



**Checklist for medical practitioners
involved in the monitoring of cholinesterase inhibition
of pesticide handlers**

**Procedures for use of the
Test-Mate™ ChE handheld spectrophotometer**

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**Wim C. Mullié
James W. Everts**

**CERES-Locustox Foundation
Regional Centre for Ecotoxicology and Environmental Safety
Km 15 Route de Rufisque
DAKAR-Sénégal**

INTRODUCTION

Organophosphate insecticides (OP-esters) are commonly used in Locust and avian control. Between 1988 and 1995 alone, Senegal's national Plant Protection Service (DPV) conducted about 11,000 phytosanitary treatment operations, especially against Grasshoppers, using 500,000 liters Ultra Low Volume formulations. In most of these treatments, organophosphates were used (Dossou & Mullié 1997). During the invasions of Desert Locust *Schistocerca gregaria* the doses of pesticides used are greater. For example, between November 2003 and June 2004 1.7 million hectares were treated against the Desert Locust in Morocco, >90% with organophosphates (CNLAA data). The Locust Pesticide Referee Group states that operators must be well trained and subject to mandatory health monitoring (FAO 2021).

Exposure to organophosphates or carbamates, can intoxicate people by inhibiting the activity of cholinesterase enzymes. Therefore personal protection is necessary to avoid exposure through one of the common routes: oral, dermal or by inhalation (FAO 1990).

Exposure to cholinesterase inhibitors can be detected by an acetylcholinesterase (AChE) assay in erythrocytes or a butyrylcholinesterase (ChE) assay in plasma or blood serum. If blood levels of AChE decrease significantly, the operators must be given rest or alternative tasks until they have fully recovered (FAO 2021).

Cumulative cholinesterase inhibition, as observed with OP-esters, does not occur with carbamates. For the latter, the inhibited enzymes are being reactivated quickly and completely. Thus, a cholinesterase assay after exposure to carbamates may underestimate actual exposure (Jeyaratnam & Maroni 1994).

Effects on cholinesterase activity in plasma and erythrocytes have different toxicological significances. The former is an indicator of exposure to cholinesterase inhibitors, the latter indicates the existence of acute toxicological effects. Because some organophosphates cause higher cholinesterase inhibition of activity either in erythrocytes or in plasma, it is strongly advised to assess both activities (OEHHA, 2017). Chlorpyrifos and carbamates, for example, are specifically inhibitors of plasma activity.

GUIDELINES FOR MEDICAL PRACTITIONERS¹

Pre-exposure medical examinations

During the first examination of a worker, the doctor must have information about previous exposures (Form 1 in the appendix) and conduct a physical examination. Because of the high risks for individuals suffering from respiratory, hepatic, or cardiovascular diseases, the doctor will inquire about the worker's health history such as active peptic ulcer and bronchial asthma, which can be negatively affected by cholinergic reactions. Other conditions such as anemia, degenerative diseases of the central nervous system, chronic colitis, psychosis, and diseases like myasthenia gravis and glaucoma treated with cholinesterase inhibitors, may lead to complications. Individuals with congenital cholinesterase deficiency, show exceptionally low ChE activity in plasma. Even so, these people can handle organophosphates and carbamates normally.

Monitoring of cholinesterase levels ("Monitoring")

Monitoring of handlers of OPs and carbamates who continually work with pesticides belonging to WHO classification categories I, II or III (WHO, 2020) is indicated when exposure may

¹ For more complete instructions, see OEHHA, 2017

have a cumulative effect. This is the case with applicators in locust or bird control. The cholinesterase test is the only instrument available to the doctor for the medical supervision of workers. Like any biological monitoring, the cholinesterase test has two functions. First, the detection of serious individual exposure *before* the person gets sick. Then the detection of adverse working conditions, induced by a slight decrease in average levels among all workers in each workplace as is the case, for example, for a treatment team.

Blood sampling and cholinesterase tests are done by a trained agent, with a small handheld spectrophotometer, the Test-mate™ ChE kit (EQM Research, Inc.), suitable for field use. Instructions for its use will be given in the procedures annexed to this document. Test-mate ChE is currently used in Senegal, Mauritania, Morocco, and Niger as part of locust control monitoring. See Mamadou et al. (2008) for an example of its application in Niger.

The ChE Test-mate has also been validated with the blood of cattle: goats, sheep, oxen, horses, and camels. For monitoring procedures and constraints see Mullié & Touré (2000). Sheep are particularly useful for monitoring. A prototype of a Test-mate analyzer specifically adapted for animal blood was successfully used by Cheke et al. (2012) on Red-billed Quelea *Quelea quelea*. However, this analyzer has never been commercialized.

The individual baseline level and sampling

For the monitoring of ChE activity in an applicator, the Individual Baseline Level is established first. For this we take blood, before the person starts using pesticides. That is, the person must not have been exposed to a cholinesterase inhibitor for at least 30 days. If this is not possible, a *Preliminary Baseline Level* will be established and information on the previous exhibition will be included in the personal file of the person concerned.

The baseline level is established by taking blood samples twice, with an interval between a minimum of three days and a maximum of 14 days, and then the average of the two values for cholinesterase activity is calculated. If the difference between the two values is of the order of 15% or more, a third sample is taken. Then the average of the two closest values is used as the base level.



Blood collection after finger puncture

The test is extremely sensitive to the presence of pesticides. Contamination of the blood with residues present on the skin can give unreliable results. To eliminate this risk, venous blood can be used, instead of capillary blood that is obtained by puncture on the fingertip. The use of venous blood also has the advantage of being able to do the analysis in a laboratory, which reduces errors and thus gives more reliable results. Because of the inconvenience that this can be for patients, one can choose to use capillary blood. That is why the Test-mate is supplied with micro-capillary tubes. In this case, it is particularly important that the people to be tested have clean hands, especially fingers, and well washed with soap and water, before blood is taken.

Permissible inhibition levels and exposure spacing

According to WHO recommendations, an inhibition of 20% from the baseline level means exposure and a marked lack of protection. Sustained monitoring is sufficient to limit exposure, as treatment is not required.

An inhibition of 30% in the level of activity of erythrocyte AChE, or 40% in plasma PChE, is considered a biological limit, and the persons concerned should be excluded from the source of exposure, especially if a second test after 24 hours confirms these results. These people can only

return to work when cholinesterase activity returns to a value of 80% compared to the individual baseline level. With an erythrocyte turn-over of about 1% per day, normally these people can resume their activities after 7-10 days. A new test is required to confirm this decision.

An inhibition of 60% or more of AChE means serious intoxication and it is often, but not always, accompanied by symptoms of poisoning (see below). The victim must be placed under medical observation and excluded from exposure for the remainder of the spraying season **CAUTION:** In the case of chronic exposure, symptoms are sometimes absent, even with a significant inhibition.

The employer shall keep data for three years on the dates of exclusion due to exposure to a cholinesterase inhibitor and of the employee's return to work.

Frequency of cholinesterase tests

During the campaign, a period when pesticide handlers are exposed every day to organophosphates and carbamates, cholinesterase tests will be done at intervals as indicated below: The first test for a given person is done ten days after the start of the treatments in which he participated. In the case of daily exposure, the following tests are done with intervals of 10 to 14 days. If the results of the first three tests do not show a decrease in cholinesterase activity, the doctor may decide to increase the interval between the tests that follow. On the other hand, if the results show that serious exposure occurs, presenting a danger to health, the doctor may reduce the sampling intervals.

A computerized treatment recording system, incorporating information on the quantities and types of pesticides used daily by a given applicator, can help show relationships between the amounts of pesticides handled (as an estimate of exposure) and the effect on the rate of cholinesterase activity. Based on a study conducted in Senegal where this relationship was shown (Mullié *et al.* 1997), a database was developed for monitoring applicators in the field. The advantage of such an *ad hoc* approach, compared to *routine* monitoring that is done at fixed intervals, is that cholinesterase tests are done based on actual exposure. Especially during the invasions of Desert Locust, an *ad hoc* follow-up can mean better protection of the applicators. For an indication of the costs of an *ad hoc* monitoring program in Mauritania, see Aston (2000) and Aston *et al.* (2000).

Cholinesterase tests should be repeated whenever a worker becomes ill while handling organophosphates or carbamates, or after symptoms develop within 12 hours of the last exposure.

Recommended treatments in the case of poisoning²

Organophosphate insecticides

SIGNS AND SYMPTOMS OF POISONING: Exhaustion, weakness, confusion, vomiting, abdominal pain (cramps), cold sweats, salivation, oppressed chest, muscle contractures of the eyelids and tongue, followed by greater contraction of muscles, muscle weakness, diarrhea, bronchial hypersecretion, bronchospasm, bradycardia, narrowed pupil.

IN SEVERE CASES: convulsions, cyanosis, excessive sweating, incontinence, mental rambling, heart and respiratory failure, coma.

SPECIFIC TREATMENT: Organophosphate compounds are cholinesterase inhibitors and treatment should be conducted quickly. So, it is necessary to keep the patient strictly at rest!

² Based on GIFAP (1984). See USEPA (1989) and Plestina (1986) for comprehensive guidelines.

1. Support respiratory and heart functions. Give oxygen, if necessary.
2. Immediately give intravenous atropine sulfate at a dose of 2-4 mg (two to four milligrams) for an adult, and repeat the injection at intervals of 5-10 minutes, until signs of atropinization appear (for example, dry mouth, tachycardia, and normally dilated pupils). Maintain atropinization for at least 24-48 hours and carefully monitor the patient when the treatment is finished: it may be necessary to restart treatment in case of recurrence of symptoms of poisoning.
3. If organophosphate poisoning is observed within 12 hours, slowly give a 1 g oxime preparation (for example, pralidoxime chloride) by the venous route. This medicine should NOT be administered alternately or as a replacement for atropine, the use of atropine is ESSENTIAL.
4. Monitoring of the evolution of the patient's condition is imperative. Drug treatment may be necessary for up to 10 days, in severe cases.
5. Anxiety can be treated by administering 5 to 10 mg of diazepam intramuscularly.

Insecticides: carbamates

SIGNS AND SYMPTOMS OF POISONING: The same as those observed in the case of organophosphate poisoning, however, the manifestations tend to be faster and of a shorter duration.

TREATMENT: Atropine therapy, described for organophosphate compounds, is necessary.

N.B. OXIMES ARE NOT INDICATED IN THE CASE OF CARBAMATE POISONING.

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Rapid procedure for the determination of erythrocyte and plasma cholinesterase with the Test-mate™ ChE kit (EQM Research, Inc.)³

The Test-mate™ ChE kit is developed for **the field** determination of exposure to cholinesterase inhibitor pesticides. With this test, the values of erythrocyte cholinesterase (AChE), plasma cholinesterase (PChE) and hemoglobin (Hgb) can be measured with a whole blood sample. Results must be evaluated by qualified individuals, such as trained physicians, nurses, or public health officials.

The Model 460 AChE Assay Kit is used for the measurement of erythrocyte cholinesterase and the Model 470 PChE Assay Kit is used for measuring plasma cholinesterase. The type of cholinesterase inhibitor to which the patients have been exposed determines which assay kit to use. Often monitoring both types of cholinesterase is useful. The Test-mate ChE system is initially supplied with the AChE assay kit.

Content

Each test kit contains three boxes and a package insert. Boxes No. 1 and No. 2 contain 48 test tubes each, containing the buffer solution. Box no. 3 has a 96-well plate containing the substrate, 100 microcapillary tubes of 10 µl volume, 100 filter papers (to clean the microcapillaries after sampling), a small bottle filled with 18 ml of distilled water (to dissolve the substrate), two transfer pipettes and a 9-volt battery.



The plate with the substrate in the AChE test kit has a red label showing "Erythrocyte", while the plate in the PChE test kit has a blue label saying "Plasma". Also, the transfer pipettes in the AChE kit wear a red ring, and those in the Plasma kit wear a blue ring.

Never interchange the reactivist plates and transfer pipettes between the different kits.

Reagents

1. **Buffer:** 2 ml per test tube. Contains phosphate, surfactant and EDTA as preservative.
2. **Reactive solvent:** 18 ml of distilled water and preserving EDTA in a gutter bottle.
3. **Reagents for erythrocyte cholinesterase:** ("AChE Erythrocyte Cholinesterase Assay Kit"), lyophilized substrate, 96 per plate. Store the freeze-dried substrate between 15 and 30°C, protect against light. Reconstitution with three drops of reactive solvent (distilled water with EDTA). Stable for 72 hours between 15 and 30°C after reconstitution. Final test included : 1

³  Text mainly from: Test-mate ChE Cholinesterase Test System (Model 400). Instruction Manual. EQM Research, Inc. 2814 Urwiler Avenue Cincinnati, Ohio 45211 USA Manual-E (27-APR-03) © 2003. The Manual must be downloaded here: <http://www.eqmresearch.com/Manual-E.pdf>

mM AcTC, 0.3 mM DTNB, 20 µM As1397, 50 mM Phosphate de Potassium et 0.1% Triton X-100, pH 7.6.

4. **Plasma cholinesterase reagents:**("PChE Plasma Cholinesterase Assay Kit"), lyophilized substrate, 96 per plate. Store the freeze-dried substrate between 15 and 30°C, protect against light. Reconstitution with three drops of reactive solvent (distilled water with EDTA). Stable for 72 hours between 15 and 30°C after reconstitution. Final test included: 2 mMBuTC, 0.3 mMDTNB, Potassium Phosphate and 50 mM 0.1% Triton X-100, pH 7.6.

The Testmate ChE

1. Install the 9-volt battery in the bottom compartment of the Testmate ChE. The battery is in box no. 3. Replace the battery after each 96 scans (whole kit).
2. Turn on the device with the key on the right side of the device. A sound "Beep" announces the display, for about three seconds of the version number of the internal software. At this moment, the microprocessor makes the internal diagnosis to ensure the correct operational conditions. The battery saver feature of the analyzer will turn the instrument off should it be left unattended for more than 30 minutes.
3. After the internal test, the screen shows the current test mode, ambient temperature (°C) and battery voltage. "AChE" indicates that the device is ready for erythrocyte cholinesterase analyses; "PChE" indicates that the device is ready for plasma cholinesterase analyses. The device corrects the results automatically for room temperature. Try to use the device between 20 and 30°C (optimum). 25°C The operation automatically freezes when temperatures are at < 15°C or > 35°C.
4. To control the Testmate ChE, three keys are at your disposal. With the "MODE" key we make the selection between the AChE and PChE functions. Always check the mode, before starting a work session: the device remembers the last mode used. To return to the basic screen, after performing a full scan and recording the results, the "DONE" key is pressed. With "DONE" you can also interrupt a test procedure, in case of error. To start a test, we use the "TEST" button.

The test procedure

I Start

Anyone subjected to this test should wash their hands and especially their fingers with soap and water to avoid contamination of the blood sample with pesticide residues that may be on the skin.

1. Turn on the Testmate ChE. Use the "MODE" key to select the AChE or PChE procedure. Press test to begin the analysis. The screen displays "INSERT NEW TUBE" followed by "PRESS TEST".
2. Insert a new test tube (with the pad) into the Testmate. *Remove the sticker first.* The outside of the test tube shall be clean and dry. Clean with a cloth as needed and handle the tube with its cap. Press "TEST." The sequence for the "Blank" ("BLANKING") begins for about 10 seconds.
3. After a sound "Beep" the screen displays a request to remove the tube ("REMOVE TUBE") and then press "TEST". Place the tube in the rack. The screen now displays "ADD BLOOD", "INSERT TUBE" and "PRESS TEST".

II Blood sampling

*Always wear vinyl gloves, such **as no talcum powder**, when handling blood.*

1. Dispense a single capillary tube by inverting and gently shaking the container of capillaries (before the first capillary in a new assay kit is being used, the white shipping plug under the silver screw cap must be removed and discarded).
2. Hold the capillary horizontal between the thumb and first finger and then insert the end of the capillary into the drop of blood on the patient's finger. Slowly the blood will fill the capillary.
3. Once the capillary is completely full, withdraw the capillary. Carefully wipe any blood from the outside of the capillary by rolling the end of the capillary over a filter paper.
4. Place the sterile gauze pad on the patient's finger and have the patient hold it in place until the lanced finger stops bleeding. Apply a small round bandage to the puncture site.
5. Insert the capillary into the vial of the assay tube and then screw the cap back on tightly. Hold the assay tube by its white cap and shake it vigorously for 15 seconds to disperse the blood.

III Test

1. Presser "TEST" et un message "READING" sera affiché pendant 10 secondes, suivi par "REMOVE TUBE" et "PRESS TEST".
2. Remove the test tube, place in the rack, and remove the cap. Press "TEST". The screen asks to add the reagent ("ADD REAGENT").

3. Dissolve the substrate with three drops of distilled water and mix with the transfer pipette. *Check the color of the ring around the pipette!* Then just the substrate in the open tube using the transfer pipette, **without touching the solution in the tube** with the pipette. After transferring the substrate, squeeze "TEST" immediately and close the tube with its cap. The screen displays "SHAKE TUBE" and "PRESS TEST".
4. **Invert** the tube gently for 5 seconds to mix the substrate with the blood and tampon. *Align the capillary towards the black spot on the device* and press "TEST".
5. After the first "PRESS TEST", the device begins to count for 80 seconds an incubation period ("INCUBATION"), then the reading ("READING") is done for 50 seconds, followed by an audible "beep" and a message asking to remove the test tube ("REMOVE TUBE"). Remove the tube for later destruction (blood!). Press "TEST".

IV Records

- 1, Record the results, including room temperature, on a form. With the "TEST" key the different results will be displayed successively. When you are done with the results, press "DONE" to finish the test. Now the device is ready for the next test.

Note: Sometimes, three beeps indicate errors in the test. Carefully read the displayed messages (such as "BLOOD DELAY", "REAGENT DELAY", etc.) and redo the test, as needed.



ANNEX

List of equipment (the material coming with the Test-mate™ Models 460 and 470 is indicated in *italics*).

Purpose	Quantity
<i>For blood sampling by finger puncture:</i>	
<i>hardware in kit</i>	96
sterile lancets	100
<i>10µl heparinized capillary tubes</i>	100
<i>For venous sampling (also for veterinary use):</i>	
Sterile vacutainer 4.5-5.0 ml (3 ml) heparin/lithium (cap color green)	100
Sharpeners for use with vacutainer (e.g., 0.8x38 mm, green)	100
Picking rack with vacutainer	1
<i>Tubes rack</i>	1
Surgical alcohol 70%	250 ml
Cotton	package
Non-sterile gauze of 5x5 cm (packs of 100)	200
<i>Preferably:</i> cooler with cooling elements (for storing blood tubes)	1
Self-adhesive labels for vacutainers	100
<i>Other needs:</i>	
<i>Battery 9 volts, alkaline, "Type Duracell"</i>	2
Plastic bags for disposing of contaminated material with blood	10
<i>Buffer solution (in test tubes)</i>	96
<i>Reagents for 96 plasma cholinesterase tests</i>	1
<i>Reagents for 96 plasma cholinesterase assays</i>	1
<i>Reagents for 96 erythrocyte acetylcholinesterase tests</i>	1
<i>Spectrophotometer (Test-mate™ ChE analyzer)</i>	1
<i>Transfer pipette (with red and blue rings)</i>	2
<i>Wooden or plastic rack</i>	1
200 ml bottle for waste	1
Non-sterile and powder-free vinyl gloves,	10 pairs
<i>Container containing the Test-mate™ ChE kit</i>	1
<i>Manual Test-mate™ ChE kit in English</i>	1
Absorbent paper (kitchen roll)	2
Sheets for cholinesterase assay results	10
Individual records	number of people to be assessed

**Control of erythrocyte cholinesterase activity
in treatment teams**

Tracking sheet 1

Historical

Region: **Personal code:**

Name: **First name(s):**

Date of birth:

Size: m **Weight:** Kg

How long have you been spraying: months/years.

Training received: **Yes / No**

If yes, duration + year: Type of training:

Have you sprayed or had any contact with pesticides in the last three months? **Yes / No**

If so, what pesticide: Type of contact:

Did you participate in the breakup this year? **Yes / No**

If so, when:

Have you ever had a poisoning problem with a pesticide **Yes / No**

If yes, when:

Pesticide used:

Type of formulation:

Treatment apparatus:

Symptoms observed:

General practitioner:

Place of care:

Evolution of symptoms:

Comments:

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**Control of erythrocyte cholinesterase activity
in treatment teams**

Tracking sheet 2

Applications

Region:

Personal code:

Name:

First name(s):

Date of treatment:

Treatment site: **Coordinates:** N O

1. Work performed:

2. Task: **Applicator / Driver /**

Team composition (names):

.....

3. Product + formulation used:

4. Quantity applied: Liters

5. Departure time from base: Time of return to base:

6. Start time of treatments: End of treatments:

1. 1.

2. 2.

7. Type of treatment gear:

8. Protection used:

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9. Weather: *Temperature:*°C

Windspeed: m/s

Relative humidity:%

Miscellaneous observations:

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