Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1961, **24**, 509-517

Methods for Measuring Insecticide Susceptibility Levels in Bed-bugs, Cone-nosed Bugs, Fleas and Lice

JAMES R. BUSVINE [1] & J. LIEN [2]

A standard kit is prepared and distributed by WHO for testing insecticide resistance in adult mosquitos, and it would seem advantageous to be able to use the filter papers impregnated with DDT and dieldrin contained in this kit for testing resistance in other insects. Experiments have been successfully conducted with a view to developing methods based on the use of these papers for testing susceptibility levels in bed-bugs, cone-nosed bugs, fleas and lice. The designs of the various test methods and the results obtained are described in this paper. The tests for bed-bugs and fleas have been adopted as provisional methods by the WHO Expert Committee on Insecticides; those for cone-nosed bugs and lice have been designated tentative methods, requiring further investigation prior to the drawing up of specifications.

INTRODUCTION

The methods for testing insecticide resistance in adult mosquitos sponsored by the World Health Organization have been remarkably successful. Approximately 650 adult test kits have been assembled and distributed to field workers in various parts of the world. Standard impregnated papers, for use in the tests, are supplied as required. Therefore, in the development of test methods for other types of insect, it would seem sensible to make use of these standard papers, prepared in Switzerland and distributed throughout the world.

We have experimented with such tests, based on the use of impregnated papers, for bed-bugs, cone-nosed bugs, fleas and lice. These techniques were considered by the WHO Expert Committee on Insecticides in September 1959. The tests for bed-bugs and fleas were adopted as provisional methods and are described in the Committee's report (WHO Expert Committee on Insecticides, 1960); the tests for cone-nosed bugs and lice were designated as tentative methods, requiring further investigation prior to drawing up specifications.

[1] London School of Hygiene and Tropical Medicine, London, England.

[2] Provincial Malaria Research Institute, Chao-Chow, Ping-Tun, Taiwan. The work was carried out during a visit to London made possible by the author's tenure of a WHO Fellowship.

METHOD FOR BED-BUGS

The method is substantially as described by Busvine (1958) and by Smith (1959). Small pieces (about 2 × 5 cm) are cut from the standard impregnated papers prepared for the WHO test for adult mosquitos. They are folded and dropped into ordinary test-tubes. Batches of about 10 adult bugs are put into the tubes; the bugs cling to the papers except when paralysed and moribund. Bugs are used five days after a blood meal; they are exposed for five days in an incubator at 25°C and mortalities are then determined.

Busvine (1958) gives some information on relations between exposure time and kill and on the relative susceptibility of the sexes, and notes that "even normal bugs have a low susceptibility to DDT immediately after feeding. After about a week, they became easier to kill with DDT, though this effect was not noted with dieldrin". We have examined the effects of certain other factors on results obtained by this method. In a few of the first of these additional tests, controls were maintained; but the deaths were always 5% or less, as found by Busvine (1958), and controls were therefore omitted from subsequent tests with bed-bugs.

Age of bugs and sex differences

Tests were made with DDT and dieldrin, using bugs one, two, four and eight weeks after emergence.

Results are shown in Table 1. It will be seen that susceptibility is roughly constant for the first two weeks of adult life, increases at four weeks and is considerably higher at eight weeks.

The relatively greater susceptibility of females, noted by Busvine (1958), has been confirmed; it is especially marked with DDT. This is anomalous, since in most tests with insecticides, female insects are more resistant (see Busvine, 1957, p. 29). A possible explanation which occurred to us is that female bugs might move about more under the stimulus of DDT than males and so pick up a larger dose. To investigate this, special tests were conducted in which the bugs were individually confined in small pieces of glass tubing, standing vertically on the treated paper, so that they were unable to walk about. (This was done by Barnes, 1945.) The results are shown in Table 1 under the heading " Bed-bugs immobilized ". It will be observed that this treatment considerably reduces the difference between susceptibility of males and females to DDT, so that there is some support for our suggestion.

TABLE 1

PERCENTAGE KILLS OF BED-BUGS AT DIFFERENT AGES, USING DDT AND DIELDRIN [a]

Concentration (%)	Sex	Percentage kill of bed-bugs						
		Normal tests				Bed-bugs immobilized		
		1 week	2 weeks	4 weeks	8 weeks	1 week	2 weeks	4 weeks
DDT								
2.0		95	85	95	100	85	90	65
1.0	M	10	5	50	84	35	15	15
0.5		0	0	20	55			
LC_{50}		1.4	1.5	0.95	0.47	1.2	1.4	1.6
DDT								
2.0		100	95	100	100	95	100	100
1.0	F	76	90	100	96	45	35	80
0.5		80	0	80	95			
LC_{50}		0.66	(0.5)	(0.3)	(0.25)	1.0	(1.1)	(0.75)
Dieldrin								
0.2		100	90	80	100			
0.1	M	20	20	57	81	45	65	50
0.05		0	0	32	34	5	5	5
LC_{50}		0.12	0.13	0.085	0.060	0.11	0.09	0.10
Dieldrin								
0.2		100	100	100	100			
0.1	F	30	60	90	96	35	75	100
0.05		5	0	39	46	0	0	20
LC_{50}		0.12	0.09	0.055	0.052	(0.12)	(0.085)	(0.065)

[a] 20 bed-bugs per concentration. The LC_{50} values are estimated graphically; those in parentheses are rough approximations based on one point with a line drawn parallel to other curves.

Temperature

For the tests on the effect of temperature, groups of bugs were kept in incubators at 20°C, 25°C and 30°C respectively for a week before testing at the same temperature. In other respects, tests were conducted on standard lines.

The results are shown in Table 2; they are quite interesting. It will be seen that temperature has comparatively little effect on the susceptibility of bed-bugs to DDT, except for a slight lowering of the LC_{50} at the lowest temperature tested. In contrast, susceptibility to dieldrin shows a strong positive correlation with temperature. The explanation may be as follows.

The kills obtained in any test in which the insects " dose themselves " by crawling on a surface is the resultant of two factors: (*a*) the dose picked up, which depends on activity of the insects; and (*b*) the intrinsic toxicity of the insecticide. It is presumed that bugs become more active at higher temperatures and so pick up more insecticide. The intrinsic toxicity of DDT, however, is found to have a negative correlation with temperature (see summary in Busvine, 1957, p. 35). Therefore, the two factors oppose each other to maintain susceptibility roughly constant. With dieldrin, however, both factors tend to greater susceptibility at higher temperatures.

Tests with organo-phosphorus insecticides

A certain number of tests was conducted with diazinon and malathion to determine whether this general method would be suitable for tests with organo-phosphorus insecticides. Risella oil is not a suitable solvent for these compounds; therefore they were dissolved primarily in di-octyl phthalate, which is an excellent solvent and in its physical properties appears to resemble Risella oil. The primary solutions were applied to filter papers with a volatile solvent (chloroform) exactly as described by Busvine & Nash (1953). Control tests showed no mortality (25 bugs). The results with the insecticides are shown in Table 3. It appears that the method is

TABLE 2
RESULTS OF SUSCEPTIBILITY TESTS WITH BED-BUGS AT DIFFERENT TEMPERATURES, USING DDT AND DIELDRIN [a]

Concentration (%)	Percentage kills of bed-bugs					
	Males			Females		
	20°C	25°C	30°C	20°C	25°C	30°C
DDT						
4.0	100			100		
2.0	80	84	88	96	96	88
1.0	12	8	4	72	40	40
0.5	8	0	0	36	8	32
0.25			0			0
LC_{50}	1.3	1.5	1.5	0.65	0.95	0.9
Dieldrin						
0.4	100			100		
0.2	60	92		76	100	
0.1	0	20	88	4	32	92
0.05		0	20		2	56
0.025			4			4
LC_{50}	0.18	0.13	0.065	0.16	0.11	0.050

[a] 20 bed-bugs per concentration. LC_{50} values estimated graphically.

TABLE 3

RESULTS OF SUSCEPTIBILITY TESTS WITH BED-BUGS, USING ORGANO-PHOSPHORUS INSECTICIDES [a]

Concentration (%)	Males		Females	
	No. used	% kill	No. used	% kill
Diazinon				
0.3	20	100	20	100
0.1	40	100	40	100
0.03	80	58	80	54
0.01	60	22	60	5
LC_{50}	0.025		0.028	
Malathion				
0.5	60	100	60	100
0.25	40	90	40	87
0.10	60	70	60	68
0.05	23	13	27	22
LC_{50}	0.07		0.07	

[a] LC_{50} values estimated graphically.

quite adequate, provided the papers are prepared freshly. One interesting result is the absence of a difference in susceptibility between the sexes.

METHOD FOR CONE-NOSED BUGS

It seemed reasonable to use a similar test for large blood-sucking bugs of the genera *Triatoma*, *Rhodnius*, etc. Such insects would require a larger exposure chamber than a test-tube and, as a first choice, the WHO exposure tubes for adult mosquitos were tried. It was found that, if these were lined with impregnated papers in the usual way, the large bugs crawled on to the wire-screen end. To prevent this, the papers were cut short about half an inch from the top, leaving a ring of bare plastic; this successfully kept the bugs on the paper.

Tests were made, at different concentrations of DDT and dieldrin, to determine susceptibility levels of adult *Triatoma protracta*. The results are shown in Table 4.

Although the numbers available for these tests were small, it seems virtually certain that a method of this type is perfectly suitable. It is evident that females are less susceptible than males and that nymphs (3rd to 5th stages) were even more difficult to kill.

Comparing the results with those obtained with bed-bugs, it will be seen that the order of susceptibility of the sexes is reversed and that *Triatoma* adults have about the same level of resistance to DDT but are more susceptible to dieldrin.

METHOD FOR FLEAS

Several workers have used impregnated papers for measuring susceptibility levels in fleas. Shawarby (1953), Sen (1958) and Smith (1959) used filter papers impregnated as described by Busvine & Nash (1953). In Shawarby's method, 10 small discs (5 mm in diameter) were punched out of each treated paper and put into a 3 × 1-inch (7.5 × 2.5-cm) glass tube with a flat bottom. After one hour, the fleas were moved to clean tubes containing a little dry sawdust and examined for mortality 24 hours later. Sen merely states that his experiments were done " by following the Busvine-Nash technique of exposing the insects to treated filter papers of known strength for one hour and by recording the mortality after 24 hours ". Smith put the fleas into 3 × 1-inch specimen tubes lined with treated papers and recorded the mortality after 24 hours' exposure.

Shawarby and Sen used *Xenopsylla cheopis*; Smith worked with *Pulex irritans*.

TABLE 4

RESULTS OF SUSCEPTIBILITY TESTS WITH *TRIATOMA PROTRACTA* EXPOSED TO DDT OR DIELDRIN FOR 5 DAYS AT 25°C [a]

Concentration (%)	Percentage mortality		
	Males	Females	Nymphs
DDT			
4.0		100	92
2.0	100	75	23
1.0	46	45	
0.5	28		
Dieldrin			
0.4			100
0.2			77
0.1	100	100	58
0.05	100	78	0
0.025	62	50	
0.012	13	0	

[a] Each point based on 12-14 individuals. Control mortalities: males 7 %, females 9 %, nymphs 0.

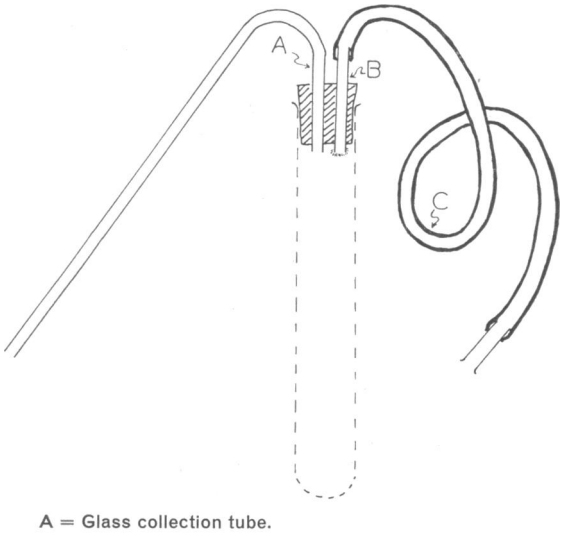
Suggested method

In order to simplify matters, we have tried setting up the test in the same way as for bed-bugs. A small piece (about 3 × 2 cm) is cut from each impregnated paper, folded twice in the form of a Z and dropped into an ordinary test-tube. Batches of fleas are added with the aid of the simple suction device shown in Fig. 1, which is fitted to the top of each tube in turn. The fleas are collected from a large glass accumulator jar, into which the contents of a flea culture jar have been shaken. (Any vessel with polished sides over 15 cm high will retain *Xenopsylla* fleas.) The fleas in the prepared tubes climb up the papers and sometimes jump from the tops; but if the tubes are kept upright, they do not escape. The exposure (at 25°C) is made in darkness, which reduces the activity of the fleas.

In some of the tests, the exposure to treated papers was one hour, after which the fleas were transferred to clean tubes, which contained clean paper slips, and kept for 24 hours before mortality counts. In other, later, tests a 24-hour exposure was given and mortality estimated at the end of it. To examine the fleas, the paper slips were tipped out into a glass bowl and the live fleas collected by the suction apparatus and killed with chloroform vapour. Dead fleas were collected in another tube and the two lots examined under a binocular microscope for sexing.

FIG. 1

SUCTION DEVICE FOR COLLECTING FLEAS IN TEST-TUBES



A = Glass collection tube.
B = Aspirator tube, with gauze-covered end.
C = Rubber tube ending in mouthpiece (or attachment to suction pump).

The tests with DDT and dieldrin were done with WHO impregnated papers. Additional tests were done with malathion and diazinon dissolved in di-octyl phthalate instead of Risella oil and applied to the papers as described by Busvine & Nash (1953).

Results

Some preliminary tests were done with unfed fleas. The lack of food resulted in high control mortalities, especially in male fleas. Therefore all the tests were done subsequently with recently blood-fed fleas. This was achieved by placing a mouse in the flea culture jar overnight before the tests.

The results of tests with a one-hour exposure are given in Table 5 and those for 24-hour exposures in Table 6. The experiments with 24 hours' exposure were more convenient than those with a one-hour exposure, because one operation (transfer to clean tubes) was omitted. It is considered that the lower LC_{50}'s for the 24-hour tests would also be an advantage, in that they allow more room for comparison with possible resistant strains or for tests with less susceptible species.

The results of the one-hour tests show the same relationships as the bed-bug tests, though less clearly. Thus, (1) females are slightly more susceptible than males, and (2) while the tests with DDT are comparatively similar over the temperature range 20°-30°C, the results with dieldrin show a distinct lowering of susceptibility with increasing temperature.

In the 24-hour test, the difference between the sexes is lost (or perhaps slightly reversed).

Comparing our one-hour results with other published work we have the following LC_{50}'s: for DDT—Shawarby, 0.37%; Sen, 0.4%; this paper, 0.5%-0.64%; for dieldrin—Shawarby, 0.032%; this paper, 0.14%.

AN ALTERNATIVE METHOD FOR LICE

The existing WHO test method for resistance in body lice was one of the earliest introduced; it has been quite widely used and has given some useful information. On the other hand, there are certain inherent defects in this type of test.[1] Insecticidal

[1] Rao, R. T. (1958) *Development of test methods for other insects of public health importance* (paper presented at the PAHO/WHO Seminar on the Susceptibility of Insects to Insecticides, Panama, 1958).

TABLE 5

RESULTS OF SUSCEPTIBILITY TESTS WITH *XENOPSYLLA CHEOPIS* AT DIFFERENT TEMPERATURES WITH 1-HOUR EXPOSURE [a]

Concentration (%)	Percentage kill of fleas					
	Males			Females		
	20°C	25°C	30°C	20°C	25°C	30°C
DDT						
4.0	100	100	100	100	100	100
2.0	94	79	90	100	96	88
1.0	80	68	78	73	70	80
0.5	18	41	41	38	54	31
LC_{50}	0.70	0.64	0.60	0.62	0.50	0.60
Dieldrin						
1.6	94			100		
0.8	87	100	100	93	100	100
0.4	61	100	100	87	91	100
0.2	27	62	90	44	79	97
0.1		25	42		25	35
LC_{50}	0.35	0.14	0.11	0.22	0.14	0.11

[a] Approximately 25 fleas of each sex per concentration. LC_{50} values estimated graphically. C ntrol mortalities: at 30°C, males 16%, females 0; at 20°C, males 7%, females 7%.

TABLE

RESULTS OF SUSCEPTIBILITY TESTS WITH *XENOPSYLLA CHEOPIS* AT 25°C WITH 24-HOUR EXPOSURE [a]

Concentration (%)	Percentage kill		Concentration (%)	Percentage kill	
	Males	Females		Males	Females
DDT			Malathion		
1.0	100	100	1.0	100	100
0.5	100	100	0.5	100	100
0.25	80	69	0.3	83	82
0.1	23	29	0.2	32	13
LC_{50}	0.14	0.16	LC_{50}	0.23	0.25
Dieldrin			Diazinon		
0.2	100	100	0.1	100	97
0.05	92	100	0.05	50	25
0.025	76	82	0.03	8	0
0.01	31	18	0.01	0	0
LC_{50}	0.015	0.015	LC_{50}	0.05	0.07

[a] LC_{50} values estimated graphically. Control mortalities: males 3%, females 0.

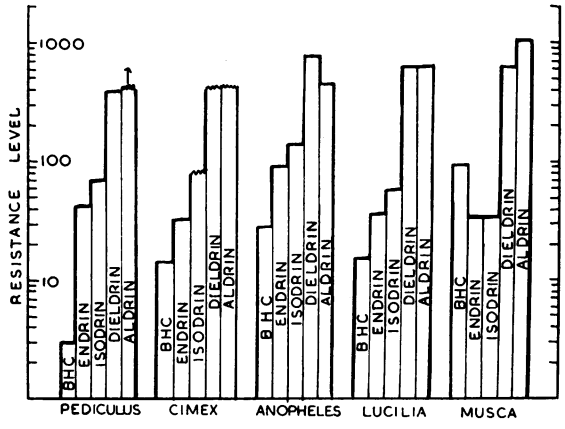
TABLE 7

RESULTS OF SUSCEPTIBILITY TESTS WITH TWO LOUSE STRAINS AT 25°C WITH 24-HOUR EXPOSURE

Tanganyika strain			Cairo strain		
Concentration (%)	No. lice	% kill	Concentration (%)	No. lice	% kill
DDT			DDT		
2.0	30	86	2.0	19	68
1.0	34	62	1.0	24	38
0.5	17	6			
Dieldrin			Dieldrin		
4.0	44	25			
1.6	32	3			
			0.05	29	97
			0.025	30	48
			0.01	21	5
γ-BHC			γ-BHC		
0.3	20	95			
0.2	24	79			
0.1	25	60			
0.05	23	13	0.05	34	94
			0.025	27	18
Endrin			Endrin		
3.0	32	81			
2.0	39	69			
1.0	31	10			
			0.05	44	66
			0.025	35	23
Aldrin			Aldrin		
16.0	19	0			
			0.10	12	100
			0.05	21	90
			0.025	12	0
Isodrin			Isodrin		
10.0	12	100			
4.0	36	80			
2.0	20	15			
			0.05	20	65
			0.025	10	10
β-chlordane					
12.0	35	20			
8.0	14	0			
Control	30	3			

FIG. 2

RESISTANCE SPECTRA FOR STRAINS OF VARIOUS INSECTS RESISTANT TO BHC-DIELDRIN GROUP



Pediculus humanus: see text.
Cimex lectularius: after Busvine (1958).
Anopheles gambiae: data of Davidson (1958).
Lucilia caesar: unpublished data of Busvine & Shanahan.
Musca domestica: after Busvine (1954).

TABLE 8

RELATIVE RESISTANCE OF CAIRO AND TANGANYIKA LOUSE STRAINS TO VARIOUS CHLORINATED HYDROCARBON INSECTICIDES

Insecticide	Cairo strain LC_{50}	Tanganyika strain	
		LC_{50}	Resistance
DDT	1.30	0.9	× 0.7
γ-BHC	0.033	0.1	× 3
Endrin	0.035	1.4	× 40
Isodrin	0.040	2.8	× 70
Dieldrin	0.025	(7)	× 280
Aldrin	0.035	⩾16	⩾× 450
β-chlordane	—	⩾12	—

tests with dusts, in which the insects are dosed by making them walk over a fixed deposit, do not show a good correlation between concentration of toxicant and mortality. The results summarized by Wright & Brown (1957) show that in many cases a fivefold increase in concentration does not give much increase in kill. The data are not suitable for a calculation of LC_{50} values, and therefore the degree of resistance cannot be measured. This test method was introduced before the general availability of standard impregnated papers, which have generally given steep concentration/kill regression lines for susceptible strains of various insects. Such papers have been used for measuring susceptibility levels of lice (by Busvine & Nash, 1953) and for detecting resistance (by Busvine, 1953; and by Smith, 1959). We have used a simplified and standardized form of the test to delineate a resistance spectrum for the dieldrin-resistant strain of lice, found by Dr A. Smith in Tanganyika, of which a subcolony was sent to us and maintained by one of us in London, worn daily on the leg as described by Buxton (1947). For comparison, a colony was obtained from the Insect Control Section, Ministry of Public Health, Cairo, which appears to be normally susceptible except for a possible slight resistance to DDT (i.e., the LC_{50}'s for various insecticides approximate to those given by Shawarby, 1953, for a susceptible colony).

The papers used were WHO standard papers (prepared for the adult mosquito test) for DDT and dieldrin; papers containing other compounds were prepared as described by Busvine & Nash (1953). Adult lice were confined on the papers by 5-cm diameter inverted glass funnels (or glass rings for gamma-BHC) and kept in darkness at 25°C for 24 hours. Batches of approximately 10 were used, males and females together, each test being replicated once or twice from different generations. Mortality counts were made at the end of the exposure, the criterion of death being inability to stand. The results are shown in Table 7.

Approximate LC_{50}'s were estimated from these data and are set out in Table 8, which also shows the resistance levels for various compounds. The resistance spectrum for these lice is shown in Fig. 2, in comparison with those for other strains of insects resistant to the BHC-dieldrin group. The similarity will be at once apparent; it presumably indicates a similar resistance mechanism.

RÉSUMÉ

La trousse standard mise au point par l'OMS pour évaluer la résistance des moustiques adultes aux insecticides a été largement répandue dans le monde et a fait ses preuves. Il a paru souhaitable d'ajouter à cette trousse des papiers imprégnés de DDT et de dieldrine, destinés à l'étude de la résistance d'autres insectes — punaises de lit, triatomes, puces et poux. Les auteurs ont essayé diverses méthodes utilisant des papiers filtres

pour éprouver la sensibilité de ces groupes d'insectes. La sensibilité des punaises de lit adultes est constante durant les deux premières semaines de vie, mais s'élève notablement entre la quatrième et la huitième semaine. Les femelles sont plus sensibles, peut-être parce que, plus actives, elles absorbent plus d'insecticides. Un changement de température de 20° à 30°C abaisse de beaucoup la DL_{50} pour la dieldrine. Il semble que la méthode pourrait être étendue à l'étude de la résistance aux organo-phosphorés. Pour les puces, le temps d'exposition doit être réduit (1-24 heures). La sensibilité au DDT n'est influencée ni par le sexe ni par la température; la sensibilité à la dieldrine en revanche, comme pour les punaises de lit, augmente avec la température.

Le test OMS pour évaluer la sensibilité des poux convient aux travaux sur le terrain, mais il est insuffisant pour la recherche. Les auteurs proposent une méthode applicable aux poux, maintenus en observation pendant 24 heures. Ils l'ont appliquée à l'étude d'une souche de poux dont la résistance aux insecticides du groupe HCH/dieldrine semble obéir au même mécanisme que celle de punaises, moustiques et mouches. La recherche des méthodes satisfaisantes pour les triatomes et les poux doit être poursuivie avant que des normes puissent être proposées.

REFERENCES

Barnes, S. (1945) *Bull. ent. Res.*, **36**, 273

Busvine, J. R. (1953) *Nature (Lond.)*, **171**, 118

Busvine, J. R. (1954) *Nature (Lond.)*, **174**, 783

Busvine, J. R. (1957) *Techniques for testing insecticides*, London, Commonwealth Bureau of Entomology

Busvine, J. R. (1958) *Bull. Wld Hlth Org.*, **19**, 1041

Busvine, J. R. & Nash, R. (1953) *Bull. ent. Res.*, **44**, 371

Buxton, P. A. (1947) *The louse*, London, Arnold

Davidson, G. (1958) *Bull. Wld Hlth Org.*, **18**, 579

Sen, P. (1958) *Bull. Calcutta Sch. trop. Med. Hyg.*, **6**, 14

Shawarby, A. A. (1953) *Bull. ent. Res.*, **44**, 377

Smith, A. (1959) *Bull. Wld Hlth Org.*, **21**, 240

World Health Organization, Expert Committee on Insecticides (1960) *Wld Hlth Org. techn. Rep. Ser.*, **191**

Wright, J. W. & Brown, A. A. W. (1957) *Bull. Wld Hlth Org.*, **16**, 9