NR 2003:3

Neurotoxicity after poisonings with organophosphate pesticides in Nicaragua

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ARBETE OCH HÄLSA | VETENSKAPLIG SKRIFTSERIE

ISBN 91-7045-668-2 ISSN 0346-7821 http://www.niwl.se/

Universidad Nacional Autónoma de Nicaragua
Facultad de Ciencias Médicas
UNAN-León, Nicaragua, América Central

Arbetslivsinstitutet
National Institute for Working Life

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ARBETE OCH HÄLSA
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National Institute for Working Life
S-112 79 Stockholm
Sweden

ISBN 91–7045–668–2
ISSN 0346–7821
http://www.niwl.se/
Elanders Gotab, Stockholm

To :

María José,

Ana Sofía

and Amanda

List of papers

This thesis is based on six papers, which will be referred to in the text by their Roman numerals:

I. McConnell R, Delgado E, Cuadra R, Torres E, Keifer M, Almendarez J**, Miranda J,** El-Fawall H, Wolf M, Simpson D, Lundberg I. Organophosphate neuropathy due to metamidophos: biochemical and neurophysiological markers. *Arch toxicol* 1999;73:296-300.

II. **Miranda J**, Lundberg I, McConnell R, Delgado E, Cuadra R, Torres, Wesseling C, Keifer M. Onset of grip and and pinch strength impairment after acute poisoning with organophosphate insecticides. *Int J Occup Environ Health* 2002;(8):19-26.

III. **Miranda J**, McConnell R, Delgado E, Cuadra R, Torres E, Wesseling C, Keifer M, Lundberg I. Tactile vibration thresholds after acute poisonings with organophosphate insecticides. *Int J Occup Environ Health* 2002(8):214-221.

IV. **Miranda J**, McConnell R, Delgado E, Cuadra R, Torres E, Wesseling C, Keifer M. Lundberg I. Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticides. Submitted.

V. **Miranda J**, Cuadra R, McConnell R, Borg K, Delgado E, Torres E, Wesseling C, Keifer M, Lundberg I. Nerve conduction velocity after poisoning with organophosphate insecticides: A two-year follow-up study. Manuscript.

VI. **Miranda J**, Borg K, Wesseling C, McConnell R, Lundberg I. Occurrence and severity of neuropathy during two years after organophosphate pesticide poisoning. Manuscript.

Abbreviations

OP	Organophosphate pesticide
OPIDP	Organophosphate-induced delayed polyneuropathy
NTE	Neuropathy target esterase
LNTE	Lymphocyte neuropathy target esterase
IMS	Intermediate syndrome
NCV	Nerve conduction velocity
NFs	Autoantibodies to neurofilament triplet proteins
IgG	Immunoglobulin G
IgM	Immunoglobulin M
GFAP	Glial fibrillary acidic protein
MBP	Myelin basic protein
ELISA	Enzyme-linked immunoabsorbent assay
PAHO	Pan-American Health Organisation

Contents

Introduction

Pesticides have been used since ancient times but only during the last 50 years, with the discovery and manufacture of synthetic pesticides, has pesticide production become a successful industry. Current global production estimates range between 2 and 8 million tons of formulated pesticides (1)(2). The use of pesticides in developing countries is around one fourth of world production (1), but poisonings are far more frequent and 99% of the more than 200,000 annual deaths in the world due to acute pesticide poisonings occur in the developing world (2). Pesticide exposure in developing countries is more widespread and higher than in industrialised countries (3). Pesticide regulations in developing countries are less strict and less widely enforced. In addition, industrialised countries export many restricted and prohibited pesticides to developing countries, (4-12). Many pesticides classified by the World Health Organisation as highly or extremely toxic are in widespread use, among these organophosphate pesticides (OPs).

OPs are a particular public health concern in developing countries, because they cause the majority of acute pesticide poisonings (3, 7, 11, 13, 14). Besides the fact that such poisonings can be life-threatening, severe poisonings caused by particular OPs have been associated with a persistent neurological impairment that may ensue a few weeks after the recovery from acute poisoning, so-called Organophosphate-induced Delayed Polyneuropathy (OPIDP). OPIDP is a sensory and motor neuropathy which is a result of axonal degeneration and secondary demyelinization occurring in long and large-diameter axons in peripheral nerves, the spinal cord and the anterior horn cells (15-17). The distal portions of the lower limbs are primarily affected, but in the most severe poisonings the upper limbs may be also affected (18). OPIDP has been recognised as a clinical entity since 1953 (19) and a substantial body of experimental and epidemiological evidence has been published on the subject. However, many aspects of OPIDP are still unclear. All previous studies have been performed as single cross-sectional examinations after the poisoning episodes (20). Some studies have reported mixed sensory-motor neuropathy (16, 19, 21-23), while others have found predominant or exclusive motor neuropathy (24-26) after severe poisonings with some organophosphate insecticides. The intermediate syndrome (IMS) has been identified as another OP-induced neurotoxic illness that appears after the acute cholinergic crisis but before the expected onset of OPIDP (18). The pathogenesis of IMS has not been well characterised but is suspected to involve a combination of pre- and post-synaptic dysfunction of neuromuscular transmission as a result of prolonged acetylcholinesterase inhibition (27).

The occurrence and pathogenesis of three types of effects caused by poisonings by OPs (acute, delayed and intermediate) have been well characterised in experimental settings. Clinically observable cases of IMS and OPIDP have confirmed these experimental findings. Epidemiological findings are restricted to descriptive surveys of acute OP poisonings, one prospective study on the occurrence of IMS

among OP poisoned patients, and a few cross-sectional studies on peripheral sensory effects years after OP poisoning. However, no prospective studies have been carried out so far to assess the onset and evolution of neurotoxic symptoms among OP poisoned subjects. Quantitative measurements of the course of symptoms of neurological disease have not been performed in follow-up studies. Moreover, the possibility of predicting the development of neuropathy after human acute poisoning as well as how the neurological findings relate to the time between poisoning and examination, to the type of OP that caused the poisoning, or to the severity of the poisoning are additional uncertainties.

This thesis presents the results of evaluations of the effects on the peripheral nervous system after acute OP poisonings in Nicaraguan patients: the predictive value of serial lymphocyte neuropathy target esterase (LNTE) measurements in the development of OPIDP (Paper I), the onset of motor and sensory impairments (Papers II and III), and the evolution and persistency of motor and sensory effects in the long term (Papers IV to VI). The studies involve observations of the individual courses of the neurological disease, which could also help to elucidate the questions above.

Background

Nicaragua

Nicaragua is a developing country located in the Central American isthmus, with a population of 5 million, and a labour force of 1.7 million, 42% of whom are agricultural workers. Development indicators are among the lowest in Latin America, with 50% of the population below the poverty line (population without the possibility of buying food or items to satisfy essential needs: nutrition, clothing, housing and energy) (28), an infant mortality of 33 per 1000 live births, a life expectancy of 69 years, and a literacy rate of 68% in 2001. Official figures for unemployment were 10.7% (29). However there is an extensive informal labour sector producing an underemployment rate over 70% of the economically active population (30). This results in massive migration patterns, mainly to neighbouring Costa Rica (31).

Extensive exposure to pesticides in developing countries like Nicaragua is a public health concern. During the last four decades pesticide poisonings and pesticide-related illness had been an important problem in Nicaragua (32) (33) (34) (35) (36) (37) (38) (39). Organophosphate insecticides are the major cause of these poisonings (38, 40). Poisoning incidence rates in Nicaragua are among the highest in the world. In the same geographical region that produced the cases studied in this thesis incidence of poisoning was estimated to be 7.5/100 person years of pesticide use among individuals between 10 and 79 of age (20). Underreporting of poisonings was estimated to be around 65% based on the contrast between information on poisoning episodes obtained from the surveyed population and the official poisoning registry (20). A recent national survey estimated almost 68,000 symptomatic poisoning episodes in a one-year period in the Nicaraguan population, i.e. 2.4 per 100 inhabitants (38, 40).

According to the official poisoning registry for 1999, 49% of the poisonings were suicide attempts, 32% occupational, 18% non-occupational accidents and 1% homicide attempts (40). OPs caused 38% of these poisonings, 17% were caused by herbicides, 13% by fumigants and 12% by carbamates. Unidentified pesticides, organochlorines, pyrethroids and fungicides caused the rest of the poisonings. However, the above-mentioned national survey has demonstrated that poisonings due to suicide attempts are enormously over-represented compared to the occupational poisonings in the official registry. Since these poisonings are frequently the most severe, the likelihood of ending up in hospital and being reported is greater than for the less severe poisonings (39).

Difficulties during the research

Conditions for research in Nicaragua are very different from those in developed countries. Research infrastructure is insufficiently developed both in terms of material and human resources. Although experience of research has increased

during the last years, it is still limited. In addition, researchers have to deal with the consequences of frequently occurring natural hazards such as volcano eruptions, hurricanes, landslides or flooding. Poverty and natural disasters oblige people to emigrate in search of minimal survival conditions. During this research, many of the above-mentioned situations were encountered. There were plenty of times in which interruptions in the supply of electric current prevented us from completing examinations. When the electromyograph failed, it took months to import a new one. Basic services for communication, i.e. telephones and mail services, do not exist in rural communities. The researchers had to visit the participants at their homes or working places, on many occasions on horseback, by tractor or on foot. Sometimes, after intense rains, the researcher could not contact the participants because of flooded rivers. During the study, a hurricane changed the ecological conditions of coasts in the Pacific sea, and fishing was not sufficient to supply the nutritional needs of fishermen's families. A number of members of the fishing co-operatives participating in the research as controls moved to places where the impact of the hurricane was less dramatic. Others left the country searching for a better life.

Organophosphate pesticide poisonings

Organophosphate insecticides are used in agriculture to kill soft body insects (41). More than 20,000 organophosphate agents have been developed since they were first synthesised in 1854 (41). Human OP poisonings may occur as a result of occupational, accidental or intentional exposure. Absorption of OPs may occur through the skin, mucus membranes, gastrointestinal system or by inhalation (42). Acute organophosphate poisoning causes well-known peripheral and central nervous system manifestations (43). Exposure to OPs causes inhibition of the acetylcholinesterase enzyme. Acetylcholinesterase regulates the concentration of acetylcholine at the interneuronal and neuroeffector junctions. Acetylcholine is a neurotransmitter that mediates electrical activity between neurones and the nerve endings and the effectors. Muscle contraction or gland secretions occur when acetylcholine is secreted in adequate amount. Inhibition of acetylcholinesterase produces accumulation of acetylcholine at the site of the effector junctions (44) resulting in increased cholinergic activity: miosis, gastrointestinal stimulation, stimulation of sweating and salivary glands, cardiovascular effects, bronchoconstriction, bronchial secretion, muscle fasciculation and cramps. The cholinergic manifestations on the central nervous system include dizziness, mental confusion, headache, weakness, convulsions and coma (45).

Some OPs have been associated with peripheral nerve diseases that appear a few weeks after a poisoning event (neuropathic OPs). However, based on some anecdotal cases there seems to be certain recognition that OPs previously reported by the WHO as non-neuropathic (46) also may produce delayed neurological effects (47). The most widely recognised long-term effect from acute OP poisoning is Organophosphate-induced Delayed Polyneuropathy (OPIDP), which produces persistent sensory-motor impairment. OPIDP has been defined as a

distal symmetrical mixed sensory-motor peripheral and central neuropathy that appears 2-4 weeks after poisoning after the acute cholinergic crisis has disappeared. OPIDP mainly affects the lower limbs, but in severe cases, upper limbs may also be involved. Clinically, OPIDP is usually resolved some months after poisoning. Most of the case reports have reported that OPIDP mainly, and in some cases exclusively, involves motor impairment (19, 24-26, 48), but some studies have reported OPIDP with significant sensory impairment (16, 23). Also two epidemiological studies found elevated vibrotactile thresholds more than 2 years after the acute poisoning (36, 49).

The proposed mechanism for the pathogenesis of OPIDP is independent of acetylcholinesterase inhibition. Inhibition of a nervous system enzyme, known as neuropathy target esterase (NTE), has been pointed out as responsible for its onset (16). This mechanism of onset, studied experimentally in animals, is claimed to be initiated when an OP has inhibited NTE activity by more than 70%, generally within 48 hours after the poisoning episode (18). Some neuropathic OPs promote an "aging" process in NTE which was considered essential for the development of OPIDP(18). Aging involves the loss of an alkyl group from the phosphoryl residue attached to NTE leaving a negatively charged phosphorylated NTE. However, according to some evidences, the development of OPIDP may be possible, even in the absence of NTE aging. It has been observed that in poisoning with methamidophos and with a mixture of a carbamate and a sulfonate compound neuropathy may occur when NTE aging is absent. (16). After the NTE inhibition/aging phase, a progression phase involving selective reduction of the retrograde axonal transport has been demonstrated, but the series of events that finally determine the OPIDP onset are still unclear (16, 18). NTE may be monitored in peripheral lymphocytes, and in peripheral red blood cells as surrogates for acetylcholinesterase activity in the nervous system. Lymphocytic neuropathy target esterase (LNTE) has been proposed as a biomarker for the occurrence of OPIDP. There is little information about LNTE inhibition thresholds for predicting the development of neuropathy in humans. In fact, there are only two previous case reports, one by Lotti et al (16) who observed inhibition of 60% in LNTE measured one month after a chlorpyrifos poisoning that resulted in neuropathy and the other by Moretto et al (50) who reported a LNTE inhibition greater than 90% immediately after an acute methamidophos poisoning also resulting in neuropathy.

Another peripheral neuropathy related to acute OP poisoning is the "intermediate syndrome" (IMS). IMS, reported to appear after the acute cholinergic crisis and before the eventual onset of OPIDP, is characterised by muscular weakness, affecting mainly the proximal limb muscles and the neck flexors, and cranial-nerve palsies. IMS has been regarded as life-threatening since respiratory depression may occur (51). It has been suggested that IMS occurs as a result of prolonged acetylcholinesterase inhibition that leads to a pre- and post-synaptic dysfunction of neuromuscular transmission. IMS is considered primarily independent of the type of OP (27). Reports on the time of recovery of IMS vary from 2 to 38 days (27, 51-53).

Objectives

Main objective

The main aim of this thesis is to describe the neurotoxic effects of acute OP poisonings in relation to the type of OP agent and severity of poisoning in a cohort of Nicaraguan subjects.

Specific objectives

1. To assess the predictive value of serial LNTE measurements in the development of OPIDP in a case of severe acute methamidophos poisoning (paper I).
2. To test the hypothesis that OP poisoning is associated with persistently impaired motor function, and to characterise the onset and evolution of such potential impairment as measured by pinch and grip strength among OP poisoned men (papers II and IV).
3. To test the hypothesis that OP poisoning results in chronically impaired sensory function, and to characterise the onset and evolution of such potential impairment as measured by quantitative cutaneous vibration thresholds in the hands and the feet among OP poisoned men (papers III and IV).
4. To assess whether acute OP poisoning is related to long-term slowing of sensory and motor nerve conduction velocities (paper V).
5. To evaluate the association of acute OP poisoning with long-term motor-sensory symptoms and clinical evidence of neuropathy among OP poisoned men (paper VI).

Subjects and methods

Subjects

The studies included in this thesis were based on 90 individuals (77 men and 13 women) admitted for acute OP poisoning to two hospitals in the cities of León and Chinandega, Nicaragua between July 1, 1992 and December 15, 1996. A nurse from each hospital contacted the co-ordinator of the research team each time a patient suspected of having been poisoned by OP arrived at the emergency room. The co-ordinator then visited the patient, explained the study and asked whether the person wanted to participate. When the patients were about to be discharged from the hospital, the nurse contacted the co-ordinator again and a time was set for a first examination within the next few days (examination I). The second examination (examination II) was scheduled for around 7 weeks after poisoning. Around 2 years after poisoning the patients were revisited and given an appointment for a third examination (examination III).

A control subject was contacted and scheduled for examination every time a poisoned patient was about to be examined for the first time. Examinations lasted for about 2 hours and were carried out at the university hospital in León. At each examination, all participants signed a consent form containing detailed information on the project and they were given the results of the tests, transported from and to their domiciles, and compensated for lost wages.

The control group was composed of 74 individuals, 20 working at a cattle farm co-operative and 54 working at two fishing co-operatives. There was only one woman in the cattle farm and one in the fishing co-operatives. A control subject was contacted and scheduled for examination approximately every time a poisoned patient was about to be examined for the first time. By the end of 1992, severe climatic conditions limited fishing off the Pacific Coast, which gradually forced a considerable number of members of the fishing co-operatives to migrate out of the area, causing a substantial loss of controls in Examination II.

Table 1 shows the number of participants examined and included in the different papers. Paper I concerns the analysis of one case of severe intentional OP poisoning with serial LNTE measurements as a predictor in the development of OPIDP. Paper II and III are based on the subjects with grip and pinch strength and vibratory threshold tests, respectively, for examination I and II. Paper IV is based on the subjects who performed these tests at all three examinations. Paper V is based on the subjects with nerve conduction velocity for examinations I and III. This is the only paper that includes the poisoned women, because there was not a sufficient number of women in the control group and all outcomes included in this thesis apart from nerve conduction velocity are related to gender. Malfunctioning of the equipment caused the loss of data on an additional number of subjects. Paper VI is based on all subjects that were clinically examined. Fewer individuals than in the other studies were included because the protocol of clinical examination was introduced when a fair part of the field work had already been completed.

Table 1. Number of participants examined at each examination and included in the studies.

Examinations	Number of participants examined						Number of controls/and poisoned subjects included in each study
	After hospital discharge		7 weeks after poisoning		Two years after poisoning		
	Controls	Poisoned	Controls	Poisoned	Controls	Poisoned	
Hand grip	74	66	39	59	28	48	II 39/59, IV 28/48
Hand pinch	74	64	39	57	28	48	II 39/57, IV 28/48
Vibrometry	74	57	39	56	28	48	III 39/56, IV 28/48
Nerve conduction velocity	72	74	37	57	20	55	V 17/48
Clinical sensory and motor testing	17	36	15	28	29	49	VI 29/49

Methods

Exposure assessment

The agent responsible for the poisoning was ascertained at the emergency room by means of testimonies from patients, co-workers or relatives. Field visits to confirm the poisoning agent were made to the homes or workplaces for 64% of the patients. During the visits the agent was confirmed by checking the label on the containers. If containers were unlabeled, information was obtained from the local dealer or, in a few cases, by means of chemical analyses. The agreement between testimony and field or laboratory confirmation was 100%. The types of OP agents were categorised into neuropathic or non-neuropathic according to a report by the WHO (46). The severity of the poisoning was classified as moderate or severe according to the clinical features, and further subclassified into occupational or intentional.

Testing

The room temperature at which all the examinations were performed was between 28 and 33 degrees centigrade.

For a patient with severe intentional poisoning by methamidophos, serial LNTE was assessed according to the method described by Maroni and Blecker 1986 (54) and autoantibodies to neurofilament triplet proteins (NFs), to glial fibrillary acidic protein (GFAP) and to myelin basic protein (MBP) were measured by an enzyme-linked immunoabsorbent assay (ELISA) (Paper I).

All participants were administered a detailed questionnaire investigating a number of factors that could affect the study outcomes. A set of standard tests was assembled to assess motor and sensory functions. Grip and pinch strength measurements were performed according to a previously recommended standardized procedure using an adjustable-handle Jamar dynamometer and the B & L Engineering pinch gauge, respectively. The mean of three successive trials was used as the outcome for each strength modality (Paper II and IV). Vibration thresholds were measured using a Vibraton II (Sensortek, Inc: Clifton, NJ) for dominant index and big toe. Five readings (trials) were made for each finger and toe. The first, the lowest, and the highest readings of vibrometry were discarded. The mean of the remaining two readings was the vibration threshold used as the outcome (Paper III and IV). NCVs in motor nerves (ulnar and tibial) and in sensory nerves (ulnar and sural) were measured first using a TECA electromyograph and later using a CADWELL 500 electromyograph (Paper V). A clinical examination with a fixed protocol for investigating neuropathy was carried out. This examination investigated neurological symptoms (numbness, par- or dysaesthesia, neuralgic pain, muscle cramps and muscle weakness in the limbs). The limbs were also tested for tactile, graphaesthesia and temperature perception.

Atrophy was analysed in the digitorum brevis muscle, anterior tibial muscles and in the intrinsic muscles of the hands. Biceps, knee and Achilles' tendon reflexes were tested bilaterally.

Statistical analysis

The exposure (poisoning) was divided into 4 categories for the analyses: moderate and severe non-neuropathic poisonings and moderate and severe neuropathic poisonings in studies II and III. In studies IV-VI the groups of moderately and severely poisoned by non-neuropathic OPs were pooled together because of small numbers. The vibration threshold distribution was normalised by log-transforming amplitude (into log microns). Since grip and pinch strength, and NCVs, were normally distributed, no transformations were needed.

Associations between exposure and hand strength and vibratory sensitivity were assessed by means of multiple linear regression analysis (Papers II – IV). Confounding factors were evaluated by entering the relevant exposure variable together with one potential confounder variable at a time, in the multiple linear regression analyses. Regression coefficients with 95% Confidence Intervals (CI) were calculated for the various categories of poisonings. Coefficients with a p value of <0.05 were taken as being statistically significant. For hand strength, also a trend for means for ordered categories was evaluated through a Jonckheere-Terpstra test (55) (Paper II). The changes over time within the exposure groups were evaluated through paired-sample t-tests and the differences of mean change between the control and the exposure groups were evaluated through t-tests for independent samples.

Fisher's exact test, one-tailed, was used for testing differences in numbers of individuals with NCV values below the 10^{th} percentile among the controls, between the different groups (Paper V). Fisher's exact test (one-tailed) and prevalence ratios with 95% confidence intervals were used to determine the likelihood that the proportions with symptoms or clinical signs in the different groups of intoxicated individuals were different from the proportions in the control group (Paper VI).

Results

The results of the studies in this thesis in comparison with findings in the literature are shown in Table 2.

Prediction of development of OPIDP by LNTE (Paper I)

LNTE activity of a male patient who attempted suicide by ingesting methamidophos was 77% inhibited when measured 3 days after poisoning. Two weeks after poisoning the patient developed a motor neuropathy. M (IgM) and IgG against the NFs triplet proteins, GFAP (IgG only), and MBP were present in serum 3 and 52 days after poisoning, when neuropathy was well established. IgM to GFAP and of both isotypes to NF68 were slightly decreased (Paper I).

Motor function (Papers II, IV, V and VI)

A statistically significant grip and pinch strength reduction was found for examinations performed at hospital discharge and around 7 weeks after poisoning through all exposure categories as they were ordered according to organophosphate type and poisoning severity. In order to give an estimate of the magnitude of the effect, the regression coefficients with confidence intervals presented in the separate papers have been recalculated to percent reduction of grip strength, etc. Significantly impaired grip strength, [a reduction of 20%, 95% confidence intervals (CI) 10% -30%], and pinch strength (reduction of 16%, CI 5% - 27%) were found at examination I among those individuals severely poisoned by neuropathic organophosphate insecticides as compared to the controls. Grip strength was decreased by 25% (CI 16 % - 34%), at examination II in this group, while pinch strength, was reduced by 22% (CI 12% - 32%) as compared to the controls. When dividing the subgroup with severe poisonings by neuropathic OPs into occupational and suicidal poisonings, a 18% reduction in grip strength (CI 4% - 32%) and a 25% reduction in pinch strength (CI 8% - 42%) were found among those who attempted suicide, at examination I as compared to the controls. In this group strength was further reduced by the second examination resulting in a 33% decrease in grip strength (CI 19% - 46%) and a decrease in pinch strength of 41%, (CI 27% - 55%) as compared to the controls (paper II). Two years after poisoning, grip and pinch strength of men with poisonings due to non-neuropathic OPs and with less severe poisonings had practically recovered. However, grip strength was still reduced by 15% (CI 6%-24%) and pinch strength was reduced by 15% (CI 6%-25%) among those severely poisoned by neuropathic OPs (Paper IV). NCVs in the tibial motor nerve were found to have slowed by 20% (CI 10%-29%) at hospital discharge and were still found to be slowed two years after

poisoning by 18% (CI 7%-29%)among individuals poisoned by non-neuropathic OPs. Those moderately poisoned by neuropathic OPs had slowed tibial NCVs at hospital discharge by 14% (CI 6%-23%) which persisted two years after poisoning. Those severely poisoned by neuropathic OPs had tibial motor NCVs slowed by 15% (CI 5%-24%) at hospital discharge, and persisted two years after poisoning (paper V).

Sensory function (Studies III, V and VI)

To assess sensory neuropathy, quantitative vibration perception thresholds were measured in dominant index fingers and big toes. Men with severe poisonings due to attempted suicide by neuropathic organophosphate insecticides had doubled their toe vibrotactile thresholds at examination II as compared to controls. A significant increase of toe vibrotactile thresholds from examinations I to II was also found in this group. No evidence of impaired vibrotactile thresholds was found in the index finger or big toe in any of the other exposure categories neither in the first nor the second examination (paper III). Two years after poisoning, index finger and toe vibration thresholds were found elevated among those poisoned regardless of the OP type or severity of the poisoning (paper IV). Sensory NCVs measured at hospital discharge and two years after poisoning did not appear to be affected in any exposure group (paper V). Two years after poisoning, self-reported symptoms of neurological impairment were increased and abnormalities in sensory testing were found among all poisoned individuals, more evidently in those severely poisoned by neuropathic OPs (paper VI).

Table 2. Summary of results in comparison with findings in the literature

Study/type	Findings	Findings in other studies	Contribution
I/Case report	Inhibition of LNTE was a good predictor of motor neuropathy. Serum autoantibody titers to nervous system may be markers of neuropathy	Moretto et al. 1994 (50) reported inhibition of LNTE >90% after acute poisoning with methamidophos. Lotti et al. (16) reported LNTE inhibited by 60% when measured one month after chlorpyrifos poisoning, resulting in OPIDP two weeks later.	Further evidence of LNTE inhibition as predictor of OPIDP. Serum autoantibodies to nervous system examined for the first time as biomarkers of neuropathic effect in a patient that developed OPIDP
II/Cohort	Development of muscular weakness related to severity of poisoning and type of OP agent.	No studies with comparable semi-objective measurements available	Evidence of motor impairment for all OP types, but clearer for neuropathic OP-poisonings
III/Cohort	Development of elevated toe vibrotactile thresholds weeks after poisoning among the most severely poisoned by neuropathic OPs	No studies with comparable objective measurements available	Evidence of sensory neuropathy only from the most severe poisonings by neuropathic OPs
IV/Cohort	Persistent weakness and some indications of vibration threshold impairment 2 years after poisoning regardless of the type of OP or severity of the poisoning, impaired strength more evident among those severely poisoned by neuropathic OPs	De Jager et al. (25), Stamboulis (48) and Vacilescu et al. (56) reported distal weakness 1 to 1.5 years after poisoning with parathion, mecarban and thrichlorphon respectively. Steenland et al. (49) and McConnell et al. (36) found increased vibration threshold between 10 and 34 months after poisoning	Hand strength improved in all poisoned groups. The extent of the improvement was related to poisoning agent and poisoning severity
V/Cohort	Slowed tibial motor NCVs at hospital discharge that persisted 2 years after poisonings regardless the OP type. More noticeable among those severely poisoned by neuropathic OPs	De Freitas et al. (57) found diminished motor but also sensory NCVs in a patient examined 3 months after poisoning. Wadia et al.(58) reported normal motor ulnar and lateral popliteal NCVs among 101 patients poisoned by different OPs examined during hospital admission who developed paralytic signs. Hierons et al. (24) found normal median motor NCV in the forearm of a patient that developed a progressive neuropathy 2 to 8 weeks after acute poisoning by trichlorphon when examined 4 months after the neuropathy onset	Possible motor effects regardless of the OP type
VI/Cohort	Increased symptoms and sensory abnormalities in all poisoned groups after 2 years, most evidently among those severely poisoned by neuropathic OPs.	No similar studies available	Symptoms and signs related to sensory neuropathy two years after poisoning. Further evidence of effects from OPs not reported previously as neuropathic

Discussion

Internal validity

Selection bias

The exposed group was identified and contacted after admittance to the two participant hospitals due to acute poisonings with organophosphate insecticides. However, an unknown number of mild or moderate cases of poisonings do not reach hospitals. Some of these poisonings, and often those occurring on private farms, are taken care of at primary care and private practice level and they are not reported to the health care system. In addition, it is also feasible that some hospitalised poisonings were not reported, and in consequence not recorded. Thus, a certain number of poisoned patients could not be included in this study, especially the milder poisonings. It is unlikely that the inclusion of poisonings in the study was related to the type of poisoning agent. In any case, since the poisonings were stratified by severity and type of OP agent and the results presented by categories, this selection should not have distorted the relationship between the poisoning groups and the outcomes, but numbers became smaller. The considerable loss of control subjects for examination II and III was mainly due to economic reasons. A possibility is that the healthiest and strongest subjects left their homes in search of job opportunities elsewhere. However, the results in the first and second examination among those who left the study and those who remained for examination III, were similar and no such bias seems to have occurred.

Misclassification

Exposure misclassification. Since the hospital information on insecticides causing the poisonings agreed excellently with information obtained through field visits and laboratory analysis, it seems reasonable to rely on the hospital information also for the 36% of the study participants for whom field visits were not possible. For this reason, it seems unlikely that the effects observed among subjects poisoned by non-neuropathic OPs are due to misclassification of exposure to neuropathic OPs.

The categorisation of the poisonings by severity was based on clinical evidence. It is possible that some signs and/or symptoms were neither evaluated nor recorded or underreported or overreported by mistake by the hospital personnel. Since the symptoms and signs were recorded by means of a checklist, it is likely that, if this occurred, it was not related to the severity of the poisoning or the type of OP agent. Such misclassification would be non-differential over the exposure categories, resulting in an underestimation of the effect for each of the categories and obscuring of a possible dose-response relation. A differential misclassification would be a possibility if some signs and/or symptoms were more frequent among the poisoned subjects, not from the poisoning but as a consequence of pre-existing

neurological illness which increased their likelihood to be hospitalised. This is very unlikely since previous neurological condition was carefully evaluated and there were no cases of subjacent neurological illness among the participants.

Outcome misclassification. Two examiners performed grip and pinch strength testing and they could often guess the exposure status. The devices to assess grip and pinch strength were rather simple and the examiners gave the same instructions to the examined subjects. Both tested poisoned as well as control subjects. The participation of the two examiners was evaluated in multivariate analyses and was not associated with the outcome and did not change the coefficients for the exposures of interest.

The same equipment was used for all measurements of grip and pinch strength but it was not calibrated. An evaluation of the measurements performed at four different points in time in examinations I and II did not detect any particular time-dependent trend for any of the strength measurements. The vibrometer was calibrated twice during the data collection period. The NCVs were recorded with two different electromyographs by the same person. However, the NCV measurements obtained with the two devices were compared within each exposure category and no differences were observed. The clinical sensory and motor testing using a fixed examination protocol was performed by the same person. Also here, the examiner was aware of the exposure in most cases. However, it is possible that the design of the protocol of examination may have reduced the risk of bias since it was applied in the same way to all participants.

Confounding

Among all potential confounders evaluated in this study, only age affected the outcome and was controlled for in the statistical analyses. Height was treated as a confounder in the analysis of NCV since it is recognised as affecting electromyographic parameters. However, probably due to the small range of heights it was not related to the outcome in this material

Estimates of alcohol consumption based on self-reporting, have been reported to be generally reliable (59, 60). However, inaccuracy, and more often under-reporting, have been reported in relation to heavy drinking (60-62). Presumed underestimation of alcohol consumption would influence all groups of heavy drinkers to the same extent. Thus, it is unlikely that this factor would have an important effect on the risk estimates.

Long-term exposure to OPs without intoxication could have confounded the results, if those with long term heavy exposure were also the most severely intoxicated subjects. This was not the case since many of the severe poisonings were suicide attempts. It is feasible that, overall, poisoned subjects remember their previous use of pesticides better than controls, or the opposite, that controls using less pesticide have less difficulty recalling their lifetime pesticide use. In both scenarios the misclassification of this confounder is non-differential and its effects on the outcomes would have been underestimated. In any case, except from the

intentional poisonings, those poisoned had higher average long term exposure than controls. Hence, it can not be excluded that at least part of the effect attributed to acute intoxication may in fact have been due to long-term exposure. However, no effect from cumulative exposure to OPs was detected for any of the outcomes evaluated in this thesis.

It was also examined whether the period of time between the poisonings and examinations I and II had any effect on the results, but it seemed of little importance. Manifestations of neurotoxicity were found already at examination I among those examined early (less than 7 days after poisoning) as well as among those examined late (more than 7 days after poisoning). The results were not affected either among those patients whose examination II was performed early (less than 7 weeks after poisoning) or late (more than 7 weeks after poisoning).

Both motor and sensory impairments were more pronounced among men with severe intentional poisonings by neuropathic OPs than among those with severe occupational poisonings by neuropathic OPs. This could be due to the fact that severe intentional poisonings were actually more severe than the severe occupational poisonings. But another difference between the suicidal subjects and the occupationally poisoned subjects is also possible: a possible depressive condition among the suicidal subjects could be a cause of their progressive weakness. However, these patients underwent a psychiatric evaluation and none of them had signs of depression. All of them had committed the act in an impulse of anger. Besides, the influence of a possible depression on the vibrotactile thresholds is more difficult to explain since the suicidal individuals only showed sensory impairment in the second examination, when they were likely to be, on average, less depressed than when they committed their suicidal act.

The small size of the study and possibilities to draw conclusions

The results from the second, and particularly, the third examinations presented in this thesis were based on a small number of subjects. The control group in examination III consisted of 28-29 individuals in the studies of grip and pinch strength, vibration thresholds (paper IV) and clinical examinations (paper VI). In the study of nerve conduction velocities there was only 17 controls in examination III.

Small study samples increase the margins for random errors which is reflected in the wide confidence intervals for the effect estimates. This has diminished the possibilities to detect effects that may still have been of clinical significance.

However, not only groups of poisoned individuals and controls were compared. Changes in different symptoms and signs could also be followed over time in the same individual. In general this reduces confounding problems and increases possibilities to detect effects compared to when only cross-sectional comparisons are made between groups.

The original hypothesis behind the study was based on what was at that time known or anticipated concerning OPIDP symptomatology and signs. It suggested that little or no effect would be observed on most outcome variables in the first examination, that the effect would be most pronounced in the second examination

and that injury would to a variable extent resolve until the third examination. In study V, for example, an effect was detected in the tibial nerve where an effect was primarily expected while the effect in the poisoned groups was similar in all examinations and no change could be detected over time in the individual participants. Compared to the original hypothesis the findings are somewhat contradictory which complicates the interpretation. However, given that these findings were true, the interpretation would have been complicated also in a larger study since the interpretation must be based on information about mechanisms of injury.

Comparison with previous investigations

There are no studies to our knowledge that have used dynamometry to assess motor impairment from pesticide exposure. However, clinically evaluated motor impairment is often reported in association with severe poisonings with certain organophosphate pesticides, and as a common feature of OPIDP (18, 19, 56, 63). Thus, the weakness found in the group with the most severe poisonings (suicide attempts) is in agreement with previous studies. Among the five severe suicidal poisonings by a neuropathic OP, in fact, four developed clinical OPIDP. The magnitude of the motor impairment among these patients and latter clinical diagnosis, confirmed the development of OPIDP. Besides this clear effect, our results showed a significant trend in reduced strength as the exposure categories were ordered by type of OP and severity of the poisoning, in examinations I and II. This finding suggests that all poisonings caused motor impairment and that impairment depended on the severity of the intoxication and on the type of organophosphate. Our findings in the subgroup of severe occupational poisonings were not very different from those for the intentional poisonings. However not a single case of clinical OPIDP appeared in the former subgroup. Persistent motor impairment among the occupational poisonings, as determined by sensitive grip and pinch tests, could be explained as subclinical manifestations of OPIDP. Another possible explanation for the findings at hospital discharge and around 7 weeks after poisoning among the occupational and less severe cases may be the development of an IMS. IMS transitory weakness, mainly in proximal limbs, has been reported to be resolved in about 40 days (51, 53, 64, 65).

The findings of persistently slowed motor NCVs two years after poisoning regardless of the OP type seem to confirm the findings of motor impairment. To the author's knowledge, there are no findings in the literature, except for one case by De Freitas et al (57), reporting slowed NCVs long after poisoning. A cautious interpretation of the NCV results as well as more research is warranted. The findings of persistently elevated vibration thresholds in the group with the most severe poisonings are an indication of long myelinated fiber damage. They are in agreement with previous findings (36, 49) and indicate that sensory neuropathy appears more frequently in the very severe cases. However, sensory abnormalities of different modalities indicating damage of nerve fiber of different diameters were found using a fixed protocol for clinical examination, which may indicate

that the detection of sensory impairments may be a matter of methods. Unfortunately no follow-up studies are available for comparison of the findings of unexpected increased sensory abnormalities two years after poisoning in all the exposure groups (study VI). Nevertheless, it is acknowledged that explanations other than the poisonings may have influenced the results. Confounding control was not possible due to the reduced size of the groups. However, it is very unlikely that age may have accounted for the results since its distribution was similar among controls and poisoned. Moreover, the controls drank somewhat more than the poisoned. Long-term OPs-exposure was higher among the poisoned than among the controls, but no effect from the estimates of long-term OPs-exposure used in this thesis was observed on any of the other outcomes.

Finally, the findings in this thesis of certain motor and sensory impairment among those poisoned by OPs with non-documented neuropathic effects further add to the growing suspicion against the alleged lack of neurotoxicity of many OPs (42).

Interpretation of the results

The results in this thesis suggest that poisonings by organophosphate insecticides have resulted in several different manifestations of neurotoxicity. Strength impairment found at hospital discharge among all poisoned subjects may be related to diminished excitability of the muscle membrane caused by sustained depolarisation during the acute phase of the poisoning (depolarisation blockade). However, if restoration of acetylcholinesterase had already occurred by hospital discharge, it is probable that unexpected weakness may be related to manifestations of an IMS or an early OPIDP. The findings of persistent hand weakness around seven weeks after poisoning in the groups with less severe poisonings may be attributed to either an unresolved IMS or to a mild or subclinical form of OPIDP. However, the extremely severe cases developed serious motor and sensory impairment, which is in accordance with an OPIDP. Sensory and motor impairment still found two years after poisoning in the group with severe poisonings by neuropathic OPs may be an indication of insufficient recovery from OPIDP.

It was not possible to pinpoint the onset of motor neuropathy since the severely poisoned patients had already shown weakness by examination I, probably caused by depolarisation blockade. The weakness found at examination II among the severely poisoned was probably due to axonopathy involved in the development of neuropathy. The mechanisms causing axonopathy involve alterations of the transport mechanisms in the axon membrane, loss of the ionic balance across the membrane, abnormal water uptake, axonal swelling and subsequent degeneration of the axon. Slowed NCVs found at hospital discharge may indicate that axonal degeneration and secondary demyelination of long myelinated nerve fibers may have been occurred. Demyelinization occurs trough similar mechanisms than those producing axonal degeneration. However, slowed NCVs two years after poisoning may indicate incomplete remyelinization. There was some indication of

sensory neuropathy at examination II among the subjects with severe intentional poisonings by allegedly neuropathic pesticides, but the evidence was stronger two years later. The sensory dysfunction seems to have affected mostly sensory modalities mediated by small diameter myelinated or unmyelinated nerve fibers. It seems improbable that the findings in the suicidal subjects could have been influenced by factors other than the OP poisoning, since their results of motor and sensory testing in the first examination were similar to the rest of the groups in the study.

The results of this thesis support the occurrence of organophosphate-induced delayed polyneuropathy among men with severe poisonings due to those insecticides classified as neuropathic and the occurrence of a certain degree of sensory and motor impairment also among those with poisonings due to OPs not reported previously as neuropathic. Determination of the onset of the motor impairment in the course of the neurological illness was not possible because different pathophysiological conditions were suspected to produce effects of similar magnitude at different points in time. It is difficult to determine whether the early motor effects were due to acute toxicity, to an early OPIDP or to IMS. However, among those most severely poisoned by neuropathic OPs, the test results and clinical course were clearly compatible with the onset of OPIDP a few weeks after the cholinergic crisis.

The present findings seem to indicate that poisoning with OPs produce persistent sensory and motor impairments affecting nerve fibers of different diameters. It was also found that, although there is some recovery of motor impairment after two years, a deterioration of sensory functions, mainly associated with the severity of poisoning, may have occurred.

Implications for prevention in Nicaragua

Nicaragua's economy is founded on agriculture. Pesticides have been used since the 1950s to improve profits from food and cotton production. The import of pesticides increased by 25% annually between 1992 and 1995 without improving the food production (66). Pesticide-related illnesses are one of the main occupational health problems in Nicaragua (67-69).

Important initiatives in Central America designed to reduce pesticide use and pesticide-related problems have been taken since the 1980s. Experience with surveillance of pesticide poisonings in Nicaragua and Costa Rica in the 1980s demonstrated that the number of cases of pesticide-related illnesses was overwhelming in comparison with the official figures of the recordings of the health systems (33). In the 1990s the surveillance systems in the seven Central American countries, reinforced by a regional program of the Pan-American Health Organisation (PAHO), PLAGSALUD, were focused on high-risk regions. The number of cases of pesticide-related illness has increased even more since then (70). However, those cases are still only a small fraction of cases that actually occur (38). As an answer to the growing concern among different sectors of society about the

effects of pesticides on health and the environment, the legislation on regulation of toxic substances was improved in 1998 (71). The content of the present law involves regulation of all activities related to the imports, exports, distribution, sales, use, handling and destruction of pesticides and other toxic substances (71). However, in practice there is little restriction of the use and widespread availability of hazardous pesticides. This is mainly due to the powerful influence of the pesticide industry over the ministry of agriculture (39). The pesticide industry opposes policy reforms to regulate pesticides, and the government authorities end up prioritising commercial interests over health and environment protection (39). Moreover, the potential of the data gathered by the current surveillance systems is not sufficiently exploited because the health authorities have difficulties in the interpretation, management and reporting of this data (39).

The use of non-chemical alternatives reduces the dependence on pesticides for the control of pests. An intervention study by Hruska and Corriols among Nicaraguan peasants demonstrated that the training of pesticide users in integrated pest management is an effective method for reducing pesticide exposure (67). In general, this method involves education on pesticide hazards and on the use of non-chemical methods to control pests (38). The pesticide industry recommends the use of protective equipment. However, this is in most cases inadequate for the climate of the tropics (72).

It is also of great importance to improve the training of the hospital staff in the diagnosis and treatment of poisoned patients in order to reduce mortality due to pesticides and to enhance the quality of the data on pesticide-related illnesses.

Scientific evidence is necessary to support the demands for control of pesticides. The results in this thesis provide further indications that poisonings by OPs produce different short and long-term effects on the peripheral nervous system. Furthermore, it was found that probably there are no OPs that lack adverse effects during months after poisoning.

Acute poisonings by OPs also affect the central nervous system producing impairment of neuropsychological functions, as demonstrated by Rosenstock et al. in a study of 36 Nicaraguan men (34). There is an unknown number of Nicaraguans suffering from functional limitations as a consequence of the effects of OP-poisonings on the peripheral and central nervous system.

It is possible to reduce the use of pesticides and their consequences on public health and the environment. There are a lot of difficulties, due in part, to the limited resources inherent to the developing world. However, it is possible to maximise the efforts to consolidate the surveillance systems of pesticide-related illnesses. The health authorities should be more active in solving problems and counterbalancing the influences of the pesticide industry. Alternatives to manage pests should be supported and promoted. The relevant legislation should be strictly applied. The research groups should continue providing scientific evidence of the effects of pesticides. This in fact, may have an important role not only at the problem-solving levels but also in the education of general population.

Abstract

In Nicaragua rates of acute human poisonings caused by pesticides, in spite of significant underreporting, are among the highest in the world. Hence the unrestricted availability of pesticides and their misuse, as well as the continuous increase in annual imports of pesticides are reasons for public health concern. Organophosphate insecticides (OPs) are responsible for more than 45% of pesticide poisonings in Nicaragua. Severe poisonings caused by certain OPs have been associated with Organophosphate-induced Delayed Polyneuropathy (OPIDP), a persistent neurological impairment that may ensue a few weeks after recovering from acute poisoning.

Neuropathy was evaluated in relation to acute poisonings with some organophosphate pesticides (metamidophos, chlorpyrifos or fenthion) in Nicaraguan individuals hospitalised 1992 and 1996. Serial Lymphocyte Neuropathy Target Esterase (LNTE) was measured in a case of acute OP poisoning in order to evaluate this enzyme as a predictor of subsequent neuropathy. Motor function, as in grip and pinch strength, was evaluated among 62 patients immediately after hospital discharge and around 7 weeks later. Sensory function, as in vibrotactile thresholds, was evaluated among 56 at the same two occasions. Hand strength and vibrotactile thresholds were re-evaluated 2 years after poisoning among 48 patients. Nerve conduction velocity was measured on 3 occasions during a two-year period among 44 patients. Finally, long-term sensory neurological impairment was evaluated through a fixed protocol of clinical examination among 46 patients at the end of the follow-up. A variable number of never poisoned controls were examined at corresponding points in time.

Inhibition of LNTE was found to be a good predictor of the development of OPIDP in one poisoned individual. Hand strength was found to be reduced at examinations I and II in all exposure categories regardless of the OP type, but weakness was more marked among those with poisonings due to neuropathic OPs. Among these patients, those with intentional poisonings were the weakest and worsened with time, an unequivocal sign of OPIDP. Toe tactile vibration thresholds were impaired 7 weeks after poisoning among patients with severe intentional poisonings. This finding confirmed OPIDP. Two years after severe occupational and attempted suicide poisonings by neuropathic OPs patients had persistent sensory and motor impairment, predominantly motor impairment, which served as an indication of remaining OPIDP. In addition, slowed conduction velocity in the tibial motor nerve that persisted two years after poisoning was found among all individuals poisoned by OPs regardless of whether the poisoning pesticide was considered as neuropathic or non-neuropathic, and this served as a possible indication of insufficient recovery from OPIDP. An increased number of reported symptoms and signs was detected among all patients, more evidently among those severely poisoned by neuropathic OPs two years earlier, which served as an indication of altered sensory function in contrast with our earlier findings of some recovery from motor dysfunction.

Sammanfattning (Abstract in Swedish)

Nicaragua är förekomsten av akuta förgiftningar med bekämpningsmedel, trots betydande underrapportering, bland de högsta i världen. Därför är den obegränsade tillgången till bekämpningsmedel, den stadiga ökningen av importen och överanvändningen av bekämpningsmedel en viktig folkhälsofråga. Organiska fosforföreningar (OP) står för mer än 45% av bekämpningsmedelsförgiftningarna i Nicaragua. Allvarliga förgiftningar orsakade av vissa OP har visats kunna ge upphov till en speciell form av neuropati (OPIDP). OPIDP är en bestående neurologisk funktionsnedsättning, som kan uppstå några veckor efter tillfrisknandet från den akuta förgiftningen.

Förekomsten av neuropati studerades hos personer inlagda på två sjukhus i Nicaragua mellan 1992 och 1996 efter akut förgiftning med OP (metamidophos, chlorpyrifos eller fenthion) Enzymet LNTE studerades i ett fall av akut OP-förgiftning för att bestämma enzymets förmåga att förutsäga neuropati. Motorisk funktion, i form av greppstyrka, mättes hos 62 patienter omedelbart efter utskrivning från sjukhus och ungefär sju veckor senare. Sensorisk funktion, i form av vibrationströsklar, mättes hos 56 patienter vid samma tillfällen. Handstyrka och vibrationströsklar mättes åter hos 48 av patienterna två år efter förgiftningen. Nervledningshastigheter mättes hos 44 patienter vid samma tre tillfällen under en tvåårsperiod. Vid slutet av uppföljningen mättes slutligen långvarig sensorisk, neurologisk funktionsnedsättning hos 49 patienter med hjälp av ett standardiserat protokoll för klinisk undersökning. Ett varierande antal kontrollpersoner som aldrig blivit förgiftade med OP undersöktes också vid motsvarande tidpunkter.

Hämning av LNTE konstaterades vara en god prediktor för utvecklingen av OPIDP hos den förgiftade personen. Handstyrkan var reducerad vid båda de första undersökningarna hos alla exponeringskategorierna oberoende av OP-typ, men svagheten var mer uttalad hos dem som hade förgiftats med OP som tidigare ansetts kunna framkalla OPIDP (neuropatiska OP). Bland dessa patienter var de med avsiktlig förgiftning de svagaste, och de försämrades över tid som ett tecken på OPIDP. Vibrationströsklarna i stortårna var nedsatta sju veckor efter förgiftningen hos patienter med allvarlig, avsiktlig förgiftning. Detta resultat styrkte att OPIDP förelåg. Två år efter allvarlig förgiftning av neuropatiska OP i arbetet eller som självmordsförsök hade patienterna bestående sensoriska och framför allt motoriska skador, vilket indikerade bestående OPIDP. På samma sätt konstaterades nedsatt ledningshastighet i nervus tibialis (motorisk nerv) två år efter förgiftningen hos alla personer förgiftade av OP, oberoende av om det förgiftande bekämpningsmedlet betraktades som neuropatiskt eller icke-neuropatiskt. Detta kan vara ett tecken på ofullständigt tillfrisknande från OPIDP. En ökning av antalet rapporterade symtom och fynd i neurologstatus tecken upptäcktes hos alla patienter men mest tydligt hos dem med allvarliga förgiftningar med neuropatiska OP två år tidigare. Detta tydde på förändrad sensorisk funktion i motsats till våra tidigare fynd av viss restitution av de motoriska skadorna.

Resumen (Abstract in Spanish)

Las tasas de intoxicaciones humanas causadas por plaguicidas en Nicaragua, a pesar de importante subregistro, se encuentran entre las mayores del mundo. Debido al contínuo aumento de las importaciones, su irrestricta disponibilidad y su mal uso, los problemas generados por los plaguicidas son de gran interés en la salud pública. Los organofosforados (OPs) provocan mas del 45% de las intoxicaciones por plaguicidas en Nicaragua. Las intoxicaciones graves causadas por ciertos OPs han sido asociadas con una neuropatía tardía (conocida como OPIDP por sus siglas en inglés) un daño neurológico persistente que puede instalarse unas pocas semanas después de la recuperación de una intoxicación aguda.

Se evaluó la presencia de neuropatía en relación a intoxicación aguda con algunos OPs (metamidofos, clorpirifos o fention) en individuos Nicaragüenses hospitalizados por intoxicación aguda con OPs entre los años 1992 y 1996. La enzima esterasa neuropática seriada (LNTE, por sus siglas en inglés) se midió en un caso de intoxicación aguda con el fin de evaluar esta enzima como predictora del posterior desarrollo de neuropatía. La función motora, se evaluó a través de las fuerzas de apretar y de pinzar en las manos de 62 pacientes, inmediatamente después del alta hospitalaria y alrededor de 7 semanas después. La función sensorial se evaluó por medio de la determinación de los umbrales de sensibilidad vibrátil en los dedos índice y gordo del pie de 56 pacientes en las mismas dos ocasiones. La fuerza manual y los umbrales vibrotáctiles fueron re-evaluados 2 años después de la intoxicación en 48 pacientes. La velocidad de conducción nerviosa en las extremidades superiores e inferiores fue evaluada en 3 ocasiones durante un período de 2 años en 44 pacientes. Finalmente, se evaluó la persistencia de alteraciones neurológicas por medio de un protocolo fijo de examinación clínica en 46 pacientes, al final del período de seguimiento. Un número variable de controles nunca intoxicados fueron examinados en momentos correspondientes.

La LNTE resultó ser buena predictora del desarrollo de OPIDP en un individuo intoxicado. La fuerza de la mano se encontró reducida en las evaluaciones relizadad inmediatamente después del alta hospitalaria y alrededor de 7 semanas después en todas las categorías de intoxicación, sin importar el tipo de OPs, pero la debilidad se encontró mas marcada en los intoxicados con OPs considerados neuropáticos. Entre estos pacientes, los que sufrieron intoxicaciones intencionales estaban mas débiles y empeoraron con el tiempo, lo que constituye un signo inequívoco de OPIDP. El umbral de sensibilidad táctil del dedo gordo del pie se encontró alterado alrededor de 7 semanas después de la intoxicación en pacientes con intoxicaciones intencionales severas. Este hallazgo confirma la presencia de OPIDP. Dos años después de severa intoxicación ocupacional e intencional con OPs neuropáticos los pacientes presentaron alteraciones motoras y sensoriales persistentes, predominando las alteraciones motoras, lo cual es una indicación de OPIDP residual. Además, la velocidad de conducción en el nervio tibial se

encontró persistentemente disminuída dos años después en los individuos intoxicados con OPs considerado como neuropáticos y como no neuropáticos. Esto puede indicar una insuficiente recuperación de una OPIDP. Un número elevado de síntomas reportados y signos se detectó en todos los pacientes, pero fue mas evidente en los severamente intoxicados con OPs neuropáticos dos años antes. Esto puede ser una indicación de alteración de la función sensorial, en contraste con nuestros anteriores hallazgos de cierta recuperación de la alteración motora.

Acknowledgements

Thanks a lot to:

My main supervisor and co-author, Ingvar Lundberg, for all his wise guidance through many years, for his opportune advice when I got stuck, for all his support, patience and friendship at good and bad moments.

My co-supervisor and co-author, Rob McConnell, for showing me the way to occupational health, for a friendship challenged by time and distance, for his enthusiasm and always welcome advice. This work would not have been possible without his initiative.

My co-supervisor and co-author, Catharina Wesseling, for always being there when I needed her valuable advice, for friendship and understanding and for sharing her experience.

My co-authors, Ricardo Cuadra, Edgar Delgado, Edmundo Torres, Mathew Keifer and Kristian Borg, for everything we shared when collecting data, for their support, enthusiasm and friendship.

The victims of the pesticides, the fishermen and cattle farmers for their participation in this research.

The staff of the epidemiology departments of the Hospital Escuela Oscar Danilo Rosales Arguello (HEODRA), León and Hospital España, Chinandega for giving us access to patients' records

Per Arne Håkansson, for his unlimited support, for his understanding, even when not having any vacations, and for always listening to me.

To my daughters, María José, Ana Sofía and Amanda, for forgiving me for all the time we did not share, do you?

This research was financed by the Department for Research Cooperation of the Swedish International Development Cooperation Agency (Sida/SAREC) and the United States National Institute for Occupational Safety and Health (NIOSH).

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