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PROTECTIVE MEASURES CONCERNING THE USE OF PESTICIDES

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Risks which arise from the use of pesticides could be diminished if certain rules are observed. In this respect five important points are discussed: (1) education of pesticide users, (2) advisory service and information, (3) regulations, (4) the equipment used and (5) alternatives to pesticides.

Having in mind the necessity of further use of pesticides a research project concerning the use of pesticides in Sweden has been started by the National Board of Occupational Safety and Health. The project is planned to focuss on four aspects: (1) attendance, (2) testing, (3) enquiry and (4) medical aspect.

The use of pesticides may involve risks to the personnel if they are not familiar with the risks or have not got sufficient education in handling pesticides. Risks will also occur if the user fails to follow the regulations and rules for correct handling.

In order to show which measures have to be taken to prevent diseases resulting from the use of pesticides the following five points of special importance will be discussed:

1 education
2 advisory service and information
3 regulations
4 equipment and
5 alternatives to pesticides

Education

Due to economic reasons the farmer and the gardener as well as any other manufacturer has to produce as much as possible at a lowest possible rate of costs. The farmer must know that high yields (crops) cannot be achieved unless the soil is properly tilled and harrowed. Furthermore he must use the right kind and amount of fertilizers as well as seed. The importance of soil drainage and rotation crops must not be neglected.

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Sažetak

METODE ZA MJERENJE EKSPOZICIJE ANTIKOLINESTERAZNIM INSEKTICIDIMA

Od mnogih metoda za mjerenje aktivnosti kolinesteraze razmjerno je malo prikladnih za rutinsku kontrolu profesionalne ekspozicije antikolinesteraznim spojevima. U radu su prikazani činioci od kojih zavisi izbor i primjenljivost pojedine metode s osobitim obzirom na razlike između organofosfornih i karbamatnih spojeva. Također su izneseni rezultati vlastitih istraživanja na dobrovoljcima i radnicima zaposlenim u proizvodnji spomenutih insekticida.

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Four monomethyl carbamate insecticides were used as inhibitors. The plasma cholinesterase values were found the same when measured by the Acholest or spectrophotometric method. The degree of the whole blood cholinesterase inhibition was found up to 11% lower when measured by the tintometric method compared with the level registered spectrophotometrically by the *Ellman's* method. The results obtained with one carbamate insecticide — propoxur are presented in Fig. 4.

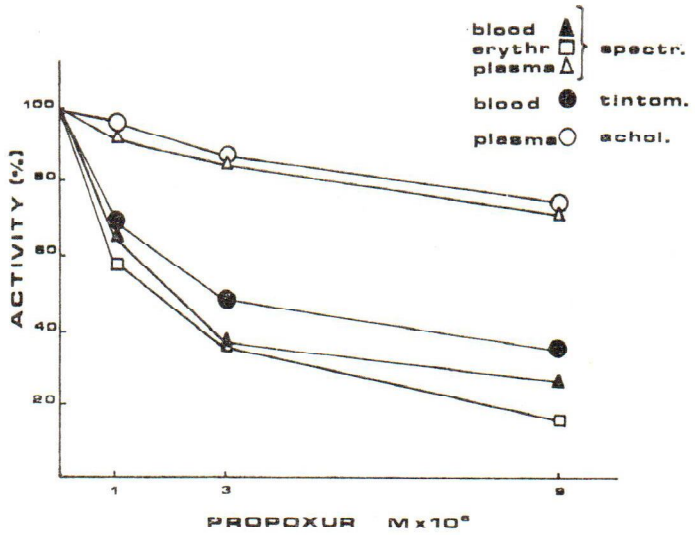


Fig. 1. *Activity of human blood cholinesterases measured by the acholest, tintometric and spectrophotometric methods after inhibition by propoxur. Each point represents the mean value of four determination*

Svetličić et al. (27) reported the results of the trial in a group of formulating workers exposed alternatively to OP and OP plus DDT component of the product. The fall in the enzyme activity during OP compound exposure and the reverse to the normal values during the exposure to the combined product was attributed to the hepatic enzymes induction.

The fact that it is possible to follow the biochemical lesion produced by anticholinesterase agents before the critical fall in the enzyme activity, i. e. before the appearance of clinical picture of poisoning, offers the following practical advantages within the security scheme of occupationally exposed personnel:

(1) to verify the efficiency of protective devices,
(2) to classify the working places according to the health hazards for workers,
(3) to select the individuals »chronically« careless as regards the safety measures and
(4) to prevent the intoxication by stopping the exposure and rotating the worker to the safe job.

rose. One hour after ingestion the subject felt better. The rapid disappearance of symptoms was consistent with the further rapid recovery of erythrocyte cholinesterase activity.

In a subject who was given 5 doses of 0.20 mg of propoxur per kg of body weight, at 30 min intervals, erythrocyte cholinesterase activity decreased continually down to 60% of the normal value. Plasma cholinesterase was practically unaffected. By receiving the total dose of 1 mg/kg the volunteer complained of only slight mental confusion and difficulties in the pronunciation of some words. Spontaneous reactivation of cholinesterase started after the ingestion of the last dose.

The studies in volunteers have clearly shown that, while a single, relatively small oral dose (0.36 mg/kg) of propoxur may produce symptoms of short duration, higher doses may be tolerated without symptoms, if they are divided into fractions and taken within a relatively brief period.

It may also be noted that whole blood cholinesterase levels determined by the tintometric method, a field method designed originally for measuring the exposure to OP compounds (18), were in reasonably good agreement with the erythrocyte cholinesterase levels determined by the *Ellman's* spectrophotometric method (about 11% higher). Similar results were obtained when the tintometric and Acholest methods were compared with the spectrophotometric method under laboratory conditions (26).

In order to determine the condition which precedes clinical poisoning we assessed cholinesterase activities in blood of workers employed in the production, formulation and application of pesticides.

The measurements of blood cholinesterase activity were performed in the laboratory, while the sampling was done either in the laboratory or in the field when samples were kept cool and transferred to the laboratory. Cholinesterase activities were measured by the spectrophotometric method (25).

Over a period of two years, the number of workers engaged in the production of pesticides was 291 and of those applying them was 63 (spraymen and aircraft personnel). The workers handled the following compounds: dimethoate, thiometon, phosalon, dichlorvos, bromophos, demeton, parathion and malathion. Besides anticholinesterases, the workers were also in contact with some herbicides, rodenticides, chlorinated hydrocarbons and fertilizers.

Out of 354 subjects, 56 showed an activity of blood cholinesterases below 50% of their control value. In only 14 of these workers, the following cholinergic symptoms were observed in addition to blood cholinesterase inhibition: weakness, salivation, sweating and vomiting. These cases came from a pesticide production plant where it was observed that some work places are particularly exposed and certain technological procedures inadequate. All cases were observed during the first year of our survey, while during the second year only one subject had blood cholinesterase activities less than 50% of the normal value, and this coincided with improved work conditions.

The spectrophotometric method described by *Ellman* and co-workers (25) provides adequate experimental conditions and yields reliable results. The method is based on the measurement of the enzyme activity by determining the rate of thiocholine formation due to enzyme hydrolysis of acetylthiocholine. The method is characterized by a relatively low substrate concentration, a comparatively short time required for measurement, a small quantity of enzyme preparation necessary for measurement and by the fact that the initial enzyme activity is being measured.

All these advantages have led us to choose this particular method as a reference method for the determination of cholinesterase activities in our laboratory experiments.

Useful as they are, experiments on laboratory animals are of limited use in evaluating a method for measuring exposure to anticholinesterase insecticides. There exist at first the species differences in cholinesterase activities, then different conditions of exposure and different factors of absorption to a given compound. Besides, the data recorded in the course of accidental poisonings, field studies on persons occupationaly exposed to insecticides, manufacturers, formulators and applicators, as well as studies on volunteers may provide the answer to many practical problems.

During the last few years, several studies have been carried out (some of them are still going on) in our laboratory related to human exposure to anticholinesterase insecticides. These include volunteers, occupationally exposed workers and accidental poisonings.

Some studies in volunteers have been carried out with the particular objective of evaluating methods for measuring blood cholinesterase levels in persons exposed to carbamate insecticide — propoxur (24, 6). Propoxur (95% pure, recrystallized before use) was given orally to healthy subjects, — investigators actively involved in the study — either in a single or repeated dose, the latter imitating the occupational exposure to insecticides. Two methods for cholinesterase assay were compared; signs and symptoms if any, were correlated with enzyme activity; the persistence of the inhibitor in the blood was studied; and the excretion of phenyl derivatives in the urine was determined.

In spite of a marked fall in erythrocyte cholinesterase activity down to 27% of the normal value after ingestion of 1.5 mg of propoxur/kg of body weight, no depression in plasma cholinesterase was observed. Both enzymes were determined spectrophotometrically. This was found consistent with the differences observed in the affinity of propoxur for the two enzymes *in vitro*, the I_{50} values being 4.6×10^{-7} M (erythrocyte), and 2.3×10^{-5} M (plasma).

The symptoms such as blurred vision and nausea appeared 20 min after ingestion. Within the next 10 min pronounced nausea with repeated vomiting and profuse sweating developed. Pulse rate and blood pressure

In Table 1 is presented the process of acetylcholine decomposition due to the action of cholinesterase on alcohol and acetic acid as well as the methods based on the determination of the remaining substrate (acetylcholine or other esters) i.e. of the substrate which was left unreacted after incubation with the enzyme. The activity measured with these methods is calculated from the difference in the amount of the substrate at the beginning of the reaction and at the end of a fixed time interval.

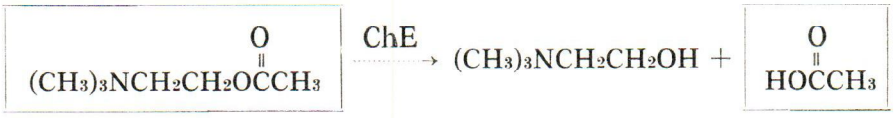
In the same figure are presented the methods based on acid formation by enzyme hydrolysis of acetylcholine or other choline esters.

Two methods *(Winteringham's* and *Reed's)* are based on the radiometric determination of cholinesterase activity, with radioactive acetylcholine as substrate.

In Table 2 presented by the same equation are the methods based on alcohol formation. They are used to measure the amount of alcohol (or thioalcohol , like the above mentioned methods) formed by enzyme hydrolysis of thiocholine and noncholine esters.

Table 1

»Remaining substrate« method and methods based on formation of acid

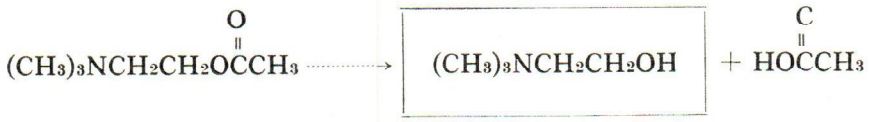


»Remaining substrate«:
Hestrin (1949)[13]
Winteringham and Disney (1962)[19]

Acid formation:
Ammon (1933)[14]
Michel (1949)[15]
Jensen-Holm (1961)[16]
Herzfeld and Stumpf (1955)[17]
Edson (1958)[18]
Reed et al (1966)[20]

Table 2

Methods based on formation of alcohol



Alcohol formation:
Ellman (1959)[21]
Fišerova-Bergerovà (1964)[22]
Guilbault and Kramer (1965)[23]

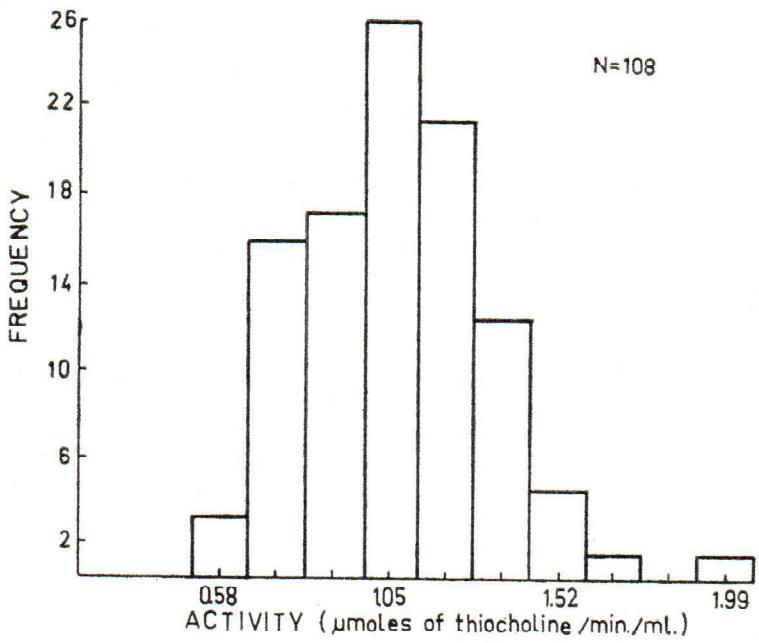


Fig. 1. *Distribution of plasma cholinesterase activity in 108 healthy persons (7)*

se activity decreases by 50% from well established preexposure value. It also seems advisable that workers who show the lowered enzyme activity from the beginning should not be recruited for the work in which the exposure is likely to occur.

The measurement of blood cholinesterases in the course of routine surveillance of individuals exposed to anticholinesterase insecticides may give the misleading results due to changes which occurred in the interval between sampling and measurement. The inhibitor present in the sample may favor the subsequent inhibition or the enzyme in the non-aged state of inhibition may show the spontaneous recovery. On the other hand, no change in cholinesterase activity was observed in blood samples of persons poisoned by OP compounds when stored dry at the temperature below 4°C (10). According to the *in vitro* findings of *Wilhelm* and *Reiner* (11), it is presumed that dilution of 1:300 into buffer of pH 5 and storage at 4°C is necessary to keep the cholinesterase activity unchanged in samples obtained from persons exposed to monomethyl carbamate insecticides.

Many methods for cholinesterase determination have been developed and critically evaluated, but only few of them have been found appropriate for the field purposes. *Holmstedt* reviewed briefly the most common groups according to the principles on which they are based (12).

so far to be »direct« inhibitors of cholinesterase. The spontaneous reactivation of carbamylated enzyme is much more rapid than that observed in the phosphorylated cholinesterases; thus the cumulative inhibitory effects on cholinesterase due to daily exposure to insecticidal carbamates would be extremely improbable. Rapid and complete recovery from illness is an outstanding feature of carbamate poisoning. Accordingly, routine cholinesterase determination has little or no practical value in assessing over-exposure to some carbamate insecticides (3). Furthermore, there is another important characteristic of this group of anticholinesterases which distinguishes them from OP compounds. In the case of over-exposure to carbamates the incapacitating symptoms (headache or nausea) would develop rapidly and prevent further exposure by stopping the operator working long before a dangerous dose had been absorbed. Owing to the existence of an »early warning system«, no severe poisonings after occupational exposure to carbamates have been reported (4).

Cholinesterase activity determination can be used as an index of absorption of anticholinesterases, but the interpretation of enzyme depression is complex and requires more information. The analysis of OP compounds or their metabolites in blood and urine will give a different kind of information than the measurement of cholinesterase activity, because excretion occurs rather rapidly, while enzyme activity recovers more slowly. Thus, the finding of a compound or metabolite indicates a very recent exposure, while the fall in enzyme activity, especially that of erythrocytes, represents the sum of the physiological effects of repeated exposure lasting sometimes for weeks or months.

Although the measurement of cholinesterase activity may give an indication of the dose of the inhibitor absorbed, it can not be used alone to predict the clinical sequence of events. A sudden slight enzyme depression is often associated with the moderate illness, while a gradual severe depression may be compatible with good health because the body has been adapted to the high levels of the accumulated acetylcholine. Therefore both, the degree and rate of enzyme depression should be taken into account. In almost all instances severe poisoning by an OP insecticide is accompanied by severe depression of blood cholinesterases, especially of that of the plasma. On the other hand, some widely used carbamate insecticides such as propoxur, show much greater activity against erythrocyte cholinesterase (5). This is relevant when making a decision about the type of cholinesterase to be measured and accordingly the choice of the adequate method (6).

The great distribution range of normal cholinesterase activity values, from one person to another (Fig. 1) (7), as well as the significant genetic differences between coloured, asiatic and white populations (8) makes the individual preexposure enzyme activity determination extremely important. The WHO Expert Committee on Pesticides (9) suggested that spray operators be withdrawn from the work if their blood cholinestera-

in evaluating the relative hazards of different working procedures, protective devices and different routes of poison absorption. Total occupational exposure may also be estimated from direct measurement of the concentration of pesticides in the air.

The *indirect* methods of measurement include the detection of the pesticide compounds or their metabolites in body tissue or excreta, or the registration of some biochemical changes caused by the absorbed product. The fact that these changes may be discovered before the appearance of clinical effects offers the chance of preventing the over--exposure and accounts for a more accurate diagnosis. Thus, the data obtained by indirect measurements are preferentially used as indices of dosage at tissue level giving, as a rule, a reasonable correlation with the clinical picture of poisoning.

Until recently the determination of cholinesterase activity was used more than any other method for measuring the exposure to anticholinesterase pesticides, organophosphates and carbamates. It was feasible indeed to measure *p*-nitrophenol in the urine of people exposed to parathion, methyl-parathion and EPN, as well as metabolites, such as phosphorus, of a limited number of organophosphorus (OP) compounds. Undoubtedly the method developed by *Shafik* and *Enos* (1), by which the metabolites of the most common OP insecticides can be measured, permits the large scale monitoring of exposure to these insecticides. The metabolites of at least some carbamates can also be measured in the serum and urine of occupationally exposed persons (2). Regardless of these developments in analytical techniques, the cholinesterase activity determinations will continue to be used as a valuable index of exposure at least to organophosphorus insecticides.

It is generally accepted that the toxic action of *OP compounds* is closely linked with their ability to inhibit cholinesterases and thus to interfere with the proper functioning of the nervous system by allowing acetylcholine to accumulate. Their toxicity, however, depends on a number of factors, each of which may play a relatively different role in insects and mammals and so determine the selectivity of the compound. Such factors are: (1) the conversion of the original compound into an active inhibitor, (2) the relative affinity of inhibitor for different cholinesterases, (3) the speed of reactivation of the inhibited enzyme and (4) the hydrolysis of the inhibitor by independent enzyme systems.

The remarkably low toxicity of some OP compounds (e.g. bromophos, malathion, ronnel) may be explained by a combination of the slow rate of oxidation of these compounds to the active inhibitor and the relatively rapid recovery of inhibited enzyme. Consequently the rate of reversal of inhibition may keep pace with fresh inhibition by the newly formed inhibitor.

Although according to their action *carbamates* belong to the group of anticholinesterase insecticides, their properties differ markedly from those of OP compounds. All monomethyl carbamates have been shown

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METHODS OF MEASURING EXPOSURE TO ANTICHOLINESTERASE INSECTICIDES

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Many methods for cholinesterase activity determination have been suggested and compared, but only a few of them have been found suitable for the routine monitoring of occupational exposure to anticholinesterase compounds. In the present paper the factors determining the choice and applicability of a method are discussed with particular reference to the differences between organophosphorus and carbamate insecticides.

Some results of own studies on volunteers and workers exposed to anticholinesterases are also presented.

The sequelae of the widespread use of pesticides can be classified into two categories: (1) toxicological effects being reflected in morbidity and mortality rates, and (2) storage of some compounds as expressed by the presence of residues in the environment, food of plant and animal origin and in human body itself. The former effects are definitely injurious, the latter are still the subject of many controversies.

The integral pesticide safety programme includes the knowledge of the actual situation, the legislation adequate for the country, effective control and fiscalisation, education and permanent monitoring of exposure.

The monitoring studies may include occupationally exposed workers in manufacture, formulation and field application of pesticides, as well as the general population. However, the most useful data on the effects of pesticide exposure can be obtained by scheduled trials on volunteers or from clinical case reports of accidental poisonings. Each group of the above mentioned studies does not give an all-round picture, but all together may provide sufficient data on the matter.

The exposure to pesticides may be measured *directly*, by means of absorbant pads and clothing, washes and similar techniques. The amount of the chemical trapped or removed is then a direct measure of the exposure. The results of direct measurements of exposure may be of help

Sažetak

KLINIČKA SLIKA, DIJAGNOSTIKA I TERAPIJA OTROVANJA ORGANOFOSFORNIM INSEKTICIDIMA

Izneti su opšti principi delovanja organofosfornih insekticida na ljudima. Posebna pažnja posvećena je kliničkoj slici trovanja, dijagnozi i terapiji. U radu su razmotreni problemi kao što su: vrednost znaka trovanja, aktivnost holinesteraze i naročito terapija upotrebe reaktivatora holinesteraze — oksima, i to na osnovu stranih i naših podataka.

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of poisoning which has to be treated. Thus for example we have found that trimedoxime is even superior as an antidote in experimental poisoning by organophosphorous compounds of GD—42 and GD—7 types (52).

Finally, opinions on the therapeutic efficiency or inefficiency in the treatment of poisoning differ depending on way of penetration of the poison in the body, time of application of the oxime after poisoning and the kind of organophosphorous compounds which should be treated.

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On the other hand some authors (30, 35, 36) propose in an early phase of poisoning one or two administrations of an oxime in an appropriate dose. They stress that reactivation of cholinesterases by oximes is most pronounced early after inhibition and that further injections could only have minor effects. If on the contrary the oximes have no effect at the beginning of therapy of organophosphorous poisoning they remain ineffective.

In our opinion the absence of activity as well as the absence of reactivation by oximes (pralidoxime, trimedoxime and obidoxime) in vitro and in vivo indicates the ineffectiveness of prolonged oxime therapy and the »ageing« of the enzyme-inhibitor complex.

In some cases hepatic damage was seen which was imputed either to the oxime therapy or to organophosphate poison or to both (27, 30, 31, 32, 36).

Animal experiments had shown that high doses of oximes did not produce liver damage, whereas high doses of organophosphate did (36, 37, 38). Also, our results of investigation of oximes (pralidoxime, trimedoxime) in healthy men-volunteers have shown that therapeutic doses of mentioned substances did not affect liver function (22, 25, 26). These controversial facts suggest that further studies are needed in order to reach a clear conclusion about the relationship concerning the usage of oximes and liver damages.

In the past few years, in some cases obidoxime and trimedoxime have been used in the treatment of human organophosphorous poisoning instead of pralidoxime. The explanation lies in the fact that obidoxime and trimedoxime are in many respects superior to pralidoxime as antidotes in animal poisoning with organophosphates (e.g. stronger and more rapid reactivation of inhibited cholinesterase) (39—47). It is interesting to point out that until today obidoxime and trimedoxime have not replaced pralidoxime for use in man, probably because on a weight basis in animals obidoxime and especially trimedoxime show a higher toxicity than pralidoxime.

Comparing obidoxime and trimedoxime it could be very often found in literature that obidoxime is given preference on account of low toxicity in animal experiments on weight basis. However, animal studies have failed to show any superiority of obidoxime over trimedoxime as an antidote in animal poisoning (48—50). On the contrary our results (25) show that 28% of the dose of trimedoxime injected intramuscularly in healthy men is excreted in urine in two hours. This is only approximately half of the amount excreted in the urine after the injection of obidoxime (51). Under conditions where the duration of the oxime level in blood is a critical factor, any advantage which obidoxime might have because of its slightly lower toxicity in animals, is diminished by its shorter persistence in blood and its faster urinary excretion.

It is really very difficult to give an advantage to any of the oximes mentioned because the antidotal action very often depends on the kind

Cholinesterase activity

What relationship, if any, exists between general tissue levels of cholinesterase and erythrocyte enzyme activity is difficult to determine since patients may have blood cholinesterase levels as low as 15% of normal without toxic signs (28, 29). Estimation of erythrocyte cholinesterase activity is theoretically preferred, since it would reflect the degree of inhibition of synaptic cholinesterase (1) and also it is a better parameter for the efficiency of oxime therapy than are plasma cholinesterase activities (30, 31, 32). On the other side, the estimation of plasma cholinesterase has an advantage because the measurement is simpler and more accurate than the estimation of erythrocyte cholinesterase.

However, it should be kept in mind that plasma (serum) cholinesterase, whether tested by sensitized paper or a more elaborate method, is subject to problems of interpretation in view of the changes of pseudocholinesterase found with liver disease, influence of phenothiazines and under other conditions. Another problem concerns what constitutes a normal range of cholinesterase for the method in use. Most studies are based upon standards of activity derived from fresh adult venous (or capillary) blood. These standards of comparison may not be applicable to blood obtained from different age groups not already standardized. To avoid difficulties which may appear in diagnosis of poisoning, especially in the workers occupationally exposed to organophosphorous insecticides, it is necessary to have their enzyme values checked at regular time intervals.

The individual who has been acutely poisoned and has shown marked cholinesterase depression, should not be allowed to return to work with organophosphate insecticides until his cholinesterase activity levels have returned to approximately 75—85% of the normal.

Finally, one conclusion could be drawn from the facts mentioned: that in practice the cholinesterase activity test is more valuable as a confirmatory, rather than a diagnostic procedure. This is why clinician should set on his clinical impression and on the history of exposure rather than wait for the laboratory results.

Oximes therapy

Although the therapeutic principles of poisoning with organophosphate insecticides are largely accepted, several points still remain open to discussion. These points mainly concern the oxime therapy where in some cases reactivation of inhibited cholinesterase was found, whereas it was absent in others. Also, some authors propose a repeated injection of oximes and others give only two injections early in intoxications. Many authors state that the oxime must be given in repeated doses especially in cases when the poison has to metabolize before acting, as for instance nitrostigmin (33) or where a redistribution from other depots (34) or a continuous absorption from the intestinal tract (1) may occur.

3. *Maintenance of patient airway and artificial ventilation*

According to the degree of poisoning picture severity the treatment may also include:

— removal of fluids collected in the mouth and pharynx before medical treatment (atropine, etc.) by suction or by sweeping with cloth-covered fingers and postural drainage;

— a prone, head-down position of the patient;

— elevation of the mandibulae of a patient in the supine position and pulling forward of the tongue;

— insertion of an endotracheal catheter if practicable.

In the moderate to severe case of poisoning, because of pulmonary deficiency, artificial respiration may be needed using a positive-pressure method. As soon as cyanosis has been overcome, atropine and oxime should be promptly given intravenously or intramuscularly. Atropine is contraindicated in a cyanotic patient because of the possibility of inducing ventricular fibrillation.

4. *Other general measures*

When the patient is unconscious, a catheter should be used to observe the urinary flow for early detection of oliguria.

In cases with convulsions a short action barbiturate (e.g. thiopenthal sodium) may be administered intravenously, or after intubation d-tubocurarine, which counteracts the nicotine-like manifestations of organophosphorous poisoning. Morphine, aminophylline and phenothiazines are specifically contraindicated.

Other measures of non-specific therapy should be also undertaken depending on the course of illness (e.g. intravenous fluids, antibiotics, cardiotonics, etc.).

COMMENTS

Signs of poisoning

The most helpful signs in diagnosis of organophosphorous poisoning are miosis (especially due to the local exposure to poison) and muscular fasciculations, which are almost always present in moderately severe or severe poisoning. Other signs that are helpful in diagnosis include excessive sweating, salivation, lacrimation and bronchial secretion. The ingestion of organophosphorous compounds is often followed by severe abdominal pain, diarrhoea and vomiting.

As a guide for the efficiency of antidotal therapy pupil diameter seems to be a misleading parameter (4, 5, 27) because there is no correlation between the appearance of mydriasis and an improvement in the clinical condition. Therefore, dryness of the mouth or red flush of the face could be more useful signs as a guide in the efficiency of antidotal therapy.

ological (primary) function of destroying the accumulated acetylcholine; and second, a complex of the reactivator substance with the anticholinesterase agent is formed which will »neutralize« anticholinesterase before it reaches the active site on the enzyme.

Several substances which accelerate the reactivation of cholinesterase have been established for human use (5, 7—26). They are known as oximes, and among the best known for the treatment of human organophosphorous poisoning are:

— *PAM-2, P2AM, PAM, Pralidoxime, Protopam*
(Pyridine-2-aldoxime methiodide or methchloride)

— *P²S, Contrathion*
(Pyridine-2-aldoxime methyl methane sulfonate)

— *TMB-4, Trimedoxime, Dipiroksim*
(1,1'-trimethylene bis-/4-pyridinium-4-aldoxime dibromide or dichloride)

— *LüH6, Obidoxime, Toxogonin*
(1,1'-oxydimethylene bis-/4-pyridinium-4-aldoxime dibromide or dichloride).

In comparison with pralidoxime, the experience in using trimedoxime and obidoxime in humans poisoned by organophosphorous compounds is relatively insufficient. Although trimedoxime and obidoxime are effective in smaller doses for the treatment of organophosphate poisoning, they are more toxic than pralidoxime.

It is essential for successful management of the treatment that these substances be administered soon after poisoning. This is because phosphorylated enzyme passes rapidly from an unstable phase, when it can be reactivated, to a stable form after which it cannot be reactivated (»ageing«).

The intravenous or intramuscular injection of oximes produces the effect in 5 to 15 minutes. Oximes are also effective when administered orally, but previously mentioned routes are preferred for prompt effect. Pralidoxime should be administered in the dose of 1 to 2 g intravenously or intramuscularly. This should be repeated in one hour if respiration weakens or muscular fasciculation recurs. We would like to stress that a continuous infusion of oxime at rates up to 0.5 g per hour is more effective than repeated single injection of 0.5 or 1.0 g, probably because of the maintenance of continuously higher concentration of this substance in the blood.

Trimedoxime and Obidoxime should be administered in the dose of 0.25 g intravenously or intramuscularly, and all procedures described for pralidoxime could be applied for these oximes as well. However, the combined use of an oxime and atropine is more effective than the application of either of them alone.

TREATMENT

The treatment of acute poisoning by organophosphorous compounds should be started without waiting for the results of laboratory tests. In order to be effective the treatment of poisoning by organophosphorous insecticide should consist of combinations of *antidotal therapy* and *general measures:*

1. *Decontamination*

Generally speaking, a wide range of chemicals could be used as decontaminants, the choice depending upon the particular agent which has to be neutralized, the type of surface that needs to be treated, the extent of contamination and the amount of the time available. In any case the termination of exposure by removal of the patient or application of a protective mask will be necessary if the atmosphere is contaminated.

a) Skin — organophosphate by which skin was contaminated can be destroyed and removed by using solutions of peroxides, hypochlorous acid (Clorox), dilute alkali, soap and water or plain water. By removal of contaminated clothing further contamination is also terminated.

b) Eyes — if organophosphate insecticides have splashed into the eyes, they should be immediately irrigated with water or with physiological saline, or sodium bicarbonate if at hand.

2. *Antidotal therapy*

Atropine — in sufficient dosage antagonizes very effectively the muscarinic-like manifestations of poisoning at periphery and to a moderate extent the central respiratory paralysis and other central actions. It is comparatively ineffective against the autonomic ganglionic actions and it has virtually no effect against the peripheral neuromuscular paralysis. Atropine acts by blocking directly the parasympathetic receptor sites from acetylcholine.

Atropine should be given in »heroic« doses until muscarinic-like symptoms are relieved and signs of mild atropinization (mydriasis, dry mouth and dry flushed skin, heart rate over 140 beats/min.) appear. Following an initial injection of 2 to 4 mg given intravenously if possible, otherwise intramuscularly, the 2 mg dose should be repeated every 5 to 10 minutes until muscarinic-like symptoms disappear, and also, if they reappear. As much as 10 to 50 mg of atropine may have to be administered over a twenty-four hour period (3, 4, 5, 5). A mild degree of atropinization should then be maintained by the oral administration of 1 to 2 mg at intervals of several hours, as long as such symptoms are in evidence.

Cholinesterase reactivators (Oximes) — the therapeutic action of reactivator substances may be explained in two ways: first, reactivation of inhibited cholinesterase will allow the enzyme to perform its physi-

POISONING PICTURE

Organophosphate insecticides may exert their influence locally and generally. Signs and symptoms of such poisoning are attributable mainly to the accumulation of acetylcholine at the cholinergic synapses and may be traditionally classified into muscarine-like (parasympathetic), nicotin-like (sympathetic and motor) and central nervous system manifestations according to the site of action of acetylcholine (Table 2). This division has also some importance in understanding the treatment of poisoning caused by organophosphates.

Signs and symptoms of acute organophosphate poisoning shown in Table 2 are generalized and could serve to recognize the degree of severity of poisoning picture. The most serious manifestation and the usual cause of death is respiratory insufficiency which is a result of the following progression: a) bronchoconstriction; b) depression of the respiratory center; and c) paralysis of respiratory muscles. Cardiovascular function is usually maintained until the terminal stage.

Chronic poisoning by organophosphorous compounds most frequently presents a very complex clinical picture. Thus prolonged exposure to small doses of organophosphorous insecticides (e.g. laboratory workers) may provoke appearance of symptoms referable to the central nervous system (psychic symptoms), peripheral nervous system (paresis, possibly paralysis) and digestive system. These effects may cause serious symptoms, but some of them are reversible upon drug withdrawal and some of them can persist for weeks, months or years.

DIAGNOSIS

The diagnosis of illness or death caused by an organophosphorous insecticide is not simple. Such diagnosis depends upon a great deal of information:

1. *Case history* (For acute poisoning evidence of exposure to organophosphates within the previous 24 or exceptionally 48 hours. For subacute or chronic poisoning evidence of exposure for last weeks or months in connection with occupation.)

2. *Clinical observation* (Signs and symptoms of poisoning shown in Table 2.)

3. *Laboratory tests* (Cholinesterase activity of the blood. Detection of organophosphate or its metabolites in gastric aspirate, skin, blood, urine or clothing.)

4. *Effectiveness of antidotal therapy* (Improvement or recovery from signs and symptoms of poisoning.)

Inhibition of cholinesterase in blood is a specific test for proving systemic absorption of organophosphate compounds. Normal cholinesterase activity of the blood excludes poisoning by these compounds.

It could be very often seen in literature that severity of poisoning, beside clinical manifestations, is classified on basis of the degree of cholinesterase inhibition in blood (plasma and erythrocytes). Also, the level of cholinesterase inhibition serves as a guide for prognosis of poisoning and efficiency of therapy. Generally, signs and symptoms of acute poisoning occur when more than 50% of the plasma or erythrocyte cholinesterase is inhibited (1, 2).

Table 2

Signs and symptoms of acute organophosphate poisoning

Muscarinic-like manifestation:	
Ocular symptoms	Miosis, occasionally unequal, blurring of vision, headache (ciliary pain);
Glandular symptoms	Increased sweating, lacrymal, salivary, nasopharyngeal and bronchial secretion;
Bronchial tree and pulmonary symptoms	Sensation of constriction, possibly light pain in the thorax, increased secretion with coughing, possibly pulmonary edema, dyspnea, cyanosis;
Cardiovascular symptoms	Bradycardia, fall in blood pressure, initially dilatation of blood vessels of skin and mucosa;
Gastrointestinal symptoms	Anorexia, nausea, vomiting, tenesmus, abdominal pain, diarrhoea, involuntary discharge of feces;
Urinary tract symptoms	Pollakisuria, possibly involuntary discharge of urine;
Nicotinic-like manifestation:	
Striated musculature symptoms	Fatigue, flaccidity, twitching, fasciculation, convulsions;
Sympathetic ganglia symptoms	Tachycardia, initial elevation of blood pressure, pallor (constriction of blood vessels of skin and mucosa);
Central nervous system manifestations:	
	Giddiness, anxiety, tension, emotional lability, excessive dreaming, insomnia, nightmare, headache, tremor, apathy, difficulty in concentration, confusion, slurred speech, ataxia, phobia, hallucinations, generalized weakness, convulsions, coma with absence of reflexes, Cheyne-Stokes respiration, depression of respiratory and circulatory centers;

inhibition, noncompetitive inhibition, or a combination of the two. Inhibitors with reversible effects may be removed by dialysis; a free equilibrium exists between enzyme and inhibitors, which is not the case with inhibitors of irreversible effects. They form loose or firm complexes with enzyme which may be reactivated in certain cases. Thus the term »irreversible« is correct in a limited sense of the word.

Several of organophosphorous insecticides are rather weak anticholinesterases in themselves. In the organism, however, they are transformed into very strong inhibitors (e.g. parathion into paraoxon, malathion into malaoxon, etc.).

Cholinesterases are classified into two types: acetylcholinesterase (true cholinesterase, specific cholinesterase) and butyrocholinesterase (pseudo cholinesterase, non-specific cholinesterase).

In man, synapses, erythrocytes, nerves and skeletal muscles contain mainly acetylcholinesterase; and serum (plasma) and liver contain mainly pseudocholinesterase. The function of non-synaptic cholinesterase is not known. Clinically, reduction of serum cholinesterase (synthesized in the liver) is used as an indicator of liver disfunction. A genetically inherited syndrome resulting in reduction serum cholinesterase occurs, and individuals with this syndrome are hypersensitive to succinylcholine, a cholinesterase inhibitor, used as a muscle relaxant in anesthesia.

Very low concentrations of organophosphorous compounds inhibit the activity of both types of cholinesterases. Inhibition of these enzymes by organophosphate is a result of firm binding of phosphate radicals of organophosphates to the active sites of the enzymes, forming phosphorylated enzymes. The pharmacologic and toxicologic effects of organophosphates are primarily, if not entirely, due to inhibition of acetylcholinesterase of the nervous system, resulting in accumulation of acetylcholine at the synapses. The over-abundance of acetylcholine initially stimulates and then paralyzes transmission in cholinergic synapses, sparing adrenergic synapses.

Today acetylcholine is supposed to be of importance for:

— transmission of the nervous impulse,
— neurohumoral transmission,
— permeability of membranes,
— local hormonal function (e.g. in the heart).

Phosphorylated cholinesterase complex is inactive and very stable in contrast to acetylated cholinesterase which is a normal complex in organism produced during decomposition of acetylcholine. Spontaneous hydrolysis of the phosphorylated enzyme takes place only to a very short extent. Consequently cholinesterase activity does not reach the normal level till after the formation of new enzyme. This process takes several weeks, possibly months, depending upon the kind of organophosphate which produced inhibition of cholinesterase.

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CLINICAL PICTURE, DIAGNOSIS AND TREATMENT OF POISONING BY ORGANOPHOSPHATE INSECTICIDES

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General principles of organophosphorous insecticide poisoning in humans have been reviewed in this paper. Special attention has been paid to the clinical picture of poisoning, its diagnosis and treatment. Discussion of such problems as the importance and evaluation of the signs of poisoning, cholinesterase activity and oximes therapy has been based upon foreign and our experience.

During the Second World War chlorinated hydrocarbons and organophosphates were introduced as insecticides. These compounds enjoyed wide use, particularly DDT which has low toxicity. Recently chlorinated hydrocarbons have attracted public attention as a possible ecological hazard, because these compounds have accumulated unchanged on the earth and in the adipose tissue of mammals. Chlorinated hydrocarbons are stable and very slowly metabolized. Although there is not enough evidence at present that DDT or other chlorinated hydrocarbons in the tissues of human beings are harmful, there are opinions that the concentration may eventually become intolerably high. This concern was dramatically portrayed by Rachel Carson in her book »Silent Spring«. Further apprehension for the ecological problem in general led the USA and some states in Europe to take measures to reject DDT except for uses essential to human health and welfare.

Since future and civilization are greatly dependent on insecticides in agriculture, forestry and vector control, particularly in malaria control, substitutes for chlorinated hydrocarbon insecticides are required. Presently organophosphate insecticides are considered to be the most practical substitutes because they are potent insecticides and are relatively rapidly hydrolized after application. Many organophosphate compounds have been synthesized in the past 30 years and many of them have been widely used as agricultural insecticides. Useful as they are, organophos-

The following relevant facts should be pointed out. The workers were not informed about the origin of pesticide or about the danger of intoxication. The self protective measures were very poor. The wind blowing in their faces brought a large amount of pesticides in their nose, throat and lungs. All these facts led to acute intoxication, as it happened.

CONCLUSIONS

A correct evaluation of the degree of occupational risk in the production of pesticides by the classical methods of industrial hygiene control is hardly possible.

It is necessary to establish the maximum allowable concentrations concerning the mixtures of the powdered toxic dusts.

In the application of pesticides, the medical and technical protective measures ought to be more effective and strictly carried out. Better information about the toxicity of pesticides is important. Correct use of protective devices is necessary.

In patients poisoned by organophosphorus and organochlorine pesticides an evident but transitory lesion of liver parenchyma was found. This phenomenon could be a consequence of the simultaneous influence of various pesticides since no correlation between clinical signs and the activity of cholinesterase was established.

Ophthalmological control is very useful in workers exposed to pesticides. Therefore the influence of pesticides on the eye ought to be investigated thoroughly.

Further investigations are necessary regarding a possible synergetic effect of toxic compounds on the liver and bone marrow during the production and application of pesticides especially in the course of chronic exposure.

Sažetak

OPASNOST ZA ZDRAVLJE U PROIZVODNJI I PRIMJENI PESTICIDA

Prikazani su rezultati višegodišnjeg praćenja radnih uvjeta i zdravstvenog stanja radnika jedne industrije pesticida u Srbiji. Izvještaj obuhvaća i podatke oftalmoloških pretraga jednog dijela izloženih radnika kao i deset slučajeva otrovanja u toku primjene pesticida. Istaknute su teškoće pri ocjeni opasnosti kada se radi o istodobnoj izloženosti komercijalnim pripravcima različita mehanizma djelovanja.

U zaključcima su dani prijedlozi za djelotvorniju preventivu profesionalnih otrovanja kao i za temeljitiji studij mogućih kombiniranih učinaka pesticida pri kojem se, po mišljenju autora, valja koristiti i oftalmološkim nalazima.

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