RECOVERY AFTER KNOCKDOWN AND NON-CONTACT TOXICITY OF CARBAMATES CARBAMULT AND ARPROCARB

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Georghiou (1962) discussed the significance and implications of the speed of knockdown of mosquitoes and consequent recovery after exposure to insecticides. He also showed that carbamates as a group produced fastest knockdown. It was considered that the phenomena of recovery after knockdown may not be of serious consequence in the case of residual application of insecticides because the toxicant stays on the walls for several months and the chances of recovery after repeated cases of knockdown were very remote. However, this may not be true for DDT-resistant strains of house flies as shown by Barber and Schmitt (1949). Perry (1964) discussed this question in detail and suggested that the ability to recover from knockdown involves removal of the toxicant from the site of action, as also shown by Ikeshoji and Suzuki (1959). With the carbamate insecticides, recovery from knockdown may also result from incomplete and reversible enzyme inhibition (Kolbezen *et al.*, 1954).

Since knockdown and subsequent recovery are important factors influencing the effectiveness of residual insecticides in interrupting transmission of disease, these phenomena require study in connection with the evaluation of new insecticides.

During 1966, while testing the efficacy of the carbamates arprocarb [2] and carbamult [3] as wall deposits and on cheesecloth against *Anopheles gambiae* and *An. funestus* in Nigeria, some observations were made on the knockdown and recovery aspects of these compounds in the field and in the laboratory. These observations were also extended to non-contact toxicity exerted by these carbamates.

MATERIALS AND METHODS. Three-day-old, blood-fed *An. gambiae* reared in the laboratory were used in these experiments. The conical chambers of transparent plastic as supplied in the WHO Bio-assay Kits [4] were used for exposure in the knockdown-recovery investigations. The time when the mosquitoes were introduced into the cones and when they were knocked down was recorded. As soon as a mosquito was knocked down, it was removed from the chamber to prevent further contact with the toxicant and transferred to a non-waxed paper cup and

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[2] O-isopropoxyphenyl-N-methyl carbamate also known as Bayer 39007, Baygon and OMS-33.

[3] Methyl-5-isopropylphenyl-N-methyl carbamate also known as Schering 34612 and OMS-716.

[4] Wld. Hlth. Org. techn. Rep. Ser., No. 265, 1963, p. 140.

kept for 24-hour recovery. During the recovery, the mosquitoes were provided with a 5 percent glucose solution.

For investigating the non-contact toxic effect of the insecticides, the conical chamber of the WHO Bio-assay kit was fitted with a double ring of hard-board with mosquito netting to prevent the mosquitoes from coming into direct contact with the insecticide deposit (Fig. 1). The non-contact effect was studied only in the laboratory.

In the laboratory studies, technical carbamult and arprocarb dissolved in acetone were sprayed on glass plates at 2 g, 0.2 g, and 0.02 g/m^2. For field studies, two surfaces of mud (one smooth, rendered partially with cement, and the other rough) sprayed with Carbamult wdp 50 percent and cheesecloth inpregnated with carbamult technical dissolved in acetone at the rate of 2 g/m^2, were used.

All the experiments were conducted at room temperature (25°–28° C). The 24-hour variation was in the range of 25°–35° C. and 60–80 percent humidity.

RESULTS. Laboratory findings by exposing *An. gambiae* directly on glass plates sprayed with Carbamult at the rate of 2 g/m^2 and 0.2 g/m^2 and 0.02 g/m^2 using a WHO Bio-assay chamber.

It will be noted (Table 1) that immediately after spraying there was a fast knockdown on all plates, and nil to only a few recoveries at all dosages. Observations after 2 months of storage showed

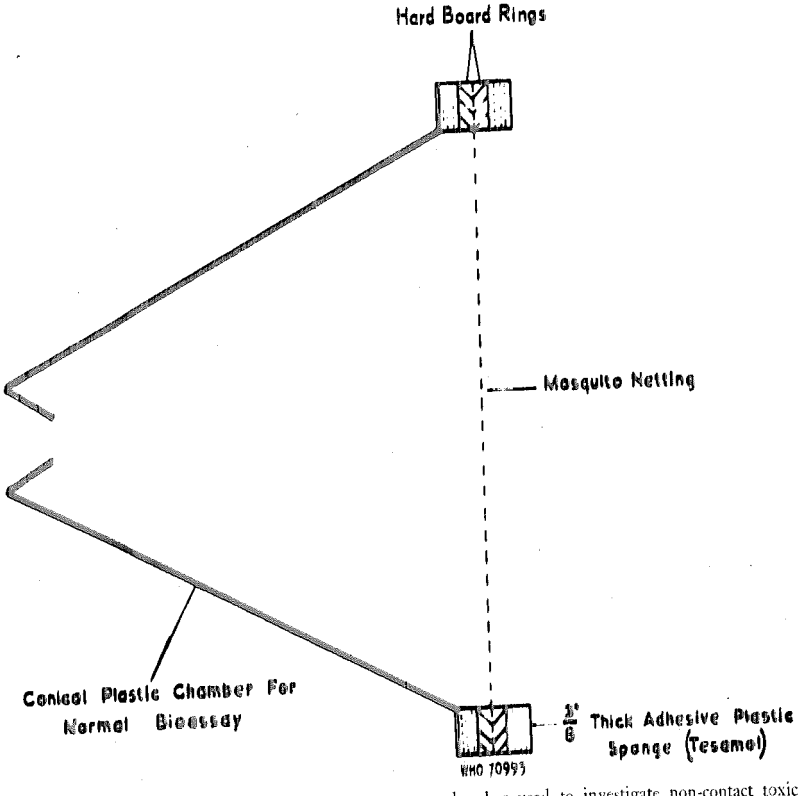

FIG. 1.—Cross section of a modified bioassay exposure chamber used to investigate non-contact toxic effect of insecticides.

that 2g/m^2 produced fast knockdown and no recoveries but at the lower dosage there was no knockdown and even after three hours of exposure negligible mortality.

Field studies were made on aging deposits of carbamult sprayed on walls and on impregnated cheese-cloth at 2 g/m^2.

Fig. 2 and Table 2 give the results of this series of investigations. Although the rough and the smooth surfaces were sprayed with the same dosage of insecticide there were differences in the results obtained. It appears that the effective dosage is not the same on rough and smooth surfaces and there may also be a difference in the rate of loss of the residue. It was noted that 49 minutes were required to produce a 50 percent knockdown (KD_{50}) on exposure to rough mud surfaces seven days after treatment, whereas only 12 minutes were required at 4 days on the smooth surfaces and only 7 minutes at 5 days on cheesecloth. As more time passed after treatment, the time required to produce 50 percent mortality also increased at a greater rate on the rough mud. The rate of increase in time required to achieve KD_{50} on cheesecloth is negligible up to 46 days after treatment (Table 2). Between 46 days and 72 days,

TABLE 1.—Knockdown and recovery of *A. gambiae* exposed directly to carbamult on glass plates.

Days after Treatment	Time in minutes in which 50% mosquitos were knocked down	Time range required for knockdown (minutes)	% 24 hours recovery
		0.02 g/m^2	
1*	17	11-28	0.0
1	11	7-18	0.0
3	13	9-19	15.0
60	No K.D. during 180 minutes**	180	91.0
		0.2 g/m^2	
1	8	11-12	1.5
1	7	4-12	1.5
2	10	6-14	0.0
60	No K.D. during 180 minutes	180	95.0
		2.0 g/m^2	
0	9	4-12	0.0
1	9	4-12	0.0
2	9	4-13	0.0
60	9	5-14	0.0

* On this day A. gambiae raised from wild caught larvae were used.

** K.D. = Knockdown.

however, there is a sharp increase in time required to produce KD_{50}.

Thus, although there is a loss of the toxicant from the surface, the amount available is above the critical level up to 46 days. Recovery following knockdown which was zero through 46 days, increased to 67 percent at 72 days after treatment. On the smooth surface, the rate of increase in time to achieve KD_{50} is gradual up to 151 days after treatment. This shows that the amount of toxicant remains much longer on the smooth surfaces than on rough surfaces. The percentage of knockdown mosquitoes recovering after exposure to the three treated surfaces is shown in Fig. 3. The percentage recovery increased up to a certain point in relation to the time exposure occurred after treatment with the insecticide. The percentage recovery, however, decreased after 67, 123 and 72 days for the rough, smooth and cheesecloth surfaces respectively. Later increases again oc-

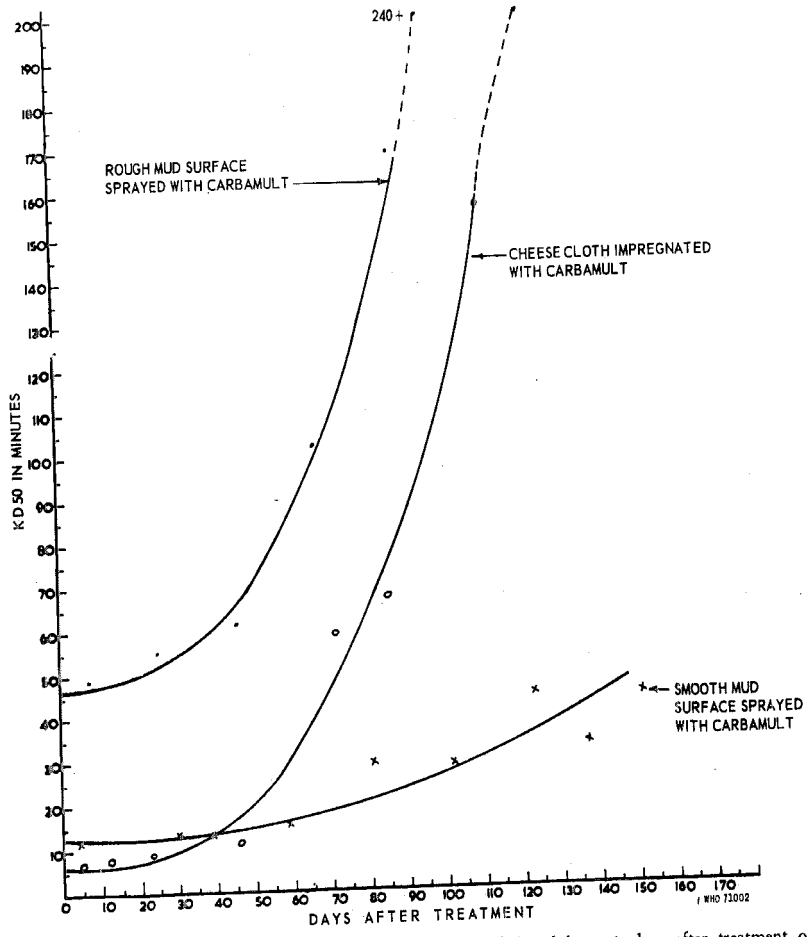

FIG. 2.—The relation between time required to produce 50% knockdown & days after treatment of two mud surfaces & cheesecloth treated with carbamult at 2g/m².

TABLE 2.—Knockdown and recovery of *A. gambiae* exposed to carbamult in Akowonjo village near Lagos: mosquitoes exposed in WHO bio-assay chambers.

2 g/m^2 on rough mud				2 g/m^2 on smooth mud (cement rendered)				2 g/m^2 acetone cheese-cloth			
Days after treatment	Time (min) 50% knockdown	Range K.D. period (in min)	% 24 hours recovery	Days after treatment	Time (min) 50% knockdown	Range K.D. period (in min)	% 24 hours recovery	Days after treatment	Time (min) 50% knockdown	Range K.D. period (in min)	% 24 hours recovery
7	49	22-78(86)	19.7	4	12	4-24(134)	0.0	5	7	3-10(84)	0.0
25	55	27-105(141)	23.4	30	13	6-23(145)	0.7	12	8	5-12(56)	0.0
46	61	38-130(66)	24.2	39	13	5-24(134)	0.0	23	9	5-14(140)	0.0
67	102	71-130(33)	51.5	59	15	5-30(121)	0.8	46	11	5-20(140)	0.0
88	169	13-240(71)[a]	38.0	81	29	16-60(96)	13.5	72	59	33-83(85)	67.0
109	240+	81-240(113)[b]	38.1	102	28	19-60(101)	23.5	86	67	24-189(84)	52.4
				123	44	29-128(97)	39.2	111	156	68-232(77)[c]	63.9
				137	32	18-76(84)	11.9	128	240+	76-240(105)[d]	66.7
				151	43	31-109(83)	48.2				

In parenthesis, number of insects exposed

Control Mortalities below 5%

K.D. = Knockdown

a = only 55 mosquitos K.D. after 240 minutes

b = only 45 mosquitos K.D. after 240 minutes

c = only 59 mosquitos K.D. after 232 minutes

d = only 32 mosquitos K.D. after 232 minutes

curred on smooth mud and cheesecloth surfaces. No explanation of this result is attempted. It is noted, however, that not all of the mosquitoes were knocked down during their exposure to the rough surfaces on the 88th and 109th day after treatment and to the cheesecloth on the 111th and 128th day after treatment.

Vapour and/or microparticulate effect of carbamult and arprocarb and a study of knockdown and recovery phenomenon.

This study was made in the laboratory, using two dosages of carbamult and of arprocarb on glass plates. The mosquitos were prevented from directly contacting the sprayed surface (Fig. 1). Results are shown in Table 3 and Figs. 4 and 5.

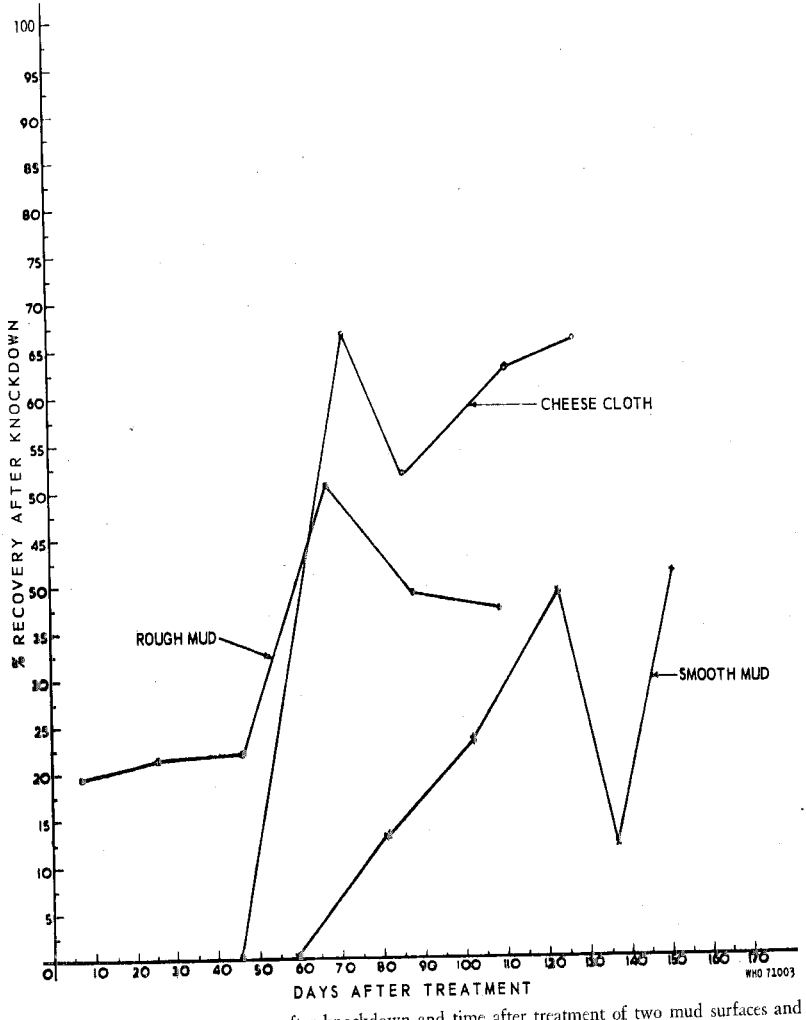

FIG. 3.—Relation between recovery after knockdown and time after treatment of two mud surfaces and cheese cloth treated with carbamult at 2g/m².

It will be seen that immediately after treatment there was only a slight difference in time required for KD_{50} for the two dosages of the same insecticide indicating an almost equal vapour or particulate effect regardless of surface dosage. The time required to attain KD_{50} for carbamult and aroprocarb at 0.2 g/m^2 increased within 16 days from 13 minutes and 9 minutes respectively, to more than 120 minutes. After the first week there was a sharp increase in the percentage of recovery.

At the higher dosage, i.e. 2 g/m^2, within 41 days there was an increase in KD_{50} value from 16 minutes (carbamult) and eight minutes (arprocarb) to 31 and 16 minutes respectively. The percentage of recovery after knockdown increased gradually. It is evident therefore that the air-borne lethal effect of these insecticides is lost rather rapidly but extends for a much longer period from higher dosages of residual spray.

DISCUSSION. The speed of knockdown produced by the insecticides may indicate the relative speed with which the site of action is reached and the intrinsic severity of the biochemical lesion produced. It is evident that for carbamates there is a fast absorption by contact and from vapor or microparticles. Soon after treatment the vapor and/or microparticulate effect especially may accelerate the speed

Carbamult				Arprocarb			
Days after treatment	Time (min) in which 50% mosquitos K.D.	Range of K.D. in minutes	% 24 hours recovery	Days after treatment	Time (min) in which 50% mosquitos K.D.	Range of K.D. in minutes	% 24 hours recovery
			0.2 g/m^2				
0	13	8-23(40)	0.0	0	9	5-15(41)	0.0
2	22	12-75(43)	6.9				
4	22	12-56(33)	6.1				
7	18	12-34(48)	4.2	8	15	70-45(64)	7.8
14	71	24-120(28)[a]	64.3	14	120+	75-120(45)[c]	80.0
16	120+	83-120(28)[b]	78.6	16	120+	90-120(50)[d]	84.0
			2 g/m^2				
0	16	10-22(48)	0.0	0	8	5-16(32)	0.0
5	20	11-69(42)	0.0				
17	14	9-27(45)	0.0	14	12	9-17(40)	0.0
23	20	9-30(51)	9.1	23	12	8-18(60)	3.3
28	27	21-65(36)	25.0				
34	23	14-48(44)	13.9	31	15	11-30(41)	14.6
41	31	19-105(33)	48.4	41	16	8-26(43)	23.2

Note: In parenthesis - No. of mosquitos exposed
K.D. = Knockdown
a = only 18 mosquitos K.D. after 120 minutes
b = only 10 mosquitos K.D. after 120 minutes
c = only 5 mosquitos K.D. after 120 minutes
d = only 6 mosquitos K.D. after 120 minutes

TABLE 3.—Knockdown and recovery of *A. gambiae* exposed to carbamult and arprocarb on glass surface in the laboratory. Exposure carried out in special bio-assay chamber to preclude contact of mosquitoes with insecticide.

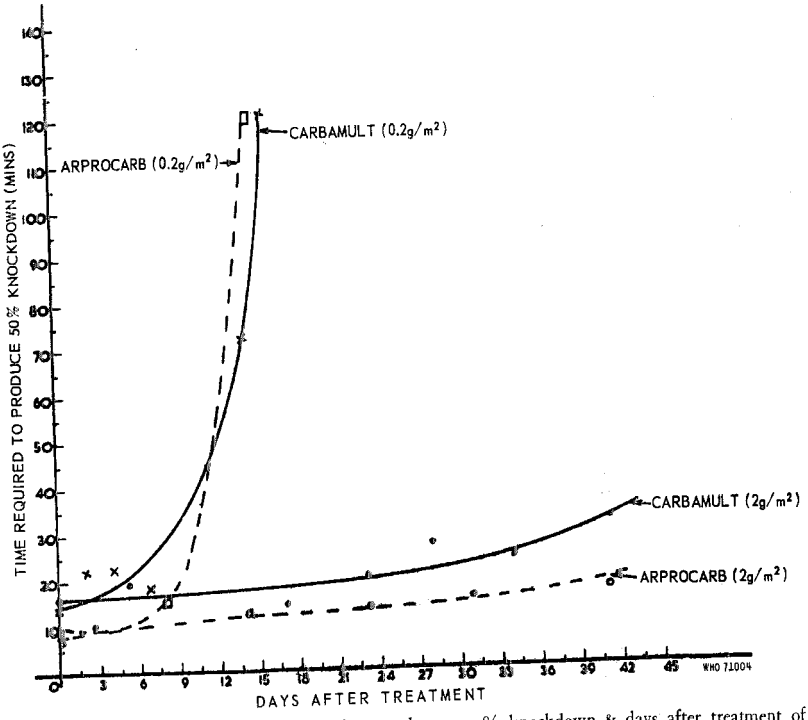

FIG. 4.—Relation between time required to produce a 50% knockdown & days after treatment of glassplates treated with carbamult & arprocarb, when *A. gambiae* adults were exposed in special bioassay chambers to preclude direct contact of mosquitoes with the insecticide film.

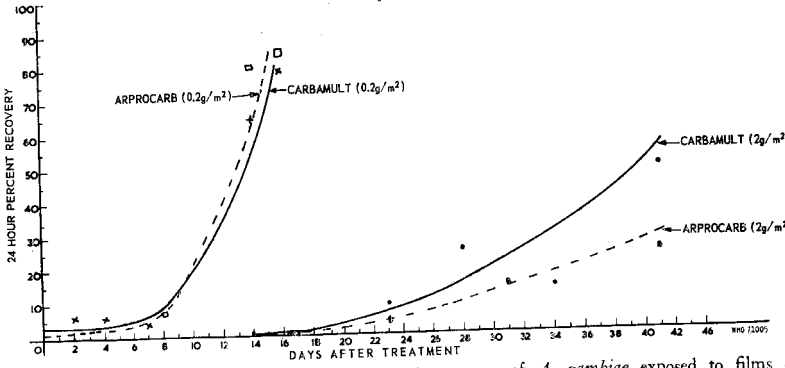

FIG. 5.—Relation between time after treatment & recovery of *A. gambiae* exposed to films of carbamult & arprocarb using special bioassay cones to preclude direct contact of mosquitoes with the insecticide.

of knockdown. Recovery after knockdown may result from incomplete and reversible enzyme inhibition as is apparently the case with carbamult and arprocarb, or by metabolism of the toxicant. A critical dosage has to be received by the insect before death can be produced. As more time after spraying elapses, the dosage received by the insect decreases due to decomposition of wall deposits and there is more and more recovery after knockdown. Evaporation or microparticulate loss accounts for this in addition to the normal processes of sorption and chemical breakdown. The significance of recovery after being knocked-down following a single exposure to the insecticide, cannot be evaluated in terms of control of disease transmission since the effect of multiple exposures and how these affect vector capacity are not known.

A significant percentage of recoveries after knockdown of *An. gambiae* on single exposure to wall deposits, impregnated cheesecloth and glass plates treated with arprocarb and carbamult within a few weeks of treatment were recorded. This phenomenon was also observed when mosquitoes were not allowed to come in direct contact with the insecticide film.

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