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HUMAN URINARY METABOLITES OF ORGANOPHOSPHATE INSECTICIDES FOLLOWING MOSQUITO ADULTICIDING

FREDERICK W. KUTZ AND SANDRA C. STRASSMAN

Ecological Monitoring Branch (WH-569), U. S. Environmental Protection Agency
Washington, D. C. 20460

ABSTRACT. Adult mosquito control practices generally employ chemical toxicants, and thus, unavoidably expose inhabitants of treated areas to insecticides. Human exposure to most organophosphate insecticides results in the excretion of specific urinary metabolites. In Dover, Delaware, urine specimens were collected from people residing in and adjacent to an area treated with naled for adult mosquito control. Chemical analysis of the spray solution revealed that it was contaminated with traces of temephos, another organophosphorous insecticide used in mosquito control. Urine samples from the same individuals were taken prior to and just after aerial spraying and analyzed for organophosphate insecticide metabolites. Levels of these metabolites varied; increases in dimethyl phosphate (from exposure to both temephos and naled) and dimethyl phosphorothionate (solely from temephos exposure) in post-treatment samples could be attributed to this spraying. Metabolite levels observed in this study did not approach concentrations normally associated with cholinesterase inhibition or other clinical repercussions. Other aspects of human exposure to insecticides are discussed.

Intensive mosquito suppression activities are practiced in many communities throughout the United States. The actual methods and procedures employed vary greatly from one place to another and, in some cases, take advantage of behavioral or ecological phenomena indigenous to specific locales. One characteristic which most adult mosquito abatement programs share is their reliance on chemical pesticides. These insecticides are applied by numerous aerial and ground means. Treated areas are usually in or near population centers. Therefore, these practices unavoidably expose the general population of the treated, and perhaps adjacent, areas directly to certain amounts of pesticides.

Human exposures to organophosphate insecticides result in the excretion of urinary metabolites which are generally characteristic of the type of pesticide used. The identity and rate of excretion as well as the amount excreted vary and are functions of the specific pesticide, the exposure, and the intrinsic and extrinsic factors influencing individual metabolism.

Changes in cholinesterase levels have also been noted as a sequela of mosquito control treatments. In several published reports from other countries, the use of fenthion, an organophosphate insecticide used for mosquito control, has produced moderate depression of whole blood and plasma cholinesterase in spraymen and in inhabitants of sprayed dwellings (Taylor 1963, Elliott and Barnes 1963). During studies of the exposure of mosquito control workers to fenthion in the United States, Wolfe et al. (1974) found that there was no important change in erythrocyte cholinesterase activity. However, there

was some decrease in plasma cholinesterase activity in certain workers.

During the St. Louis encephalitis epidemic in Corpus Christi, Texas, in the fall of 1966, malathion was applied by aerial spraying over the city and outskirts to control the vector mosquito. Gardner and Iverson (1968) studied a group of 119 volunteers who received varying degrees of exposure to this spray. This study involved comparing pre-spray and post-spray cholinesterase activities and compiling histories of exposure and symptoms. A 5% incidence of mild and transient symptoms such as headache, nausea and weakness was noted in the exposed volunteers, but there were no pathognomonic signs. There was no correlation of symptom frequency or severity with enzyme activity and no statistically or clinically significant change in enzyme activity related to time of spraying. It was concluded that there was negligible risk to human health involved in aerial applications of malathion.

Although the measurement of cholinesterase activity in humans has been used in the control of occupational exposure to organophosphate insecticides, it is a nonspecific method and requires high levels of exposure for detection of significant decreases in cholinesterase activity. This is especially apparent in the case of those pesticides which are weak cholinesterase inhibitors. In addition, the levels of cholinesterase vary greatly, thus making interpretation difficult.

A more precise method for determining exposure to the newer biodegradable pesticides, such as organophosphorous and carbamate insecticides, is the determination of their metabolites in body fluids. Studies in laboratory animals indicate that the determination of their metabolite concentration in body fluids, especially urine, provides a specific and more reliable index of exposure (Hunter et al. 1972 and Mattson and Sedlak 1960).

The objectives of this article are to report the incidences and levels of certain organophosphorous metabolites in human urine before and after exposure to the insecticide naled, applied for adult mosquito control. Metabolite determinations were made on urine from the general population and from occupationally-exposed mosquito control workers. Additionally, information was collected regarding the location of the person sampled at the time of spraying and about the accidental or intentional protective measures used.

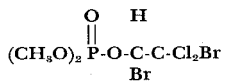
METHODS AND MATERIALS

An area near Dover, Delaware, was selected for this experiment. This area of approximately 3,400 acres was chosen because it is routinely treated for adult mosquito control whenever mosquito biting counts and other measures of mosquito activity reach pestiferous levels. The insecticide used was naled (Fig. 1) applied by a twin-engine aircraft. The spray plane was calibrated to deliver 2 quarts of No. 2 fuel oil per acre (0.05 lb. actual). The droplet characteristics, as measured prior to application, were as follows:

Mass Median Diameter = 120μ
Range = 35 275μ
Average Deposit = 15 droplets/in^2

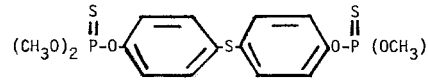
The actual application was made under the direction of the Mosquito Control Section of the Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control, and in all respects conformed to label directions.

A sample of the prepared insecticide was analyzed by a U.S. Environmental Protection Agency Laboratory at Beltsville, Maryland. Thin layer chromatography and gas chromatography—flame photometry systems were employed. The results indicated that naled was present in the solution at a concentration of about 1%. The tests also showed that another insecticide, temephos, was also present in trace amounts (less than 0.04%). Temephos is an organophosphate insecticide also used in mosquito abatement. Its chemical structure and exact nomenclature are given in Figure 2. No investigation was made to determine the sources or causes of this contamination.



(1,2, Dibromo- 2,2-dichloroethyl dimethyl phosphate)

Fig. 1. Chemical structure and nomenclature of naled.



0,0,0',0'-Tetramethyl 0,0'-thiodi-p-phenylene phosphorothioate

Fig. 2. Chemical structure and nomenclature of temephus.

SURVEY DESIGN. The area of Dover, Delaware, which was used for this project is shown in Figure 3. The area was divided into 2 parts. The central area (unmarked) represents the actual spray target. The surrounding area (stippled) represents a 1 mi. margin around the treated portion. Approximately 100 residents were identified in each of the 2 areas. The criteria for selection were presence in the appropriate area at the time of spraying, and willingness to participate voluntarily in this project.

Two urine specimens were requested from each of the identified individuals. The 1st specimen was taken several hours prior to the beginning of spray operation. The 2nd specimen was taken within 3 hr after the spray operation. All specimens were deposited in a clean bottle furnished by the program and were frozen immediately. Each donor completed a questionnaire indicating his/her location at the time of spraying. The urine collections and completion of the information survey were accomplished in cooperation with the Dover Lions Club.

All urine specimens were then subjected to chemical analysis following the method of Shafik et al. (1973). The actual analysis was completed at the laboratory of the Colorado Epidemiologic Studies Center, Ft. Collins, under contract with the National Human Monitoring Program for Pesticides. Shipment from the Dover area to Colorado was made under dry ice to insure that the specimens remained frozen at all times.

The chemical method detected the presence of 6 human metabolites of organophosphate insecticides. Detectable residues are listed in Table 1.

COMPUTATIONS. The following descriptive statistics were used to characterize the data obtained from this study:

Sample Size—the total number of samples analyzed.

Percent Positive or Frequency—the percentage of samples showing quantifiable levels of a specific metabolite.

Percent Trace—the percentage of samples showing concentrations of a specific metabolite which could not be quantified, but could be identified.

Maximum Value—the highest metabolite value detected.

Arithmetic Mean—Calculated according to standard statistical formula. In the case of these data, the arithmetic mean always closely approximated the geometric mean.

95% Confidence Interval—the range containing the true mean with 95% confidence ($p = .05$).

Table 1. List of dialkyl phosphate abbreviations

Abbreviation	Chemical composition
DMP	o,o—Dimethyl phosphate
DEP	o,o—Diethyl phosphate
DMTP	o,o—Dimethyl phosphorothionate
DETP	o,o—Diethyl phosphorothionate
DMDTP	o,o—Dimethyl phosphorodithioate
DEDTP	o,o—Diethyl phosphorodithioate

RESULTS AND DISCUSSION

Pre- and post-treatment urine samples were obtained from 107 people who were present in the spray area at the time of application. The average age of the volunteers was 34 years, with a range from 4 to 68 years. This study group had a male/female ratio of 54:46, and 5% of the people were from races other than Caucasian.

A total of 100 people present in the adjacent area at application time donated pre- and post-treatment urine specimens. The mean age of the donors was 33 years, ranging from 6 to 83 years. This study group had a male:female ratio of 54:46; all were Caucasians.

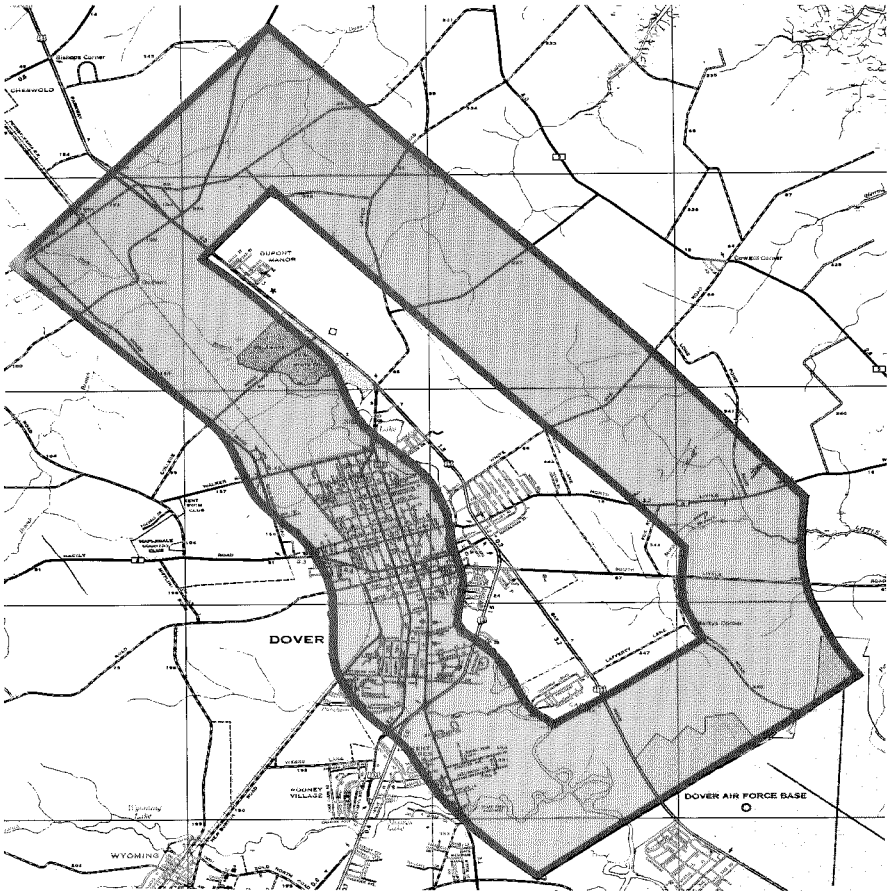


Fig. 3. Map of Dover, Delaware, spray area.

The occupation of the volunteers in both areas varied widely. However, most were employed in higher socio-economic categories or were students. None of the donors in either area had occupational association with pesticide manufacture or use.

The results of the urine analyses for human metabolites of organophosphate insecticides are presented in Tables 2 through 5. All 6 detectable metabolites were present in specimens taken prior to and after spraying. This finding indicates that the general populace of the Dover area was exposed to a variety of organophosphorous insecticides or their major degradation products at low levels during the course of this experiment.

Human exposure to naled and temephos will result in the metabolic breakdown of these chemicals to dimethyl phosphate (DMP) and dimethyl phosphorothionate (DMTP), respectively. The dimethyl phosphorothionate (DMTP) would be further metabolized by the human body to dimethyl phosphate (DMP). Therefore, special attention should be focused on the results of these 2 compounds. The other metabolites are presented as a matter of general interest.

Table 2 presents the results of analysis of urine from people residing within the treated area at the time of spraying. Mean levels of DMP and DMTP increased in samples taken after spraying. The levels of the other metabolites remained constant. The increase in the mean level of the DMP metabolite was statistically significant at the .05 level, while the increase in the DMTP metabolite just failed to show significance at that confidence level. The frequency of detecting these 2 residues remained at about 50% both before and after spraying. These results indicate that people residing within the treated area are exposed to the insecticide during spray operations. The extent of exposure, however, was low.

Table 3 presents the results of analysis of urine from people residing in the 1-mile perimeter area around the spray area. No statistically significant variation in the mean levels was observed. The increases in the mean levels of diethyl phosphorothionate (DETP) and diethyl phosphate (DEP) could not be attributed to the metabolism of the insecticides used in this experiment and, therefore, are unexplained. It was interesting to note that the residue levels of the diethyl phosphate (DEP) were not statistically significant when the data were analyzed after geometric transformation. These results indicated that people residing near, but

Table 2. Results of analysis of urine from people inside spray area,[1] Dover, Delaware, 1973.

Dialkyl Phosphate	Time of Spray	Percent Positive	Percent Trace	Concentration in PPM		
				Maximum Value	Arithmetic Mean	95% Confidence Interval
DMP	Before	20	21	0.10	.004	.002 – .007
	After	25	18	0.50	.009	
DEP	Before	56	21	0.18	.014	.010 – .019
	After	59	22	0.07	.014	
DMTP	Before	22	13	0.30	.011	.004 – .018
	After	31	16	1.60	.017	
DETP	Before	56	13	0.30	.029	.019 – .039
	After	64	11	0.30	.028	
DMDTP	Before	5	5	0.20	.002	.001 – .006
	After	4	8	0.20	.002	
DEDTP	Before	31	17	0.09	.007	.004 – .010
	After	32	21	0.10	.007	

[1]Sample size – 107.

Table 3. Results of analysis of urine from people within adjacent area,[1] Dover, Delaware, 1973.

Dialkyl Phosphate	Time of Spray	Percent Positive	Percent Trace	Concentration in PPM: Maximum Value	Concentration in PPM: Arithmetic Mean	Concentration in PPM: 95% Confidence Interval
DMP	Before	28	16	.07	.006	.003 – .008
	After	28	24	.80	.005	
DEP	Before	55	24	.08	.013	.010 – .017
	After	63	18	.01	.018	
DMTP	Before	22	19	.12	.006	.002 – .009
	After	25	14	.04	.005	
DETP	Before	67	12	.24	.027	.019 – .035
	After	68	11	.18	.034	
DMDTP	Before	2	7	.02	0	
	After	1	4	.01	0	
DEDTP	Before	39	17	.09	.010	.006 – .012
	After	37	21	.11	.010	

[1] Sample size=100.

not within, the treated area were not exposed to measurable amounts of these 2 insecticides. It does appear likely that several people within this group were exposed to naled, as evidenced by the increase in the maximum value detected (0.8 ppm).

Table 4 shows the results when the data obtained from people within the spray area at the time of treatment were divided on the basis of location at the time that the insecticide was applied. This information was part of the questionnaire completed by each donor. No statistically significant increase in metabolite level was observed for people who remained inside their houses during the treatment. However, the greatest increase in mean levels of DMP and DMTP occurred in the group of people who were outside in the treatment

Table 4. Results of urine analysis from people within spray area by location at time of spray, Dover, Delaware,,1973.

Dialkyl Phosphate	Time of Spray	Percent Positive	Percent Trace	Concentration in PPM: Maximum Value	Concentration in PPM: Arithmetic Mean	Concentration in PPM: 95% Confidence Interval
	Inside during spray (sample size=51)					
DMP	Before	12	16	.10	.003	.001 – .007
	After	20	14	.07	.004	
DMTP	Before	16	18	.17	.007	.001 – .014
	After	25	22	.03	.004	
	Outside during spray (sample size=56)					
DMP	Before	27	27	.06	.005	.002 – .008
	After	30	21	.50	.014	
DMTP	Before	29	9	.30	.014	.002 – .026
	After	36	11	1.60	.041	

area at the time of application. Changes in these metabolites were statistically significant at the p= .05 level. The mean levels of these 2 metabolites were among the highest observed in this study and were only exceeded by the levels reported in samples taken from mosquito control employees.

Results of other questions answered on the information form indicated that most of the participants were uncertain of their exposure to pesticides from other sources, i.e., pest control at offices, etc. Also, no correlations between metabolites detected and medicines taken prior to sample collection were apparent.

Table 5 indicates the results of urine analysis of employees of the Mosquito Control Section and the aircraft pilot. These individuals have almost continual exposure to many insecticides and, as would be expected, showed the highest levels of detected metabolites during the course of this study. These results are remarkably low when compared to other occupationally-exposed individuals and are a tribute to the safety precautions exercised by these workers.

Studies relating naled and temephos exposure as measured by urinary metabolites to clinical cholinesterase depression or inhibition have not been conducted in humans to our knowledge. Experiments using rats exposed to closely related organophosphate insecticides have been continuing for over a year (D. E. Bradway, 1976, personal communication). When rats were fed insecticides metabolized to dimethyl phosphate (DMP) and dimethyl phosphorothionate (DMTP), plasma cholinesterase depression did not occur until these two metabolite levels exceeded 2 to 3 ppm in the urine. Although the results of these animal experiments cannot be directly extrapolated to human effects, they do provide some type of guide to interpreting these human monitoring studies. Most of the metabolite levels found in this study were several orders of magnitude lower than levels indicative of clinical repercussions. The highest levels of metabolites observed in this study were 1.6 ppm of dimethyl phosphorothionate (DMTP) and 0.80 ppm of dimethyl phosphate (DMP). Even these extremes were lower than cholinesterase inhibiting levels in rat studies.

It appears that the urinary metabolite levels detected in this study did not approach levels generally accepted as indicative of cholinesterase depression, inhibition or other clinical repercussions.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of the following: Darrell E. Louder, Director of the Delaware Division of Fish and Wildlife; Mr. A. W. Wheatley and Mr. C. Stachecki of the Mosquito Control Section of the Delaware Division of Fish and Wildlife; and Dr. Frank J. Murphey of the Department of Entomology and Applied Ecology, University of Delaware. The Colorado Pesticides Epidemiologic Studies Center, Ft. Collins, performed the urine analyses and Mr. R.

Table 5. Results of urine analysis of mosquito control employees,[1] Dover, Delaware, 1973.

Diakyl Phosphate Metabolite	Frequency (%)	Maximum Value	Arithmetic Mean
		Concentration in PPM	
DMP	78	0.07	0.024
DEP	67	0.05	0.019
DMTP	56	0.16	0.032
DETP	67	0.14	0.030
DMDTP	22	0.05	0.007
DEDTP	67	0.03	0.009

[1] Sample size was 9.

F. Thomas of the U. S. Environmental Protection Agency, Beltsville, MD, analyzed the spray formulation. Mr. Daniel Cirelli of our branch provided the automated data processing and analysis. Dr. E. W. Cupp of the Entomology Department at Cornell University critically reviewed the manuscript.

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EVALUATION OF SEVERAL INSECTICIDES FOR THE CONTROL OF LARVAL *AEDES SIERRENSIS* (LUDLOW)

M. L. OLDHAM, E. E. LUSK AND D. J. WOMELDORF

M. L. Oldham, Tehama County Mosquito Abatement District, Red Bluff, CA 96080, and E. E. Lusk and D. J. Womeldorf, California Department of Health, Waste Management Section, Redding and Sacramento

ABSTRACT. Chlorpyrifos, temephos, fenthion, and propoxur were applied to naturally occurring treeholes at the rate of 1 gm AI liter of treehole water. Inspections for reinfestation and bioassays of water samples from the treeholes were performed at intervals of approximately 1 year. Field infestations of *Ae. sierrensis* occurred during the 2nd year in trees treated with propoxur. Temephos and fenthion treated trees were infested during the 4th year. Larvae of *Ae. sierrensis* were found in treeholes treated with chlorpyrifos during the 6th year. Fenthion, temephos, and propoxur began to fail in bioassay at 2 years and chlorpyrifos at 5 years.

Aedes sierrensis, the western treehole mosquito, is a ubiquitous pest throughout many residential and wooded areas of California from near sea level to the high mountains. It is an exceedingly annoying biter in outdoor shade. Additionally, the adults often are so small that they are able to crawl through ordinary window screen and attack people indoors. During late spring and early summer, the species is responsible for many complaints received by mosquito control agencies. Weinmann and Garcia (1974) have discussed the potential of *Ae. sierrensis* as a vector of canine heartworm, *Dirofilaria immitis* (Leidy), a parasite found frequently in dogs but rarely in humans.

Ae. sierrensis breeds almost exclusively in treeholes, usually a rothole formed at the site of a broken-off or pruned limb and filled during the fall and winter by rainwater. Permanent control can be achieved by