P,p′-DDT and p,p′-DDE in Blood Samples of Occupationally Exposed Workers

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THE IDENTIFICATION and quantitation of certain chlorinated hydrocarbon pesticides and their metabolites have drawn increasing attention recently (*1–3* and unpublished paper by J. E. Davies and co-workers, "An Epidemiologic Application of the Study of DDE Levels in Whole Blood"). Rapid development of methods for measuring these chemicals in the parts per billion (ppb) range and the availability of gas chromatographic equipment have led to a proliferation of data.

In this paper, we have summarized the whole blood level findings of p,p′-DDT and one of its metabolites p,p′-DDE found in groups of persons in south Florida who are occupationally exposed to pesticides. (All references in this paper to DDT and DDE are to the p,p′-isomers.) These findings are compared with DDT and DDE values of persons nonoccupationally exposed to these chemicals (unpublished paper by J. E. Davies and co-workers). Although other pesticide chemicals and their metabolites, in addition to DDT and DDE, were discovered in these blood samples, these data will not be presented in this paper.

Dr. Edmundson is medical director, and Dr. Davies is principal investigator, Community Studies on Pesticides, Public Health Service. Mrs. Nachman is a chemist, Miami Pesticides Laboratory, and Mr. Roeth is senior sanitarian, Florida State Board of Health, Miami. *This research was supported by the Pesticides Program, Food and Drug Administration, Consumer Protection and Environmental Health Services, Public Health Service, through the Florida State Board of Health under contract No. PH 86–65–26.*

Joseph Freal and Dr. Ana Barquet, chemists, Miami Pesticides Laboratory, Florida State Board of Health, and Miss Iris Keim and Mrs. Janet Cassady, biostatisticians, University of Miami, assisted in the chemical analyses and preparation of these data.

Methods and Materials

A total of 350 observations were made of 154 men, an average of 2.3 blood samples per person. The study population was divided into groups by occupation and was compared with a group of 60 men who said that they had no present or past occupational exposure to pesticides. Twenty were employed by the local health department, and 40 were selected from a group of prisoners and a group of food handlers. The persons nonoccupationally exposed were designated as controls. Occupational groups included 26 floral sprayers, 16 pesticide formulators, 16 agricultural sprayers and mixers, 16 lawnsprayers, 13 structural pest control operators, and seven insect control sprayers of interiors of aircraft.

Venous blood was collected in Becton-Dickinson vacutainers containing dry heparin at the various worksites of the occupationally exposed persons and the controls. These whole blood

samples were analyzed at the Miami Pesticide Laboratory, Florida State Board of Health, by a modified Dale, Curley, and Cueto method (*4* and unpublished paper by G. A. Nachman, J. J. Freal, A. Barquet, and C. Morgade, "A Simplified Method for the Analysis of DDT and DDE in Blood for Epidemiologic Purposes"). In comparing results derived from serum and plasma with those from whole blood, one should remember that in plasma or serum these chemicals are in approximately twice the concentration contained in whole blood (*4*). In our laboratory, we consider the sensitivity of this method to be reliable with the amounts of DDT detectable in levels of 7 ppb or more and 1 ppb of DDE.

The sensitivity of electron capture detectors varies from day to day; these detectors are less sensitive to DDT than to DDE. The sensitivity varies from just measurable amounts of 2 to 7 ppb of DDT and from 0.5 to 1 ppb DDE in the method described. For statistical use, the sensitivity to DDT for accurate quantitation is taken as 7 ppb or more. The amounts recorded are as observed, not extrapolated, and are expressed to the nearest part per billion.

Results

The means and ranges of the results of DDT and DDE observations in the whole blood samples were compiled (table 1). The data are given by occupational group and race, including data on the nonoccupationally exposed controls. The results are listed separately by race, since there were obvious and significant differences in the findings associated with race. Significant differences in these observations have been reported in groups of nonoccupationally exposed men (unpublished paper by J. E. Davies and co-workers). The means of DDE results derived from this table were used for figure 1, which shows graphically the differences in the various groups and races.

Figure 1. Mean p,p′-DDE levels in blood samples of white and nonwhite men in seven exposure groups

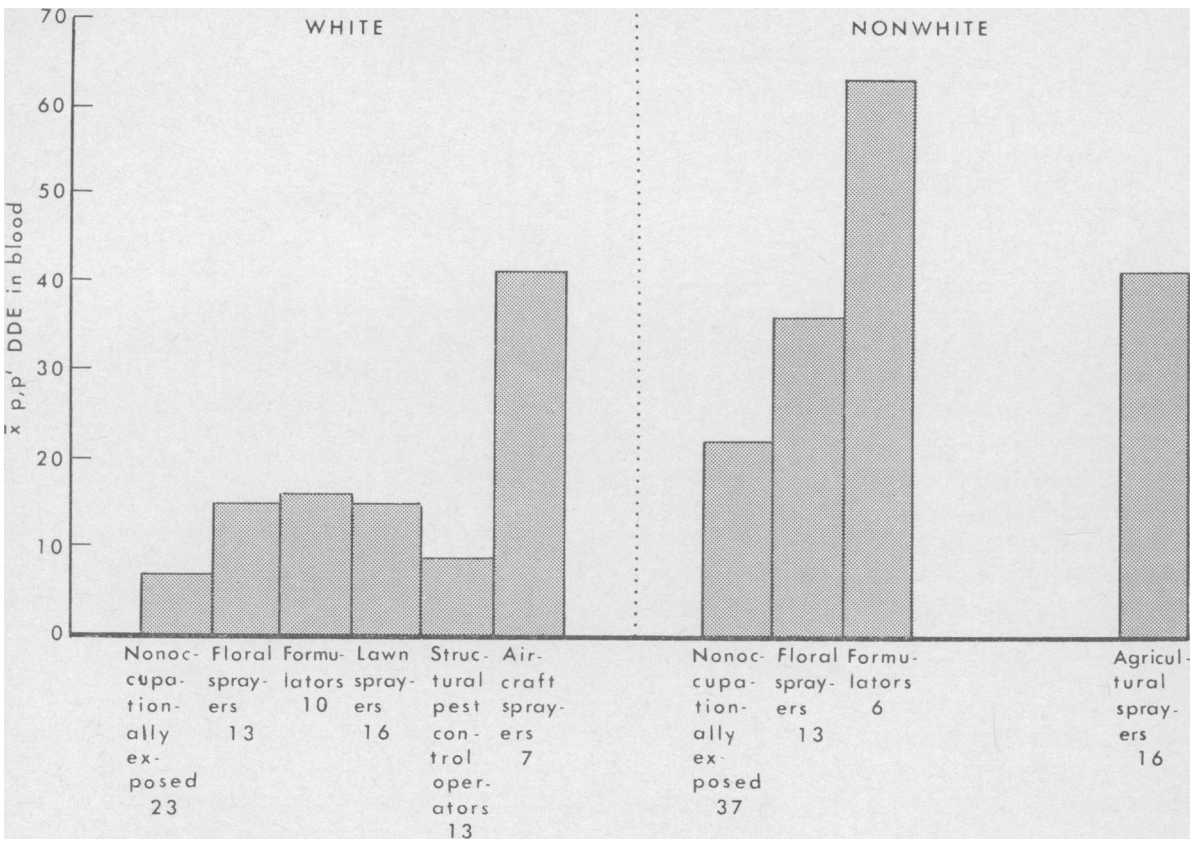

Figure 2. Relation of sequential observations of DDE levels in blood of 78 occupationally exposed workers taken at an interval of 1 to 2 months, south Florida, 1967

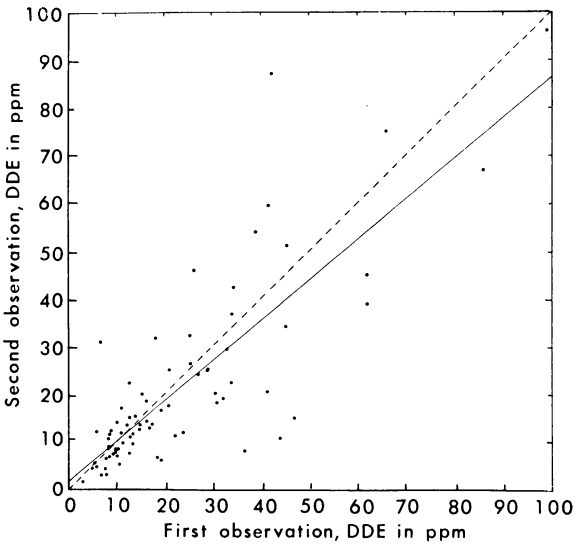

Table 2 shows the relation of time to DDT and DDE values in the whole blood of five formulators, 3 and 29 days after formulation of DDT dust. Figure 2 is a comparison and correlation of DDE values obtained from analyses of blood from 78 persons in the occupational groups as a whole from whom samples had been taken within 30 to 60 days of one another.

Comments

From the data in table 1 it became apparent, when we noted the means and ranges of DDT and DDE, that these values in the occupational groups differed from those of the controls when the results were compared within racial groups. The occupational groups then made up a universe separate from the control group consisting of nonoccupationally exposed men.

The degree of difference of each occupational group from the control group, as measured by the mean heights of DDE levels for each group, can be related to the extent and frequency of the exposure to DDT of members of each group. For example, the structural pest control workers and the lawnsprayers rarely use DDT, the floral sprayers occasionally use DDT for special horticultural problems, the agricultural workers use DDT on corn and certain other crops, the formulators work with DDT at irregular intervals depending on the (decreasing) demand, and the aircraft sprayers use 3 percent DDT aerosol exclusively.

The frequency of usage, in addition to the years occupationally exposed, was reflected in the mean DDE values obtained (table 1). Also, it should be noted that the nonwhite controls and the nonwhite occupationally exposed had higher mean values and greater ranges of these blood values than the white persons in either group. These elevated values would seem to correlate with three main factors of varying weights: (*a*) the nonwhite group would be expected to have higher initial blood levels when entering the occupation since nonwhite controls also had higher DDE blood levels, (*b*) the nonwhite group tends to have more laborers than supervisors and, therefore, they are exposed to DDT for longer periods of time, so that exposure to DDT would be expected to be greater, and (*c*) the mean length of time was greater for the nonwhite workers in occupations, especially those involving spraying and mixing of pesticides.

The factor of length of time in the occupation relative to blood values appears to be most important in the person's first few years in the trade; Laws and co-workers (*5*) have shown this factor to be of no importance in men working more than 11 years in the production of DDT. This observation is interesting when it is compared with the length of time (approximately 10 years) that children require to reach adult levels of DDE in their adipose tissue (*6*).

DDT levels. According to J. E. Davies and coworkers (unpublished paper), the DDT levels in the control group were, in more than 80 percent of the persons, below consistently measureable levels, which was considered to be 7 ppb. In the persons occupationally exposed, the mean DDT levels of the white workers were also less than 7 ppb with the exception of the white formulators (21 ppb) and the aircraft sprayers (25 ppb). DDT mean values of the only groups of nonwhite workers were 10 ppb in the floral sprayers, 40 ppb in the formulators, and 32 ppb in the agricultural sprayers.

DDT in blood is transient, as shown by the data in table 2. The blood levels of DDT and

DDE of five formulators were measured at 3 days and at 29 days after formulation of DDT dust. The DDT values 29 days after formulation were below measurable limits, but 3 days after formulation the men showed relatively high levels.

In these studies of the occupationally exposed, elevated DDT values are considered to reflect recent exposure to the chemical. The finding of DDT depends on the proximity of random blood sampling to exposure because greater frequency of exposures and the extent of absorption increase the probabilities of discovering quantities of DDT. Sampling after a period of time, during which metabolism of DDT and its storage or excretion could take place, would reduce the probabilities of detecting DDT in measurable quantities in a blood sample.

DDE levels. DDE has been shown to be a stable value in the studies of the nonoccupationally exposed. DDE levels were also constant in the blood sampling of members of these occupational groups when repeated sampling was carried out at 30- to 60-day intervals on the same persons. Figure 2 demonstrates the correlation of sequential DDE values in the blood of 78 persons in the occupational groups taken 1 to 2 months apart; the coefficient of correlation of these sequential values was 0.81, $P < 0.001$. This degree of correlation was almost identical with that of similar sequential values in the nonoccupationally exposed, which was 0.83, $P < 0.001$. The scatter of values in the occupational group was understandably greater, though the correlation was generally the same as the control group. This high correlation would not be expected if blood were regularly drawn within a few days of exposure to large concentrations of DDT, that is, before preexposure levels of DDE could become reestablished.

DDE is a more accurate measure of degree of

Table 2. Comparisons of effects of time on whole blood levels (ppb) of p,p′-DDT and p,p′-DDE in five formulators 3 and 29 days after DDT dust formulation, south Florida, 1967

Formulator No.	3 days after formulation [1]		29 days after formulation [2]	
	DDT	DDE	DDT	DDE
1	26	57	<7	63
2	40	62	<7	60
3	81	81	<7	106
4	117	59	<7	49
5	140	225	11	220

[1] Exposed to 675 pounds of DDT dust; expressed as 100 percent DDT, 1-day exposure.
[2] Exposed to 5,152 pounds of DDT dust; expressed as 100 percent DDT, 1-day exposure.

Table 1. Means and ranges of p,p′-DDT and p,p′-DDE in whole blood samples of men, by occupation and race, south Florida, 1967

Occupation and race	Persons	Mean years' experience	Mean DDT (ppb)	Mean DDE (ppb)	Observations	Range of observations (ppb)	
						DDT	DDE
Nonoccupationally exposed controls:							
White	23		<7	8	57	<7–12	3–16
Nonwhite	37		<7	22	49	<7–15	9–98
Exposure groups by occupation:							
White structural pest control operators	13	8.4	<7	9	21	<7–60	3–23
White lawnsprayers	16	3.6	<7	15	37	<7–13	6–48
White floral sprayers	13	3.7	<7	16	35	<7–36	2–44
Nonwhite floral sprayers	13	7.5	10	36	38	<7–60	6–87
Nonwhite agricultural sprayers	16	6.5	32	41	26	<7–142	14–82
White formulators	10	2.9	21	17	12	<7–55	8–29
Nonwhite formulators	6	4.8	40	64	38	<7–128	13–250
White aircraft sprayers	7	6.0	25	41	37	<7–87	10–113
Total	154				350		

exposure for a prolonged period in an occupation than is the more transient DDT, especially when blood sampling is done randomly, that is, at long irregular intervals. The degree of absorption of DDT relative to continuing occupational exposure can be assessed by sequential DDE blood determinations, and comparisons can be made with nonoccupationally exposed workers. Comparisons should be made with a carefully selected control group of similar race, age group, sex, and background.

Of special interest are the mean DDE levels in blood samples taken from the aircraft sprayers. These white workers had the highest mean DDE values (41 ppb) of the white groups; the means of the other white groups did not exceed 17 ppb. It should be noted that the aircraft sprayers worked exclusively with 3 percent DDT aerosol (with pyrethrum) regularly, whereas the persons in all the other groups were usually more sporadically exposed to various concentrations of DDT dust. We believe that the respiratory absorption of DDT from aerosol and the greater frequency of exposure for longer periods of time had important influences on the demonstrated DDE values.

The ranges of the observations (table 1) made on men in the various groups indicate that DDE values, as well as those of DDT, varied considerably among the persons in the groups; variations within persons also occurred with sequential sampling (fig. 2). Absorption of DDT, with resultant levels of DDT and DDE, depends on many factors which are far from constant—such as seasonal changes and special situations requiring DDT usage, concentration of the chemical, the amounts and physical characteristics of the compound to which the worker is exposed, as well as protective measures and industrial hygiene practices. Recency and extent of exposure in relation to the time of blood sampling have obvious effects on DDT levels. Importantly, DDE levels seem more related to the length of time the worker has been occupationally exposed and the repetitiveness of his exposure to DDT.

We believe DDE levels can be used as a surveillance tool in some occupations; the relative values offer an index of the degree to which DDT is being absorbed under existing working conditions. The heights of the DDE levels can be interpreted as reflecting the degree of failure that has occurred in the efforts to avoid absorption of DDT and thus they relate to efficiency of protective measures and industrial hygiene practices in a general way. Additionally, studies of DDE levels in the occupationally exposed appear important to understand the meaning of these levels as epidemiologic tools. These studies can also serve as models for pharmacodynamic studies of chemicals similarly encountered and metabolized by man.

During the more than 2 years that these people were studied, no occurrences that could be related to the clinical toxicity of DDT have been observed. Persons handling DDT or other potentially toxic chemicals, however, should take care to reduce the possibilities of their absorbing the chemical.

Summary

Data consisting of levels of p,p′-DDT and p,p′-DDE in whole blood samples taken from 94 men occupationally exposed to pesticides in south Florida were examined. The amount of DDT and DDE in whole blood samples was measured by gas chromatography with a modified Dale, Curley, and Cueto method.

DDT levels in blood samples were shown to be transient and related to the recency of exposure of the worker. The DDT and DDE levels in blood samples of nonwhite members of the study groups were consistently higher than their white counterparts in the same occupational groups. The length of time (less than 10 years) the worker was employed in the industry, as well as the relative intensity of his exposure, is considered important in interpreting this observation. DDE levels in sequential blood samples of persons in the exposed occupational groups, taken 1 to 2 months apart, had a coefficient of correlation of 0.81, $P < 0.001$.

We believe sequential sampling for DDE levels in blood could be a tool for surveillance of persons occupationally exposed to DDT and the results could be related to the efficiency of measures taken to avoid its absorption over prolonged periods. Studies of groups of persons occupationally exposed to DDT are considered important in shedding light on the pharmacodynamics of this chemical and perhaps on other

chemicals which man similarly encounters and metabolizes. No toxicologic inferences are made in these studies.

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Dr. Nirenberg Awarded Nobel Prize

Dr. Marshall W. Nirenberg, chief of the National Heart Institute Laboratory of Biochemical Genetics, National Institutes of Health, has become the first Federal employee ever to receive the Nobel Prize for Medicine.

Dr. Nirenberg shares the $70,000 prize with NIH grantees, Dr. Gobind Khorana of the University of Wisconsin and Dr. Robert W. Holley of the Salk Institute of California. The scientists are being honored for "discoveries concerning the interpretation of the genetic code and its function in protein synthesis."

The three geneticists, working independently, have made major advances in understanding the chemical mechanisms by which genetic language or information is translated into various proteins that determine the nature and characteristics of all living things. Their work has cleared the path for investigators throughout the world to examine in detail many aspects of protein synthesis.

The thousands of different proteins found in nature are composed of only about 20 basic building blocks, called amino acids. The exact characteristics of each protein molecule are determined by the number, kinds, and arrangement of amino acids within the molecule. The production of proteins is directed by the genes, which are made up largely of DNA (deoxyribonucleic acid).

DNA does not act directly on protein synthesis but goes through an intermediary, RNA (ribonucleic acid), implanting the code in RNA. The subunits of DNA and RNA, called nucleotides, in turn contain still simpler substances belonging to the chemical classifications of purines and pyrimidines combined with sugar and phosphoric acid. The names of the nucleotides in RNA are adenine (A), guanine (G), uracil (U) and cytosine (C). It is the composition and arrangement of these nucleotides that dictate the final composition of the protein molecule.

After receiving an M.S. from the University of Florida, Gainesville, and a Ph.D. from the University of Michigan, Ann Arbor, Dr. Nirenberg began studies at NIH in 1959 on protein synthesis in cell-free systems. With amazing rapidity, he reported a series of observations which are now known throughout the world.

Dr. Nirenberg showed for the first time that messenger RNA is required for cell-free synthesis. Subsequently, he discovered that when a synthetic RNA containing only a single pyrimidine, uracil, was used as a template for cell-free protein synthesis, the result was a protein containing only one amino acid, phenylalamine. This single experiment can be said to have "cracked" the genetic code for it led to the completion of the "dictionary" of triplate "words" which act as messages for each of the body's 20 amino acids.

As a postdoctoral fellow of the American Cancer Society from 1957–59, Dr. Nirenberg worked at the National Institute of Arthritis and Metabolic Diseases, and from 1960–62 he was a postdoctoral fellow in the Section of Metabolic Enzymes. In 1962 he joined the Heart Institute staff as head of the Section of Biochemical Genetics.