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PESTICIDES EPIDEMIOLOGICAL FIELD STUDIES

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PESTICIDES EPIDEMIOLOGICAL FIELD STUDIES

by

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Abstract

Quantitation of pesticide exposure is an essential feature of occupational health insofar as the safety of the pesticide worker is concerned. When accurate pesticide exposure can be obtained surveillance will protect the pesticide applicator and has the potential for evaluating human pesticide residue exposures and the reliability of current re-entry standards. Since worker exposures are usually multiple, the use of the multiresidue technique for the analyses of urinary metabolites can provide the key to quantitation of mixed organophosphate and carbamate human exposures. With this philosophy in mind, occupational studies of pilots and loaders were measured. Aircraft loaders were found to be receiving excessive pesticide exposure as evidenced by the occurrence of several acute pesticide intoxications, a chronic state of cholinesterase inhibition and a high turnover in this category of work. The study of urinary di-alkyl phosphates and phenols confirmed the excessive exposure of these two working groups and the amounts of di-ethyl phosphate (DEP), di-ethyl thiophosphate (DETP), di-methyl phosphate (DMP) and di-methyl thiophosphate (DMTP) and paranitrophenol (PNP) were significantly inversely correlated with the red blood cell cholinesterase at the p 0.001 level. Thus, the lower the red blood cell cholinesterase, the higher the urinary metabolites.

The four di-alkyl phosphates were the derivatives constantly identified in these occupational exposures. Following an eight hour exposure to a single organophosphate both the di-alkyl phosphates and the appropriate phenolic derivatives persisted in the urine for thirty six hours or longer. The impression was gained that the optimum time for urinary surveillance based on a single sample was nine hours after the last exposure. These two groups of metabolites were also exceedingly informative not only in the confirmation of acute pesticide poisoning but also in the monitoring of the poisoned victim with regard to his need for anti-dotal therapy.

Organophosphate poisoning is usually thought of as being a abbreviated episode wherein the clinical manifestations and outcome are usually resolved within 48-72 hours. Recently we have encountered a much more protracted illness following acute exposure to certain halogenated organophosphates, this was especially noticeable following intoxication of VC-13(R) which is a dichlorophenol di-ethyl phosphorothioate. The presence of the halogen derivatives attached to the phosphate moiety resulted in the delayed occurrence of acute intoxication and a protracted clinical crisis. This was the case in the poisoning reported herein, and emphasizes the need for a changed appraisal of human subjects intoxication by these fat soluble organophosphates. Contrary to past experience VC-13(R) and Dursban(R) were regularly detected in the air sample and DDT was conspicuously absent. Qualitatively the air samplings reinforced the greater use of organophosphates in South Florida.

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CONTENTS

FIGURES

TABLES

ACKNOWLEDGEMENTS

The investigators acknowledge with gratitude the cooperation and assistance offered us to conduct these studies by Allied Helicopter Service Inc., the Dade County House and Urban Development Program, the Eveready Pest Control Company. Without the enthusiastic support of the management and staff of these institutions, these evaluations of human pesticide exposure under normal working conditions could not have been possible.

SECTION I

CONCLUSIONS

Important conclusions were reached in three of the four areas of the research conducted this last year. In the area of the study of urinary alkyl phosphates and phenolic derivatives, there is no doubt that recent multi-residue analytical techniques have provided a greater opportunity to measure human occupational exposure to pesticides. The data facilitates the quantitation of mixed pesticide exposures from workers involved in pesticide application, and from workers exposed to dislodgable leaf residues in re-entry situations. Their measurement also significantly contributed to the accurate diagnosis of acute pesticide poisoning and in the investigation of suspect "pesticide associated" illnesses. They will be useful to the physician as well, for their presence is informative during the clinical management of the poisoned victim; they provide an indication for the continued need for atropine and oxime therapy.

In the area of occupational pesticide exposures, our preliminary data although based upon small numbers suggested that the alkyl phosphates are significantly inversely correlated with red blood cell cholinesterase levels. When the pesticide exposures of the aircraft loaders were compared with the pilots, the excessive exposure of the former was substantiated by a) the number of acute poisoning episodes, b) an 88% employee turnover rate, c) very low cholinesterase levels particularly the red blood cell values, d) high paranitrophenol averages, and e) by DEP, DETP and DMP alkyl phosphate residues. The association of DMP with red blood cell cholinesterase values was particularly impressive and was an association which possibly emphasized the special toxicity problems of Phosdrin(R). As was the first experience of Dr. Shafik and his colleagues, in animal studies, DEP, DETP, DMP and DMTP were the only alkyl phosphates found under these conditions of mixed organophosphate exposures. The monoethyl and monomethyl phosphoric and phosphorothioic acids are not discussed herein because Dr. Shafik stated that they are found in acute pesticide poisonings only and even then their sensitivity is poor, and therefore, there is no need to look for other alkyl phosphates other than those herein described. Another significant finding was the possible significance of DETP/DEP ratio; greater amounts of the former were observed in workers when their exposure was to ethyl and methyl parathion. At this point in time, this observation must be considered as an impression rather than a conclusion and it certainly warrants further study.

Since exposure to parathion was the predominant organophosphate exposure in the applicator group this exposure promoted an evaluation of the relative merits of the alkyl phosphate data compared to the paranitrophenol levels. Since grab samples of urine rather than 24 hour urine outputs were studied, firm conclusions cannot be reached on the relative merits of either type of metabolite. During the daily studies of alkyl phosphate and paranitrophenol levels in the urine of one loader, the impression was gained that the former particularly DEP plus DETP were less subject to the wide variations observed with paranitrophenol values. One conclusion which can be made with some degree of certainty is that more field research is necessary so that diurnal variations of both types of metabolites can be studied especially following work exposure to a single pesticide; a parathion urinary profile is the one most urgently needed. Another important conclusion was that these metabolites promote the study of organophosphate and carbamate exposures at levels not necessarily producing cholinesterase inhibition. With such features, therefore, they are ideal for occupational surveillance and for future use as epidemiologic instruments.

The special studies of Baygon and Dursban made it possible to conclude that future surveillance programs with these pesticides, if reliance must be placed on a single grab sample of urines, then 6 to 9 hours after exposure is the ideal time to collect such a specimen. With regard to urinary pesticide metabolites concentrations should be expressed in terms of micrograms per hour rather than by correcting for urine osmolality. Pesticide residues were much greater than expressed in this way then when corrected for an osmolar correction factor.

From the epidemiological studies of acute pesticide poisoning, several conclusions were reached. The number of confirmed cases and the growing problem of pesticide suspected illness emphasize the future need for Pesticide Poison Control Centers in those areas were pesticides are heavily used. New pesticides are appearing on the scene and without a system for monitoring the acute incident it is impossible to anticipate generalized effects in an area before they occur. VC-13 is a case in example--the different clinical consequences of over exposure to this fat soluble pesticide was first appreciated as a result of the investigation of an acute poisoning. The more halogenated ions are in the pesticide moiety the more delayed are the effects. Our experience with VC-13 poisoning confirms that chronic organophosphate pesticide poisoning has now become a reality.

New pesticides are now found in the South Florida ambient air such as VC-13, Dursban and some of those identified are fat soluble and the whole picture is changing from an incidental organochlorine potential to an incidental organophosphate exposure. One must conclude that an ongoing meaningful air sampling program is essential.

SECTION II

RECOMMENDATIONS

The potential of the urinary pesticide metabolite needs to be further explored in the occupational environment of the pesticide applicator, in the work situation following exposure to dislodgable residues and as an evaluatory parameter of proposed field re-entry studies. In the presence of these needs further studies on urinary metabolite excretion patterns under normal working conditions should be conducted. Special emphasis should be given to single pesticide exposure situations - 36 to 48 hour urinary profiles. Methodologic research of urine metabolites in the direction of a simplified and accelerated method of testing is urgently needed. These should be applicable for testing in the field, what is really needed is a sort of dipstick test for occupational exposure. Until this is developed grab sample urines, a system which requires knowledge of the prior voiding time, seems to offer the only acceptable type of specimen in a large scale surveillance program. Based on the findings of this first year's work the metabolites must be evaluated for as long as 48 hours after a six hour exposure. We have no information on the different rates of excretion of alkyl phosphates which are the result of differences of fat solubility. We do not know whether there are diurnal variations in the concentrations that are excreted following a single exposure nor have we confirmed that these metabolites are present in the general population. It is recommended that these studies be directed to answer some of the aforementioned questions to be conducted as soon as possible if the merits of the urinary metabolite is to be measured in human exposure studies.

In addition to the development of metabolite information, our emphasize this year has been the need to establish selected pesticide poison control centers in appropriate areas where pesticides are extensively used in the United States. Secondly, with the change of pesticide use patterns an ongoing air monitoring program should be instituted in the areas of high pesticide usage in the United States.

SECTION III

INTRODUCTION

SCOPE AND PURPOSE OF THE PROJECT

This project entitled "Pesticide Field Epidemiologic Unit" has as its primary objective to implement field epidemiologic studies of in-house research areas recently developed in the U.S. Environmental Protection Pesticides Laboratory in Perrine, Florida. The subordinate objectives reflective of in-house research areas which were ready for field testing included:

A. Urinary metabolite studies of pesticide workers
B. Air Monitoring studies
C. Field testing of a portable EEG apparatus
D. Pesticide poisoning investigations.

This was a new three year project, and in the first year called for the relocation and establishment of a new pesticide laboratory in the University of Miami School of Medicine. This annual which covers pertinent findings in the first year in these four areas of pesticide field epidemiologic investigations must serve as a final report since owing to budgetary cuts in projects, the second and third years of the study were renegotiated on a contractural basis.

SECTION IV

URINARY METABOLITE STUDIES

A. Urinary metabolite studies - Background Information. Traditionally, the occupational health surveillance of the occupationally exposed worker has relied upon the red cell and plasma cholinesterase changes observed under conditions of pesticide exposure as an indication of human exposure to organophosphate and carbamate insecticides. Experience has shown that there are several serious limitations of the interpretability of these indices. These included the wide range of normal cholinesterase values in the non-exposed individual, the absence of a definitive cholinesterase level which is diagnostic of the occurrence of overt illness, and the necessity of obtaining pre-exposure baseline data in occupationally exposed studies. Because of this, many workers in occupational health have investigated the use of pesticide urinary metabolites as an alternate human pesticide exposure index. Parathion, carbaryl and propoxur are examples of some of the more commonly used pesticides in this group whose metabolites have been studied under conditions of occupational exposure. Recently Shafik, Enos and their colleagues have developed a residue procedure for halogen and nitrophenols in urine.[1, 2] Their preliminary data suggested that these newer techniques lend themselves to a more specific study of pesticide exposure in man, and may well prove to be the key to the quantitation of mixed organophosphates and carbamate exposures.

In a preliminary study of six samples from persons having no exposure and from occupational workers exposed to Dasanit (Fensulfrothion), Thimet (Phorate) and Di-Syston (Disulfoton), Shafik found that DMP, DEP, DETP and DMTP alkyl phosphate metabolites were identified. In the non-exposed group urinary metabolite concentrations ranged from 0.005 - 0.04, from 0.003 - 0.08, from 0.02 - 0.10 and from Not Detected - 0.01 ppm respectively for these four alkyl phosphate metabolites. The average concentrations for these metabolites in this non-exposed group was 0.01, 0.05, 0.06 ppm and Not detected respectively. Similarly from six samples of the occupationally exposed, the ranges were 0.005-0.06, 0.34-2.41, 0.06-0.22, and 0.16 and 1.62 ppm respectively. Average concentrations in these six samples from the exposed were 0.02, 0.97, 0.10, and 0.60 ppm respectively. These very preliminary data suggested that these metabolites reflected occupational exposure to organophosphate pesticides and were now ready for field testing.

Our studies have sought to take over from this point and in a logical stepwise progression to explore in greater detail the qualitative and quantitative profile of these pesticide metabolites in several occupational groups in South Florida. In addition, we have attempted in a preliminary way to explore the relationship of these metabolites to cholinesterase findings.

Description of Phases - As soon as we received the grant award, we started to prepare our study as follows:

Phase I - Making contact with the several companies in this area and investigating what is their season, types of pesticides that are being used and whether they would participate in our study. It also required the specialized training of our

chemists with Drs. Shafik and Enos in the multiple residue and alkyl phosphate analytical procedures. Plans were also made to equip and relocate the pesticide laboratory to the University of Miami Medical Research Building.

Phase II - Related to the qualitative and some quantitative examinations of urines from occupationally exposed pesticide workers.

Phase III - Called for the relation of these exposure indices to cholinesterase findings.

Phase IV - Called for individual 24 to 36 hour urinary pesticide metabolite profiles for each individual pesticide.

Phase V - Called for the identification of the optimal time between and after exposure for the meaningful ongoing surveillance of the pesticide worker.

Phase VI - The final phase called for the identification of urinary concentrations of individual pesticide metabolites which were premonitory of cholinesterase decline and the occurrence of acute symptoms. In order to systematically implement the above experimental design, the following studies were conducted in a group of pesticide workers in a helicopter spray outfit in the Belle Glade area and in selected exposure studies in structural pest control operators.

Materials and Methods

The preliminary qualitative and quantitative urine studies acquired from occupational pesticide exposure were first conducted in cooperation with the Allied Hellicopter, Inc., in Belle Glade, Florida. This is a crop dusting company which contracts with South Bay Growers to aerially apply a wide variety of chemicals to the fields in the area. The individual growers select and purchase the pesticides which they want to have applied and the helicopter company is merely responsible for its application. The helicopter company operates from a modern helicopter pad situated two miles outside of Belle Glade and they have constructed a new hanger on the site as well as adjoining administrative offices. Six multiple rotary blade Bell helicopter aircraft are used these are fitted with twin multiple jet spray beams and twin loading tanks. When not in use, the helicopters are tethered outside the hanger. Inside the hanger there is a repair shop and a mechanic is employed full-time. Showers and changing rooms with individual lockers are provided for the four regular pilots and their loaders. Agricultural chemicals are stored away from the hanger on a separate site. During the season the pilots and loaders work seven days a week. Two sorties are made daily, the first at 6 a.m. until about 10 or 11 a.m. and the second at 2 p.m. to 6 p.m. The pilots do not mix the chemicals themselves and wear coveralls, a crash helmet, respirator mask, gloves and flying boots. They are partially enclosed in a plastic bubble and each pilot works with one loader. The loader decants the agricultural chemical from 55 gallon drums of the pesticide concentrate in the airport into the drum of a large tank truck. In addition, five gallon drums of a wide variety of pesticide concentrates are also stored and transported on the vehicle to the various operational sites in the field. The planes are loaded and reloaded at these sites. Water is obtained from ad-

joining canals and after being pumped into the tank the pesticide concentrate is added and the mixture agitated. The diluted material is then pumped through a 50 foot hose into the twin tanks on each side of the helicpter. These are filled and refilled three or four times an hour. The loaders are provided with gloves, masks and rubber boots. Additionally, oceralls were provided during the last six months of the study period. A wide variety of pesticides are applied and the materials used last year included: parathion 8-E, parathion 6-3 (ethyl-methyl mixture), parathion-toxaphene mixture, Cygon (dimethoate), AAtrex (atrazine), Trithion (carbophenothion), Phosdrin (mevinphos), Dithane (maneb), Lannate (methomyl), Dipel(R), Azodrin (monocrotophos), Monitor(R), Dibrom (naled), Guthion (azinophos methyl), and 2,4-D(R). After obtaining a signed consent form from sixteen employees of the Company a study was conducted on four pilots, five loaders and three controls. Periodic blood cholinesterase and urine samples were obtained from this occupationally exposed group. Ten cc. of blood were collected in a heparinzed vacutainer tubes, transported over 100 miles in an icebox to the University of Miami Pesticides Laboratory were they were analyzed 24 hours after collection. Periodic grab sample urines were collected in labelled hexane washed jars stored in dry ice for up to four days and then shipped to Miami where they were frozen at -15° C and at a later date analyzed both for phenolic derivatives and alkyl phosphate metabolites.

The Michel method was used for red blood cell and plasma cholinesterase determinations, and the Shafik, Bradway, Enos & Yobs modification for the gas liquid chromatography analyses of alkyl phosphate metabolites in urine.[3, 4] The Shafik, Sullivan and Enos procdure was used for the analyses of halo and nitrophenols in urines[1] and the Shafik, Sullivan and Enos procedure for 1-Napthol in urine was adapted to measure Iso-propoxyphenol (IPP).[5] Grab sample urines were corrected to an osmolality of 800 ml/Osm/l prior to analysis.

Urine and blood studies were conducted in two groups of structural pest control operators exposed to a single organophosphate or a single carbamate insecticide. Blood and grab urine samples were obtained from six structural pest control operators (SPCO) working for the Dade County Housing and Urban Development program who had sprayed tenement building eight hours a day with a 2% solution of Dursban and a 1.5% Baygon (propoxur); search was made for 3,5,6-TC pryidinol and IPP during these exposures. Continuous urinary metabolite excretion profiles over a 36 hour period were obtained from two SPCOs of the Eveready Pest Control Company selectively exposed to 0.5% Baygon, 2% Dursban and 0.75% DDVP.

Results

Table 1 lists the total number of urinary metabolite analyses and cholinesterase studies in the several occupational groups. Cholinesterase studies were not always obtained on every urine specimen collected. In addition, 118 cholinesterases were analyzed from pesticide exposure groups including agricultural inspectors from the U.S. Department of Agriculture, sprayers from the Dade County Department of Parks, etc. Clinical surveillance of and pesticide metabolite data from the helicopter group of workers revealed that pesticide exposure in this group of workers, particularly the aircraft loaders, was extensive. Three episdoes of acute pesticide intoxications, all in pesticide loaders, occurred in the study period; 34% of 54 red blood cell cholinesterase determinations in this group gave a delta pH per hour of 0.40 or less. A high turnover rate was observed during this study period; 88% of the loaders and 28% of the pilots changed their jobs during a period of

Table 1. NUMBER OF URINARY METABOLITE ANALYSES AND BLOOD CHOLINESTERASE STUDIES IN DIFFERENT PESTICIDE EXPOSED GROUPS IN SOUTH FLORIDA

Analyses	Aircraft Applicators (n=21)	SPCO HUD (n=6)	SPCO Eveready (n=2)	Others	Total
Urinary Phenols	87	13	32		132
Urinary Alkylphosphates	39		19		58
Blood Cholinesterase (red blood cell and plasma)	54	11	6	118	189

12 months and were lost to follow-up. To the observer it was obvious that significant loader exposure occurred during the refilling of the aircraft with pesticides; an effect which was due to the down draft produced by the rotary wings causing an aerosol exposure from pesticide spillage on the ground. This spillage could be avoided if an automatic nozzle cutoff valve were a mandatory requirement in all aircraft spray booms, a suggestion which was reported to the Project Officer. Sequential data was available on twelve subjects, these were 5 loaders, 4 pilots and 3 controls. The mean and ranges of the red blood cell cholinesterase, the urinary alkyl phosphate and paranitrophenol concentrations of these subjects are shown in Table 2. These subjects during the study period are typical of high, intermediate and non-organophosphate exposures. Both the RBC and urinary pesticide metabolite data emphasized the excessive exposure of the loaders. The four di-alkyl phosphates (DEP, DETP, DMP and DMTP) were the only ones tested in these samples. Except for DMTP all were found in each of the 27 urines analyzed in the loaders; some of these specimens included pre-exposure 6:00 a.m. voidings. Paranitrophenol (PNP) was likewise found in every specimen in this occupational category. DMTP was not found in 14 of the 27 urines from the loaders, a deficiency which probably reflected early analytical problems of separation of the metabolite and which was subsequently cleared up. In the pilots, these biological indices predominately of ethyl and methyl parathion exposure was considerably less and on four occasions DMP was not identified. DEP was identified on every occasion as was also PNP. The correlation coefficients of PNP, RBC and Pl. ChE with these urinary metabolites are shown in Table 3. Significant exposure to organophosphates resulted in the lowering of the RBC and Pl. cholinesterases and also exposure to parathion as high levels in PNP were found. Thus, if urinary alkyl phosphates were equally good measures of occupational exposure to anti-cholinesterase, one would expect significant inverse correlation of them with RBC and Pl. ChE and a highly significant correlation with PNP. Conventionally excessive exposure to organophosphates is reflected by the red blood cell and plasma cholinesterase inhibition. If the organophosphate exposure is from parathion, urinary paranitrophenol levels is an additional index of pesticide exposure and high levels of these metabolites are found. This indeed was the case and DEP, DETP and DMP were significantly correlated with these known biological indices of pesticide exposure at the highly significant level of $p < 0.001$. The only exception was in the correlation of DMTP data which almost certainly reflected initial analytical difficulties with this specific metabolite. The inverse correlation of red blood cell cholinesterase with paranitrophenol on the basis of 15 samples from ten subjects in this study group was -.76. Although the occupational exposure to this group were to a wide variety of pesticides, ethyl and methyl parathion (6-3 mixture) were the preparations most frequently used. The highly significant correlations herein shown strongly support the potential of these metabolites in occupational studies of organophosphate expsores, although the smallness of sample size should be emphasized. The highly significant inverse correlations of DMP with RBC ChE is especially noteworthy. It is possible that Phosdrin(R) exposure which is known to be highly toxic and which would give DMP urine metabolites only might be contributory to the significance of this association.

Figure 1 illustrates the monthly levels of urinary metabolites obtained on a grab sample basis from a pilot who was studied over a three month period of excessive spraying. Monthly red blood cell and plasma cholinesterase are also depicted. Figures 2 and 3 are first expressed with urinary di-alkyl phosphate and phenolic data detected in these helicopter pilots and swampers on a grab sample basis. Corresponding red blood cell and plasma cholinesterases were not obtained on every occasion that the urine samples were collected.

Table 2. COMPARISONS OF URINARY ALKYLPHOSPHATE CONCENTRATIONS IN HIGH, INTERMEDIATE EXPOSED WORKERS AND CONTROLS IN SOUTH FLORIDA.

Exposure groups	Cholinesterase*		Alkylphosphates (ppm)**					Paranitrophenol (ppm)**	
	No. tests	RBC mean, range	No. tests	DEP mean, range	DETP mean, range	DMP mean, range	DMTP mean, range	No. tests	Mean, range
High-Loaders (n=5)***	15	0.3 (.09-.72)	27	0.48 (.10-1.37)	0.49 (.11-1.7)	0.25 (.005-.62)	0.09 (0-.36)	61	0.8 (.04-4.36)
Intermediate-pilots (n=4)	9	0.7 (.5-.8)	11	0.26 (.07-.66)	0.11 (.001-.3)	0.06 (0-.26)	ND 0	11	0.3 (.04-.74)
Controls (n=3)	3	0.8 (.80-.82)	3	0	0	0	0	3	0

* pH/hr
**corrected to 800 mOsm/l
***includes 3 acute illnesses

Table 3. CORRELATION COEFFICIENT (R) OF PARANITROPHENOL, RED BLOOD CELL, PLASMA CHOLINESTRASE WITH URINARY ALKYLPHOSPHATES FROM PESTICIDE EXPOSED WORKERS

Biological indices	n	Alkylphosphates (ppm)						
		DEP	DETP	DMP	DMTP	DEP + DETP	DMP + DMTP	DEP, DETP, DMP, DMTP
PNP (ppm)	39	.75	.75	.5	.54	.80	.66	.79
RBC ChE	15	-.72	-.64	-.89	-.11	-.72	-.86	-.78
Plasma ChE	15	-.52	-.48	-.75	.04	-.53	-.68	-.58

Figure 1. Monthly red blood cell and plasma cholinesterase, paranitrophenol and alkylphosphate metabolites observed in a helicopter crop duster (F.B.), Belle Glade, Florida 1973

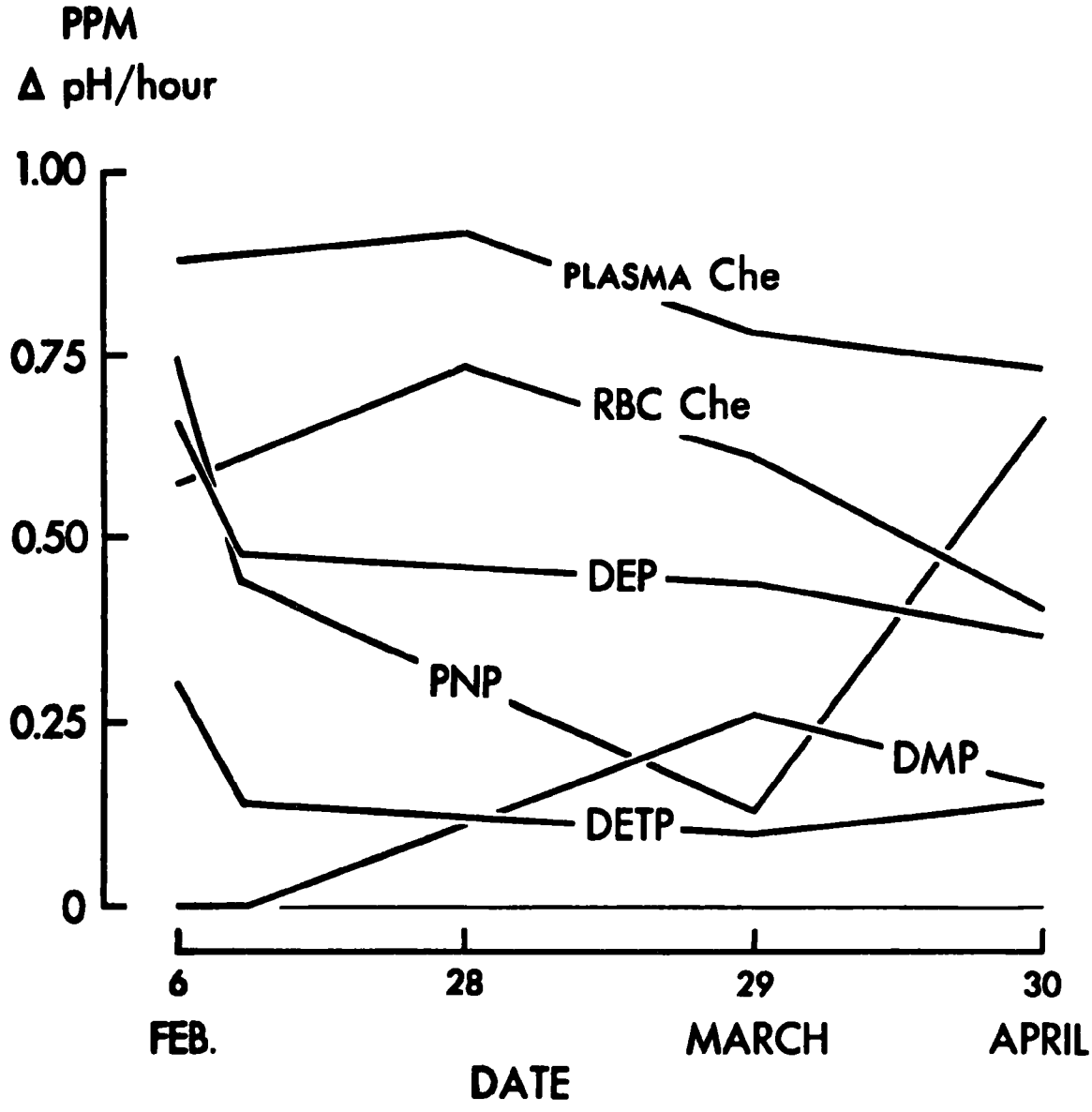


Figure 2. Comparisons of cholinesterase and paranitrophenol excretions observed in an aircraft loader (F.R.), Belle Glade, Florida 1973

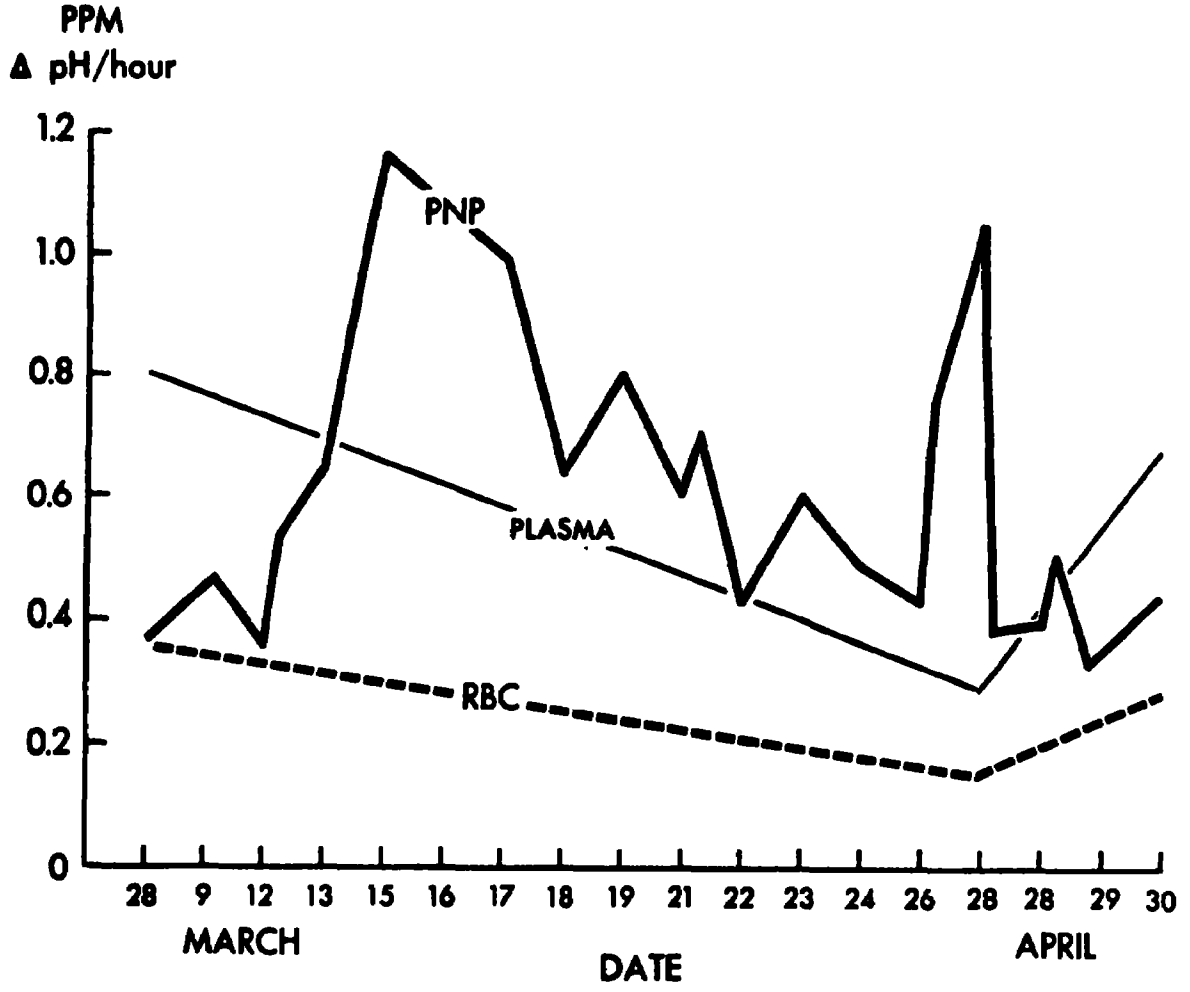
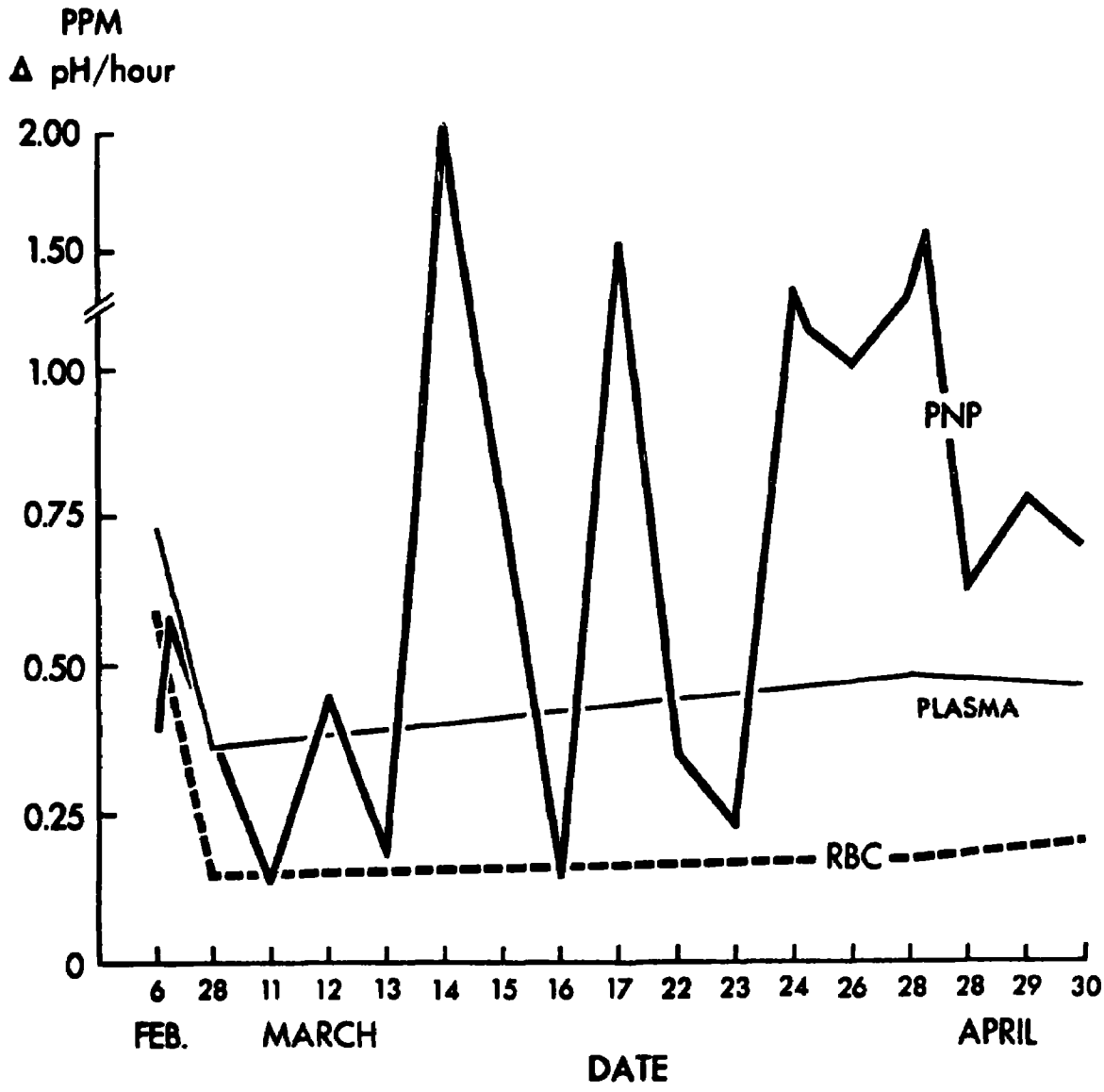


Figure 3. Comparisons of cholinesterase and paranitrophenol excretions observed in an aircraft loader (K.D.T.), Belle Glade, Florida 1973



These figures are presented merely to indicate the interpretative complexities that were first encountered when urines were obtained from persons whose pesticide exposures were multiple and where the data was from individuals who received pesticide exposure daily. In addition they illustrated the need first to obtain baseline information on the magnitude and duration of the urinary metabolite excretion following a single exposure to a single pesticide so that information could be obtained of the duration of excretion of the metabolite and the nature of the diurnal fluctuations.

In order to better understand some of these, daily urines were collected from an aircraft loader (Maynard W.). This man had overt symptoms of anticholinesterase intoxication which had developed from occupational exposure to parathion. He was hospitalized and given atropine (this point is shown in Figure 4). His cholinesterase levels at that point in time were RBC ChE 0.09 and Pl. ChE was 0.13 ΔpH/hr; during the next month he mixed and loaded parathion 8E and ethyl-methyl parathion 6-3 mixtures on the following days:

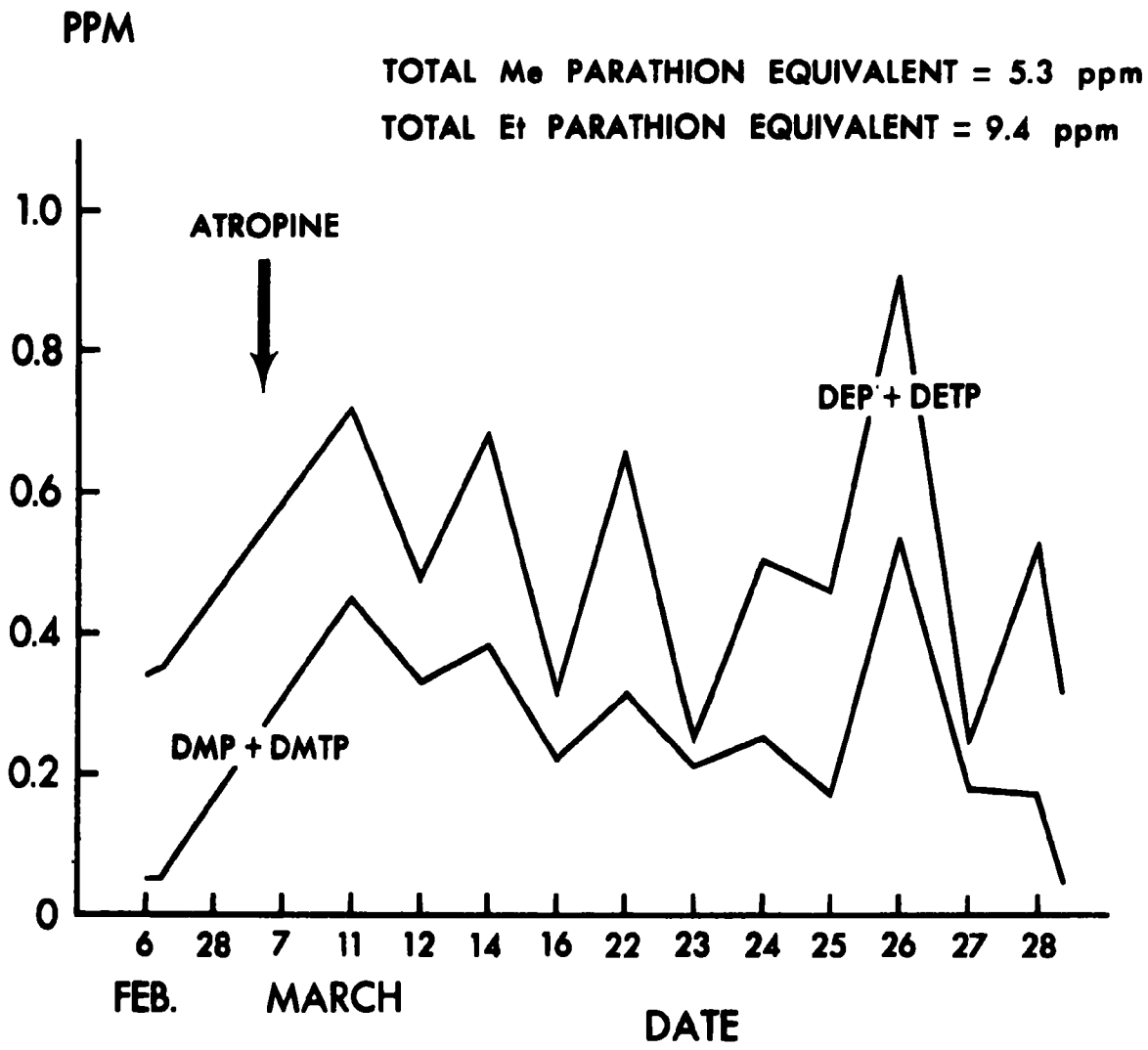
March 13th, 18th, 19th, 20th, 21st, 23rd, 24th, 27th and 28th

He was unable to give us his pesticide exposure on the other days during this month. Figure 4 illustrates that based upon the combined di-ethyl and di-methyl alkyl phosphate levels in daily grab samples there was indeed significant pesticide exposure to ethyl methyl yet at the end of the study period a repeated cholinesterase showed that the red cell had risen from 0.09 to 0.35 pH/hr and the plasma had risen from 0.13 to 0.32 ΔpH/hr.

One observation which may prove significant in future studies concern the ratio of DETP to DEP, as already mentioned, we have noted that the loaders were more exposed than the pilots and since the predominant organophosphate exposure was to ethyl-methyl parathion these would be reflected by the excretion levels of the di-ethyl alkyl phosphate metabolites. Both pilots and loaders were representative and reflective of persons chronically exposed to this pesticide and we noted that 66% of the urines from the loaders had higher DETP levels than DEP levels whereas the urines from the pilots only 39% had higher DETP levels than DEP. Very useful quantitative information was also obtained from the study of the phenolic derivatives utilizing the Shafik et al. multi-residue procedure. Paranitrophenol was identified in all of the urines of the pilots and loaders which means that however examined these high and intermediately exposed group of people always had qualitative evidence of parathion exposure. Trace amounts of this parathion metabolite were also identified in the mechanic in 7 out of 9 specimens. Pentachlorophenol (PCP) traces were found in all 87 urines; the mean concentration was 0.01 ppm with a range of 0.004-0.024 ppm. These indicate minimal incidental exposure to this wood preservative and these may be indicative of general population exposure rather than work exposure.

The identification of 2,4,5-trichlorophenol was probably reflective of incidental exposure to Ronnel since there was no history of this insecticide being used by Allied Helicopters Inc. Similarly, 2,4-DCP and 3,5,6-TC pryidinol which are phenolic derivatives of VC-13 and Dursban and must have reflected incidental exposure to these pesticides in a domestic setting or even in the ambient air (as will be seen in another section of this report both insecticides have been regularly identified in the air sampling program).

Figure 4 - Combined alkyl phosphate excretion in an aircraft loader (Maynard W.) during ethyl-methyl parathion exposure.



Thus the combination of data from the urinary alkyl phosphates and the phenolic derivatives provide highly informative qualitative data of the occupational and incidental exposure of the agricultural worker, and qualitatively indicate the potential of the multi-residue surveillance of different occupational groups. Quantitative interpretation is much more complex as will be described hereinafter.

Discussion - The ultimate long term goal of these studies is to try and find qualitatively and quantitatively a urine profile of the di-alkyl phosphates which is prognostic of excessive exposure, significant cholinesterase inhibition and incipient cholinesterase inhibition. If a screening test can be identified which can be used to monitor the pesticide exposed worker than pesticide handling can be put on a more rational basis and the worker and industry will have the potential for a routine industrial hygiene program. It is our hope that the urinary metabolite will become the ultimate occupational surveillance parameter. The studies reported so far have confirmed, a) the potential of the di-alkyl phosphates and phenolic derivatives as diagnostic indices of organophosphate and carbamate pesticide poisoning. Collectively the can provide very specific information as the nature of the intoxicant in acute poisoning, b) they have also been shown to be very useful toxicological monitors during an acute poisoning. They provide the essential information as to the degree of the organophosphate intoxicant on any point in time during the acute phase in illness and they provide toxicological data as to when specific antidotes can be discontinued. This potential is ill understood and ill recognized by physicians having to handle long drawn acute organophosphate intoxications. c) based on a small sample the di-alkyl phosphates appear to have good correlation with cholinesterase inhibition and these findings confirm Shafik's earlier observation, however, much more information is needed on the dynamics of these metabolites under chronic exposure before there is any likelihood of identifying a quantitative or qualitative profile which is sufficiently indicative of excessive exposure that can be relied upon as an occupational surveillance index. Perhaps, it will be the DETP-DEP ratio under chronic or occupational exposure to parathion which might provide the clue.

In order to explain the next thrust of our studies next year it is perhaps timely to list some of the unknown variables, these include:

1. The duration of metabolite excretion following a single non-toxic exposure to a single pesticide.
2. The effects of a single exposure to multiple pesticides.
3. The quantitative and qualitative urinary profiles of non-occupational exposure, i.e., how many of these metabolites, if any, can be detected in the general population--does food, house dust or the use of insecticide strips or sprays in the home environment result in the pesticide excretion?

It is to these unknown areas that we will turn our study design and the remainder of this report describe single exposures to single and multiple pesticides.

The first group to be studied was a group of six structural pest control operators working in the Dade County Housing and Urban Development Program. Two of these (C.L. and M.H.) were sprayers, and the four others were maintenance men and so were peripherally exposed to pesticides. This group was first studied while working with Baygon (propoxur). A 1.5% Baygon solution was used during a period of 8 hours. Grab samples of urine and blood for cholinesterase

levels were obtained to see if Iso-propoxyphenol (IPP) could be identified by the Shafik et al method in this type of exposure. The cholinesterase and Iso-propoxyphenol are shown in Table 4. These preliminary findings confirmed that IPP could be readily identified in this type of work exposure. The same group was similarly tested after they switched from a 1.5% Baygon solution to a 2% Dursban application. Blood cholinesterases and eight sequential urines were obtained from three spraymen (C.L., M.H., and M.K.). Following the switch to Dursban, the average plasma cholinesterase was noted to have declined by 50% though the average red blood cell levels remained unchanged in the group. 3,5,6-TC-pyridinol, the phenolic metabolite of Dursban was readily identified both during and after this occupational exposure. Urinary concentrations ranged from 0.29-1.03 ppm. Peak concentrations were reached between nine and twelve hours after exposure which suggested that this might be the optimal time for surveillance by this technique if grab urine samples were to be used.

This information prompted us to try and identify structural pest control operators with single pesticide exposures and whose work practices promoted the study of pre-exposure baseline urines. We have also sought operators who could be studied on the basis of single work exposures. Eveready Pest Control Company was a firm who employed two operators whose work practices fulfilled these experimental design requirements. Each operator worked six to eight hour shifts on Saturdays only; spending the rest of the week attending college. Both agreed to participate in the study and to provide sequential urines.

The first study was a Baygon(R) study. Pre-exposure urines and bloods were collected. Max P. used a hand spray and Edward S. a power spray. Ed wore rubber gloves and respirator; both mixed their individual spray solutions reducing a 14% Baygon concentrated solution to a 0.5% solution for application. They sprayed from 8:00 a.m. to 4:00 p.m. The sequential IPP excretion over this period is shown in Table 5 and Figures 5 and 6.

Results - Firstly the men had negative urines at the commencement of the study thus confirming their non-exposure history of the previous day. Secondly, Max sustained more exposure than had Edward, a fact which may be attributed to the different amount of exposure from the hand spray versus the power spray and also the use of protective clothing. Thirdly, in both the peak exposure time occurred five hours after completion of work. Fourthly, in one spray operator (Max P.), his excretion of the metabolite was completed within 16 hours after his last exposure; in the other sprayman (Edward S.) 36 hours was insufficient time for the total excretion of the metabolite, trace amounts still being detected at the end of this time. The total amount of IPP excreted in the 30 hours for Max was 1,550 ug which is equivalent to 2,139 ug of Baygon. In the other, Edward, S., 712 ug of IPP was excreted in 29 hours which is the equivalent of 982 ug of Baygon. It is possible to relate this metabolite's excretions to the original exposure of the intact pesticide. In order to obtain a rough estimate of the percentage of the intact pesticide that is applied the micrograms of IPP are multiplied by a factor of 1.38 to convert them to the intact pesticide of Baygon based on their molecular weight.

A second thirty six hour study was conducted in these spraymen following their single 4½ hour exposure to 2½% Dursban and 0.75% of DDVP. Urine levels of DMP would be indicative of DDVP exposure and Dursban would be identified by DEP and DETP alkyl phosphate excretion and also by 3,5,6-TC prydinol excretion. The excretion pattern of these metabolites in the two spraymen are shown in Table 6 and Figures 7 and 8. Here again several interesting findings were noted. Firstly, excretion of the metabolite from Dursban and DDVP were still occurring 36 hours after this single 4½ hour exposure. Secondly, the maximum excretion of DMP was noted 6 to 9 hours after exposure. Thirdly, Max never excreted DEP in contrast to Edward. This was also observed in a sub-

Table 4. CHOLINESTERASE AND URINARY ISOPROPOXYPHENOL LEVELS IN BAYGON (0.5%) IN STRUCTURAL PEST CONTROL OPERATORS AND ASSISTANTS

Identification	Work classification	Cholinesterase pH/hr RBC	Cholinesterase pH/hr Plasma	Iso-propoxyphenol (ppm)
C.L.	Spray operator	0.96	1.04	7.99
M.H.	" "	0.99	1.33	0.98
H.L.	Maintenance	0.90	0.91	0.32
C.J.	"	0.89	0.90	<0.10
R.D.	"	0.72	0.98	0.11

Table 5. TWENTY FOUR HOUR EXCRETION OF ISOPROPOXYPHENOL IN TWO SPRAYMEN OCCUPATIONALLY EXPOSED FOR SIX HOURS TO 0.5% BAYGON SPRAY SOLUTION

Operator	Time of Voiding	IPP (ppm) ug/ml*	IPP ug/hour	Baygon (ug/hour) equivalent
Max P.	8:15 a.m.	N.D.	N.D.	N.D.
	12:30 p.m.**	0.07	1.69	2.33
	4:10 p.m.	0.26	18.38	25.4
	6:05 p.m.	0.63	56.6	78.1
	9:30 p.m.	2.3	249.1	343.8
	11:30 p.m.	2.5	149.4	206.2
	8:00 a.m.	0.56	22.8	31.5
	2:30 p.m.	0.12	3.5	4.8
Edward S.	8:20 a.m.	N.D.	N.D.	N.D.
	3:00 p.m.	0.10	4.8	6.6
	9:45 p.m.	2.4	70.8	97.7
	3:28 a.m.	0.54	5.9	8.1
	1:00 p.m.	0.56	16.6	23.0

*corrected for 800 mOsm/l
**exposure period

Figure 5 - Urinary excretion of Iso-propoxyphenol (Baygon 0.5%) in a structural pest control operator (Max P.) following six hours of exposure.

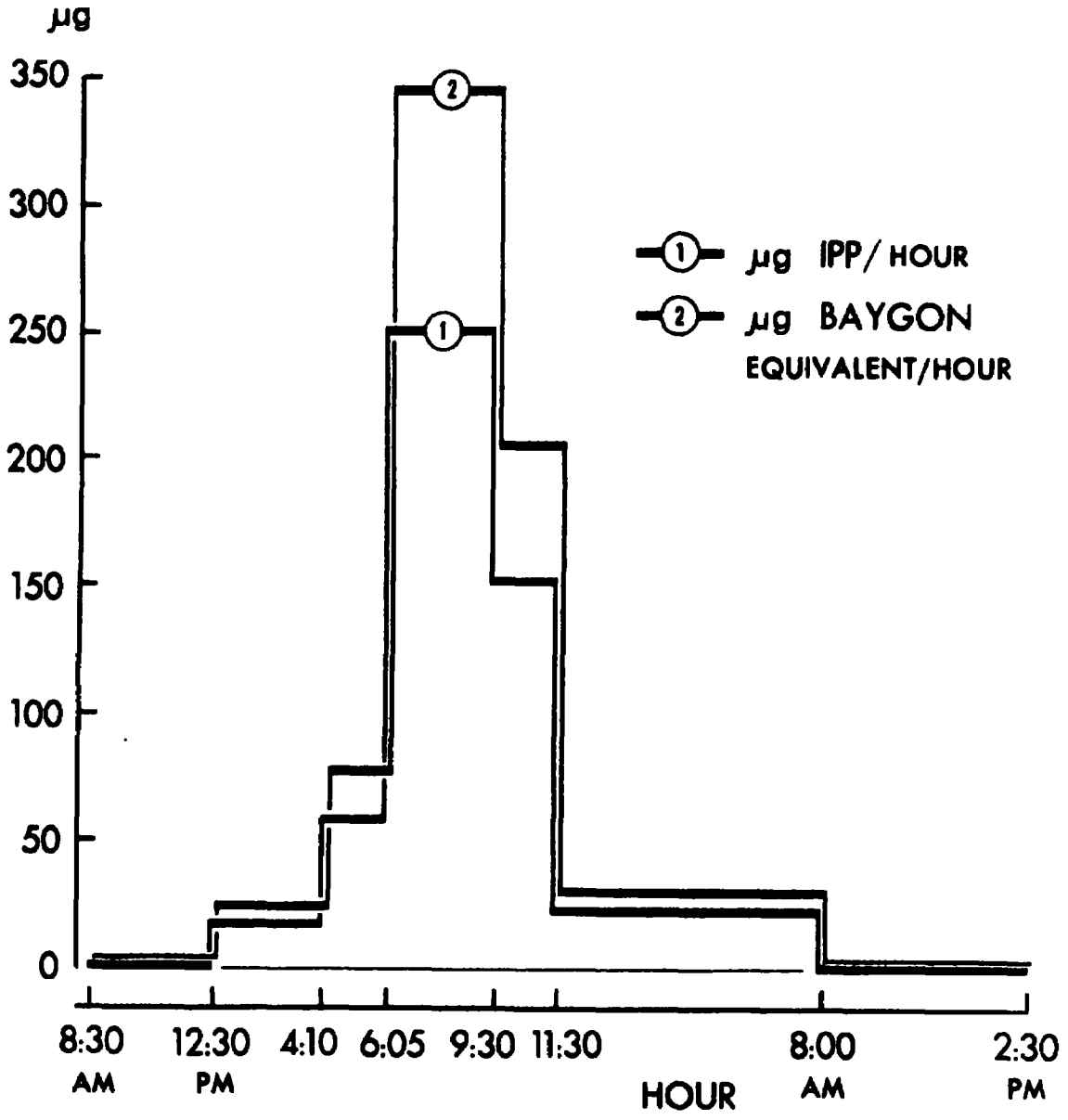


Figure 6 - Urinary excretion of Iso-propoxyphenol (Baygon 0.5%) in a structural pest control operator (Edward S.) following six hours of exposure.

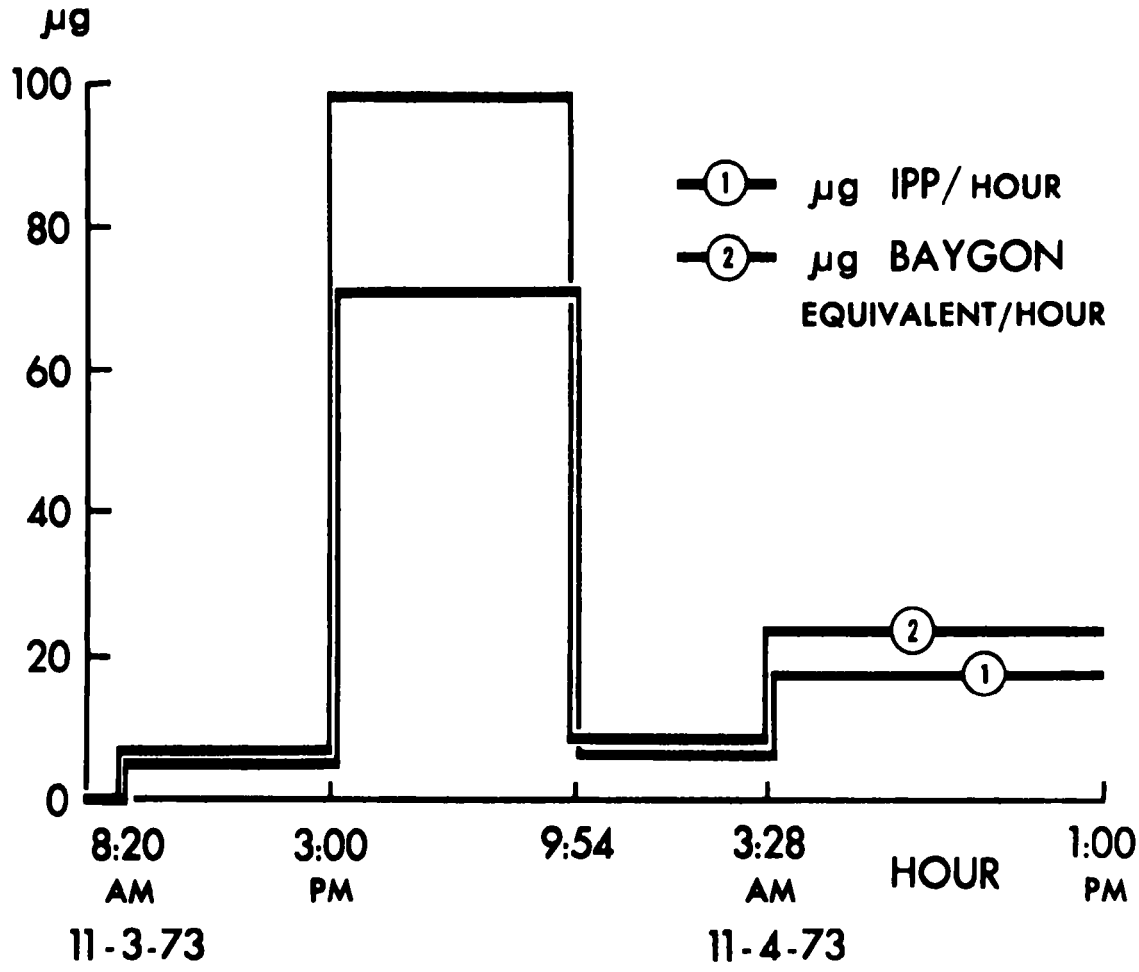


Table 6. THIRTY-TWO HOUR URINARY EXCRETIONS OF 3,5,6-TC PYRIDINOL AND DMP, DEP, DETP IN TWO STRUCTURAL PEST CONTROL OPERATORS

Operator	Time of Voiding	3,5,6-TC Pyridinol ug/hour	Dursban equivalent	DMP ug/hour	DEP ug/hour	DETP ug/hour
Max P.	8:15 a.m.	N.D.	N.D.	N.D.		N.D.
	2:45 p.m.	1.1	1.95	5.6		8.5
	6:20 p.m.	2.1	3.72	19.9		9.6
	8:00 p.m.	3.4	6.02	20.7		16.9
	9:45 p.m.	2.4	4.25	20.1		16
	11:45 p.m.	3.0	5.31	16.1		11.0
	12:30 a.m.	3.3	5.84	11.1		13.8
	8:42 a.m.	2.7	4.78	5.1		9.6
	10:50 a.m.	3.2	5.66	Tr.		12.4
	1:30 p.m.	2.2	3.89	N.D.		3.8
Edward S.	8:27 a.m.	N.D.	N.D.	N.D.	N.D.	N.D.
	2:49 p.m.	2.4	4.25	9.5	N.D.	1.4
	6:35 p.m.	3.5	6.20	33.9	N.D.	9.1
	11:00 p.m.	4.1	7.26	29.0	2.1	11.0
	5:00 a.m.	3.7	6.55	14.7	1.2	8.6
	8:20 a.m.	4.3	7.61	6.2	1.7	4.8
	3:45 p.m.	4.1	7.26	5.8	2.0	1.3
	6:25 p.m.	7.1	12.6	6.8	3.3	9.3
	8:00 p.m.	7.5	13.3	7.1	2.8	11.2

Figure 7. Comparisons of urinary 3,5,6-TC pyridinol metabolite and alkyl-phosphate metabolites observed in a structural pest control operator (M.P.), Miami, Florida 1973

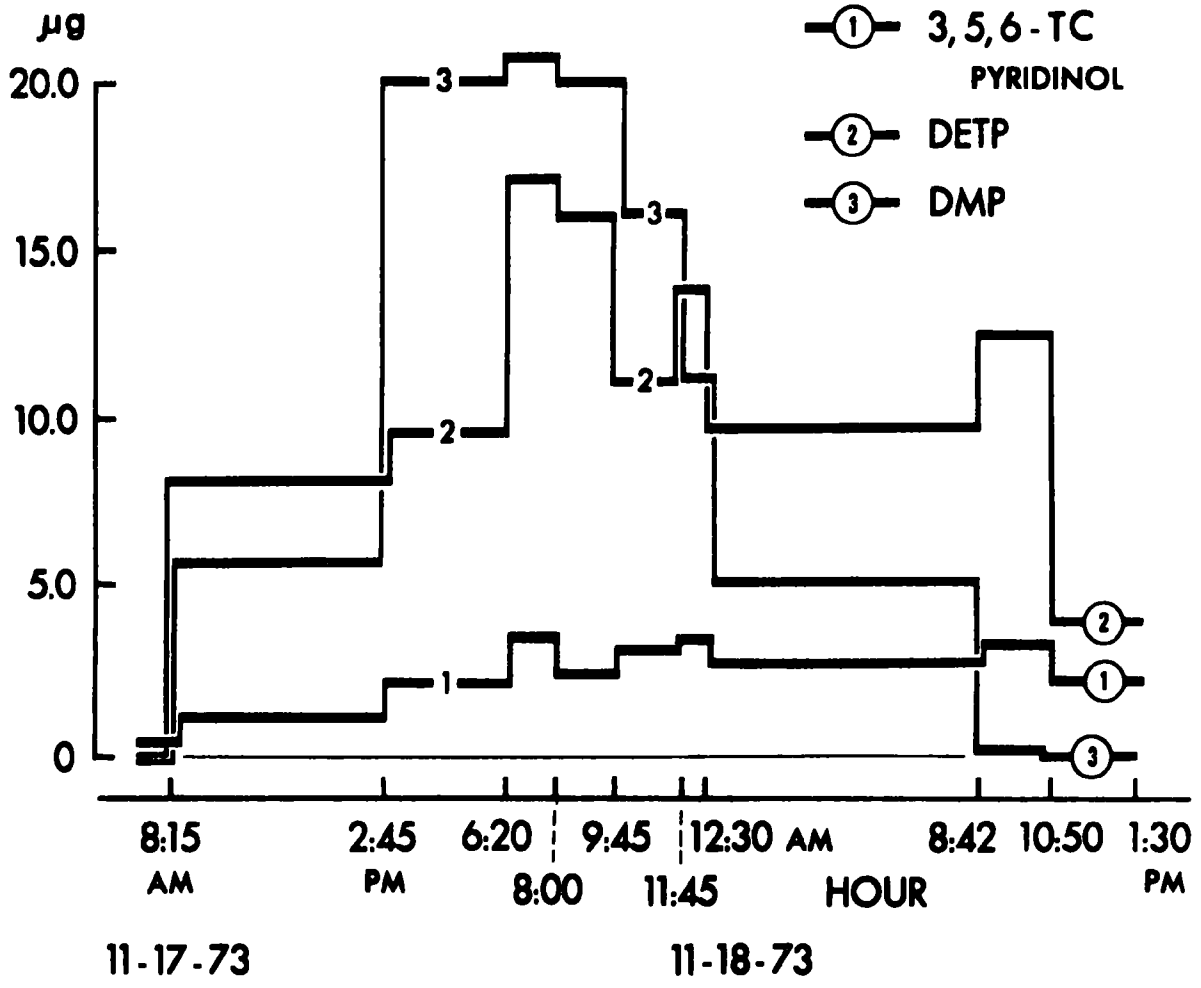
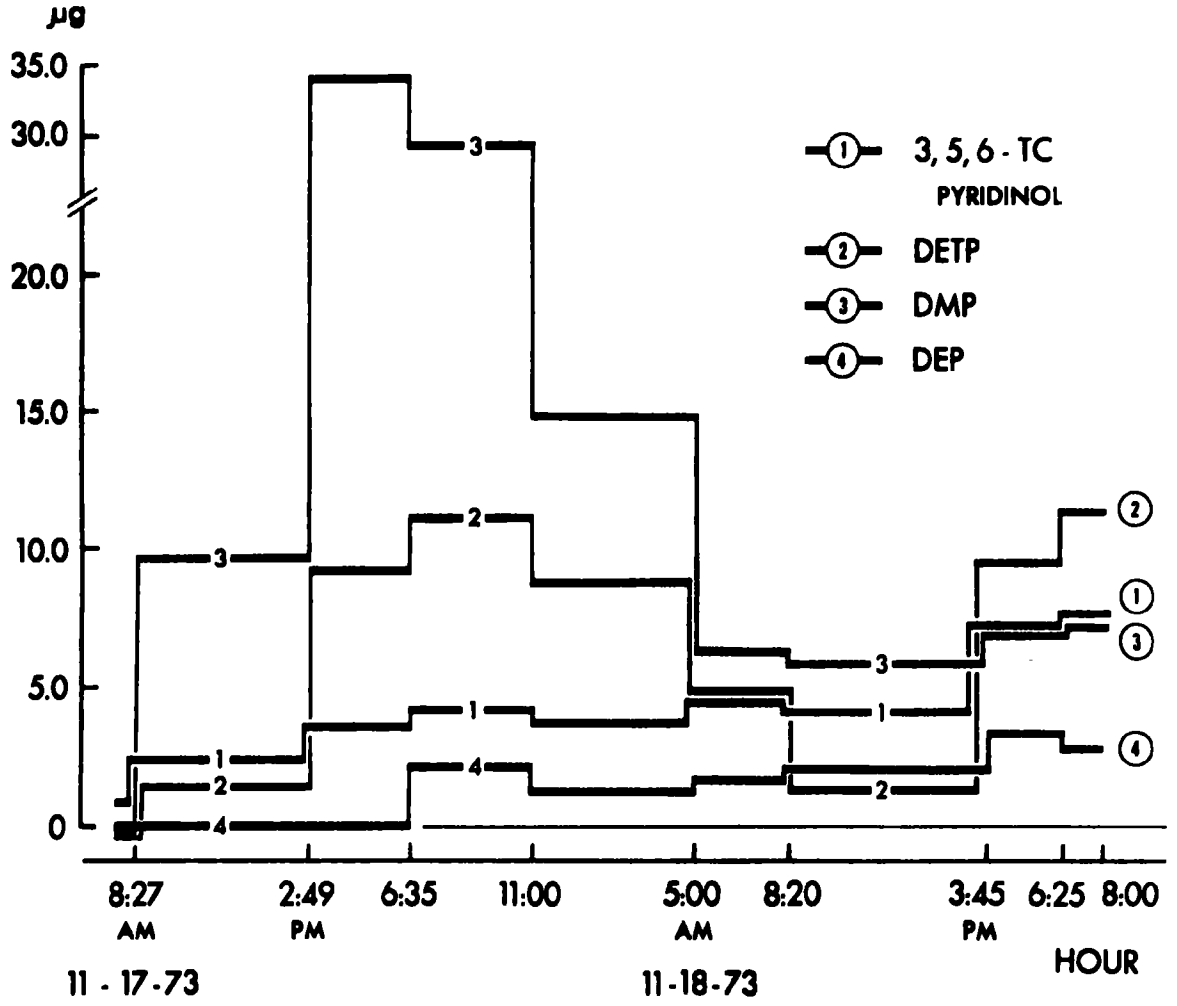


Figure 8. Comparisons of urinary 3,5,6-TC pyridinol metabolite and alkyl-phosphate metabolites observed in a structural pest control operator (E.S.), Miami, Florida 1973



sequent experiment not herein reported; it was found that once again the same sprayman (Max) had DETP but no DEP. Whether this reflects an inherent enzyme defect is conjectural and is under further study. Fourthly, maximum excretion of DETP was 6 to 9 hours after exposure but 3,5,6-TC pyridinol reaches a peak much later. Fifthly, both men had detectable amounts of these urinary metabolites even after 36 hours of study.

The Dursban and DDVP equivalent of the total exposure of the total exposure of each sprayman was calculated on the basis of the di-alkyl phosphate metabolites. In Edward S., 211 ug of DETP was excreted in 36 hours which is equivalent to 357 ug of Dursban. In addition, 50 ug of DEP was excreted in 36 hours which is equivalent to 91 ug of Dursban. Together, therefore, this alkyl phosphate metabolites reflect a total exposure of 448 ug of Dursban. In Max P., 293 ug of DETP only were excreted which is equivalent to 496 ug of Dursban. Thus, on the basis of the di alkyl phosphate the mono alkyl phosphate cannot be measured as such, therefore the calculated intact pesticide is perforce of an accured expression of the total exposure of these workers to the pertinent pesticide.

SECTION V

AIR SAMPLING STUDIES

B. Air Sampling - Background Information

While the routine monitoring of air for pesticides and other environmental pollutants has assumed increased importance, available information on the qualitative and quantitative profiel of air pollution has suffered from inadequacies in the air samplers being used, earlier analytical procedures and the number and location of existing sample sites. In-house research in the Environmental Protection Agency had addressed themselves to these problems on chemical analyses and improvement in air sampling techniques and have made significant progress in both areas. A new analytical method needed to be evaluated and the introduction of a double impinger system into the air sampler needed to be tested. The objectives of this project sought to evaluate these new approaches. First study were conducted in the laboratory to check the recovery of 25 pesticides, and secondly in the field-to evaluate and test the double impinger sampler system in different locations in South Florida.

Description of the various phases -

Pursuant to the objectives, the following phases were planned:

Phase I - In-house training of the chemists in new methodologies.

Phase II - To measure the percent recovery of 25 pesticides in a double impinger system.

Phase III - To measure trapping efficiency and percent recovery of 25 pesticides in ethylene glycol.

Phase IV - To field test the equipment in three different locations of South Florida.

Materials and Methods

In order to evaluate a new analytical method an investigation of several substrates (cottonseed oil, etc) were first analyzed. Cottonseed oil, silica gel and ethylene glycol were the materials selected to assess the trapping potential of each. Duplicate experiments were also conducted with ethylene glycol. 50 ml. of ethylene glycol was placed in each impinger, the first impinger was spiked with 25 pesticides and the percentage recovery of each pesticide in each impinger measured. The second experiment tested the trapping potential of ethylene glycol by drawing ambient air through a U shaped tube in an oil bath spiked with the same 25 pesticides. Air was drawn in at 0.82 cubic feet per minute for a 12 hour period giving a total volume of 16.4 m^3 of air. After 12 hours the pesticide concentrations in each impinger were analyzed. Midwest Research Institute air samplers were placed in a Miami suburb, at the Miami International Airport and at a remote site in the Everglades, 40 miles west of Miami. A double impinger system was used in each sampler and 100 ml. of ethylene glycol placed in each impinger. During the first 12 hour period, air was drawn through the first double impinger system and at the completion of this 12 hour period, a second sample of air was drawn through the second double impinger system. The vacuum pump was run at full capacity during the 24 hour period. The 24 hour volume of air was recorded as was the wind direction and wind velocity.

This 24 hour sample was transferred to the University of Miami laboratory and analyzed without delay. The analytical method used to measure these air samples was by the Sherma and Shafik method.[6]

Results

Ethylene glycol was found to be the most suitable trapping material. The results of retention of 25 pesticides using ethylene glycol following a 12 hour run are shown in Table 7. Table 8 describes the trapping efficiency of ethylene glycol for the 25 pesticides. Table 9 presents the air concentrations of pesticides identified in the three sampling sites. Simultaneous samplings were done at the Everglades and suburban sites.

Discussion

The reproducibility of pesticide recovery in both spiked samples is testimony of the reproducibility of the analytical method. Similarly, maximum entrapment occurred in the first impinger and in the second impinger between 12 - 22% of the various pesticides trapped. The types of the several pesticides identified in the various ampling sites emphasized the changing profile of ambient pesticides in these areas. It will be observed that VC-13 was identified on four occasions in the Miami suburbs, as also was Dursban. These pesticides are currently being extensively used in homes and gardens and their presence is illustrative of predominant organophosphate profile of the Miami area.

The qualitative profile of the pesticides identified is strikingly different to air sampling studies conducted in previous years by virtue of the predominance of minimal traces of these new type of organophosphates in contrast to the usual presence of the organochlorine pesticides. The following pesticides were not detected in the seven samples sites: p,p'-DDT, o,p'-DDE, B-BHC, HCB, Endrin, Trithion, Ethion, Ronnel, Parathion, and methyl parathion. DDT was not found in any sample though p,p'-DDE was still present in trace amounts. The traces of malathion are almost certainly reflective of mosquito control practices. The Everglades sampling site which is 40 miles away from the two other sites in the downtown Miami area and the nearest agricultural area is more than 20 miles away, the preponderance of αBHC related to γ BHC in this site is difficult to explain. The identification of Dursban in the Everglades is most surely reflective of aerial transport from the urban coastline areas since Dursban is not agriculturally applied.

In addition to the 20 pesticides listed in Table 9, VC-13 and Dursban were identified in the second fraction; the percentage recovery through silica gel of these two pesticides were 99 and 100% respectively.

Table 7. RETENTION OF PESTICIDES IN ETHYLENE GLYCOL
(12 hr. run-Flow .82 CFM (16.4 m^3)

Concentrations in nanograms	Pesticide	Percent recovery S Impinger 1	S Impinger 2	S Total	Percent recovery S2 Impinger 1	S2 Impinger 2	S2 Total
840	Ronnel	73	11	84	76	10	86
1620	Methyl Parathion	85	Tr	85+	93	Tr	93+
2070	Ethyl Parathion	91	Tr	91+	96	Tr	96+
3765	Trithion	93	Tr	93+	97	Tr	97+
2400	Ethion	96	Tr	96+	102	Tr	102+
545	Diazinon	88	7	95+	87	8	95
2300	Malathion	97	Tr	97+	94	Tr	94+
80	BHC	62	21	83	70	22	92
100	Aldrin	23	39	62	17	38	55
200	pp' DDE	94	10	104	81	11	92
600	op DDT	84	Tr	84+	81	Tr	81+
800	pp' DDD	80	--	80	82	--	82
800	pp' DDT	90	--	90	89	--	89
80	Lindane	91	11	102	97	7	104
801	B BHC	79	--	79	81	--	81
180	Heptachlor Epoxide	81	10	91	82	7	89
400	Dieldrin	96	Tr.	96+	98	Tr	98+
800	Endrin	93	Tr.	93+	93	Tr	93+
200	Baygon	70	Tr.	70+	88	Tr	88+
200	2,3,5-Landrin	74	16	90	83	10	93
400	Carbofuran	73	--	73	83	--	83
800	Matacil	69	10	79	77	10	87
800	Zectran	65	--	65	70	--	70
400	Carbaryl	99	--	99	98	--	98
400	Mesurol	85	--	85	98	--	98

Table 8. ETHYLENE GLYCOL TRAPPING EFFICIENCY
(12 hr. run=Flow .82 CFM (16.4 m^3 of air)

Concentrations in U-tube (ng)	Pesticide	Percent Recovery AR Impinger 1*	AR Impinger 2*	Total*	U-Tube	Percent Recovery AR2 Impinger 1	AR2 Impinger 2	AR2 Total
840	Ronnel	52	19	71		62	23	85
1620	Methyl Parathion	53	15	68		76	16	92
2070	Ethyl Parathion	63	17	80		78	18	96
3765	Trithion	53	13	68	7%	74	16	90
2400	Ethion	58	14	72	12%	77	17	94
545	Diazinon	46	16	62		68	18	86
2300	Malathion	56	16	72		73	14	87
80	α BHC	43	22	65		64	28	92
100	Aldrin	23	22	45		17	30	47
200	p,p'-DDE	74	20	94		78	25	103
600	o,p'-DDT	71	17	88		75	19	94
800	p,p'-DDD	73	16	89		78	19	97
800	p,p'-DDT	73	16	89		81	19	100
80	Lindane	59	20	79		96	25	111
801	β-BHC	53	15	68		64	11	75
180	Heptachlor Epox.	64	21	85		67	20	87
400	Dieldrin	73	17	90		77	16	93
800	Endrin	78	18	96		78	18	96
200	Baygon	38	18	56		65	20	85
200	2,3,5-Landrin	40	21	61		65	24	89
400	Carbofuran	40	16	56		65	16	81
800	Metacil	26	12	38		44	17	61
800	Zectran	18	Tr	18+		28	TR	28+
400	Carbaryl	60	13	73		91	19	110
400	Mesurol	49	12	61		73	15	88

*(4 hour power failure)

Table 9. AIR CONCENTRATIONS OF PESTICIDES (ng/m^3) FROM DIFFERENT SAMPLING SITES IN SOUTH FLORIDA 1973

Pesticide	Suburban				Everglades		Miami Airport
	Sample #1 ng/m^3	Sample #2 ng/m^3	Sample #3* ng/m^3	Sample #4* ng/m^3	Sample #5* ng/m^3	Sample #6* ng/m^3	Sample #7 ng/m^3
VC-13	1.09	1.21	0.76	0.63	----	----	2.95
Dursban	1.68	1.82	2.04	1.47	0.87	0.77	6.10
Diazinon	1.02	0.78	2.27	0.30	----	0.30	1.00
Malathion	----	----	7.24	----	----	----	1.68
α BHC	0.51	0.65	0.95	1.37	0.85	1.32	0.97
Heptachlor	1.00	0.52	0.51	0.58	----	----	2.65
Aldrin	0.25	0.13	0.18	0.10	----	----	0.89
Lindane	0.68	0.41	0.93	0.62	0.18	0.19	0.42
Dieldrin	0.40	0.35	0.42	0.46	0.18	0.20	0.67
p,p'-DDE	0.18	----	----	0.19	----	----	0.62
M^3 Collected	43.15	48.92	45.87	53.71	45.81	47.74	49.94
Wind Direction:							
Start	N-NE	SW	NE	ESE	NE	E-NE	SE
Finish	S-SE	NW	N-NE	SE	NW	E-SE	NW

*simultaneous samples (#3 and 5, #4 and 6)

SECTION VI

EEG STUDIES

C. EEG Studies - Scope, purpose and background information

Changes in the electroencephalograph following human pesticide exposure are dependent upon the type of pesticides involved and the nature of the exposure. In acute poisoning, the duration of cerebral anoxia has been shown to produce EEG changes. Hypothalamic spikes were noted by Holmes which persisted three years after a parathion exposure. Brown reported persistent EEG changes similar to those seen in temporal lobe epilepsy following mild acute organophosphate exposure. Metcalf and Holmes reported unusual EEG changes in organophosphate exposures. Hunter and Robinson conducted EEG studies in three groups of volunteers fed 0.01, 0.05 and 0.211 mg of dieldrin per man per day, no abnormal EEG changes were noted with this amount of dieldrin intake. EEG changes were prognostic of incipient convulsions due to over exposure to the diene group of insecticides. The occurrence of this degree of incipient toxicity is nowadays avoided by relying entirely on threshold blood levels of dieldrin.

Animal studies recently conducted in the U.S. Environmental Protection Agency Perrine Primate laboratory by Dr. John Santolucito and others in the Pharmacological Branch have compared chronic and low level exposure effects of Carbaryl on the EEG of monkeys. Similar abnormalities have been noted following parathion, dieldrin and DDT exposure. Using a portable EEG machine suitable for battery operation in the field it was planned to conduct EEG studies in human volunteers who were occupationally exposed to pesticides.

Description of the various phases

The first phase was the completion and in-house evaluation of the portable EEG apparatus. The second phase was the training of project personnel in EEG techniques and a medical review of the equipment and its application by the Division of Electroencephalography, University of Miami School of Medicine. The third phase was the implementation of any necessary modifications suggested by this Division and pre-testing on non-exposed volunteers, and the fourth and final phase, field application of the machine.

Materials and methods

The EEG machine to be used is a portable battery operated apparatus with facilities for recording tracings on a tape unit. There will be digital conversion of electronic recordings which are transcribed from electro-magnetic tapes. These will be submitted to Dr. Santolucito in Research Triangle Park for interpretation. It was planned to use intracuticular scalp recordings from needle electrodes rather than surface electrodes. These should dimish the background interference. After obtaining signed consent forms, the technique to be used in testing was as follows: Three sterile disposable butterfly infusion needles would be inserted at three sites into the following areas of the scalp after cleaning this area with acetone. One anterior electrode placed 1½" posterior to the nasion, a second left lateral electrode 1½" to the left of the occipital, and third, a right lateral electrode place 1½" to the right of the occipital point. The subject would be examined in a recumbent position with the eyes closed and tracing would be run for a fifteen minute period.

Interim Report

Prior to the receipt of the portable unit in the second half of the project year, the field investigator received instructions on electroencephalographic techniques from the Division of Electroencephalography in the Department of Neurology of the University of Miami School of Medicine. The director of this Unit, Dr. E.T. Richey and his Biomedical Engineering representative requested a review of the specifications of the apparatus and agreed to review the procedure. Following a preliminary run on a non-exposed human volunteer, Dr. Richey expressed concern with regard to low input impedence and questioned whether the electrical tracing from the three scalp sites could be interpreted with ease. It was not understood at this time that the frequency rather than the actual wave patterns were being measured. Questions were also raised as to whether there was any hope of altering the technique to obviate the necessity of intracuticular electrodes in favor of using the conventional and acceptable surface electrodes. It was agreed to provide the EEG Division with the specifications of the apparatus from Biomedical Engineering, and to arrange a conference with Dr. Santolucito prior to moving to phase 4 - the field application. Additional safety modifications suggested by the Director of the Division of EEG have been implemented and intracuticular electrode will still be used. The machine is now ready for use in the field.

SECTION VII

ACUTE PESTICIDE POISONING INVESTIGATIONS

D. Acute Pesticide Poisoning Investigations - Scope, purpose and background information - The two objectives of this work unit were:

1. To investigate and confirm suspect pesticide poisoning cases occurring in the Dade County area, and to document the clinical and therapeutic responses observed at the bedside. These findings, with medical confirmation of suspect cases, would be made available to the Dade County Community Studies Pesticide Project.

2. To study pesticide metabolite dynamics in blood, urine and fat in acute poisoning cases hospitalized in the Dade County area and to determine the informative potential of these in the clinical management of the case.

Although pesticide poisoning has not been a reportable disease in Florida, the resources and experience of the Miami Pesticides Laboratory have become known to most hospital Emergency Rooms and pathologists in the South Florida area and so has been widely used. In the past, epdiemiologic and laboratory confirmation has been offered through the Community Studies Program and it was planned in this project to continue this function in the University and to facilitate acquisition of pesticide poisoning incidence data in the area.

Results

Table 10 lists the confirmed fatal pesticide poisoning cases investigated by this project and Table 11 lists the number of confirmed non-fatal cases. Table 12 presents the clinical and analytical features of an additional 26 investigated cases of suspect pesticide illnesses which the project investigated during this year.

In February, 1973, no further support was received at the Florida Community Pesticide Studies terminated, therefore, the data has been made available to the Pesticides Operations Office in Washington, D.C. and in the Southwest Region IV (Drs. Evrard and Kessler) so that the material could be utilized by the appropriate Federal authorities. Administratively, therefore, reports were transferred from Jacksonville, Florida to Atlanta, Georgia.

Discussion

1 - Confirmed fatal cases - All three fatalities were due to organophosphate exposure; these pesticides were identified in the various specimens and are shown in the table and all were confirmed by gas liquid chromatography in the flame photometric and electron capture detectors. One of the deaths resulted from the ingestion of VC-13. This pesticide is becoming an increasingly serious chemical problem in the area and the material is readily available in most garden stores. Illness following ingestion of this material is slow to evolve and the clinical picture is protracted in many instances. In the past, the patient has been hospitalized initially more of a precautionary measure and then what usually happens is that quite suddenly a severe and life threatening cholinergic crisis will develop calling for heroic antidotal and resuscitative management. The danger of this type of intoxication lies in the deceptively mild clinical picture at onset, a feature which might tempt the inexperience to discharge the

Table 10. CONFIRMED FATAL INTOXICATIONS IN SOUTH FLORIDA, DECEMBER 1972-DECEMBER 1973

Name	Race/Sex	Age	Date of incident	Type of Exposure	Material	Confirmed clinical and laboratory tests
1. Juan M.	W/M	20 y/o	12/31/72	Ingestion-Suicide	Parathion	RBC ChE 0.15 Pl. ChE 0.15 Parathion in blood 18.3 ppb.
2. Henry K.	W/M	76 y/o	5/9/73	Ingestion-Suicide	VC-13	Gall Bladder - VC-13 - 37.5 ppm. by F.P.D. Fat from kidney - VC-13 - 174 ppm. by F.P.D. Urine = 2,4-DCP (chloroacetate derivative) 15 Liver - VC-13 - 3.6 ppm. by F.P.D. Gastric content (294 mg. bloody) - 0.34 ppm by F.P.D. Whole blood - VC-13 - 1.0 ppm by F.P.D.
3. Walter M	W/M	51 y/o	9/7/73	Ingestion-Suicide	Diazinon 51%	9/10/73 - RBC ChE 0.20 Pl. ChE 0.07 Pre PAM- Diazinon in blood 34 ppb. by EC & I 9/10/73 - RBC ChE 0.25 Pl. ChE 0.06 Post PAM - Diazinon in blood 17 ppb. by " 9/12/73 - RBC ChE 0.29 Pl. ChE 0.09 Diazinon in blood 9 ppb. by EC & 9/13/73 - RBC ChE 0.30 Pl. ChE 0.09 by " & Diazinon in blood 10 ppb. E.C. 9/18/73 - RBC ChE 0.32 Pl. ChE 0.09 Diazinon not found (FPD & EC) p,p'-DDT <2, p,p'-DDE 7.1 and Dieldrin <1 pp respectively.

the patient home if the probabiluty of a delayed onset of illness is not appreciated. The halogenic portion of this organophosphate pesticide favors deposition in fat, and this is the same reason for the slow evolution of symptoms. The fat solubility potential also calls for the daily monitoring of blood for VC-13 and urine for the phenolic metabolite 2,4-DCP. Atropine therapy must be continued as long as the parent compound or its urinary metabolite can still be identified, and atropinization may have to be continued for several days. Experience gained in this special type of organophosphate exposure (halogenated organophosphate) emphasizes the need for further study of acute and chronic exposure to organophosphate pesticide that have fat soluble characteristics.

2 - Confirmed cases - Incidence Data - Eighteen non-fatal pesticide poisonings were confirmed in 1973. The types of poisonings, materials involved and results of the appropriate analytical diagnostic tests are shown in Table 11. Eighteen cases were due to organophosphate intoxications and ten of these were from occupational exposure. In contrast to residue problems in California, once again in South Florida, our investigations have failed to confirm an episode of "picker poisoning", a topic of current discussion of political importance and exhibiting succinct geographical distribution characteristics. Arsenic and pentachlorophenol were the other agents involved besides the organophosphate insecticides.

The case of arsenic poisoning occurred in a 6 year old white male child who was poisoned by some toys that he picked up in a garbage dump. Weed killer had been discarded into the dump and has spilled over the toys.

The pentachlorophenol intoxication was observed in a carpenter and the diagnosis was made on the basis of exposure, clinical picture and urine results which were obtained eight weeks after his last exposure. The patient was a 60 year old white male carpenter who had had extensive exposure to pentachlorophenol which he used for termite control. Besides complaining of weakness anorexia and loss of weight, the patient had severe dermatitis with fissures of the skin. He also developed an extensive generalized and painless lymphadenopathy which resembled Hodgkins Disease. Lymph node biopsy, however, did not confirm this diagnosis, the section showing only generalized chronic inflamatory changes.

Discussion 3 - Unconfirmed Cases - Twenty six cases of pesticide associated illnesses were investigated, and on the basis of clinical findings, exposure histories, pathological findings and appropriate laboratory analyses were considered not to be due to pesticide exposure. Five of the cases were fatal, and at the time of death, health or agricultural officials suggested suspect pesticide exposure. The definitive diagnosis was not made available to us in all cases but alcoholic seizures, acute asthma with chronic obstructive lung disease, methaqualone overdose and meningitis were the ultimate causes of death in four of the five unconfirmed cases. Of significant medico-legal importance was the occurrence of an acute asthmatic attack in two agricultural inspectors, both had been employed by the Florida State Department of Agriculture for many years and both had a history of chronic bronchitis, emphysema and periodic attacks of asthma. One of the inspectors died after inspecting a celery field; it was first thought that the cause of death was the result of parathion residues on celery or an exposure from the drift from aerial applications in the adjoining fields. Leaf residues were insignificant and no paranitrophenol or paraoxon were found in the liver or kidney by gas chromatography using the electron capture and flame photometric

Table 11. CONFIRMED NON-FATAL INTOXICATIONS IN SOUTH FLORIDA, 1973

Name	Race, Sex	Age (yrs)	Date of incident	Type of Exposure	Material	Confirmed clinical and diagnostic tests
1. Abraham W.	B/M	61 y/o	1/4/73	Dermal-Occupational	Phosdrin	1/4/73 - Pre-PAM - Serum ChE 0.14 Post PAM - RBC Che 0.26 Pl. ChE 0.12 1/5/73 - RBC ChE 0.16 Pl. ChE 0.12 Unable to detect Phosdrin by E.C.
2. Frank R.	B/M	57 y/o	2/6/73	Occupational	Organo-phosphate.	RBC ChE 0.21 Pl. ChE 0.49
3. Sammy G.	B/M	21 y/o	2/6/73	"	"	RBC ChE 0.11 Pl. ChE 0.15 Asymptomatic, taken off work
4. April P.	W/F	19 mos.	3/7/73	Ingestion-Accidental	Dursban and Diazinon	3/8/73 - RBC ChE 0.14 Pl. ChE 0.10 3/7/73 - Gastric contents pH 1.72 Diazinon 45 ppm. and Dursban 213 ppm by F.P.D. 3/8/73 - Serum - Diazinon 0.6 ppm. and Dursban 0.4 ppm. by F.P.D. and E.C.
5. Maynard W.	B/M	27 y/o	3/8/73	Occupational-Dermal	Organo-phosphate	3/7/73 - Post PAM RBC Che 0.09 Pl. 0.13
6. Felix S.	Ind./M	21 y/o	3/9/73	Dermal-Occupational	"	3/8/73 - RBC ChE 0.18 Pl. 0.16 Hospitalized
7. James C. P.	W/M	25 y/o		Ingestion-Suicide	VC-13	90% Inhibition colormetric method Case confirmed by clinical picture
8. Jeral F.*	W/M	18 y/o	3/23/73	Occupational-Accidental	Organo-phosphate	80% Inhibition Fleisher Pope method
9. Fernando B.	W/M	5 y/o	3/5/73	Ingestion-Accidental	Dematon	Case confirmed by clinical picture.
10. Charles P.	B/M	25 y/o	3/11/73	Occupational-Dermal	Parathion	RBC ChE 0.48 Pl. ChE 1.26 (5/16/73) Case confirmed by hospital notes and clinical picture.

*A second incident occurred on May 12th when patient was hospitalized for spilling Phosdrin on skin and inhalation. Case confirmed by clinical picture and atropine response.

Table 11 (continued). CONFIRMED NON-FATAL INTOXICATIONS IN SOUTH FLORIDA, 1973

Name	Race, sex	Age	Date of incident	Type of exposure	Material	Confirmed clinical and diagnostic tests
11. Wesley S.	B/M	30	3/3/73	Occupational Dermal	Organo-phosphate	Case confirmed by clinical picture and atropi response.
12. Johnny L.	B/M	46	1/8/73	Occupational-Inhalation	Parathion	Cholinesterase 13 ChE-tel units (Normal 45-90) Serum ChE 20 (N 45-90) 1/9/73
13. Stephen B.	W/M	18	5/9/73	Occupational-Dermal	Organo-phosphate	RBC ChE 0.35 Pl. ChE 0.32 5/10/73 RBC ChE 0.29 Pl. ChE 0.34
14. Francisco C.	W/M	14	6/20/73	Ingestion-Accidental	Spectricide (Diazinon 25%)	Pre-treatment (Hospital Notes) ChE .45 (Norma 1.90-3.0) Post-treatment Pl ChE 22 units (45-units Normal range) 6/22/73 - RBC ChE 0.70 Pl. ChE 0.08 (Michel Method)
15. William K.	W/M	60	7/2/73	Occupational-Carpenter	Pentachloro-phenol	Serum PCP .513 ppm, Urine 0.3 ppm. (Analyzed 9/21/73 p,p'-DDE 39 ppb. p,p'-DDT 8 ppb. dieldrin 1.5 ppb. 7/2/73 - RBC ChE 0.65 Pl. ChE 0.68
16. Walter B.	B/M	2	9/10/73	Dermal-Accidental	Parathion	Case confirmed by clinical picture and atropi response. 9/15/73 - RBC ChE 0.50 Pl. 0.72 (Analyzed 9/1 collected at 4:17 p.m. 9/15/73 - RBC ChE 0.62 Pl. 0.87 (Analyzed 9/1 collected at 10:16 p.m. p,p'-DDE 2 ppb., no p,p'-DDT, dieldrin 1ppb. No organophosphate found by F.P.D. and EC
17. Donald T.	W/M	6	10/17/73	Ingestion-Accidental	Arsenic	Case confirmed by M.E.'s office Urine 16 mg/ml. Gutzeit method Field investigation made.

detectors. Other mechanisms involved in unconfirmed pesticide poisonings related to 1) the occurrence of dermatitides and 2) to the chance finding of unusually low red blood cell or plasma cholinesterase. An example of the former was the occurrence of erythema multiforme in a 52 year old white male whose home had recently been sprayed with Baygon. Iso-propoxyphenol was not identified in the urine of the other members of the family.

With regard to the latter, low cholinesterase values were identified in a three week old black male infant with pneumonia and possible pesticide exposure. In order to evaluate normal physiological occurrence of low red blood cell and plasma cholinesterase in the newborn, five blood samples were collected and analyzed from healthy premature babies of the same birth weight as the case in suspect. The average red blood cell of these infants were 0.44 ΔpH/hr and for plasma cholinesterase the average level was 0.47 ΔpH/hr respectively.

At the request of Dr. Suggs and with Dr. Durham's permission, pseudocholinesterase studies were conducted in individuals with low plasma cholinesterase as well as their families. These studies were reconfirmed by Dibucaine studies by Dr. Suggs in the Center for Disease Control in Atlanta, Georgia. A ciguatera poisoning was also confirmed.

SECTION VIII

REFERENCES

1. Shafik, T.M., H.C. Sullivan and H. F. Enos. A Multiresidue Procedure for Halo and Nitrophenols-Measurement of Exposure to Biodegradable Pesticides Yielding These Compounds as Metabolites. J. Agr. Fd. Chem. 21:295-298, 1973.

2. Shafik, T.M., D. E. Bradway, H. F. Enos, and A. R. Yobs. Human Exposure to Organophosphorus Pesticides. A Modified Procedure for the Gas Liquid Chromatographic Analysis of Alkyl Phosphate Metabolites in Urine. J. Agr. Fd. Chem. In Press

3. Michel, H.O. An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity. J. Lab. Clin. Med. 34:1564-1568, 1949.

4. Method for the Determination of Metabolite or Hydrolysis Products of Organophosphorus Pesticides in Man. Perrine, U. S. EPA Office of Research and Monitoring Analytical Manual, Sec. 6, 1972.

5. Shafik, T.M., H. C. Sullivan and H. F. Enos. A Method for the Determination of 1-Napthol in Urine. Bull. of Environ. Contam. and Toxicol. 6:34-39, 1971.

6. Sherma, J. and T. M. Shafik. A Multiclass, Multiresidue Analytical Method for Determining Pesticide Residues in Air. Submitted for Publication

7. Holmes, J.H. Clinical Studies of Exposure to the Organophosphorus Pesticides. New York, Academic Press, 1965.

8. Brown, H.W. Electroencephalographic Changes and Disturbance of Brain Function Following Human Organophosphate Exposure. Northwest Med. 70: 845-846, 1971.

9. Metcalf, D.R. and J. H. Holmes. EEG, Psychological, and Neurological Alterations in Humans with Organophosphorus Exposure. Ann. N.Y. Acad. of Sci. 160:357-365, 1969.

10. Hunter, C.G., J. Robinson, and M. Roberts. Pharmacodynamics of Dieldrin (HEOD) Part II: Ingestion by Human Subjects for 18-24 Months and Post-Exposure for 8 months. Arch. Environ. Health 18:12-21, 1969.

Section IX

GLOSSARY

EEG	electroencephalograph
Dasanit (R)	(0,0-diethyl-0-p (methylsulfinyl) phenyl phosphorothioate - fensulfothion
Thimet R	0,0-Diethyl S-(ethylthio)-methyl phosphorodithioate - phorate
Di-Syston R	0,0-Diethyl S-(2-(ethylthio)ethyl) phosphorodithioate - disulfoton
DMP	0,0-Dimethyl phosphate
DEP	0,0-Diethyl phosphate
DETP	0,0-Diethyl phosphorothionate thiophosphoric acid
DMTP	0,0-Dimethyl phosphorothionate thiophosphoric acid
N.D.	Non-detectable
ppm	parts per million
parathion	0,0-Diethyl-0-p-nitrophenyl phosphorothioate
Methyl parathion	0,0-Dimethyl 0-p-nitrophenyl phosphorothioate
parathion 8E	8 pounds Ethyl Parathion in a gallon of liquid
parathion 6-3	Mixture of Ethyl and Methyl parathion
Toxaphene (R)	A mixture of chlorinate camphene compounds of uncertain identity - combined chlorine 67-69%)
Cygon (R)	(0,0-Dimethyl S-(N-methylacetamide)phosphorodithioate - Dimethoate
Aatrex (R)	2, chloro, 4, ethyl 6 iso proylamino-S, triazene
Trithion (R)	(0,0-Diethyl S-(p-chlorophenylthio) methyl phosphorodithioate)
Phosdrin R	mevinphos (0,0-Dimethyl 2 methoxycarbonyl-1-methyl vinyl phosphate)
Dithane R	manganese-Ethylenebisdithiocarbamate - maneb
Lannate R	methomyl, S-Methyl N (methyl-carbamoyl)oxy)thioacetimidate
Dipel R	Bacilluis thuringiensis (crystalline bacterial toxin)
Azodrin R	dimethyl phosphate of 3-hydroxy-N-methyl-ciscrotonamide - monocrotoph
Monitor R	methamidophos O,S-Dimethyl phosphoramidothioate
Dibrom R	(1,2 dibromo-2,2-dichloroethyl dimethyl phosphate) - naled
Guthion R	0,0-Dimethyl s-(4-oxo-1,2,3-benzotriazine-3-(4-H-yl methyl) - azinphos methyl

ml/mOsm/l	milliliter per milliosmols per liter
3,5,6-TC pyridinol	3,5,6-trichloro-2 pyridinol
IPP	2-Iso-propoxyphenol
PNP	paranitrophenol
Baygon(R)	Aprocarb 2-isopropoxyphenly-N-methyl carbamate
Dursban(R)	chlorpyrifos 0,0-Dimethyl O-p-nitrophenyl phosphorothioate
DDVP(R)	dichlorvos 2,2-dichlorvinyl dimethyl phosphate
RBC	red blood cell
Pl.	plasma
ChE	cholinesterase
n	number of persons
HUD	Housing & Urban Development (Dade County)
2,4,5-T	(2,4,5-tricholorophenoxyacetic acid)
2,4,5-TCP	(2,4,5-tricholorphenol)
2,4-DCP	2, 4-dichlorophenol
Ronnel(R)	fenchlorphos 0,0-Dimethyl (2,4,5-tricholorphenol phosphorothioate
tr	trace amounts
VC-13	dichlofenthion (O-(2,4-dichlorophenyl) 0,0-dietyl phosphorothionate)
ug/ml	microgram per milliliter
CFM	cubic feet per minute
α BHC	alpha isomer of 1,2,3,4,5,6-hexacloro-cylcohexane
γ BHC	Lindane gamma isomer of 1,2,3,4,5,6-hexachloro-cyclo-hexane of 99+% purity
m^3	cubic meter

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15. Supplementary Notes

16. Abstracts: The use of the multi-residue techniques for urinary metabolites appeared to be provide the key to worker exposures from mixed organophosphate and carbamate exposures. The findings of Shafik et al., were corroborated, and highly significant inverse correlations of cholinesterase levels with the dialkyl phosphate urinary metabolites were demonstrated under conditions of work exposure to the more toxic organophosphates such as ethyl-methyl parathion and Phosdrin. DEP, DETP, DMP and DMTP were the only alkyl phosphates regularly found in these occupational exposures. Following 8 hours of a work exposure to Dursban the appropriate dialkyl phosphate and phenolic derivatives persisted in the urine for 36 hours and longer. These two groups of metabolites were exceedingly informative in acute pesticide poisong and provided etiologic specificity and information for the continued need of atropine therapy. Severe and protracted illness was observed following intoxication by more polar and fat soluble organophosphates especially di-ethyl dichlorophenol phosphorothioate (VC-13). This pesticide and its metabolites were detected in serum and in urine 48 days after exposure. Additionally air sampling of the South Florida environment confirmed the disappearance of DDT and its metabolites.

17. Key Words and Document Analysis. 17a. Descriptors

Urinary metabolite surveillance of the occupational pesticide worker
Urinary metabolites, di-alkyl phosphate and phenolic metabolites in multi-residue techniques.
Fat soluble organophsophate intoxication - diagnosis and surveillance therapy.
The changing profile of pesticide environmental air sampling.

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group

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