralysis was probably due to the use of recycled tins and drums contaminated with residual engine oil containing TOCP. The animals at this farm became ill at the same time. Chickens were most evidently affected, but horses, pigs and cattle were also involved; rabbits and a dog were unaffected.

Tosi *et al* (1994) compiled a list of the major outbreaks of TOCP neuropathy that had occurred since the end of the previous century (Table 14):

Table 14: Major outbreaks of TOCP poisoning in man *

Years	Place	No of cases	TOCP vehicles
1898-1900	France	6	phospho-creosote
1900-28	Europe	43	phospho-creosote
1930-31	USA	30-50000	adulterated beverage
1930-35	Europe	hundreds	apiol (abortifacient)
1938	Durban	68	cooking oil
1940	Basle	80	cooking oil
1940-46	Germany	hundreds	cooking oil
1942-43	Verona	41	ground contamination ?
1945	Liverpool	17	cooking oil
1955	Durban	11	contaminated water
1956	Japan	6	cooking oil
1959	Morocco	10000	cooking oil
1960	Bombay	58	cooking oil
1962	West Bengal	400	contaminated flour
1966	Romania	12	adulterated beverage
1967	Fiji	56	contaminated flour
1971-2	Vietnam	15-20	cooking oil
1977-8	Sri Lanka	20	sesame oil

* Tosi *et al,* 1994

A more recent retrospective study examined the vibrotactile thresholds in 3 groups of subjects, namely, previously poisoned with OPs (15 subjects), poisoned with methamidophos (21) and matched controls (35) (McConnel *et al,* 1994). The conclusion was that since methamidophos is known to

cause OPIDN both in man and hens and over a quarter of the subjects previously poisoned with methamidophos had abnormal vibrotactile thresholds, then classical OPIDN was only the worst disease caused by methamidophos in a spectrum of PNS impairment which represented the sequelae of poisoning. Subjects were examined 1-3 years after the episode. Since substantial recovery of peripheral nerve function is expected over such a period it might be assumed that some subjects previously displayed frank clinical neuropathy. However such an event was not reported. In addition the toxicological and clinical assessments of the poisoning episodes were not reported. The data indicate that in some tests (fingers) there was a difference in the incidence of abnormal vibration threshold between control and OP- or methamidophos-intoxicated groups. Moreover, given the difference between abnormalities in right and left toes, causes other than toxicological ones should be sought.

The level of exposure is difficult to estimate following the unintentional exposure of humans to TOCP or other OPIDN-inducers. Lotti (1992) compiled reports on OPIDN in man, in which a variety of agents was implicated. Apart from food contamination and occupational exposure, attempted suicide was the most frequent reason for exposure. The agents were CPF, dichlorvos, EPN, leptophos, methamidophos, mipafox, omethoate, parathion, TOCP, trichlorfon and trichlornat. In most cases, toxicological evidence of exposure and other toxicological data were scarce. In an intoxicated individual, estimated to have ingested 300 mg/kg CPF, blood peak concentration of 0.7 µmol/l and high inhibition of lymphocytic NTE were observed. (Lymphocytic NTE is used for monitoring in humans where no fresh nervous tissue can be obtained). Methamidophos occurred in plasma in concentration of 5.9 µg/ml and high inhibition of lymphocytic NTE was detected. Fukuhara *et al* (1977) observed delayed neuropathy in a farmer who accidentally ingested 7 ml of 50% solution of trichlorfon.

A delayed neuropathy restricted to recurrent laryngeal nerve has been reported in 3 patients who ingested insecticides with suicidal intent. They developed symptoms of laryngeal dysfunction after 25-35 days and recovered after 4-15 weeks. The compounds incriminated were CPF, parathion, and methamidophos; neither the levels of exposure, nor the extent of NTE inhibition were known (de Silva *et al,* 1994).

According to He (1996) 218 cases of OPIDN were reported in Chinese medical journals between 1960 and 1990. These were mainly induced by exposure to methamidophos (134 cases following ingestion and 11 cases of occupational poisoning) and suicide attempts with dichlorvos (35 cases), trichlorophon (15 cases), dimethoate (7 cases), isocarbophos (4 cases) and parathion (3 cases). According to He there were also two more recent outbreaks of OPIDN (unpublished information) in 1990 and 1995 involving about 200 patients and resulting from the leakage from a flour-milling machine of lubricant oil containing TOCP. No details of exposure levels or of NTE inhibition were provided.

A.3.5 OPIDN from prolonged, low-level exposure

Several reports indicate that prolonged, apparently asymptomatic exposure of humans can produce changes compatible with OPIDN.

In the USA, Namba *et al* (1971) described a chemist who had worked with OPs, sulfons and chlorinated hydrocarbons in a laboratory for 10 years. For 17 months before the examination he gradually developed progressive weakness of the limbs, wasting of the muscles, grouped atrophic muscle fibres, contraction of the fingers and forearms and foot drop. The disease was diagnosed as polyneuropathy probably due to OP poisoning. The authors reviewed literature and quoted several similar cases of OPIDN possibly resulting from a chronic exposure.

To study the effect of occupational OPp exposure in India, Misra *et al* (1988) carried out electrophysiological examination on workers engaged in the regular spraying of fenthion during an average period of 8.5 years (range 1-19 years) and compared the values with those of a group of hospital employees who were not directly exposed to pesticides. There was no clinical evidence of peripheral neuropathy or muscle weakness in the exposed subjects. After spraying, the workers reported minor symptoms such as headache, giddiness, ocular symptoms, and paresthesia. Serum AChE was significantly decreased. Electrophysiological parameters were not significantly different from controls immediately after the spraying, but in several subjects repetitive activity was present. After withdrawing the workers from exposure for 3 weeks, peroneal nerve motor conduction velocity, terminal motor latency of median and peroneal nerve, and minimal latency of F response and H reflex were significantly affected, and the repetitive activity was absent. The motor nerve conduction velocity increased and the terminal latencies decreased, indicating an improved performance after the 3-week exposure-free period. Mean ChE values also increased after the end of exposure, but were always within normal range. Although each individual was his own control, such intra-individual comparisons were not reported. Nevertheless, the observed changes were considered a potential indicator for sub-clinical neurotoxicity. Possibly the findings reflected changes in motor end-plates rather than OPIDN.

Otto *et al* (1990) reported on the examination of workers from an Egyptian pesticide formulation plant for signs of OPIDN. In this plant, OPs reported to induce OPIDN such as trichlorfon, leptophos, methamidofos and EPN, were formulated. Findings were compared with those of workers from a fertiliser or textile plant and workers re-evaluated one year later. Lymphocyte NTE was lower (20%) in pesticide formulators. The proportion of workers with abnormal neurological findings, for involuntary tremors and vibration sense only, varied between plants. Tactile thresholds in the fingers of the non-dominant hand were higher in the pesticide plant workers; toes were not tested. No changes were detected in the neurobehavioural tests. There are problems in the interpretation of the results in that

assessment of exposure is missing, the incidence of various diseases, including neurological ones, is particularly high (in both cases and controls) and, again, upper limb neuropathy is not expected in mild OPIDN.

Kaplan *et al* (1993) reported 8 people in USA who developed peripheral sensory neuropathy weeks to months after variable and unquantified exposure to CPF, an insecticide with potent inhibitory action on AChE and high "cholinesterase inhibition index" suggesting no strong neuropathic effects. The authors commented on the possibility that repeated, low-level exposure could result in neuropathic effects. Neuropathy was reported to be mild and clinically reversible. The magnitudes of the reported electrophysiological measurements were small and the absence of accurate exposure information limits any interpretation that can be drawn from this publication. In the view of other authors (Moretto and Lotti, 1998) it is improbable that sensory peripheral neuropathy would be induced by an OPp, because OPIDN is characterised by sensory-motor neuropathy in which the motor component is predominant. These authors also suggest that other causes should be sought for peripheral neuropathies in patients who did not display severe cholinergic toxicity a few weeks before the onset of the neuropathy.

Animal experiments indicate that the potential of CPF to produce OPIDN is low. Mattsson *et al* (1996) reported no neuropathological changes in rats exposed for 13 weeks to 0.1, 1, 5 or 15 mg/kg/d CPF. The top dose produced mild signs of cholinergic inhibition and 62% inhibition of brain ChE; the animals had reduced motor activity at week 4. In hens treated orally for 20 days with 10 mg/kg/d CPF, the maximum tolerated dose level, there were no signs of OPIDN. There was only a weak, non-cumulative NTE inhibition in the brain (activity 82-99% of control) and lymphocytes (activity 85-128% of control), although brain AChE activity and plasma BChE activities were reduced to 58-70% and 49-80% of control values respectively (Richardson *et al,* 1993). In hens, single oral doses of 60-90 mg/kg CPF (levels corresponding to 4-6 times the estimated LD_{50}) resulted in NTE inhibition higher than 70% (i.e. activity $<$ 30% of control) within 5-6 days, indicating a weak neuropathic potential of this agent (Capodicasa *et al,* 1991). Unfortunately, in neither study were the results of the neuropathological examination of the nervous system presented; thus the end-point that has been shown to be optimally sensitive is missing (Classen *et al*, 1996). The ability of a supra-lethal dose of CPF to produce OPIDN has been also confirmed in cats exposed to a single dose of 300 mg/kg i.m. and repeated doses of atropine and 2-PAM to prevent death. This dose of CPF caused maximum inhibition of lymphocyte NTE to 46% of pre-exposure activity at 7 days and onset of ataxia at about 19 days (Fikes *et al,* 1992).

Stokes *et al* (1995) examined 68 male pesticide applicators licensed in New York State and compared them to 68 population-based controls, individually matched for age, sex and county of residence. Of the 11 most frequently-used OPps, guthion was predominantly used in 2 recent growing seasons. Twenty seven applicators were involved in spraying for 30 years or more, while the others were

involved for shorter periods, but at least one year. The authors concluded that OPp exposure was associated with loss of peripheral nerve function. This was based on a significant increase in the mean threshold sensitivity for hands. when compared to a matched control group; feet were not affected. Long-term exposure was determined by a questionnaire, but it is unclear whether poisoning episodes had occurred in the past. Subjective symptoms were collected on and off season; frequent headaches, weight loss and nightmares were statistically increased on season. However a toxic polyneuropathy does not affect upper limbs exclusively. The susceptibility of axons is strictly dependent on their length and lower limbs are, as a rule, affected first.

A.3.6 Concluding remarks on OPIDN

The experimental and the clinical data taken together indicate that the predominant effects in human OPIDN consist in damage to the peripheral nerves and the long ascending and descending spinal tracts, associated with denervation atrophy of skeletal muscles. There is some evidence of damage to the autonomic nervous system. There is no evidence of delayed effects on other areas of the somatic nervous system although such effects cannot be totally dismissed on the basis of selected observations in animal experiments and some clinical observations in man. The neuropathic potential of OPs can be identified and predicted in animal safety studies. However, prolonged or parenteral exposure is needed to demonstrate the neuropathic effects in less sensitive animals or with weak neurotoxicants. OPIDN is produced by lower levels of neuropathic OPs when there is repeated administration as compared with a single dose and this must be taken into consideration in human safety assessment. There is, however, no evidence that prolonged weak inhibition of NTE could have cumulative effects resulting in neuropathy. Both animal experiments and studies of poisoned humans indicate that there is a substantial threshold of NTE inhibition which must be exceeded before delayed neuropathy will be caused by OPs whether by a few high-level or prolonged low-level exposures. Such a threshold cannot be exceeded by currently-used OPps without first evoking massive cholinergic effects. This may explain why the majority of reported cases of OPIDN have resulted from non-pesticidal OPs.

Certain agents which are not neuropathic can promote neurotoxicity of neuropathic agents in animals; there are no data to indicate such effects in man. Since the epidemiological studies are mostly carried out on people exposed to low levels of mixtures of pesticides during prolonged periods, it should be possible to detect such promoting effects if they did occur. We are not aware of any reports of a neuropathy induced in man with agents, such as TPP, capable of inducing OPIDN Type II in animals.

LIST OF ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetyl cholinesterase
ADI	Acceptable daily intake
ACTH	Adenocorticotropic hormone
ASP-70	Aspartate-70
BChE	Butyryl cholinesterase
ChE	Cholinesterase
CNS	Central nervous system
CPF	Chlorpyrifos
DEF	S,S,S-Tributyl phosphorothioate
DFP	Diisopropyl fluorophosphate
ECG	Electrocardiography
EEG	Electroencephalography
EPA	Environmental Protection Agency
EPN	O-Ethyl O-4-nitrophenyl-phenylphosphonothioate
ERG	Electroretinography
GI	Gastrointestinal
IPCS	International Programme on Chemical Safety
i.m.	Intramuscular
i.p.	Intraperitoneal
KBR-2822	O-(2-Chloro-2,3,3-trifluorocyclobutyl)) O-ethyl S-propyl phosphothioate
MAP-2	Microtubule associated protein-2
NFU	National Farmers' Union
NMDA	n-Methyl-d-aspartate
NOEL	No-observed-effect level
NTE	Neurotoxic esterase or neuropathy target esterase

OECD	Organisation for Economic Co-operation and Development
OP(s)	Organophosphorus compound(s)
OPp(s)	Organophosphorus pesticide(s)
OPIDN	Organophosphorus-induced delayed neuropathy
OPIDP	Organophosphorus-induced delayed polyneuropathy
2-PAM	Pralidoxime
PMSF	Phenylmethanesulfonyl fluoride
PNS	Peripheral nervous system
PON1	Paraoxonase
PSP	Phenyl saligenin phosphate
RBC	Red blood cells (erythrocytes)
REM	Rapid eye movement
s.c.	Subcutaneous
TOCP	Triorthocresyl phosphate
TEPP	Tetraethyl pyrophosphate
TPP	Triphenyl phosphite
WHO	World Health Organization

LIST OF OPs

Common name:	Azinphos-methyl, "Guthion", "Gusathion"
IUPAC name:	S-(3,4-Dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl)O,O-dimethyl phosphorodithioate
CAS No:	86-50-0

Common name:	Chlorfenvinphos
IUPAC name:	2-Chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate
CAS No:	470-90-6

Common name:	Chlorpyrifos (CPF)
IUPAC name:	O,O-Diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
CAS No:	2921-88-2

Common name:	"DEF 6"
IUPAC name:	S,S,S,- Tributyl phosphorotrithioate
CAS No:	78-48-8

Common name:	Demeton-S-Methyl
IUPAC name:	S-2-Ethylthioethyl O,O-dimethyl phosphorothioate
CAS No:	919-86-8

Common name:	Diazinon
IUPAC name:	O,O-Diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
CAS No:	333-41-5

Common name:	Diazoxon (oxo-analogue of Diazinon)
IUPAC name:	-
CAS No:	-

Common name:	Dichlorvos
IUPAC name:	2,2-Dichlorovinyl dimethyl phosphate
CAS No:	62-73-7

Common name:	Diisopropyl Fluorophosphate (DFP)
IUPAC name:	-
CAS No:	55-91-4

Common name:	"Dimecron", Phosphamidon
IUPAC name:	2-Chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate
CAS No:	13171-21-6 (E)+ (Z)-isomers; 23783-98-4 (Z)-isomer

Common name:	Dimethoate
IUPAC name:	O,O-Dimethyl S-methylcarbamoylmethyl phosphorodithioate
CAS No:	60-51-5

Common name:	"Dipterex", "Metriphonate", Trichlorfon
IUPAC name:	Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
CAS No:	52-68-6

Common name:	Disulfoton
IUPAC name:	O,O-Diethyl S-2-ethylthioethyl phosphorodithioate
CAS No:	298-04-4

Common name:	Ecothiopate iodide
IUPAC name:	-
CAS No:	513-10-0

Common name: EPN

IUPAC name: O-Ethyl-O-4-nitrophenyl phenylphosphonothioate

CAS No: 2104-64-5

Common name: Fenitrothion

IUPAC name: O,O-Dimethyl O-4-nitro-m-tolyl phosphorothioate

CAS No: 122-14-5

Common name: Fenthion

IUPAC name: O,O-Dimethyl O-4-methylthio-m-tolyl phosphorothioate

CAS No: 55-38-9

Common name: ″Guthion", "Gusathion", Azinphos-methyl

IUPAC name: S-(3,4-Dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl)O,O-dimethyl phosphorodithioate

CAS No: 86-50-0

Common name: Isocarbophos, Malathion

IUPAC name: Diethyl (dimethoxythiophosphorylthio) succinate

CAS No: 121-75-5

Common name: IsoOMPA, Schradan

IUPAC name: -

CAS No: 152-16-9

Common name: Leptophos

IUPAC name: O-4-Bromo-2,5-dichlorphenyl O-methyl phenylphosphonothionate

CAS No: 21609-90-5

Common name:	Malaoxon (ortho analogue of malathion)
IUPAC name:	-
CAS No:	1634-78-2

Common name:	Malathion, Isocarbophos
IUPAC name:	Diethyl (dimethoxythiophosphorylthio) succinate
CAS No:	121-75-5

Common name:	Methamidophos
IUPAC name:	O,S-Dimethyl phosphoramidothioate
CAS No:	10265-92-6

Common name:	Methidathion
IUPAC name:	S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl O,O-dimethyl phos phospohorodithioate
CAS No:	950-37-8

Common name:	Methyl demeton, Demeton-S-methyl
IUPAC name:	S-2-Ethylthioethyl O,O-dimethyl phosphorothioate
CAS No:	919-86-8

Common name:	Metriphonate, Dipterex, Trichlorfon
IUPAC name:	Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
CAS No:	52-68-6

Common name:	Mevinphos, "Phosdrin"
IUPAC name:	2-Methoxycarbonyl-1-methylvinyl dimethyl phosphate
Cas No:	26718-65-0

Common name: Mipafox

IUPAC name: N,N'-Diisopropylphosphorodiamidic fluoride

CAS No: 3773-49-7

Common name: Monocrotophos

IUPAC name: Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate

CAS No: 6923-22-4

Common name: Omethoate

IUPAC name: O,O-Dimethyl S- methylcarbamoylmethyl phosphorothioate

CAS No: 1113-02-6

Common name: Paraoxon (ortho analogue of parathion)

IUPAC name: O,O-Diethyl O-p-nitrophenyl phosphate

CAS No: 311-45-5

Common name: Parathion, Parathion Ethyl

IUPAC Name: O,O-Diethyl O-4-nitrophenyl phosphorothioate

CAS No: 56-38-2

Common name: Parathion Methyl

IUPAC name: O,O-Dimethyl O-4-nitrophenyl phosphorothioate

CAS No: 298-00-0

Common name: Phorate

IUPAC name: O,O-Diethyl S-ethylthiomethyl phosphorodithioate

CAS No: 298-02-2

Common name: Pirimiphos-ethyl

IUPAC name: O-2-Diethylamino-6-methylpyridimidine-4-ul O,O-diethyl phosphorothioate

CAS No: 23305-41-1

Common name: Phosalone

IUPAC name: S-6-Chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl O,O-diethyl phosphorodithioate

CAS No: 2310-17-0

Common name: Phosphamidon, "Dimecron"

IUPAC name: 2-Chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate

CAS No: 13171-21-6 (E)+ (Z)-isomers; 23783-98-4 (Z)-isomer

Common name: "Phosdrin", Mevinphos

IUPAC name: 2-Methoxycarbonyl-1-methylvinyl dimethyl phosphate

Cas No: 26718-65-0

Common name: Phenyl Saligenin Phosphate (PSP)

IUPAC name: -

CAS No: -

Common name: Quinalphos

IUPAC name: O,O-Diethyl O-quinoxalin-2-yl phosphorothioate

CAS No: 13593-03-8

Common name: Sarin

IUPAC name: O-Isopropyl methylphosphonofluoridate

CAS No: 107-44-8

Common name: Schradan, IsoOMPA

IUPAC name: -

CAS No: 152-16-9

Common name: Soman

IUPAC name: O-Pinaconyl methylphosphonofluoridate

CAS No: 96-64-0

Common name: Sulprofos

IUPAC name: O-Ethyl-O-4-(methylthio)phenyl S-propyl phosphorodithioate

CAS No: 35400-43-2

Common name: Tebupirimfos

IUPAC No: O-(2-tert-Butylpyrimidin-5-yl) O-ethyl O-isopropyl phosphorothioate

CAS No: 96182-53-5

Common name: Tetrachlorvinphos

IUPAC No: (Z)-2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate

CAS No: 22248-79-9

Common name: Tetraethyl pyrophosphate (TEPP)

IUPAC name: O,O,O,O-Tetraethyl phosphoric anhydride

CAS No: 107-49-3

Common name: Thiometon

IUPAC No: S-2-Ethylthioethyl O,O-dimethyl phosphorodithioate

CAS No: 640-15-3

Common name:	Triorthocresyl phosphate (TOCP, TOTP) ,Triorthotolyl phosphate (TOTP,TOCP)
IUPAC name:	Tri-o-cresyl phosphate, Tri-o-tolyl phosphate
CAS No:	78-30-8

Common name:	Trichlorfon, Dipterex, Metriphonate
IUPAC name:	Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
CAS No:	52-68-6

Common name:	Trichlomat
IUPAC name:	O-Ethyl-O-2,4,5-trichlorophenyl ethylphosphonothionate
CAS No:	327-98-0

Common name:	Trimetacresyl Phosphite
IUPAC name:	-
CAS No:	-

Common name:	Trimethyl phosphate
IUPAC name:	Trimethyl phosphate
CAS No:	512-56-1

Common name:	Triorthocresyl phosphite
IUPAC name:	-
CAS No:	-

Common name:	Triparacresyl phosphite
IUPAC name:	-
CAS No:	-

Common name:	Triphenyl phosphite (TPP)
IUPCA name:	Triphenyl phosphite
CAS No:	101-02-0

Common name:	Vamidothion
IUPAC name:	O,O-Dimethyl S-2-(1-methylcarbamoylethylthio)ethyl phosphorothioate
CAS No:	2275-23-2

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