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A Study of the Safety of *O*-Isopropoxyphenylmethylcarbamate in an Operational Field-trial in Iran *

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During an operational field-trial which was conducted as a part of the WHO Programme for Testing and Evaluating New Insecticides, a study of the safety of o-*isopropoxyphenylmethylcarbamate (OMS-33) was carried out. Clinical observations associated with biochemical studies were performed. Minor reactions to over-exposure to OMS-33 were recorded among some spraymen and a few inhabitants. Their incidence was, in operators, mainly associated with heavy skin contamination and insufficient washing during work, or, in inhabitants, with entering the house while it was being sprayed. No cumulative inhibitory effect could be demonstrated on whole-blood or plasma cholinesterase in operators during the 6-week exposure. A pronounced fall in whole-blood cholinesterase activity during the work and a distinct recovery after exposure ceased was established as a daily pattern of the enzyme's activity fluctuation, erythrocyte cholinesterase being much more sensitive to OMS-33 than plasma cholinesterase. In view of the very marked symptomless daily fluctuation in cholinesterase activity and the absence of cumulative inhibitory effect, the conclusion was reached that routine cholinesterase determination has little if any practical value as an early indication of serious exposure to OMS-33. Minor complaints, from which recovery is rapid, serve as an early indication of over-exposure.*

OMS-33 can be used safely in malaria eradication programmes provided proper attention is paid to the exercise of those measures of general and personal hygiene which should be practised in any spraying programme.

In the WHO Programme for Testing and Evaluating New Insecticides, 7 stages have been established for evaluation of candidate insecticides and the hazards involved in their application (WHO Expert Committee on Insecticides, 1967). The compound, *o*-isopropoxyphenylmethylcarbamate (OMS-33) [5] has been found to be a promising residual adulticide against malaria vectors and has reached stage VI in this Programme. A 6-week operational field-trial was performed with OMS-33 in the Shabankareh area in southern Iran in May and June 1967. Apart from the entomological evaluation of this compound, investigations were carried out to verify the safety of OMS-33 for operators when the insecticide was sprayed under true operational field-conditions over a prolonged period. Its safety for inhabitants of sprayed houses, which had been established in smaller trials (Vandekar & Svetličić, 1966) [6] was also to be confirmed.

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[5] Other designations for this compound include arprocarb and Baygon.

[6] Also Arnan, A. (1964), unpublished WHO working document TOX/64/2: A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

Changes in activity of the blood cholinesterase in operators were studied by a laboratory method in the course of exposure and at the same time an evaluation was made of the adequacy and usefulness of 2 field-methods for determining cholinesterase activity.

The information gained in this trial has shown that OMS-33 can be used safely under operational conditions, provided that spraying operators wear protective clothing and observe precautions which consist mainly of good personal hygiene and a proper spraying technique.

DESCRIPTION OF OPERATIONAL AREA

The whole area, approximately 300 km², is situated on a plain, 60 km north of the Persian Gulf. Some villages are located near to one of the two main rivers that cross the area, other villages are up to 10 km from the river. The area has a long, hot, dry summer, the temperature may reach 50°C, and the rivers often dry up completely in some parts of their course.

The operational area included 33 villages with a total population of about 11 000.

The walls of the house are made of mud and the majority of the roofs are of the same material although some consist of palm thatch. Only a few houses have walls finished with plaster and chalk. The floors are of sun-dried mud bricks or clay, but may occasionally be made of stone. The houses contain 2–5 rooms. Stables are usually built close to the houses and frequently inhabitants and domestic animals share the same room. Ceilings are usually 2.5–3 m high and are made of trunks and fronds of palm. There are usually a few narrow holes in the wall for ventilation.

Near to each house are one or two summer huts constructed of palm thatch with a low mud wall; these are used by both people and their domestic animals (goats, sheep, poultry).

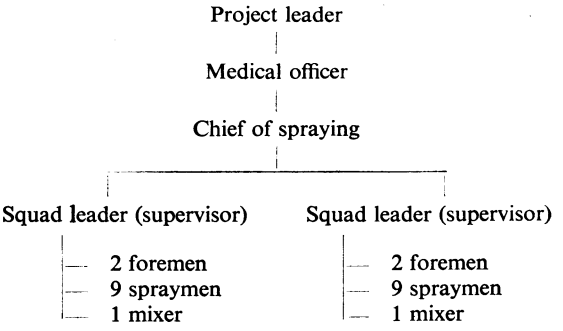
Drinking water is obtained from one or more deep wells, situated in each village. During the summer months shortage of water is a great problem in villages remote from the river.

MATERIALS AND METHODS

Planning and safety precautions

Detailed instruction sheets, translated into the Iranian language, had been prepared to provide field-personnel at all levels with information about the safe use of OMS-33.[1]

From a total of 35 candidates, 24 operators aged between 18 and 38 years were selected; 15 of these had received 6–9 years' education but only 6 had any previous experience of spraying. While the chief of spraying and 2 squad leaders were deputed by the Malaria Eradication Organization, Iran, the foremen were chosen from the most capable operators. The structure of squads and supervision was:



A 6-day training session was established for all spraying personnel, who were informed about possible hazards and were instructed in the way to handle the insecticide with the least risk to themselves and to inhabitants. Residents of each house were instructed by foremen and squad leaders in the matter of observing the precautions which would limit their exposure to the insecticide.

When working, spraymen and foremen wore overalls, a cap with an eye-shade, canvas shoes and a surgical-type mask. The mask was made from double gauze fixed with an upper band only; it covered the nose, mouth and face with the free lower edge reaching about 2.5 cm below the chin. Mixers also wore a polyethylene apron, rubber gloves and rubber boots, and their masks were fixed with 2 bands. The operators changed from overalls into their normal clothes within 1 hour of completing their day's work. The overalls were washed at least twice a week. However, whenever spraying was performed near the river, operators washed their protective clothing every afternoon. Two face masks were given to each worker; the clean one was used after the morning

[1] Vandekar, M., Hedayat, S., Pleština, R. & Ahmady, G. (1967) Annex to unpublished WHO working document VBC/67.51. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

break and the masks were washed every day. As a rule, when cleaning nozzles and handling polyethylene sheets, the foremen wore rubber gloves. Soap and water were provided close to the pump-refilling site to allow operators to wash their hands after completing the spraying of each pump-charge (i.e., 10 litres).

Some precautionary measures were intentionally relaxed during certain periods of spraying, namely, (1) during the last week, operators from the first squad worked 7–8 hours a day and washed their hands and face only twice during the day's work, and (2) during the last 2 weeks, spraymen from the second squad did not wear face masks and the mixer and foremen did not wear either masks or gloves. During 2 days of special studies, the foremen washed their hands only before the break and at the end of the day's work.

The spraying technique was supervised carefully by the squad leaders and foremen in each squad. Additional instructions given in the field were related mainly to more frequent washing of operators' hands and to complete emptying of houses before spraying. The operators were fined in several instances when obvious carelessness was encountered.

During the first week of the operation when the 2 squads sprayed villages close to each other, the medical officer was for most of the time in those villages being sprayed. Later on, however, true operational conditions were maintained as far as possible, with only occasional visits being made by the medical officer to the sprayed villages. Atropine injectors were held only by the medical officer, but each squad leader was given 100 ml of tincture of belladonna together with appropriate instructions for its use.

The insecticide, OMS-33, was stored in 4 villages in 25-kg capacity cylindrical steel barrels, and was kept in locked stores until it was used. On the same day that the barrels were emptied, they were washed in the river and stored. At the end of the spraying operation, all empty drums were washed a second time, under strict supervision and returned to store.

Exposure to insecticide

A water-dispersible powder (WDP) containing 50% of OMS-33 as the active ingredient [1] was used in this trial and applied at a target dose of 2 mg of active ingredients par m^2 of surface. The insecticide was weighed immediately before being mixed with water (1 kg of WDP to 10 litres of water). The suspension was prepared in a drum of about 200-litre capacity. The required amount of insecticide was first added to a small quantity of water to produce a homogeneous paste, then water was added to the paste until the correct dilution was achieved.

The spraying operations lasted 6 weeks and a total of 11 844 structures, including houses, stables, temporary shelters, etc., were sprayed. Spraying cycles were established in which spraying was carried out for 6 days and the equipment was checked on the seventh day; this checking lasted 2–3 hours. Usually, the spraying started between 05.00 and 05.30 in the morning and lasted until 11.00–11.30. A 30-minute break from about 08.00–08.30 was maintained throughout the spraying operations. Part of each afternoon was devoted to washing overalls and transporting equipment to the next village to be sprayed.

The duties of the foremen included protecting the villagers' immovable possessions against splashes of insecticide by the use of large sheets of polyethylene. Repeated use of these sheets throughout the day led to the upper side becoming covered with a heavy deposit of insecticide. Foremen were also responsible for cleaning the spray nozzles when it became necessary to do so.

The number of hours worked each day and the number of pump-charges sprayed (for spraymen) or prepared (for mixers) were recorded. More data relevant to the exposure of the operators were recorded during the special investigations in a smaller group of operators, when changes in cholinesterase activity were studied in the course of the daily work.

The outdoor temperature at the beginning and at the end of the daily work of both squads was recorded throughout the trial.

Clinical studies

Medical surveys of operators were made once a week by the medical officer on the day of the spraying cycle that the regular checking of spraying equipment was carried out. At the same time

[1] The acute oral toxicity of a sample taken from a batch of OMS-33 50% WDP sprayed in this trial was determined in white rats at the Institute for Medical Research, Zagreb. The toxicity obtained was in good agreement with the toxicity of the OMS-33 technical product obtained in the same laboratory several years earlier (Vandekar, unpublished report to WHO); the LD_{50} values, expressed in terms of active ingredient content, were 142 (124–163) mg per kg of body-weight and 119 (114–125) mg per kg of body-weight, respectively.

there was a half-hour retraining lecture on safety precautions for all operators.

Any suspected case of OMS-33 intoxication, either among spraymen or among residents, was reported to the medical officer. The confirmed cases were investigated thoroughly, particular regard being given to the nature of the exposure which had caused the over-absorption of insecticide.

In two villages, Nazar-Aghai and Barmak, with a population of 1052 and 310, respectively, closer observation of any possible adverse effect of OMS-33 on inhabitants was carried out. All houses were visited on the day after spraying and inquiries were made regarding any possible complaints. Whenever complaints which could be related to OMS-33 were encountered, further investigations were carried out to find any cause of over-exposure.

Determination of cholinesterase activity

The method described by Ellman et al. (1961) was adapted to measure plasma and whole-blood cholinesterase activity under semi-field conditions. Two fields-methods, the tintometric (Edson, 1958) and the acholest (Herzfeld & Stumpf, 1955; Sailer & Braunsteiner, 1959), for measuring whole-blood and plasma cholinesterase activity, respectively, were also employed.

A total of 2 or 3 determinations had been performed on all operators during the week before the start of the spraying operation, their means being taken as the initial (pre-exposure) value. The cholinesterase activities found in the course of the spraying operation are expressed as percentages of these initial values.

Blood sampling. Unless otherwise stated, blood sampling was performed in the field. Except for tintometric assays, which in all cases were done immediately after sampling, the determination of cholinesterase activity was performed in a laboratory, which was a room without air-conditioning in the research station at Borazjan, a town adjacent to the operational area. When samples were transferred from the field to the laboratory, 2–3 hours elapsed between sampling and assay. Blood samples were always obtained from a finger-prick and special care was taken to ensure that the operator's finger was properly cleaned.

For determining plasma cholinesterase activity, either by the acholest or the adapted Ellman method, samples were collected in glass capillaries as described by Stubbs & Fales (1960). The sealed capillaries were kept cold in vacuum flasks containing Jel-Ice. Plasma was separated by centrifuging the capillaries in an electrically driven centrifuge or, when assays were carried out in the field, by a hand-driven centrifuge.

For determining whole-blood cholinesterase activity by the adapted Ellman method, 20 µl of blood were collected in the field, in Sahli pipettes which had been heparinized by rinsing with a 2% heparin solution immediately before the collection of samples. The blood sample was then transferred, without dilution, into a 30-ml glass container with a plastic stopper and the containers were kept in a vacuum flask with Jel-Ice during transportation.

In a special study, when repeated blood-sampling was performed on certain operators during the course of the day's work, the determinations of plasma and whole-blood cholinesterase activity by the adapted Ellman method was performed in an improvised laboratory in the field.

Determination of plasma cholinesterase activity by the adapted Ellman method. A battery-powered Linson 3 photometer was used together with a "410" light-filter and a cuvette having a 1.27-cm light-path.

Amounts of 71.628 g of sodium dibasic phosphate dodecahydrate ($Na_2HPO_4 . 12 H_2O$)[1] and 6.900 g of sodium monobasic phosphate monohydrate ($NaH_2PO_4 . H_2O$)[1] were weighed in Geneva and sealed in suitable containers (15 of each). From some of these samples, 0.1-M buffer solutions were prepared, as described below, in Geneva, one before the departure for Iran and the other after returning. The pH values of the 2 solutions were checked with a Beckman pH meter[2] and were found to be 7.40 and 7.37, respectively, and thus in good agreement with the theoretical value of 7.40. Amounts of acetylthiocholine iodide[1] (0.175 g), dithiobisnitrobenzoic acid[1] (0.158 g) and sodium bicarbonate[1] (6.600 g) were also weighed and sealed in suitable containers in Geneva.

Redistilled water prepared in Borazjan was used for making up buffer solutions and reagents as follows: 0.2-M stock solutions of sodium dibasic phosphate and sodium monobasic phosphate were

[1] Analytical grade.

[2] Facilities for measuring the pH were kindly provided by Beckman International Technical Centre, Geneva, Switzerland.

prepared each week by dissolving the weighed quantities in 1000 ml and 250 ml of water, respectively. Finally, a 0.1-M phosphate buffer solution having an expected pH of 7.4 (solution A) was prepared from stock solutions twice weekly by mixing 405 ml of sodium dibasic phosphate, 95 ml of sodium monobasic phosphate and 500 ml of redistilled water. A 0.1-M phosphate buffer solution having an expected pH of 7.0 (solution B) was prepared from the same stock solutions by mixing 12.2 ml of sodium dibasic phosphate, 7.8 ml of sodium monobasic phosphate and 20 ml of redistilled water. An acetylthiocholine iodide solution was prepared twice a week by dissolving the weighed amount of reagent in 10 ml of redistilled water and a dithiobisnitrobenzoic acid solution was made once a week by dissolving the weighed amount in 36 ml of buffer solution B and adding 4 ml of a 1.5% solution of sodium bicarbonate. The latter solution was made by dissolving the weighed amount of sodium bicarbonate in 40 ml of redistilled water.

All solutions and weighed chemicals were kept in a refrigerator at about 4°C.

A linear relationship between extinction and reaction time or amount of enzyme was found only up to an extinction value of 0.1. At higher extinctions, a marked slope of the curve was established ;[1] for this reason, 10 µl instead of 20 µl of plasma were used for each determination. The assays lasted 2 minutes. Under these conditions, the linear relationship was found to be satisfactory.

Since the temperature in the laboratory ranged from 28°C to 40°C, and was usually well above 30°C, the temperature of the buffer solution was adjusted to 30°C, to limit the change in reaction mixture temperature during the assay. Even so, at the end of a 2-minute assay the reaction mixture in the cuvette was between 35.4°C and 36.3°C. As shown in Table 1, the heat radiated from the lamp under these conditions rapidly warmed the reaction mixture.

Using a 10-µl self-adjusting micropipette, plasma was added to 3.0 ml of buffer solution A, and 100 µl of dithiobisnitrobenzoic solution and 50 µl of substrate solution were added subsequently. Immediately after adding the substrate, the reaction mixture was transferred to the cuvette, and within 15–30 seconds the galvanometer needle was set just below "zero extinction". The moment the needle reached zero extinction, the time was recorded by starting the stop-watch. After exactly 2 minutes the reading of the extinction value was made.

TABLE 1

TEMPERATURE CHANGES IN THE REACTION MIXTURE DURING PLASMA CHOLINESTERASE ASSAYS IN A LINSON 3 PHOTOMETER [a] COMPARED WITH THE CHANGES DUE TO AMBIENT TEMPERATURE [b] ONLY

Time (min)	Temperature with light on (°C)		Temperature with light off (°C)	
	I	II	I	II
0	31.5	32.0	31.3	31.4
1	34.0	34.0	32.3	32.4
2	35.3	35.6	32.9	33.2
3	36.2	36.8	33.3	33.8
4	37.0	37.8	33.7	34.2
5	37.8	38.4	34.0	34.6

[a] i.e., light on.
[b] i.e., light off; room temperature = 37.5°C.

Determination of whole-blood cholinesterase activity by the adapted Ellman method. The same apparatus (Linson 3 photometer) and the same buffer and reagent solutions as described for the plasma cholinesterase determinations were used.

The 20 µl of blood in the 30-ml sampling container were diluted with 12 ml of 0.1-M phosphate buffer solution A at immediately before the assay ; 3.0 ml of diluted blood were transferred to a test-tube and 100 µl of dithiobisnitrobenzoic solution and 50 µl of acetylthiocholine iodide solution were added successively. Immediately following the addition of the substrate, the reaction mixture was transferred to the cuvette and after about 25–35 seconds the galvanometer needle was set just below zero extinction, or as near to zero extinction as possible. At the moment when the needle reached zero extinction, or an easy-to-read extinction value, the time was recorded by starting the stop-watch. After exactly 2 minutes the next reading of the extinction was taken. Similarly, as in the plasma cholinesterase determination, the linear relationship with time or amount of enzyme or both was found only within a narrow range of the extinction, i.e., up to a value of

[1] This was almost certainly due to the non-linear relationship between the colour of the reaction mixture and the extinction as measured by this particular instrument at the wavelength of light passed by the "410" filter.

about 0.1. The readings obtained after a 2-minute reaction time occasionally exceeded this value, particularly when pre-exposure (uninhibited) activities were determined.

During the assay change in temperature of the reaction mixture was observed; this was similar to that described when plasma cholinesterase was determined (see above).

To evaluate the effect of delay in carrying the samples from the field, 23 blood samples were collected in duplicate on 1 occasion [1] and the assays were carried out in 2 separate runs, one within 5 minutes and the other 2½ hours after sampling. A mean change of –5% was observed in the latter determination, the difference being statistically significant at the 1% level.

Tintometric method. The method described by Edson (1958) and subsequently improved by Watson & Edson (as described in the instruction sheet enclosed with the field-kit) [2] was employed. A slight adjustment was made to render the method more appropriate for determining the degree of cholinesterase-inhibition induced by carbamates. To diminish the reactivation of carbamoylated enzyme, the substrate was added to the reaction mixture immediately after the addition of the blood sample.

Except for bromothymol blue, which was weighed into small glass containers in Geneva, the field-kit as supplied by the manufacturer was used. Redistilled carbon-dioxide-free water was used for preparing the solutions. A fresh acetylcholine perchlorate solution was used for not more than 2 successive days and was always refreshed by boiling on the second day of use.

All the assays (except 1 pre-exposure determination in spraymen) were performed in the field; the temperature varied from 16°C to 46°C.

Acholest method. Except for minor modifications which, according to the laboratory evaluation of this method, allow optimum experimental conditions for obtaining reliable results (Pleština, 1966), the original method described by Herzfeld & Stumpf (1955) and later by Sailer & Braunsteiner (1959) was employed. Test-papers obtained from the manufacturer [3] were used.

As the room temperature varied from 28°C to 40°C from one assay to another, the temperature-correction factor obtained by R. Kilches (quoted in Holmstedt & Oudart, 1966) was applied, the reaction time, recorded in fractions of a minute, being converted into "units of activity" (Richterich, 1962).

[1] The sampling was performed when the workers had had no contact with the insecticide for 1½ days.

[2] Supplied by Tintometer Sales Ltd, Salisbury, England.

[3] Österreichische Stickstoffwerke A.G., Linz am Donau, Austria.

RESULTS

Clinical observations in operators

During the 6-week trial with 807 man-days of exposure, 25 complaints out of a total of 34 were attributed to over-exposure to OMS-33. The symptom most frequently encountered was headache, and was sometimes accompanied by nausea or by increased sweating or both (Table 2).

TABLE 2
SYMPTOMS AND SIGNS REPORTED ON 25 OCCASIONS BY ANY OF THE 24 OPERATORS DURING 6 WEEKS OF SPRAYING OMS-33

Symptoms and signs	No. of times reported
Headache	20
Nausea	11
Giddiness	2
Blurred vision	2
Weakness	1
Increased sweating	7
Vomiting	2
Pin-point pupils	1

Symptoms were mild and, in most instances, ceased within 1–3 hours. Usually, the operators had recovered by the time they were visited by the medical officer. The only treatment, occasionally given, consisted of a few drops of tincture of belladonna. In most instances, complaints could be associated with heavy contamination of exposed skin, which usually occurred when very small rooms or houses with high ceilings were sprayed.

As shown in Table 3, 620 man-days were carried out under typical conditions of exposure, while both 61 and 126 man-days were performed under intentionally altered conditions. A much higher frequency of complaints was encountered when the hands and faces of operators were washed only twice during the 7–8-hours working day, the difference being significant at the 1% probability level (Table 3). On the other hand, no significant

difference was found in the incidence of complaints when no face masks were worn by spraymen and no masks or gloves were worn by mixers or foremen.

Half the operators in the programme registered a complaint at one time or another. The frequency distribution of complaints during the 6 weeks of spraying is shown in Table 4, and in the same table, the mean weekly outdoor temperature, which was recorded at the beginning and at the end of the day's work, is given.

Clinical observations in villagers

Nazar-Aghai village. Among 1052 inhabitants, 19 complaints (1.8%) attributable to OMS-33 were recorded in 16 adults (2 men and 14 women) and 3 children, 11–12 years old. Symptoms usually occurred within 1 hour of the house being sprayed and were of mild intensity. They consisted of headache (19), nausea (6), increased sweating (5) and vomiting (1). Headaches never lasted longer than 24 hours, and usually ceased or lessened considerably within 2–3 hours.

In most instances, the symptoms developed as a result of 1 or more entries into the house during spraying (11 cases); less frequently the symptoms could be connected with the person's entering a room immediately after spraying (3 cases) or with floor-cleaning, with or without the use of water (5 cases).

Barmak village. Out of 310 villagers, 10 complaints (3.2%) were recorded among adults only (9 women and 1 man). The complaints, which were never serious, included headache (10), nausea (4), vomiting (1) and increased sweating (1). Symptoms developed within 2 hours of spraying and were associated with entering a room during

TABLE 3

NUMBER OF MAN-DAYS WORKED AND NUMBER OF COMPLAINTS ATTRIBUTABLE TO OVER-EXPOSURE TO OMS-33 IN DIFFERENT CONDITIONS OF EXPOSURE

Deviation from typical conditions of exposure	Number of man-days	Number of complaints	Frequency (%)
None	620	13	2.1
Hands and face washed only twice during 7–8 hour working day	61	9	14.8
No face masks worn by spraymen and no masks or gloves worn by mixer and foremen	126	3	2.4

TABLE 4

FREQUENCY DISTRIBUTION OF COMPLAINTS ATTRIBUTABLE TO OVER-EXPOSURE TO OMS-33 AMONG THE WORKERS DURING THE 6-WEEK SPRAYING OPERATION, AND MEAN WEEKLY OUTDOOR TEMPERATURES

Week of spraying	Code number of spraying operator		Mean weekly outdoor temperature (°C) [a]	
	Squad 1 [b]	Squad 2 [c]	At the beginning of work	At the end of work
1	2, 6	14, 24	26	37
2	—	14, 16, 18, 24	27	39
3	6	—	20	37
4	8, 12, 12	17	20	37
5	—	17, 18	24	41
6	3, 5, 6, 8, 8, 8, 10, 10, 10	16	25	44

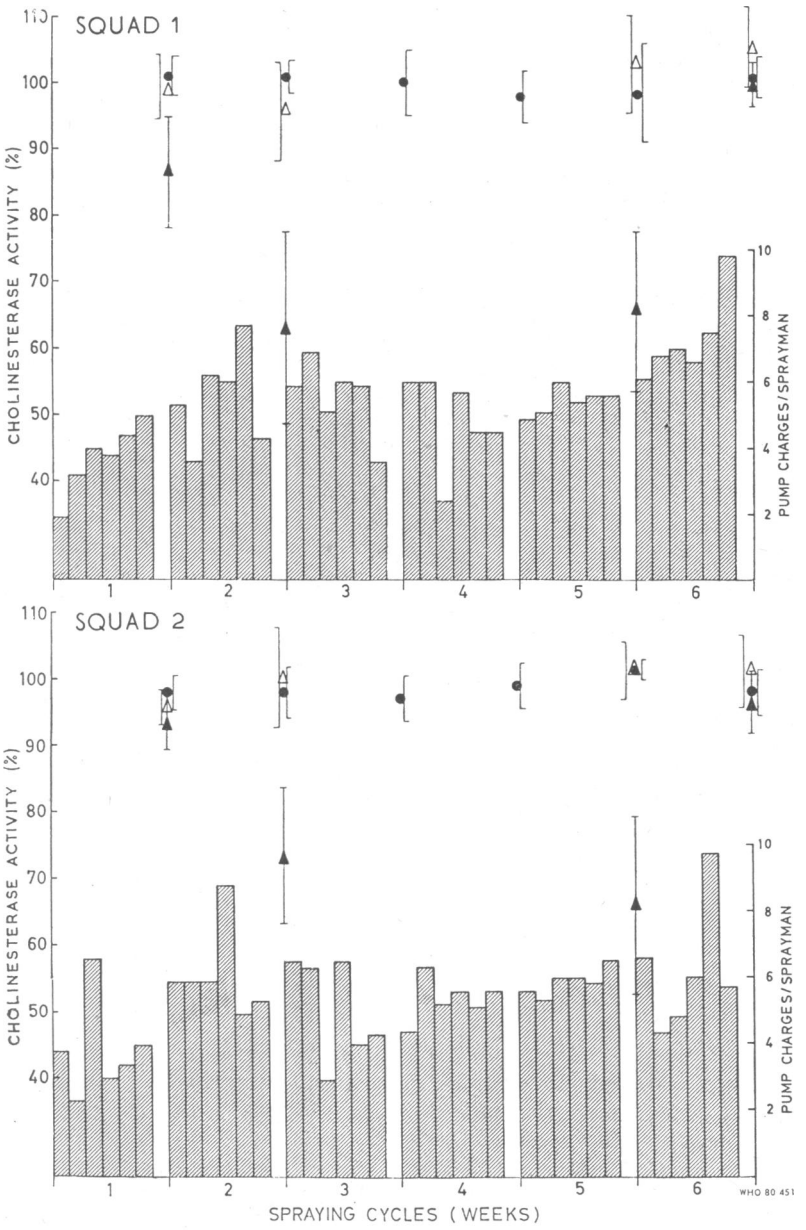
[a] Outdoor temperature was recorded daily at the beginning and at the end of work in both areas where the 2 squads were operating. Since there was little difference between the temperatures recorded in the 2 areas, the mean values of the 12 weekly recordings are shown.

[b] During the 6th week operators in Squad 1 (code No. 1–12) worked 7–8 hours a day and washed their hands and face only twice during the work.

[c] During the 5th and 6th week, operators in Squad 2 (code No. 13–24) did not wear face masks and mixer and foremen did not wear masks or gloves.

FIG. 1

WHOLE-BLOOD CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S AND TINTOMETER METHODS AND PLASMA CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S METHOD IN SPRAYING OPERATORS IN 2 SQUADS IN THE COURSE OF THE 6-WEEKS SPRAYING OPERATION



▲ Ellman's method for whole-blood cholinesterase.
● Tintometric method for whole-blood cholinesterase.
△ Ellman's method for plasma cholinesterase.

[a] The values plotted represent the means and their 95% confidence limits. The daily numbers of pump-charges sprayed by each sprayman are shown in the shaded columns.

spraying (7 cases), entering a room immediately after spraying (1 case), sleeping on a sprayed mat (1 case) or sweeping the floor without the use of water (1 case).

Two deaths among chickens were reported.

Other villages. Almost 50 complaints (about 0.5%) were reported from the remaining 9600 inhabitants living in 31 sprayed villages. With the exception of 5 cases of complaints which were recorded among children (2–3 years old) and were apparently due to rubbing against newly sprayed surfaces, all other complaints occurred among women, most of whom had entered houses during or immediately after spraying.

Assessment of possible cumulative effect on blood cholinesterase in operators

The adapted Ellman method for determining both plasma and whole-blood cholinesterase activity and the tintometric method were employed. Pre-exposure activities were determined in all operators on 2 or 3 occasions before the spraying operations started and 1 determination was made every week during the spraying operations at the start of each 6-day cycle before spraying began.

As shown in Fig. 1, moderate depression of whole-blood cholinesterase was found in operators in both squads by the Ellman method at the end of the first, second and fifth cycles of spraying. At the end of the sixth spraying cycle, whole-blood cholinesterase activity, determined by Ellman's method, was found to be within the normal range. It should be pointed out that at the end of the first 5 cycles of spraying there was a strong possibility of further exposure to the insecticide: workers checked their equipment on the seventh day of the cycle, they spent the night in the village where they were working, and on the following morning, just before blood samples were taken, they usually began preparations for the next spraying cycle. However, before the last sampling, the workers had *no* contact with the insecticide for 1½ days: on completion of the sixth week of spraying, the operators spent the night and the following day and night at their homes which were some distance away from source of contact with the insecticide.[1]

[1] The last blood sampling was performed in the laboratory; on this occasion, the operators looked particularly well-washed and wore their best clothes instead of overalls and it could be assumed that they were free from OMS-33 contamination.

No significant depression could be found in whole-blood cholinesterase levels when determined by the tintometric method (Fig. 1). Plasma cholinesterase activity, determined by the Ellman method, was not found to be depressed.

Changes in blood cholinesterase activity in operators in the course of a day's work under typical conditions of exposure

In all, 5 spraymen and 1 mixer were selected from Squad 2 for this study. The spraymen wore masks and the mixer also wore rubber boots, gloves and an apron. They washed their hands and face between each pump-charge, 6 pump-charges being sprayed each day from about 06.00 to 12.00. At about 09.00, after the spraying of the third pump-charge had been completed, the operators took a rest of 40–60 minutes for breakfast.

Apart from 1 case of short-lasting nausea and slight headache,[2] no complaints were recorded.

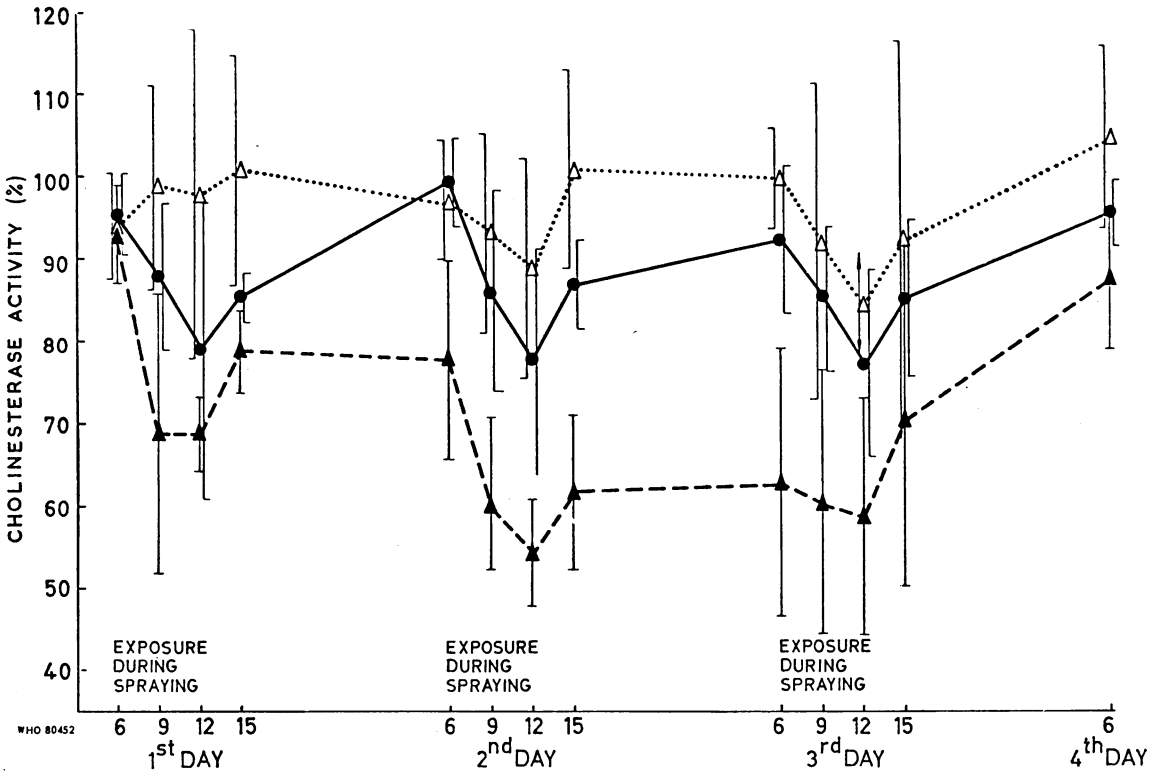
Whole-blood cholinesterase activity (Ellman method and tintometric method) and plasma cholinesterase activity (Ellman method) were determined before work began, during the break, at the end of the day's work and 3 hours after work ended throughout 3 consecutive days, and on the fourth day a single determination was made before spraying began.

Exposure to OMS-33, while spraying 3 pump-charges within 2–2½ hours or preparing about 250 l of insecticide suspension during the same period, produced pronounced depression in whole-blood cholinesterase as determined by the Ellman method, in both the spraymen and the mixer (Fig. 2). After a similar degree of exposure which followed the 40–60 minute break, further, but much less pronounced, inhibition was found. Three hours after work ended, a marked reactivation of inhibited enzyme was observed. Nevertheless, the determination next morning revealed the whole-blood cholinesterase activity well below 100% in most instances, indicating that either further absorption or additional exposure and ab-

[2] On the third day of this study, a sprayman (Code No. 18) complained of slight headache before spraying commenced. While spraying the first pump-charge, he developed nausea. The man was given 10 drops of tincture of belladonna and he recovered after a 45-minute rest. He continued working and sprayed altogether 5 instead of 6 pump-charges that day without further complaint. Whole-blood cholinesterase determined by Ellman's method revealed relatively low activity (about 40%) before the man started spraying that day.

FIG. 2

WHOLE-BLOOD CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S AND TINTOMETRIC METHODS AND PLASMA CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S METHOD IN 5 SPRAYMEN AND 1 MIXER IN THE COURSE OF A TYPICAL SPRAYING OPERATION



▲ Ellman's methods for whole-blood cholinesterase.
● Tintometric method for whole-blood cholinesterase.
△ Ellman's method for plasma cholinesterase.

[a] The values plotted represent the means and their 95% confidence limits.

sorption of insecticide had occurred between the two blood samplings. The possibility of additional contact with insecticide could not be excluded and it was found later that, in spite of instructions to the contrary, the operators taking part in the study occasionally helped the other members in the squad to prepare for the day's work just before the early-morning blood sampling.

The tintometric method revealed a similar pattern in the change in cholinesterase activity but depression was less pronounced; plasma cholinesterase activity determined by Ellman's method followed this pattern to a much smaller extent, the depression being noticeable only on the second and third days of the study (Fig. 2).

Changes in blood cholinesterase activity in operators working under different conditions of exposure

Three groups of operators were studied—(1) group A: 3 spraymen and 1 mixer (from Squad No. 1) worked 7 hours a day (7 pump-charges) under typical conditions of exposure (the spraymen wore protective masks, the mixer wore also rubber boots, gloves and an apron) but they washed their face and hands only twice during the day's work; (2) group B: 5 spraymen and 1 mixer (from Squad No. 2) worked 6 hours a day (5–6 pump-charges) without wearing masks, and the mixer worked without mask or gloves; the men washed their hands after each pump-charge;

(3) group C: 2 foremen (from Squad No. 2) worked 6 hours a day without masks or gloves and washed their hands and face before the break and at the end of the day's work.

In this 3-day study, whole-blood cholinesterase was determined by the Ellman and tintometric methods and plasma cholinesterase was determined by the Ellman and acholest methods, before and after work.

The degree of cholinesterase inhibition in groups A and B (Fig. 3) was similar to that of the group described in the section above when typical spraying conditions were adopted (see Fig. 2). On the other hand, a very pronounced but symptomless fall in whole-blood cholinesterase activity and a moderate fall in the plasma cholinesterase activity were found by Ellman's method in 2 foremen (group C) after each day's work. As pointed out above, the foremen's duties included the handling of the polyethylene sheets which became heavily contaminated with insecticide.

Both field-methods revealed a moderate depression of cholinesterase activity at the end of the day's work; the Acholest method showed a somewhat more pronounced inhibition than the tintometric method did.

Degree of cholinesterase inhibition in relation to incidence of complaints

The majority of recorded falls in cholinesterase activity, including the largest ones, were unassociated with complaints. In a total of 42 determinations performed in operators at the end of a day's work, the whole-blood cholinesterase activity ranged from 22% to 88% of its pre-exposure value with a mean of 49%. On the other hand, in 6 cases of complaints, during the course of which whole-blood cholinesterase was determined, the activity ranged from 41% to 79% with a mean of 53%.

DISCUSSION

Before an insecticide can be released for a large-scale trial, experience from smaller trials must be available: (1) to confirm the feasibility of the recommended safety measures under true operational conditions, (2) to allow adjustment, if necessary, of the safety measures to the specific conditions prevailing in the area in which the insecticide is to be applied, and (3) to assess the severity of the consequences to operators, inhabitants and domestic animals if the safety measures are partly ignored. Special studies in this trial of OMS-33 were made in order to provide, in conjunction with results obtained in other trial areas, sufficient data to meet these objectives. Although a number of special investigations were carried out in the course of the trial, in which respect the operational conditions were different from normal field operations, true operational conditions were maintained, on the whole, from the second week of spraying.

A pre-spraying period of training and the supervision of operators were organized during the trial. Instructions were given to inhabitants before their houses were sprayed. Nevertheless, reactions due to over-exposure to the insecticide were encountered among both operators and villagers on several occasions.

The transience of symptoms was one of the main characteristics of the reaction in operation caused by over-exposure to OMS-33. As a rule, complaints ceased within 1–3 hours of rest, after which spraymen were able to continue their work with no further complaint. Thus, when long-lasting complaints are encountered, it is very likely that they are due to causes unconnected with over-exposure to OMS-33.

The importance of washing the face and hands after each pump-charge was demonstrated by the significantly greater frequency of reactions among the operators who worked 7–8 hours a day under typical conditions, but washed their faces and hands only twice during the day's work (Table 3). On the other hand, when the hands and face were washed frequently, the wearing of surgical-type masks did not afford significant additional protection. A similar observation was made in a subsequent study performed during a field-trial with OMS-33 in Northern Nigeria [1] (Vandekar & Wilford, unpublished report to WHO). When the degree of cholinesterase depression and the amount of phenols excreted in urine were compared in operators working with or without respirators, no significant additional protection related to the respirators could be established. These observations are consistent with the results of the study of the health hazards involved in house-spraying with DDT (Wolfe et al., 1959) and of the exposure studies of 31 different work activities

[1] No complaints among spraying operators (121 man days) and residents (approximately 3500) were recorded in this trial. This was attributed to the comparatively cool, wet weather and to the carefully supervised washing of hands and face by operators after each pump-charge.

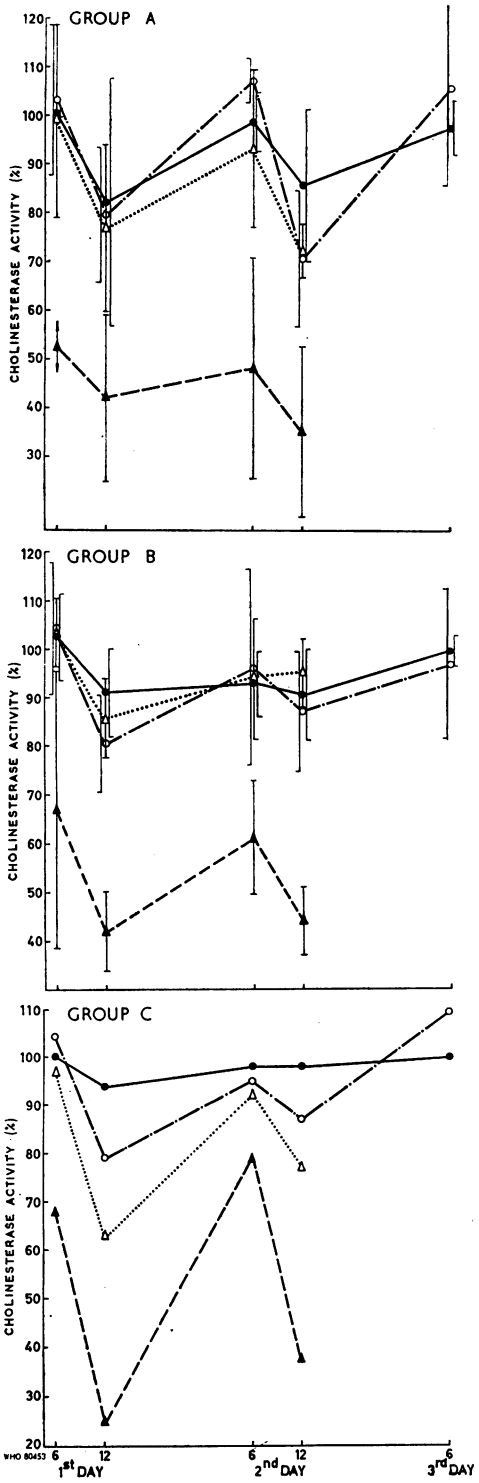


FIG. 3

WHOLE-BLOOD CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S AND TINTOMETRIC METHODS AND PLASMA CHOLINESTERASE ACTIVITY [a] DETERMINED BY ELLMAN'S AND ACHOLEST METHODS IN 3 GROUPS OF WORKMEN [b]

▲ Ellman's method for whole-blood cholinesterase.
● Tintometric method for whole-blood cholinesterase.
△ Ellman's method for plasma cholinesterase.
○ Acholest method for plasma cholinesterase.

[a] The values plotted represent the means and their 95% confidence limits.

[b] Group A: 3 spraymen and 1 mixer. The spraying operations were typical but the men washed only twice during the day's work.

Group B: 5 spraymen and 1 mixer. The men worked without masks or gloves.

Group C: 2 foremen. The men worked without masks or gloves and washed hands and face before the break and at the end of the day's work.

involving 10 different pesticides (Wolfe, Durham & Armstrong, 1967), which have clearly demonstrated that the potential dermal exposure of the workers is much greater than the potential respiratory exposure.

In some instances, however, when only a brief interval elapsed before the appearance of complaints, the inhalation of the particles of the insecticide suspended in the air probably played an important role. While it is difficult to say, without knowing the actual size of the particles, how much of the insecticide which enters the upper respiratory tract is swallowed and how much reaches the pulmonary alveoli, in both instances it will be absorbed much more rapidly than through the skin.

Inhalation of significant amounts of aerosol from the spray, especially when either small houses or very high ceilings are sprayed, is difficult to avoid, even by experienced spraymen. At the same time, insecticide dust liberated during the mixing procedure or from the deposits on the overalls or skin of the workers, as well as, in the case of inhabitants, in the dust stirred up from sweeping contaminated floors, may be inhaled or swallowed. The significance of this route of absorption should be considered in the light of the intravenous ED_{50} value of OMS-33—the dose which produces first noticeable signs of poisoning in rats—which amounts to only 0.334 mg (0.280 mg–0.398 mg) per kg of body-weight (Vandekar et al., 1965) compared with the dermal toxicity, which is more than 1000 mg per kg of body-weight (manufacturer's data).

Since the increased number of complaints (9 cases within 61 man days) which occurred among the operators from Squad 1 during the last week of spraying was almost certainly due to the altered conditions of exposure (see Tables 3 and 4), the actual frequency of complaints which corresponds to typical conditions of exposure may be obtained by subtracting these numbers from the total. Thus, during a total of 746 man days, 16 reactions (2.1%) to OMS-33 were recorded among operators. No apparent association between the number of complaints and the outdoor temperature could be established.

The analysis of the incidence of complaints in relation to operators' education and previous experience in spraying showed that, out of 15 operators with a 6-10-year school education, 8 complained on 14 occasions, while among the 9 operators with no education, only 1 complained on 2 occasions. On the other hand, among 18 operators with no spraying experience before this trial, 8 complained on 15 occasions, and of 6 operators who had had a previous experience in spraying, only 1 operator complained on 1 occasion. This comparison indicates that the operators, after being taught the precautionary measures to be observed, apparently benefited more by their experience in spraying than by their general education in avoiding unnecessary exposure to insecticide.

By far the highest proportion of villagers who complained were women (23 out of a total of 29). This may be explained by the fact that, unlike most of the men and children, they stayed near the houses while spraying was in progress, entered the house during spraying, in spite of instructions to the contrary, and cleaned the floor immediately after spraying, sometimes with an inadequate amount of water. It should be pointed out that all the symptoms attributable to OMS-33 made their appearance on the same day a particular village was sprayed, and in most instances they occurred within half an hour of the actual house being sprayed. The symptoms, which were never serious, ceased or lessened considerably within 2–3 hours, and did not lead to refusal by the residents to have their houses resprayed in the subsequent operation.[1] In addition to preventing residents from entering the house during spraying, their re-entry after spraying should be delayed for 2–3 hours, and sufficient water should be used for washing the floors.

In spite of certain difficulties met with when determining cholinesterase activity by Ellman's method under semi-field conditions, mostly due to the inadequacy of the Linson 3 photometer for this type of assay, the determination of whole-blood cholinesterase by this method proved to be the most sensitive of the 4 methods employed, and gave satisfactorily consistent results. In agreement with the results obtained both *in vitro* and in human-volunteer studies,[2] erythrocyte cholinesterase was much more sensitive to OMS-33 than plasma cholinesterase. The Ellman method re-

[1] The second round of spraying was performed after a 4-month interval (20 September to 8 October 1967).

[2] Vandekar, M., Pleština, R. & Wilhelm, K. (1967). Unpublished WHO working document TOX/67.1. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

vealed a pronounced depression in whole-blood cholinesterase activity,[1] whereas plasma cholinesterase was only slightly inhibited. By the 2 field-methods, tintometric and acholest, a relatively small degree of inhibition could be demonstrated as compared with that found in whole-blood cholinesterase determined by Ellman's method.

A typical daily pattern in cholinesterase inhibition and reactivation could be demonstrated in operators exposed to OMS-33; whole-blood cholinesterase activity showed a rapid fall soon after exposure started and a distinct recovery took place within 3 hours of the cessation of exposure. The inhibited enzyme was seldom found to be completely reactivated by the next morning. This depressed starting level, which was attributed to additional contact with insecticide just before the early-morning blood sampling, did not, however, increase the depression on the successive day's exposure. When no possibility of exposure to insecticide existed for the one day of rest after the completion of the 6-week spraying operation, the original levels of whole-blood cholinesterase activity were found to be completely recovered.

The study of the effect of delay in carrying out the assays of the whole-blood cholinesterase activity revealed a mean change of −5% in the determination performed 2½ hours after sampling as compared with the determination performed within 5 minutes of sampling, thus suggesting a consecutive inhibition of cholinesterase after the sampling. Since this comparison was made on the samples obtained from the workers who had not had any contact with the insecticide for 1½ days, the possibility cannot be excluded that a more pronounced change in cholinesterase activity might have occurred during storage in the samples which were collected immediately after exposure to the insecticide. If this is the case, the pronounced depression of whole-blood cholinesterase activity would correspond with the concentration of inhibitor in circulating blood rather than with the actual enzyme activity at the moment of sampling. At this stage, no attempt has been made to elucidate this point.

It should be pointed out that most falls in cholinesterase activity, including the largest ones, were unassociated with complaints. A lack of correlation between the degree of plasma cholinesterase inhibition as measured by the acholest method and the incidence of complaints among the operators was observed repeatedly in the course of the several rounds of OMS-33 spraying in an operational field-trial in El Salvador (J. E. Davies and G. E. Quinby, unpublished reports to WHO). In view of the very marked symptomless daily fluctuation in cholinesterase activity and the absence of cumulative inhibitory effect during exposure over several weeks, routine cholinesterase determination is of little, if any, practical value in indicating when a worker should be withdrawn from further exposure to OMS-33. Minor complaints, from which recovery is rapid, cause the operator to stop work and thus prevent further exposure. The early symptoms induced by over-exposure to OMS-33 long before a dangerous dose is absorbed (Vandekar et al., 1965; Vandekar & Fajdetić, 1966) are one of the most important properties of carbamate insecticides, in which respect they differ essentially from organophosphorus compounds.

While it cannot be expected that in a large-scale operation all precautions will be observed by all operators or inhabitants throughout the whole period of spraying, the incidence of reactions among spraymen and inhabitants can be markedly reduced by focusing attention on those measures of general and personal hygiene which should be practised in any spraying programme.

[1] The activity measured in whole blood by Ellman's method is mostly contributed by erythrocyte cholinesterase.

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RÉSUMÉ

Dans le cadre du programme OMS d'essai et d'évaluation de nouveaux insecticides, un essai opérationnel destiné à vérifier la sécurité d'emploi du méthylcarbamate d'*o*-isopropoxyphényle (OMS-33, Baygon) a été effectué dans le sud de l'Iran en mai et juin 1967. On a procédé à cette occasion à des investigations cliniques et biochi-

miques. Les opérations de pulvérisation ont duré six semaines. Trente-trois villages, comptant au total environ 11 000 habitants, ont été traités, l'insecticide étant appliqué à la concentration de 2g/m². Deux villages ont fait l'objet d'une surveillance spéciale.

Consécutivement à l'emploi de l'OMS-33, on a enregistré 25 plaintes parmi les ouvriers chargés des pulvérisations, 19 (1,8%) parmi les 1052 habitants du premier village et 10 (3,2%) parmi les habitants du second; 50 personnes environ (0,5%) sur les 9600 habitants des autres agglomérations ont également imputé à l'OMS-33 les symptômes qu'elles présentaient. Les réactions à une surexposition à l'insecticide se sont marquées principalement par l'apparition de céphalées, parfois accompagnées de nausées et/ou d'une transpiration accrue. En règle générale, ces symptômes ont été de courte durée et peu intenses. Chez les opérateurs, les plaintes ont été surtout fréquentes après une forte contamination de la peau par l'insecticide ou après des mesures de nettoyage individuel insuffisantes pendant le travail; chez les villageois, les symptômes sont apparus principalement après un séjour dans une habitation où des pulvérisations étaient en cours.

Les analyses de sang pratiquées chez les ouvriers ont permis de suivre les fluctuations journalières de l'activité des cholinestérases. On a noté un affaiblissement prononcé de l'activité cholinestérasique du sang complet pendant les heures de travail, suivi d'un retour rapide à la normale dès la fin de l'exposition. La cholinestérase érythrocytaire s'est montrée beaucoup plus sensible à l'action de l'OMS-33 que la cholinestérase plasmatique. Aucun effet cumulatif n'a été mis en évidence par les dosages effectués chez les opérateurs pendant les six semaines de travail. On n'a pu d'autre part établir aucune corrélation entre l'intensité de l'inhibition des cholinestérases du sang complet et l'expression des plaintes, la plupart des baisses de l'activité enzymatique, y compris les plus accentuées, ayant été constatées chez des sujets qui ne signalaient aucun symptôme anormal. La détermination systématique de l'activité des cholinestérases n'a donc aucune valeur pratique en tant qu'indicatrice d'une exposition notable à l'OMS-33. Les symptômes mineurs, qui disparaissent rapidement, permettent en revanche de déceler précocement une surexposition.

On ne peut espérer qu'au cours d'opérations d'envergure tout le personnel et tous les habitants appliquent l'ensemble des mesures de sécurité. La fréquence des réactions succédant à l'emploi de l'OMS-33 peut néanmoins être fortement réduite si l'on prend les précautions d'hygiène générale et individuelle indispensables.

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