

The Use of Gas Chromatography for the Quantitative Determination of Micro-Amounts of Insecticide Picked up by Mosquitos *

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The test method recommended by the WHO Expert Committee on Insecticides for determining the susceptibility or resistance of adult mosquitos to insecticides has been determined quantitatively by the use of gas chromatography to measure the amount of dieldrin picked up by adult Culex quinquefasciatus Say (= C. pipiens fatigans Wiedemann). A strain homozygous for dieldrin-resistance was used to determine the pick-up as a function of time and concentration of dieldrin. The results of experiments using electron-capture detection with gas chromatography suggest that the pick-up is a linear function of the concentration on the paper and also of the time of exposure, even of the 24-hour exposure to the 4.0 % dieldrin paper.

The test method for determining the susceptibility of adult mosquitos to insecticides recommended in the eighth report of the WHO Expert Committee on Insecticides⁴ was specifically devised to detect physiological resistance to insecticides. If physiological resistance is to be correctly diagnosed, it is necessary to know that the amount of insecticide entering the insect is a known function of the dosage and the exposure time.

MATERIALS USED

Female mosquitos, 7-14 days old, of a strain of *Culex quinquefasciatus* Say (= *Culex pipiens fatigans* Wiedemann) homozygous for dieldrin-resistance and fed only sugar-water were used.

The gas chromatograph utilized was an Aerograph Hi-Fy with electron-capture as a detector. The column a 1/8-inch by 5-foot (3-mm by 1.5-m)

stainless-steel tube packed with acid-washed 60/80-mesh Chromosorb W with a 5% SE-30 silicone coating. Measurements were made at a column temperature of 180°C and a nitrogen flow of 80 ml/minute.

The acetone in the extraction procedure was distilled over Drierite (anhydrous calcium sulfate). The petroleum ether was checked to verify that it gave no peaks in the vicinity of the dieldrin peak on the gas chromatograph recorder. Standard WHO adult mosquito testing tubes and dieldrin papers were used. All glassware was cleaned in acid dichromate.

METHOD

In the test recommended by the WHO Expert Committee on Insecticides (*op. cit.*), mosquitos are held in a tube lined with clean paper and transferred by blowing into a similar tube lined with paper treated with a known concentration of insecticide in oil. At the end of the exposure period, the insects are blown back into the tube lined with clean paper and mortality assessed after 24 hours.

In this work, at the end of the exposure period to varying concentrations of dieldrin paper, the mosquitos were blown through into a tube lined with clean paper and anaesthetized with chloroform. Twenty-five females from each test were placed in a sintered glass funnel and rinsed with 2.5 ml of

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⁴ World Health Organization, Expert Committee on Insecticides (1958) *Wld Hlth Org. techn. Rep. Ser.*, 153, 56. This method has been revised in the tenth and thirteenth reports of the WHO Expert Committee on Insecticides (*Wld Hlth Org. techn. Rep. Ser.*, 1960, 191, 15; 1963, 265).

**DIELDRIN RECOVERED FROM DIEL-R MOSQUITOS EXPOSED FOR VARIOUS TIMES
TO WHO DIELDRIN PAPERS OF DIFFERENT CONCENTRATIONS**

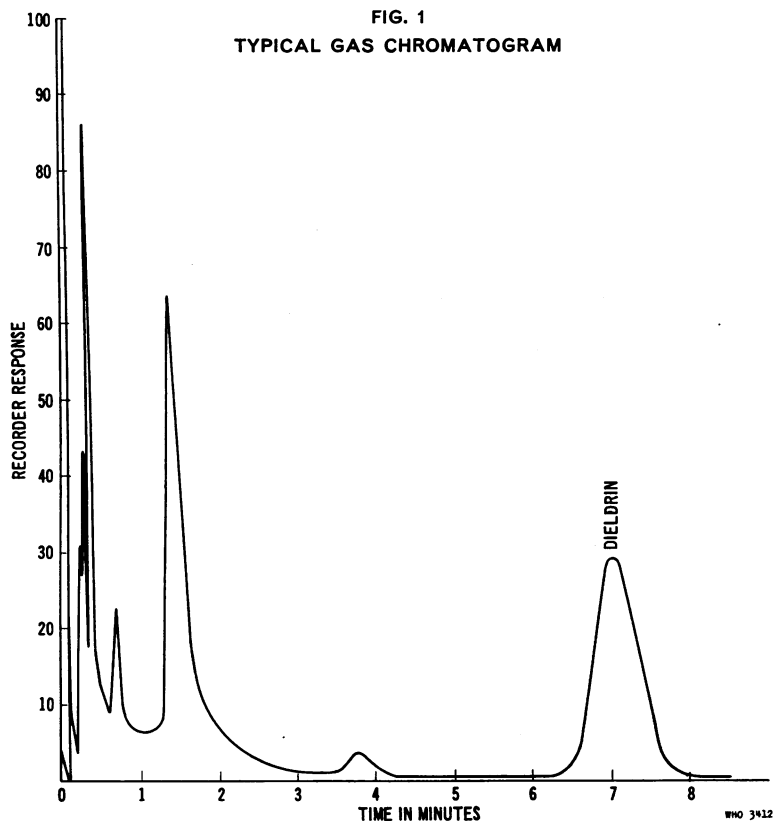
Exposure time	% Dieldrin in Risella oil	Amount of dieldrin recovered (μg per female)								
		" External "				" Internal "				" Total "
		1	2	3	Mean	1	2	3	Mean	
2 hours	0.4	0.0091	0.0097	0.0091	0.0093	0.0101	0.0118	0.0118	0.0112	0.0182
	0.8	0.0052	0.0082	0.0065	0.0066	0.0163	0.0209	0.0172	0.0181	0.0242
	1.6	0.0199	0.0166	0.0180	0.0182	0.0392	0.0364	0.0364	0.0373	0.0612
	4.0	0.0251	0.0192	0.0162	0.0202	0.0625	0.0498	0.0509	0.0544	0.0697
4 hours	0.4	0.0057	0.0096	0.0086	0.0080	0.0204	0.0194	0.0214	0.0204	0.0259
	0.8	0.0054	0.0080	0.0075	0.0070	0.0324	0.0296	0.0242	0.0287	0.0300
	1.6	0.0156	0.0210	0.0203	0.0190	0.0616	0.0638	0.0684	0.0646	0.0830
	4.0	0.0537	0.0434	0.0265	0.0412	0.1812	0.1427	0.1634	0.1624	0.2122
8 hours	0.4	0.0051	0.0056	0.0076	0.0061	0.0229	0.0197	0.0243	0.0223	0.0253
	0.8	0.0060	0.0058	0.0073	0.0064	0.0596	0.0665	0.0574	0.0612	0.0587
	1.6	0.0289	0.0227	0.0292	0.0269	0.1465	0.1559	0.1662	0.1562	0.1962
	4.0	0.0327	0.0471	0.0379	0.0392	0.1925	0.2235	0.1887	0.2016	0.2216
12 hours	0.4	0.0110	0.0100	0.0124	0.0111	0.0509	0.0392	0.0350	0.0417	0.0482
	0.8	0.0091	0.0090	0.0071	0.0084	0.1740	0.2760	0.1908	0.2136	0.1612
	1.6	0.0282	0.0284	0.0238	0.0268	0.2140	0.2319	0.1944	0.2134	0.2516
	4.0	0.0451	0.0484	0.0532	0.0489	0.3005	0.2976	0.2911	0.2964	0.3906
24 hours	0.4	0.0174	0.0165	0.0144	0.0161	0.1018	0.1048	0.1026	0.1031	0.1030
	0.8	0.0174	0.0202	0.0204	0.0193	0.2667	0.2556	0.2148	0.2457	0.2778
	1.6	0.0225	0.0280	0.0298	0.0268	0.3568	0.3324	0.3540	0.3477	0.4789
	4.0	0.0698	0.0770	0.1978	0.1149	0.7794	0.7230	0.7012	0.7345	0.9600

acetone. This volume of acetone was enough to cover them, and after gentle swirling for a few seconds, the acetone was sucked through and collected. This process was repeated, so that the 25 females were washed with a total of 5.0 ml of acetone. The rinsed mosquitos were then transferred to a homogenizing tube. The sintered glass funnel was rinsed once more with 2.5 ml of acetone, and this rinse added to the 5.0 ml previously collected. The external rinsing procedure was completed in less than one minute.

The 25 washed mosquitos were homogenized with about 5 g of anhydrous sodium sulfate and 5 ml of acetone, using a metal-bladed homogenizer. The homogenate was centrifuged until the supernatant was clear and the supernatant transferred quantita-

tively to a clean test-tube. This process was repeated three more times with the supernatant being added each time to the same test-tube. The contents of this tube were then evaporated to dryness in a stream of air and the residue dissolved in a known volume of petroleum ether. 10 μl of this petroleum ether solution were injected into the gas chromatograph to assess the amount of dieldrin present. The amount of dieldrin found in this extract is here termed "internal" dieldrin.

The 7.5 ml of acetone which had been collected from the washing procedure were also evaporated to dryness and dissolved in a known volume of petroleum ether and 10 μl of this sample injected into the gas chromatograph. The amount of dieldrin in this extract is termed "external" dieldrin.



For each concentration of dieldrin and for each time-period of exposure, four WHO testing tubes were used. Of the four tubes in each test, three were used to obtain both internal and external dieldrin values; the mosquito sample from the fourth was homogenized without washing and the dieldrin recovered called "total" dieldrin.

Only mosquitos which were alive at the end of the exposure period were used. For the 12-hour and 24-hour exposures, the tube ends were covered with a piece of moist cotton wadding. Mortality was appreciable only for the 24-hour exposure to the 4.0% dieldrin paper. Here the survivors from several tubes were combined to obtain the 25 females. The mosquitos were exposed under uniform conditions of illumination at 25°C.

METHOD OF CALCULATION

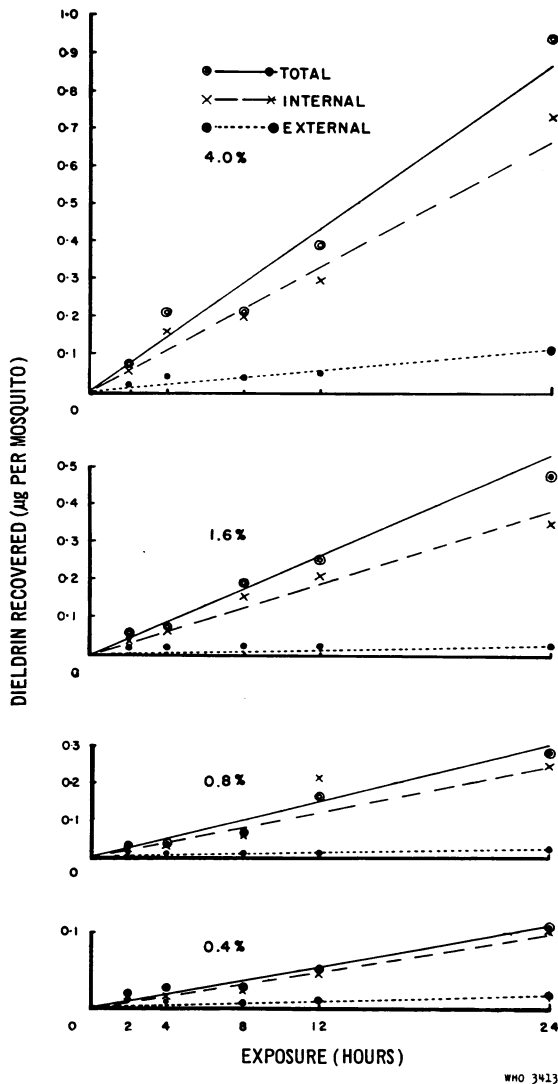
The amount of dieldrin in each sample was calculated from the area under the dieldrin peak pro-

duced when 10 μ l from the sample were injected into the gas chromatograph. A disc integrator gave this area as a certain number of units. By injecting 10 μ l of a solution containing 0.0002 μ g of dieldrin in 1 μ l of cyclohexane, the number of disc integrator units for a known amount of dieldrin was obtained.

The mean value and the standard deviation from this mean was calculated for 11 injections of this standard dieldrin solution. The 95% confidence limits did not exceed $\pm 5\%$ from the mean. The error was greater for routine analyses, where three or more injections were made from the same sample. The maximum error obtained was 17% from the mean. This error amounted to 0.004 μ g dieldrin per mosquito.

The following illustrates the method of calculation. The "total" dieldrin extracted from 25 mosquitos exposed for 4 hours to a 0.8% dieldrin WHO paper was made up to 10 ml in petroleum ether. 10 μ l of this 10-ml extract gave an average value of 162 units at attenuation 1 when injected into the gas chromato-

FIG. 2
TOTAL, INTERNAL AND EXTERNAL AMOUNTS OF
DIELDRIN RECOVERED PER MOSQUITO FOR
DIFFERENT EXPOSURE PERIODS AND
CONCENTRATIONS OF DIELDRIN PAPER



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graph. At this same attenuation, 1 unit = $\frac{0.002 \mu\text{g}}{432}$ dieldrin. Thus the 162 units of the $10 \mu\text{l}$ injected represents $0.00075 \mu\text{g}$ of dieldrin. In the entire 10-ml extract there will be $0.75 \mu\text{g}$ of dieldrin from 25 mosquitos, or $0.0300 \mu\text{g}$ per mosquito.

As a check on the effectiveness of the dieldrin extraction procedure, 0.2 ml of a solution con-

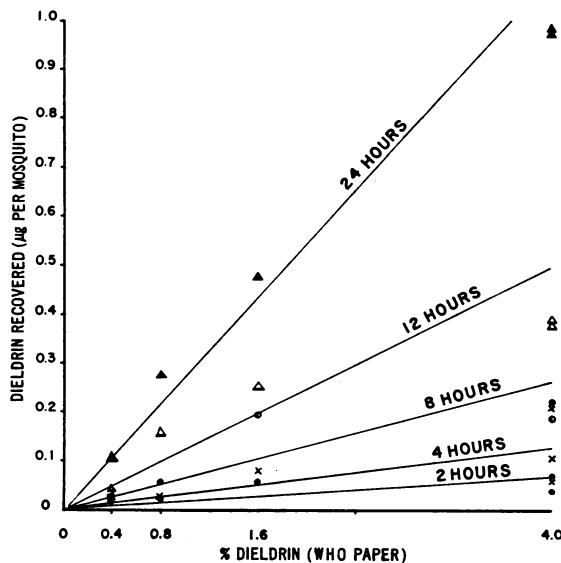
taining $0.001 \mu\text{g}/\mu\text{l}$ of dieldrin in cyclohexane was added to a 5-ml volumetric flask and the volume made up to 5 ml of petroleum ether. This solution should contain $0.0004 \mu\text{g}$ in $10 \mu\text{l}$, and an average of 3 injections of $10 \mu\text{l}$ gave $0.0004 \mu\text{g}$. Similarly, 0.2 ml of the solution containing $0.001 \mu\text{g}/\mu\text{l}$ of dieldrin in cyclohexane was added to each of two batches of 25 mosquitos which were then homogenized as described and the extract taken up in 5 ml of petroleum ether. Averages from $10\text{-}\mu\text{l}$ injections gave $0.0004 \mu\text{g}$ per $10 \mu\text{l}$ for both samples, indicating complete recovery of the dieldrin added. This is a check for "total" dieldrin.

RESULTS AND DISCUSSION

The results obtained are shown in the table. A typical gas chromatogram obtained is depicted in Fig. 1. Under the conditions stated in the text, dieldrin produces a peak about seven minutes after injection.

In Fig. 2, the mean "total", "external" and "internal" amounts of dieldrin recovered, in μg per mosquito, recorded in the table, are plotted for

FIG. 3
TOTAL DIELDRIN RECOVERED PER MOSQUITO PLOTTED
AGAINST CONCENTRATION OF DIELDRIN PAPER
FOR EACH EXPOSURE PERIOD^a



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^a Additional points have been plotted in this figure that are not recorded in the text but were obtained in subsequent work.

different exposure periods and concentrations of dieldrin paper. It will be seen that except for the 24-hour exposure to 4.0% dieldrin, the amount of "external" dieldrin remains fairly constant, while the "internal" dieldrin continues to increase as exposure time and concentration of dieldrin paper increase. This indicates that dieldrin continues to enter the insect.

The "total" dieldrin recovered per mosquito,

plotted against the concentration of dieldrin paper for each time-period of exposure is shown in Fig. 3.

CONCLUSION

In the WHO Expert Committee on Insecticides test for this strain of mosquito, pick-up is a linear function of the concentration on the paper, and also of the time of exposure, even of the 24-hour exposure to the 4.0% paper.

RÉSUMÉ

Le test de détermination de la sensibilité ou de la résistance des moustiques adultes aux insecticides, recommandé par le Comité OMS d'experts des Insecticides, a été évalué quantitativement par la mesure de la quantité de dieldrine absorbée par *Culex quinquefasciatus* Say. Des moustiques femelles, âgées de 7 à 14 jours, issues d'une souche homozygote pour la résistance à la dieldrine et nourries uniquement d'eau sucrée, ont été exposées pendant des laps de temps différents à des papiers imprégnés de dieldrine (type OMS) à différentes concentrations; la quantité de dieldrine absorbée a été mesurée par chromatographie gazeuse.

L'on a soumis au test chromatographique 25 moustiques femelles. Ces insectes ont d'abord subi deux rin-

çages à l'acétone; ils ont été ensuite recueillis à part et homogénéisés à trois reprises. La dieldrine présente dans l'extrait résultant de cette homogénéisation est appelée « dieldrine interne ». L'acétone ayant servi au rinçage des moustiques a été lui aussi soumis à la chromatographie et la dieldrine s'y trouvant a reçu le nom de « dieldrine externe ». L'on a également étudié des moustiques homogénéisés sans rinçage préalable et la dieldrine ainsi déterminée a été appelée « dieldrine totale ».

Les résultats suggèrent que l'absorption de la dieldrine est une fonction linéaire de la concentration en dieldrine du papier et de la durée d'exposition des insectes, et régulière jusqu'à une exposition de 24 heures à du papier à 4,0% de dieldrine.