TABLE 3.—Mortality of adult female *Aedes taeniorhynchus* (Wiedemann) 24 hours after exposure in various types of cages to ultra-low volume aerial sprays of insecticides.

Insecticide	Dose (pound per acre)	Percent mortality for indicated type of exposure cage						
		Screen wire cages (mesh size)			Fabric cages			
		16	32	60	Light nylon	Heavy nylon	Dacron-polyester	Cotton gauze
Malathion	0.2	92	66	..	88	..	36	..
Accothion	.05	85	29	4	70	8	4	12
Geigy GS-13005	.05	97	16	8	40	24	..	0
Average		91	37	6	66	16	20	6

allowed good penetration of the insecticide droplets.

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RESULTS OF HUMAN EXPOSURE TO THERMAL AEROSOLS CONTAINING DURSBAN ® INSECTICIDE

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INTRODUCTION. The principal active ingredient in DURSBAN ® insecticides, O,O-diethyl O-(3,5,6,-trichloro-2-pyridyl) phosphorothioate, hereafter referred to as Dowco ® 179, has shown biological activity against many species of insects as reported by Gray (1965) and Kenaga *et al.* (1965). Many authors have reported outstanding activity of Dowco 179 for control of mosquitoes; Gahan *et al.* (1966), Lewallen and Peters (1966), Ludwig and McNeill (1966), Jakob (1966), and Mulla *et al.* (1967). While aerial application is the major method of dispersing insecticides for larval control, insecticidal fogging is the preferred mode of application for control of adult mosquitoes by mosquito abatement districts. Miller *et al.* (1968), Ludwig and McNeill (1966), and Mount and Lofgren (1967) have demonstrated the efficacy of Dowco 179 when dispersed as either a thermal or cold fog.

In the use of any insecticide for thermal fog dispersion, the health hazards to man are of prime importance because the application is predominantly in urban areas.

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Ludwig and Powers (1966) found no depression in cholinesterase activity in the red blood cells or plasma in four species of animals following 4-hour exposures with dosages to 8 mg./ft.^{3} air dispensed as a thermal fog. Soule and Wolf (1967) exposed adult rats and 8–10 day old chicks to a thermally generated fog of Dowco 179 (2% w/v in diesel oil). The animals were placed at various distances from the fog generator and in the cab of the fogging truck. Air samples collected in the vicinity of the animals showed exposures ranging from <5 to 23 $\mu g/m^3$. Cholinesterase activity in blood and brain tissues from these animals showed no depression. A human exposure study has been reported by Kilian *et al.* (1967), in which two adult male subjects were exposed to a thermally generated fog of Dowco 179 (2% w/v in diesel oil) for 2 minutes. Subsequent blood cholinesterase activity was determined and no significant plasma or red blood cell depression was observed.

Jakob (1966) reported excellent control of *Aedes aegypti* (L.) and *Anopheles albimanus* (Wied.) with a thermal fog containing 1.5 oz. Dowco 179 per gallon of oil. Miller *et al.* (1968) also indicated excellent results against *Aedes aegypti* at 0.026 lb. Dowco 179 per acre, or approximately 1.5 oz. per gallon of oil. In view of these data, the dosage of 1.5 oz. per gallon of oil was selected as the dosage for the initial studies.

In order to assess more critically the potential health hazards encountered from exposures to thermal fogs containing Dowco 179 insecticide, a series of tests were conducted with human volunteers. The nature of exposures, analytical measurements, and the results of these tests are herein reported.

EXPERIMENTAL. In all tests reported here, formulation M-2995 was used. This formulation is a 63 percent concentrate of Dowco 179 in xylene. Desired insecticidal use concentrations were prepared by dilution of this concentrate with diesel oil prior to fogging operations. A Leco 120 fogger,[3] calibrated to deliver 40 gallons per hour of the insecticide mixture, was used in all experiments. This unit was loaned by the Brazoria County Mosquito Control District.

An air sampling technique was devised so that a definite measure of the exposure to Dowco 179 encountered by the human subjects could be determined. The cholinesterase activity in red blood cells and plasma was determined at various time intervals following exposure. In the latter tests, filter paper patches were attached to the clothing of the subjects in order to determine the deposition of the chemical, in an attempt to relate to potential skin exposure.

Employees of the Agricultural Department and the Texas Medical Department of The Dow Chemical Company volunteered to participate in experiments where they would be subjected to controlled exposures of Dowco 179. All volunteers signed letters of consent stating that the following criteria had been fulfilled:

1. Voluntary consent of subject.
2. Studies to be conducted under medical supervision.
3. Results of previous animal and human exposure tests related to each subject.
4. The subjects were briefed in detail on the nature of the study to be undertaken.

EXPOSURES. *Test 1.* On May 6, 1969, 13 subjects were divided into five groups of 2 or 3 each. One member of groups 2 through 5 carried an air sampling device during exposure. Details of the sampling technique are described in the Sample Collection and Preparation Section.

The volunteers were located approximately 50 feet from the path of the truck carrying the Leco 120 fogger. The fogging unit made five trips past the volunteers. The fog dispersal pattern was somewhat erratic due to variable air currents, but in general, the drift of the fog was

[3] Lowndes Engineering Co., Inc., Valdosta, Georgia.

toward and over the subjects. After the first pass Group 1 vacated the exposure area. Group 2 left the treatment area following the second pass of the vehicle. This treatment regimen was followed until all five groups were exposed. In addition to the subjects above, one volunteer simulated conditions that might occur if a child ran or rode a bicycle in the fog following a fogging truck. This subject ran in the fog, approximately 15 feet behind the nozzle of the Leco 120 unit, for 2 minutes. He carried an air monitoring unit held at face level.

The entire group was exposed to fog generated from 1.5 ounces of Dowco 179 per gallon of oil, which is equal to 0.026 lb./A. based on a 300-foot swath width.

Test 2. On July 8 between 8:30 and 9:15 a.m., three human volunteers were subjected to timed exposures of fog containing Dowco 179:

1. C.B.—female—previously exposed to 5 passes of a thermal fog in Test 1.
2. W.S.M.—male—not previously exposed.
3. R.W.S.—male—not previously exposed.

In this test, the fog truck remained stationary and the volunteers stood in the dense fog for various periods of time.

Each individual was dressed in disposable coveralls and the hair, face, and hands were protected from exposure by means of plastic covering. A hole was cut out of the protective head cover in order that the nose-mouth area was exposed. Two pieces of circular filter paper (11 cm. diameter) were affixed to the shoulders to determine how much Dowco 179 would have collected on the skin had it been exposed.

The subjects were stationed 20 feet behind the nozzle of the fogger and exposed to the insecticidal fog for 3, 5, and 8 minutes, respectively. The concentration of Dowco 179 in the fogging solution was 1.5 oz./gallon of oil.

Test 3. Three volunteers were exposed to Dowco 179 via a thermal fog. The participants had been subjected to previous exposures during Test 1.

S.L.W.—male—previously exposed to 4 passes of thermal fog (see Test 1).
D.J.K.—male—previously exposed to 2 passes of thermal fog (see Test 1).
O.C.B.—male—previously exposed to 4 passes of thermal fog (see Test 1).

In an effort to obtain greater exposure to Dowco 179, the concentration of the fogging concentrate was increased to 6.0 oz. of Dowco 179 per gallon of oil (4 times recommended level), and the subjects were instructed to stand approximately 12 feet behind the nozzle of the fogger. Since the above action would greatly increase the degree of exposure, the time periods of exposures were reduced to 1, 2, and 4 minutes.

The subjects were clothed and covered as previously noted (Test 2) and each member carried an air sampling device.

SAMPLE COLLECTION AND PREPARATION. *Air Samples.* Portable M-S-A Monitaire samplers[4] consisting of micro-air scrubbing units containing 20 ml. of n-hexane and operated from a battery pack at 0.1 ft.^{3}/min., were used in each test. The units were held in the vicinity of the breathing zone by the test subjects during the period of exposure to the fog. Following use, the n-hexane solutions from the Monitaire samplers were transferred into 125-ml. Erlenmeyer flasks by draining through 1-inch columns of anhydrous sodium sulfate. The scrubbing units and columns were rinsed with 50–70 ml. more of n-hexane, which were added to the filtered scrubbing solution. The combined extract and washes were evaporated to 15–20 ml. on a low heat hot plate and then to dryness under reduced pressure. Following removal of the n-hexane, the extracts were dissolved in 5 ml. of n-hexane and held for analysis.

Filter Papers. Two Whatman No. 1 circular filter papers, 11 cm. in diameter,

[4] Mine Safety Appliances, Pittsburgh, Pa.

attached to the clothing of each subject, were used to collect impinging particles from the thermal fogger. The papers were removed and placed into 4-ounce wide-mouth bottles with aluminum-lined caps. Thirty ml. of n-hexane were added, bottles sealed, and shaken for 30 minutes on an Eberbach reciprocating shaker set at 250 excursions per minute. The n-hexane extract was then transferred into a 125-ml. Erlenmeyer flask by draining through a 1-inch column of powdered anhydrous sodium sulfate. The extraction was repeated twice more using 25 ml. of n-hexane in a similar manner and the extracts combined. The combined n-hexane extracts were condensed to small volumes on a low heat hot plate, followed by evaporation to dryness under reduced pressure and intermittent heating. The residues were redissolved in n-hexane to a known volume and held for analysis.

Blood Samples. The blood from each subject exposed was drawn into syringes rinsed with heparinized saline and transferred into a 5-ml. glass tube wetted with a small amount of additional heparin solution. The heparinized blood was immediately immersed in an ice bath and held until separated (maximum time, 1 hour). After centrifugation at 2,000 rpm for 15 minutes at 5° C., the plasma was removed and transferred to a labeled tube for storage or for immediate analysis of cholinesterase activity. The white blood cells were removed from the red blood cells (RBCs) by aspiration. The cells were resuspended in saline by gentle agitation with a beechwood applicator stick, and the tube was stoppered. After centrifuging as before, the washing was repeated and the cells centrifuged a third time. The saline from the final wash was removed, and the cells were hemolyzed with nine volumes of distilled water. The 10 percent RBC hemolysate was allowed to stand for at least 10 minutes before assay.

ANALYTICAL METHODS. Analysis for Dowco 179 in all air and filter paper samples was by electron-capture gas chromatography. Following extraction and concentration of the extracts as described in the Sample Collection and Preparation Section, the samples were redissolved in a known volume of n-hexane and aliquots were analyzed.

A Barber Coleman Model 5360 tritium-source, electron-capture gas chromatograph was used for quantitative analysis. The parameters were: 4 ft. x ¼ inch o.d. borosilicate glass; packing SE-30 (w/w) 5 percent on 80- to 90-mesh Anakrom ABS; carrier gas, nitrogen, 60 ml. per minute; temperatures, column 190° C., injection port 215° C., detector 205° C.; electrometer sensitivity 100, attenuation 2; detector voltage 12 volts. The limit of sensitivity of Dowco 179 was approximately 0.04 μg.

CHOLINESTERASE EVALUATIONS. Determination of cholinesterase activity in plasma and erythrocytes was made using the automated pH-stat method of Nabb and Whitfield (1967). The principle of the method is fundamentally the same as that used in many of the chemical methods that have been described, and depends upon the measurement of the acid produced by the action of cholinesterase on acetylcholine. The acid production is measured in terms of ml. of standard base titrant used, and expressed as micromoles of acetylcholine hydrolyzed per minute per ml. of substrate. All measurements were performed with a pH-stat apparatus [5] consisting of: (1) a titrator with scale expander, (2) a titrigraph equipped with a 0.5-ml. syringe and micrometer, and (3) a thermostated microtitration assembly heated by a circulating water bath.

RESULTS AND DISCUSSION. Table 1 indicates that the air sampling technique was adequate to pick up submicrogram quantities of Dowco 179 in thermal aerosols. It is interesting to note that the concentration dropped approximately tenfold at 100 feet from the fogger as compared to the concentration obtained at 50 feet.

[5] E. H. Sargent & Co., Chicago, Illinois; Sargent Recording pH-Stat, Catalog No. S-30240.

TABLE 1.—Evaluation of air sampling techniques used in fogging test with Dowco 179. 4–7–69.

Sample No.	Type of fog exposure *	Distance from fogger to air sampler	Total Dowco 179 collected (μg)
2311	Intermittent (10 passes)	50 ft.	3.45
2312	" "	100 ft.	0.40
2313	Continuous (5 minutes)	100 ft.	10.02
2314	" "	150 ft.	0.85

* 1.5 oz. Dowco 179 per gallon of fogging solution.

In Test 1 (Tables 2 and 3), 13 human volunteers were exposed to a varying number of passes of a fogging machine. In Table 2 it can be seen that the repeated exposures did not cause a stepwise increase in the concentration of Dowco 179 in the solutions in the scrubbers, as would be expected. Visual observations indicated that varying wind direction and irregular drifting of the fog blanket resulted in uneven exposures. Cholinesterase values of the exposed subjects are reported in Table 3. Examination of the data shows no significant reduction in either plasma or RBC cholinesterase in any of the people.

Special attention is called to volunteer H.N.E. This subject followed behind the moving fogger for 2 minutes and carried one of the air samplers with him. The cholinesterase values for this subject show no significant reduction following exposure. This individual was exposed to a concentration slightly higher than the amount recorded from 10 passes of a fogging truck with the sampler 50 feet from the vehicle (see Table 1).

In Test 2, where three subjects were exposed for varying lengths of time in the fog emitted from a stationary fogger, the total amount of Dowco 179 collected in the air scrubber was directly proportional to the exposure time (Table 4), indicating that this was a reliable technique for measurement of exposure to Dowco 179. This also is true of the amount obtained on the two filter papers from the shoulders of the subjects.

The μg of Dowco 179 per minute obtained in the scrubbers was constant for the first two volunteers and slightly higher for the third. This increase was probably because the subject moved closer to the nozzle of the fogger.

Plasma and red blood cell cholinesterase values for these subjects indicate no depression (Table 5).

All the above tests were conducted at a dosage recommended for adult mosquito control, i.e., 1.5 oz. of Dowco 179/gallon of oil.

Since an 8-minute exposure to the insecticide-bearing thermal fog caused no depression in cholinesterase values, it was decided to increase the concentration of

TABLE 2.—Monitoring results of thermal fogs containing Dowco 179. 5–6–69.

Sample No.	Group No.	Type of fog exposure *	Distance from fogger to air sampler	μg Total **
2599	2	Intermittent (2 passes)	50 ft.	0.147
2600	3	" (3 passes)	50 ft.	0.247
2601	4	" (4 passes)	50 ft.	0.111
2602	5	" (5 passes)	50 ft.	0.268
2604	H.N.E.	Continuous (2 minutes)	15 ft.	3.93

* 1.5 oz. Dowco 179 per gallon of fogging solution.
** μg Dowco 179 per 20 ml. n-hexane scrubbing solution.

TABLE 3.—Cholinesterase values of subjects exposed to one to five passes of a thermal acrosol generator dispensing 1.5 oz. Dowco 179 per gallon of fogging solution.

Group No. & No. of Exposures	Subject	Pre-exposure Values 5–5–69 Pla *	Pre-exposure Values 5–5–69 RBC **	Cholinesterase Values (μm/min./ml.-pH-Stat. Units) 5–7–69 Pla	5–7–69 RBC	5–9–69 Pla	5–9–69 RBC	5–16–69 Pla	5–16–69 RBC	5–20–69 Pla	5–20–69 RBC
1	N.L.D.	4.17	15.52	....	.....	4.80	12.50	5.12	13.80	5.12	13.90
	A.M.	3.02	16.53	....	.....	3.52	17.38	4.00	15.8	4.00	16.80
2	M.B.	2.65	15.02	....	.....	....	.....	....	.....	3.21	13.45
	D.J.K.	3.66	15.52	....	.....	4.33	12.51	4.80	13.00 [b]	....	.....
3	E.A.F.	3.36	17.03	....	.....	3.85	16.65	4.50	15.80	4.16	15.35
	L.L.W.	2.34	15.02	....	.....	2.08	13.00	2.57	14.40	2.73	13.45
	R.E.F.	3.02	12.40	....	.....	3.52	9.16	4.00	10.10	4.00	11.50
4	E.E.	3.02	17.03	....	.....	3.52	14.42	3.67	14.40	3.69	15.35
	S.L.W.	4.17	12.52	....	.....	4.95	13.00	5.28	13.40	4.95	12.95
	O.C.B.	4.84	17.03	....	.....	5.77	15.69	5.62	14.80	5.90	15.80
5	C.B.	3.67	16.53	....	.....	4.00	14.92	4.32	14.80	3.85	14.40
	D.H.	2.34	14.52	....	.....	2.88	13.92	3.05	13.40	3.03	13.45
	P.D.L.	3.34	15.02	....	.....	4.0	13.92 [a]	3.53	13.92	3.69	14.90 [c]
Walked in fog 2 min.	H.N.E.	4.34	14.03	4.34	14.03	4.16	12.51	4.32	14.40	4.66	17.75

* Pla = Plasma.
** RBC = Red Blood Cell.
[a] 5–11–69.
[b] 5–15–69.
[c] 5–23–69.

TABLE 4.—Monitoring results of thermal fog containing 1.5 oz. Dowco 179 per gallons of fogging solution. 7–8–69.

Subject[1]	Exposure Time (minutes)	Concentration of Dowco 179 (μg) Air Scrubber[2] Total	μg/min.	μg/ft.[3]	Filter Paper[3] Total	μg/in.[2]
C.B.	3	7.67	2.56	25.6	92.5	3.14
W.S.M.	5	12.50	2.50	25.0	166.8	5.66
R.W.S.	8	25.28	3.16	31.6	235.5	8.00

[1] Distance between fogger and exposed subjects, 20 ft.
[2] Air scrubber rate = 0.1 ft.[3]/min.
[3] Filter paper, 2 sheets 11 cm. diameter: Area = 29.45 in.[2]

TABLE 5.—Cholinesterase values of subjects exposed for various time intervals to a thermal aerosol generator dispensing 1.5 oz. Dowco 179 per gallon of fogging solution. 7–8–69.

Subject[1]	Exposure Time (minutes)	Cholinesterase Levels (μm/min./ml.-pH Stat. Units) Pre-Exposure Pla.	RBC	4 Hrs. Post Exp. Pla.	RBC	22 Hrs. Post Exp. Pla.	RBC	70 Hrs. Post Exp. Pla.	RBC
C.B.	3	3.86	14.50	....	.....	3.86	14.88	3.86	14.50
W.S.M.	5	4.54	14.50	....	.....	4.54	14.50	4.65	14.12
R.W.S.	8	3.69	14.12	3.69	14.50	3.86	15.3	3.97	14.12

[1] Distance between fogger and exposed subjects, 20 ft.

Dowco 179 in the fogging concentrate to 6.0 oz./gallon of oil, or four times the effective field dose. Also, it was decided the volunteers would stand 12 feet from the nozzle of the fogger, which also would greatly increase the degree of exposure. The periods of exposure were reduced to 1, 2, and 4 minutes. Table 6 summarizes the exposure data as obtained from the air samples and filter papers.

A 100-fold increase in exposure, based on concentration of Dowco 179 in the air, was obtained by moving subjects closer to the fogger and increasing the amount of compound in the fogging solutions (see Tables 4 and 6). The first volunteer (S.L.W.) exposed to this high concentration of Dowco 179 exhibited no significant change in either plasma or red blood cell cholinesterase values (Table 7). Sub-

TABLE 6.—Monitoring results of thermal fog containing 6.0 oz. Dowco 179 per gallon of fogging solution. Human exposure data test 9–2–69

Subject[a]	Exposure[b] Time (minutes)	Concentration of Dowco 179 (μg) Air Scrubber[c] Total	μg/min.	μg/ft[3]	Filter Paper[d] Total	μg/min.	μg/in[2]
S.L.W.	1	223.9	223.9	2239	1455.0	1455	49.4
D.J.K.	2	751.2	375.6	3756	5430	2715	184.4
O.C.B.	4	911.2	227.8	2278	7968	1992	270.6

[a] Distance between fogger and exposed subjects ~12 ft.
[b] Concentration of Dowco 179 in fog was 4X insecticidal dosage.
[c] Air scrubber rate=0.1 ft[3]/min.
[d] Filter paper, 2 sheets 11 cm. diameter: Area=29.45 in.[2]

TABLE 7.—Cholinesterase values of human subjects exposed for various time intervals to a thermal aerosol fog generator dispensing 6.0 oz. Dowco 179 per gallon of fogging solution 9–2–69

		Cholinesterase Levels (μm/min/ml—pH-Stat Units)									
	Exposure Time	Pre-Exposure		24 Hrs. Post Exp.		48 Hrs. Post Exp.		72 Hrs. Post Exp.		96 Hrs. Post Exp.	
Subject [a]	(minutes)	Pla	RBC	Pla	RBC	Pla	RBC	Pla	RBC	Pla	RBC
S.L.W.	1	4.5	13.0	4.17	13.0	4.7	12.6	...	....	...	....
D.J.K.	2	4.7	13.8	4.0	14.4	4.56	13.8	4.7	13.8	...	....
O.C.B.	4	5.6	14.6	4.7	15.8	4.50	14.9	4.7	15.4	5.5	15.4

[a] Distance between fogger and exposed subjects ~ 12 ft.

ject D.J.K. showed only a slight depression (15 percent) in plasma cholinesterase at 24 hours post-treatment, with recovery to the pretreatment value by 72 hours. There was no change in the values obtained for the red blood cell cholinesterase. Subject O.C.B. demonstrated a depression of 16 percent in the plasma cholinesterase at 24 hours and a 20 percent depression at 48 hours post-exposure. By 72 hours, the plasma value was depressed only 15 percent and by 96 hours, the value had returned to the pretreatment level. A slight elevation of red blood cell cholinesterase was observed at 24 hours after exposure and remained slightly elevated for 96 hours.

These data show that it was necessary to increase the concentration of Dowco 179 in fog approximately 100-fold beyond the insecticidally effective rate to reach the threshold of plasma cholinesterase depression. Under these conditions, no red blood cell depression occurred.

This study demonstrates that inhalation of thermal fogs containing Dowco 179 at dosages effective for mosquito control would not cause depression of human plasma and red blood cell cholinesterase.

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