EFFECT OF PROLONGED SPRAYING WITH TRICHLORPHON ON BLOOD CHOLINESTERASE LEVELS OF SPRAYERS

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ABSTRACT

Two series of trials to study the effect of prolonged application of the organophosporus insecticide trichlorphon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) to cacao trees through motorized portable misting machines on the whole blood cholinesterase levels of sprayers were carried out at Popondetta, Northern District in 1968-69.

In the first series 1.5 *lb of trichlorphon (as an* 80 *per cent wettable powder formulation) in* 30 *gallons of water per acre was applied to the cacao trees.*

There was steady reduction in whole blood cholinesterase of approximately 12 *per cent between readings for the first* 2 *weeks, whereupon the readings levelled off and fluctuated between* 70 *and* 90 *units for a period of* 6 *weeks. They then dropped sharply to a predetermined 'safety' level of* 40 *to* 50 *units whereupon the sprayers were removed from exposure. This level was reached some* $9\frac{1}{2}$ *weeks after spraying commenced. The safety level set was still well above the level (*25 *per cent of normal) at which clinical symptoms of poisoning are said to become evident.*

Recovery of blood cholinesterase was rapid and had risen to 80 *per cent of normal within* 4 *weeks of removal.*

In the second series, 2 *lb of trichlorphon (as a* 70 *per cent w/v Low Volume Concentrate formulation) in* 2 *gal of water per acre was applied through the same misting machines which had been modified for Low Volume Concentrate application. The opportunity was also taken to study the effect of protective clothing—long-sleeved overalls, polythene gloves, hats and agricultural respirators—on whole blood cholinesterase levels of protected sprayers.*

Results showed that there was a steady reduction in blood cholinesterase of approximately 16 *per cent per week over the first* 2 *weeks. There was no difference between protected and unprotected sprayers during the first* 3 *weeks, but for the remaining* 7 *weeks there was a difference of from* 12 *to* 23 *per cent in blood cholinesterase levels, the protected and unprotected sprayers remaining at an average of* 20 *to* 33 *per cent and* 32 *to* 48 *per cent below normal respectively. The lowest reading obtained was a reduction of* 53.1 *per cent for an unprotected sprayer, still well above the safety level.*

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ABSTRACT

Following the introduction of trichlorphon spraying on both European and smallholder plantations in the Northern District during November-December, 1968, *a monitoring system was established in December to monitor whole blood cholinesterase levels of plantation spray team personnel. From its establishment and up until the introduction of LVC trichlorphon in August-September,* 1969 *approximately* 118 *sprayers were removed from exposure with levels ranging from* 50 *down to* 22 *units.*

With the introduction of LVC trichlorphon, however, 106 *sprayers had to be removed from exposure during the first* 4 *weeks with blood cholinesterase levels ranging from* 60 *down to* 30 *units* (*normal range*: 90 *to* 150 *units*). *This was contrary to what one would have expected from the results of the second series of controlled trials.*

In an attempt to simplify the blood sampling procedure and obviate the need to forward the blood samples to Port Moresby, a portable cholinesterase field test kit was tested in the field at Popondetta. However, the kit gave highly variable results which were often quite misleading and field use of the kit was abandoned.

No differences were recorded in whole blood cholinesterase levels for 101 *Europeans and* 103 *Papua New Guineans sampled at Port Moresby General Hospital.*

INTRODUCTION

REGULAR spraying of foliage, branches and trunks of cacao trees with the organo phosphorus insecticide trichlorphon by motorized portable misting machines was one of the Department of Agriculture, Stock and Fisheries' recommendations introduced in October, 1968 as a part of an overall programme financed by the Administration of Papua New Guinea to control *Pantorhytes szentivanyi* in the Popondetta-Sangara area of the Northern District. In the first instance a wettable powder formulation of trichlorphon was used and applied at 4-weekly intervals at the rate of 1.5 lb trichlorphon in 30 gallons of water per acre. One per cent superior white oil was added to act as a spreading agent.

In August-September, 1969 a 70 per cent w/v Low Volume Concentrate formulation of trichlorphon was introduced to replace the wettable powder formulation. Unfortunately, the lowest application rate that could be achieved with the motorized spraying units fitted with LVC adaptors was 0.5 gal per acre. As this would have resulted in 3.5 lb of trichlorphon being applied per acre, the LVC trichlorphon was diluted one in seven with water and applied at the rate of 2 gal per acre, equivalent to 2 lb trichlorphon per acre.

In Papua New Guinea, cacao forms a dense interlocking canopy at from 6 to 7 ft above ground level some 5 to 6 years after planting (see *Plate* I). It was thought that the spraying of cacao would present the same toxicological hazards as presented by spraying in a semi-enclosed environment, in that spray team personnel would be in an insecticide-saturated atmosphere for 6 to 8 hours per day 5 to 6 days per week.

Whilst trichlorphon is considered to be a relatively safe insecticide, having oral and dermal LD_{50} values in rats of 650 and 2800 mg per kg body weight respectively, no information was available on the effects of continued exposure of sprayers to trichlorphon under Papua New Guinean conditions. It was therefore thought necessary to run controlled trials to study the effect of prolonged spraying with trichlorphon on the blood cholinesterase levels of sprayers.

The opportunity was also taken (by B.K.M.) at a later date to determine the blood cholinesterase levels and range of personal variation in samples of Europeans and Papua New Guineans as we had been led to believe that Papua New Guinean levels would be lower than those for Europeans.

Plate I.—Five to six year old cacao showing dense interlocking canopy

(Photo; A. E. Charles)

METHODS AND MATERIALS

Trichlorphon

Trichlorphon (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) is a non-systemic, organophosphorus insecticide which acts mainly as a contact and stomach poison. Its solubility in water at 25 deg C is 15.4 g per 100 ml (Martin 1968). It is stable at room temperature but is decomposed by water at higher temperatures and at a pH greater than 5.5 to form dichlorvos (Muhlmann and Schrader 1957). Its activity in insects is attributed to its metabolic conversion to dichlorvos (Metcalf, Fukuto and March 1959).

Earlier work by Arthur and Casida (1957), however, failed to demonstrate the presence of dichlorvos in either *Musca domestica* L., *Periplaneta americana* (L.) or *Leucophaea maderae* (Fab.) adults poisoned with trichlorphon.

Trichlorphon has been shown to have a relatively low mammalian toxicity and to exert an anti-cholinesterase action *in vitro* and *in vivo* (Dubois and Cotter 1955). These same authors showed that rapid detoxification of trichlorphon was at least partially responsible for its low toxicity. When sublethal doses were administered to rats by intraperitoneal injection, trichlorphon disappeared from the serum rapidly and only a small percentage was excreted unchanged in the urine.

These findings were supported by the work of Robbins, Hopkins and Eddy (1956) who studied the metabolism and excretion of P^{32} labelled trichlorphon in a lactating cow following oral administration of the material at the rate of 25 mg per kg. They found that trichlorphon was rapidly metabolized by the cow and eliminated via the urine, with the peak of elimination occurring $2\frac{1}{2}$ to $5\frac{1}{2}$ hours following administration. No dichlorvos was detected in the blood, milk or urine of the cows.

Following the injection of 150 mg per kg of trichlorphon into a 9.2 kg female dog, general anticholinergic symptoms first appeared 15 minutes after administration and were followed by a marked diminution of symptoms 30 minutes later and apparent complete recovery 6 hours after administration (Arthur and Casida 1957).

It would appear that whilst acutely toxic doses of trichlorphon produce symptoms which are typical of cholinergic drugs, the outstanding difference between trichlorphon and other organophosphorus insecticides is that its duration of action is extremely brief and complete recovery of affected animals occurs within a few hours of poisoning (Dubois and Cotter 1955; Robbins *et al.* 1956; Arthur and Casida 1957; Metcalf *et al.* 1959).

Spray Team Personnel

(*a*) *Experimental Teams.*—The experimental spray teams consisted of indigenous labourers from the Northern, Chimbu and Western Highlands Districts.

(*b*) *Plantations.*—Composition of plantation spray teams varied from plantation to plantation and even between sampling dates on each individual plantation. However their composition was similar to that of the experimental teams.

Most plantation labourers had minimal formal education and very little instruction in the use of spray equipment, techniques of spray application or the dangers of pesticide application. The experimental spray teams had a slightly higher educational standard and received some instruction in the use of spray equipment and techniques of spray applications. They were also instructed in the simple safety procedures when handling or spraying pesticides—do not smoke when spraying or before washing hands; wash thoroughly after spraying; avoid gross contamination from spillage, leaking sprays, etc.

Clothing worn by sprayers varied, but generally consisted of a pair of shorts or a pair of shorts plus short-sleeved or sleeveless shirt. A typical sprayer is shown in *Plate II.*

Spraying Methods

The spraying method employed by plantations varied. The method used by the experimental spray teams was as follows:

Five sprayers were lined up at the edge of the block of cacao to be sprayed as shown in *Figure* 1. Each sprayer then proceeded down between two lines of cacao as shown (lines 1

Plate II.—Typical plantation sprayer showing spray machine and clothing usually worn under Papua New Guinea conditions

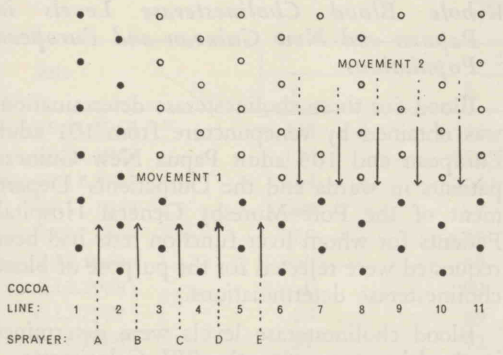

Figure 1.—Illustrates spraying method employed by experimental spray team

and 2 for sprayer A, 2 and 3 for sprayer B, etc.), spraying the half trees facing him and the canopy in between the trees as he moved. After completing the downward run (Movement 1), the sprayer then moved over five lines of cacao and sprayed rows 6 to 11 (Movement 2), and so on until the block was sprayed.

The spray units used in the controlled trials were either Solo Junior 410 or Solo Port 423 Misting Machines. All spraying on European owned/managed plantations, with the exception of one plantation, was carried out using Solo Port 423s. On the one plantation which did not use Solo Port 423s, a tractor mounted PTO misting machine was used.

Controlled Trials

1. *First Series.*—In the first series using the wettable powder formulation, 1.5 lb of trichlorphon in 30 gal of water per acre was applied through misting machines. A blood sample (1 ml venous blood) was taken from each sprayer on Mondays, Wednesdays and Fridays, and forwarded to Port Moresby for whole blood cholinesterase determinations. The Monday samples were taken before spraying commenced for the week and followed 2 days' (Saturday and Sunday) rest from spraying.

A second trial was started when it became apparent that the sprayers in the first trial were not continuously exposed. In the second trial, the sprayers were continuously exposed for 6 to 8 hours per day for 5 days a week. However there were two breaks during the exposure period—5 days between 25th and 29th December, 1968, and 1 day on 1st January 1969.

2. *Second Series.*—In the second series of trials 2 lb of trichlorphon (as a 70 per cent w/v Low Volume Concentrate formulation)* in 2 gal of water per acre was applied through the same misting machines which had been modified for Low Volume Concentrate application. Blood samples (1 ml of venous blood) were teaken as before and forwarded to Port Moresby for whole blood cholinesterase determinations.

Only one trial was conducted in this second series, and besides studying the effect of trichlorphon LVC on blood cholinesterase levels, the opportunity was taken to study the effect of protective clothing versus no protective clothing on blood cholinesterase levels. Accordingly three of the seven sprayers were outfitted with protective clothing consisting of long-sleeved overalls buttoned at the neck and wrists; elbow-length, heavy duty polythene gloves; plastic, brimmed hats; and an agricultural type respirator fitted with replaceable canisters. The unprotected sprayers were dressed in short-sleeved shirts and shorts.

Cholinesterase Determination

1. *Port Moresby Laboratory Method.*—The method used to determine blood cholinesterase in the Port Moresby laboratory was that outlined by Biggs, Carey and Morrison (1958) as modified by Varley (1962). This method relies on measuring the colour change of the indicator bromthymol blue in an EEL Colorimeter. Units of cholinesterase activity are expressed in micromoles of acetic acid liberated from acetylcholine by 1 ml of serum for 30 minutes at 37 deg C. Venous blood samples which had previously been taken into vials (at Popondetta) containing the anticoagulant lithium heparin were used.

2. *The Lovibond Comparator and Portable Kit Method.*—In an attempt to simplify the blood sampling procedure and obviate the need to forward samples to Port Moresby, a chol-

*This formulation was later replaced by a 59 per cent w/v LVC formulation, although the experimental team used the 70 per cent w/v formulation throughout.

inesterase test kit based on that developed by Gerarde (1964) and described by Simpson (1965, 1966a) using 0.01 ml of blood obtained from a finger prick, was used. The method is reported to give a combination of speed, convenience and reasonable precision for emergency or routine determinations. It relies on colour change of bromthymol blue due to the liberation of acetic acid from acetylcholine by the enzyme cholinesterase. It measures the change occurring in an 'unknown' blood sample in the time taken by a normal sample to reach the 100 per cent activity colour change. The change in colour, which is a measure of a change in pH over a fixed time, is therefore a measure of cholinesterase, reflecting the measure of exposure to the organophosphorus insecticide.

The kit was set up and tested in the Biochemistry Department of the Port Moresby Hospital. The indicator, substrate, and carbon-dioxide-free distilled water were checked each day prior to a batch of samples being tested. The kit proved quite satisfactory and was therefore taken to Popondetta to be used in the field. The first batches were run in the laboratory at Popondetta and were also fairly satisfactory.

However, as mentioned later, under both field and experimental conditions, no correlation could be obtained between the blood cholinesterase readings obtained via the kit and via the Port Moresby laboratory method, and field use of the kit was abandoned.

Whole Blood Cholinesterase Levels in Papuan and New Guinean and European Populations

Blood for these cholinesterase determinations was obtained by venepuncture from 101 adult European and 103 adult Papua New Guinean patients in wards and the Outpatients' Department of the Port Moresby General Hospital. Patients for whom liver function tests had been requested were rejected for the purpose of blood cholinesterase determinations.

Blood cholinesterase levels were determined in the laboratory using the EEL Colorimeter.

RESULTS

Controlled Trials

First Series.—The results of the first two trials are shown in *Tables* 1 and 2. It can be seen from both tables that there was steady reduction in blood cholinesterase of approximately 12 per cent between readings for the first 2 weeks, whereupon the readings levelled off. It actually rose in the first experiment because of the decreased daily exposure period, as explained previously. There was also a period during the second experiment when it appeared that cholinesterase levels had settled down at levels between 70 and 90 units for a period of 6 weeks. They then dropped sharply to a predetermined safety level of 40 to 45 units whereupon the sprayers were removed from exposure. This level was reached some 9½ weeks after spraying commenced. The safety level set was still well above the level

Table 1.—Effect of trichlorphon (wettable powder) on blood cholinesterase levels of sprayers —Trial 1

Date	Units of Whole Blood Cholinesterase				
	A	B	C	D	E
18.9.1968 (pre-exposure)	150	148	146	145	124
24.9.1968	128	128	114	134	115
26.9.1968	110	109	99	104	106
1.10.1968	104	90	92	90	93
3.10.1968	92	88	75	72	91
9.10.1968	87	88	85	80	92
10.10.1968	93	103	103	96	92
16.10.1968	108	111	112	102	103
22.10.1968	98	100	104	84	94
29.10.1968	104	105	101	96	104

Table 2.—Effect of trichlorphon (wettable powder) on blood cholinesterase levels of sprayers —Trial 2

Date	Units of Whole Blood Cholinesterase				
	F	G	H	J	K
18.11.1968	140	136	136	128	125
20.11.1968	126	128	135	110	128
25.11.1968	114	106	90	103	112
27.11.1968	80	92	97	85	97
29.11.1968	80	80	74	70	84
2.12.1968	n.d.	80	72	63	74
4.12.1968	n.d.	89	87	74	92
6.12.1968	84	89	98	81	90
9.12.1968	92	92	97	92	99
12.12.1968	64	86	88	70	91
16.12.1968	64	73	83	58	74
19.12.1968	52	n.d.	60	54	54
23.12.1968	51	69	69	48	48
30.12.1968	78	72	n.d.	n.d.	60
2.1.1969	54	64	58	48	n.d.
6.1.1969	66	64	64	66	76
8.1.1969	52	78	74	88	76
9.1.1969	70	78	72	66	76
14.1.1969	71	82	81	71	80
16.1.1969	84	71	69	71	n.d.
20.1.1969	74	82	83	74	79
22.1.1969	44	54	62	48	44
23.1.1969*	44	48	57	40	44
28.1.1969	50	86	n.d.	60	60
31.1.1969	54	80	74	68	60
10.2.1969	98	108	106	100	72
13.2.1969	100	115	86	98	95
17.2.1969	n.d.	117	110	68	117
20.2.1969	106	104	100	98	104
25.2.1969	112	114	100	106	98

*Team taken off spraying at completion of spraying on 24th January, 1969.

(25 to 30 per cent of normal) at which clinical symptoms of poisoning are said to become evident (Simpson 1966b).

Second Series.—The results of the trichlorphon LVC are shown in *Table* 3. It can be seen that there was a steady reduction in blood cholinesterase of approximately 16 per cent per week over the first 2 weeks. There was no difference between protected and unprotected sprayers during the first 3 weeks, but for the remaining 7 weeks there was a difference of from 12 to 23 per cent in blood cholinesterase levels, the protected and unprotected sprayers remaining at an average of 20 to 33 per cent and 32 to 48 per cent below normal respectively. The lowest reading obtained was a reduction of 53.1 per cent for an unprotected sprayer, still well above the safety level. This information is summarized in *Table* 4.

Use of Field Kit

A comparison between the results obtained by using the field kit and from the laboratory method for both trichlorphon wettable powder and LVC formulations is shown in *Tables* 5 and 6 respectively. The kit was used by two different operators to obtain the results presented in *Tables* 5 and 6.

Table 3.—Effect of trichlorphon on blood cholinesterase levels of protected and unprotected sprayers

Week	Units of Whole Blood Cholinesterase						
	Protected			Unprotected			
	L	M	N	P	Q	R	S
16.6.1969 (pre-exposure)	125	121	101	145	142	125	121
20.6.1969	120	116	107	125	130	118	116
27.6.1969	78	82	73	95	107	78	82
30.6.1969	80	91	78	93	107	76	n.d.
4.7.1969	70	99	73	70	84	70	87
7.7.1969	70	85	85	n.d.	79	73	88
9.7.1969	91	97	90	86	91	79	83
11.7.1969	73	83	75	75	73	73	79
14.7.1969	92	92	92	n.d.	90	87	n.d.
16.7.1960	81	88	85	83	92	83	81
18.7.1969	83	100	85	95	85	83	73
21.7.1969	77	104	95	95	85	85	73
23.7.1969	79	79	92	n.d.	85	73	75
25.7.1969	88	81	79	73	85	58	68
28.7.1969	93	97	97	85	92	75	66
30.7.1969	90	95	97	87	90	87	79
1.8.1969	87	87	99	68	81	81	68
4.8.1969	92	97	83	87	93	87	79
6.8.1969	90	93	93	77	86	75	67
8.8.1969	96	86	77	70	66	59	59
11.8.1969	98	91	95	93	70	82	70
13.8.1969	82	88	75	75	75	75	65
15.8.1969	82	90	75	73	75	68	70
20.8.1969	82	93	82	70	86	63	65
22.8.1969	70	84	80	75	70	88	65
25.8.1969	109	110	91	98	104	90	82
29.8.1969	77	82	82	70	86	68	61

Table 4.—Percentage reduction in weekly cholinesterase levels of sprayers exposed to trichlorphon LVC

Week	Protected Sprayers				Unprotected Sprayers				
	L	M	N	Average	P	Q	R	S	Average
2	37.6	32.2	28.7	32.8	34.5	24.6	37.6	32.2	32.2
3	41.6	31.4	25.7	32.9	48.3	35.4	41.6	34.7	40.0
4	33.6	17.4	15.8	22.3	34.5	43.8	33.6	39.7	37.9
5	29.6	33.1	8.9	23.9	49.7	34.6	55.4	34.8	45.9
6	30.4	28.1	2.0	20.2	53.1	34.6	35.2	43.8	41.7
7	23.2	28.9	23.8	25.3	51.7	37.7	52.8	51.2	48.4
8	34.3	25.6	25.7	28.6	49.7	49.2	45.6	42.1	46.7
9	44.0	30.6	20.8	31.8	48.3	46.2	30.6	46.3	42.9
10	38.4	32.2	18.8	29.8	51.7	33.8	45.6	49.6	45.2

Table 5.—Comparison between results for trichlorphon wettable powder using laboratory methods (column 1) and field kit (column 3)

Date	Cholinesterase Readings														
	A			B			C			D			E		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
17.9.1968 (pre-exposure)			100			86.5			70			78.5			70
18.9.1968 (pre-exposure)	150			148			146			145			124		
24.9.1968	128	83.3	87.5	128	86.5	87.5	114	78.1	68.5	134	92.4	81.3	115	92.8	50
26.9.1968	110	73.3	87.5	109	73.6	87.5	99	67.8	75	104	71.7	68.8	106	85.5	67.5
1.10.1968	104	96.3	93.8	90	60.7	81.5	92	63	68.8	90	62.1	75	93	75	56.3
3.10.1968	92	61.3	81.8	88	59.5	75	75	51.4	75	72	49.7	68.8	91	73.4	62.5

1=units of cholinesterase as determined by laboratory method

2=laboratory readings as a percentage, taking the pre-exposure level (column 1) to equal 100 per cent

3=percentage cholinesterase as determined by test kit

Table 6.—Comparison between results for trichlorphon LVC using laboratory methods (column 1) and field kit (column 3)

Date	Cholinesterase Readings																				
	L			M			N			P			Q			R			S		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
16.6.1969 (pre-exposure)	125	100		121	100		101	100		145	100		142	100		125	100		121	100	
20.6.1969	120	96	100	116	95.9	100	107	105.9	100	125	86.2	100	130	91.5	100	118	94.4	100	116	95.9	100
25.6.1969			43			59			50			78			100			66			59
27.6.1969	78	62.4	75.5	82	67.8	94	72	71.3	78.5	95	65.5	43	107	75.4	91	78	62.4	91	82	67.8	75.5
30.6.1969	80	64	49.5	91	75.2	72	78	77.2	44	93	64.1	43	107	75.4	62.5	76	60.8	69			
4.7.1969	70	56	79.5	99	81.8	75	73	72.3	65.5	70	48.3	81	84	59.2	90	70	56	78	85	71.9	78.5
16.7.1969	81	64.8	65.5	88	72.7	100	85	84.2	37.5	83	57.2	72	92	64.8	75	83	66.4	62.5	81	67	37

1=units of cholinesterase as determined by laboratory method

2=laboratory readings as a percentage, taking the pre-exposure level (column 1) to equal 100 per cent

3=percentage cholinesterase as determined by test kit

Table 7.—Blood cholinesterase levels, in units of cholinesterase, for European and Papuan and New Guinean populations, Port Moresby

	Male			Female			All		
	Number sampled	Range	Mean	Number sampled	Range	Mean	Number sampled	Range	Mean
European	51	89-150	110.5	46	94-133	107.1	101	89-150	109.1
Papuan and New Guinean	46	99-138	111.3	26	89-124	107.2	103	89-138	109.6

From both tables it can be seen that the kit gave highly variable results which in many instances bore no resemblance to those obtained by the laboratory method. In fact some of the results obtained with the kit were quite misleading.

Following the first lot of results (*Table* 5), the use of the kit for estimating blood cholinesterase levels under field conditions was abandoned.

Whole Blood Cholinesterase Levels in European and Papuan and New Guinean Populations

The results of this survey are given in *Table* 7. Contrary to expectations, there was no difference between the cholinesterase levels of Europeans and Papua New Guineans.

DISCUSSION

From the first series of trials it became evident that the use of trichlorphon on a field scale to control *Pantorhytes* spp. would have to be watched closely. In fact a monitoring programme whereby regular blood samples (each of 1 cc of venous blood) were taken from plantation spray team personnel was commenced in early December, 1968. Sampling of individual spray teams was carried out at approximately 2-weekly intervals, and the samples were forwarded to Port Moresby for blood cholinesterase determination.

From the time the monitoring programme began and up until the introduction of LVC trichlorphon, approximately 118 sprayers were removed from exposure and one sprayer whose blood cholinesterase level fell to 22 units was admitted to hospital. However he did not exhibit any clinical symptoms of poisoning and was discharged after observation.

Following the introduction of LVC trichlorphon, however, the field position assumed rather alarming proportions, and in the first 4 weeks' use 106 sprayers were removed from exposure, with blood cholinesterase levels ranging from 60 down to 30 units (normal range: 90 to 150 units). Depletion of cholinesterase was rapid and occurred mainly within the first two weeks. It was obvious that gross contamination of sprayers was occurring and investigation showed that this was mainly from leaking spray machines. However with the introduction of the more concentrated spray (10 per cent versus 0.5 per cent for the wettable powder formulation) contamination of skin and clothing could also have been important.

Accordingly, a plea was made to planters (see *Appendix*) to ensure that the correct safety precautions were adopted by both sprayers and planters. This led to a marked improvement in the field position.

Absorption of organophosphorus insecticides may occur through the lungs, gastrointestinal tract, or skin (Batchelor and Walker 1954, Culver, Caplan and Batchelor 1956, Durham and Wolfe 1962, Simpson 1966). Absorption is more rapid and complete through the first two routes although skin contamination with small amounts of the more toxic compounds has frequently proved fatal.

Respiratory exposure may be of importance wherever there is a sufficient concentration of small droplets fine enough to inhale, and this may, in part, be responsible for the increased 'poisoning' of sprayers following the introduction of trichlorphon LVC. However it is usually contamination of the skin which is responsible for the lowering of cholinesterase levels.

It is also known that multiple exposures to organophosphorus insecticides over a brief period are partially cumulative in effect (Durham and Hayes 1962). However small multiple exposures over an extended period are not

indefinitely cumulative in their effects but the cholinesterase level reaches a plateau (Hayes and Durham 1954, Sumerford *et al.* 1953). This may in part explain the levelling off of cholinesterase levels obtained in our trials. The reason for the further decrease in *Table* 2 is not known.

Recovery of cholinesterase levels as shown in *Table* 2, following removal from exposure, agreed well with that recorded in the literature. Durham and Hayes (1962) reported that following cessation of exposure to organophosphates plasma enzyme activity is increased by about 13 per cent of original activity during the first day, and more slowly thereafter, so that 30 to 40 days are required to reach the normal pre-exposure level. This would appear to be at variance with the statement by Simson, Simpson and Penney (1969) that plasma levels usually return to normal in 1 or 2 days, although the data presented by them (their Table 1) showed that a period of 32 days was necessary in the case of a person acutely poisoned with monocrotophos.

With erythrocytes, once fully inhibited, the enzyme content of a particular erythrocyte is not regenerated, and it would appear that the rate of regeneration of red blood-cell cholinesterase reflects the replacement of red corpuscles in the circulation and thus requires 90 to 100 days to return to normal after near complete depression (Durham and Hayes 1962). Ganelin (1964) quotes a recovery period of from 70 to 182 days, whilst Simson *et al.* (1969) quote a maximum figure of 42 days.

Use of Field Kit

As mentioned earlier, the results obtained when using the field kit were very disappointing, even though many attempts were made to use it and all reagents and apparatus were thoroughly rechecked. Possible reasons for its failure under field conditions are:—

1. The blood sample used with the field kit is only 0.01 ml. This is an extremely small amount to measure accurately under difficult conditions.

2. The people to be tested were often called direct from spraying. Their hands may have been improperly washed or fingers unsatisfactorily cleaned with spirit prior to sample collection, thus introducing a small amount of insecticide into the test.

3. The test is dependent upon change in pH and requires scrupulously clean glassware. This is difficult to achieve if the tubes and pipettes have to be rinsed between batches in the field.

4. The incidence of high temperatures.

The only other study of the effect of spraying with organophosphorus insecticides on blood cholinesterase levels of sprayers under tropical conditions is that reported by Marchart (1970). He studied the effects on five sprayers of routine mistblower application to cacao of 2 oz of monocrotophos per acre. Monocrotophos is reported to have oral and dermal LD_{50}s of 13 to 21 and 122 to 350 mg per kg respectively (Shell International Chemical Company Limited, London, unpublished report, 1967). Twenty-seven acres were sprayed per day and the sprayers were all fitted with protective

***Table* 8.—Mistblower application of monocrotophos: blood cholinesterase activities of spray men (from Marchart 1970)**

Sprayer	Cholinesterase Activity				
	Pre-exposure (average)	Exposure (days) 1	2	3	10 Days Post-exposure
A (Control)	123	118	140	120	127
B	114	109	90	62	87
C	102	73	85	33	80
D	111	108	61	32	134
E	146	137	134	97	132
F	110	63	85	29	81

clothing (overalls, broad-brimmed felt hats). The spray gang was cautioned that they were handling a more toxic material than usual. Results are shown in *Table* 8 (after Marchart). Although no clinical symptoms of poisoning became evident the trial was stopped on the third day. Marchart concluded that the results demonstrated the risk with heavy insecticide exposure to mistblower operators working in the confined space under dense cacao canopy.

Monocrotophos has also received some attention in Papua New Guinea where it has been aerially applied to coconuts on Lihir Island to control a complex of coconut insects. The insecticide (a 60 per cent w/v emulsifiable concentrate) was mixed with oil and water and applied at 13.5 fl oz per acre, equivalent to 0.5 lb of active ingredient per acre. The spray was mixed in bulk in closed drums, hoses and pumps being used to introduce the various components of the mixture into the drums. However, it was necessary to pour concentrated monocrotophos through a filter, and under field conditions minor spillage was probably unavoidable. All personnel wore Protector Toxiguard agricultural respirators and rubber gloves. Towels and water were available and freely used to wash any concentrate off the skin immediately. Loading of the plane was from closed drums via hose using either gravity feed or pumps. Masks and gloves were also worn throughout this operation.

Blood samples were collected before any handling of monocrotophos and immediately after spraying was completed (Lauer, unpublished data). Results of the whole blood cholinesterase determinations are shown in *Table* 9.

The levels of all the indigenous mixers subjects A to D) fell. Subject A's level fell drastically, and it was thought that this was due to his being much more active in the mixing process.

Subjects E and F were exposed in the treated area while spraying was in progress. There was virtually no change in their cholinesterase levels.

Subject G, who showed little change, had almost no contact with the spray, whilst subjects H and J were directly concerned with handling the monocrotophos concentrate.

The pilot (subject K), showed a fall of 35 per cent.

With an increasing use of organophosphorus insecticides in Papua New Guinea, some of which must be looked upon as hazardous materials for general use on plantations, it is obvious that we need to know more about the toxicological hazards for each insecticide formulation when used on specific crops. We have already had reports of methomyl (oral and dermal LD_{50}s of 17 to 23.5 (rats) and 1500 (rabbits) mg per kg respectively) producing vomiting attacks in a spray gang spraying cacao and they were apparently so severe that spraying with the application rate used had to cease.

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Table 9.—Effects on blood cholinesterase levels of exposure and posible exposure to monocrotophos (after Lauer, unpublished data)

Subject	Units of Blood Cholinesterase	
	Pre-spray Level	Post-spray Level
A	108	40
B	103	75
C	95	79
D	82	68
E	106	117
F	111	114
G	108	103
H	107	106
J	112	95
K	103	68

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APPENDIX

SAFETY PRECAUTIONS TO BE OBSERVED WHEN SPRAYING TRICHLORPHON LVC FOR THE CONTROL OF PANTORHYTES SPP.

1. The concentrate is poisonous. Avoid contact with the skin and avoid breathing the vapour. If spilled on the skin, wash thoroughly with soap and water.
2. Sprayers should spray for a 2-week period and then be rested for 4 weeks.
3. AT THE COMPLETION OF EACH DAY'S SPRAYING, SPRAYERS SHOULD WASH OR BATHE THOROUGHLY, USING PLENTY OF SOAP AND WATER.
4. Any clothing worn during spraying should be removed at the completion of each day's spraying and WASHED THOROUGHLY. AT NO TIME SHOULD THE SPRAYER SLEEP IN CLOTHING IN WHICH HE HAS SPRAYED.
5. EVERY PRECAUTION SHOULD BE TAKEN TO ENSURE THAT THE SPRAY MACHINE DOES NOT LEAK INSECTICIDE OVER THE SPRAYER, ESPECIALLY FROM THE TAP, NOZZLE OR INSECTICIDE TANK. This is very important AS IT LEADS TO GROSS CONTAMINATION, and besides, it is very wasteful of spray.
6. SPRAYERS SHOULD NOT EAT OR SMOKE WHILE SPRAYING OR IMMEDIATELY AFTER SPRAYING WITHOUT FIRST WASHING THOROUGHLY WITH SOAP AND WATER.
7. Spillage of the concentrate or mixed spray should be avoided.

8. AT NO TIME SHOULD HANDS BE USED TO MIX THE SPRAY.
9. The SECOND SMALLEST flow restrictor hole in the ULV flow restrictor slide is used.

If large holes are used, the sprayer is being exposed to higher rates of insecticide and will therefore run a higher risk of being poisoned. Use of larger restrictor holes also leads to wastage of spray mixture without any increase in kill of *Pantorhytes* adults.

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