A report is presented on the prevalence of DDE in blood and fatty tissue of stratified samples of a population. Effects of age, race, and sex on DDE levels were examined. Differences in DDE levels are considered to be associated principally with individual exposure to various environmental factors. Blood levels of DDE are regarded as measures of chronic DDT exposure.

AN EPIDEMIOLOGIC APPLICATION OF THE STUDY OF DDE LEVELS IN WHOLE BLOOD

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Introduction

PESTICIDE residues in human fat were first studied in adipose tissue of different population groups to provide information on the occurrence of these chemicals, and to compare levels in different countries and at different periods of time. More recently, pesticide levels have been associated with various states of health and disease,[1-5] and comprehensive knowledge of the prevalence of these residues in the healthy population has become imperative.

In the past, the reasons for the numerical smallness of surveys were not only the complexity of the analytical method, but also the inherent logistic problems of acquiring large numbers of adipose tissues from various samples of the population stratified by age, race, and sex. When Dale, et al.,[6] described a method of measuring chlorinated hydrocarbon levels in blood, they selected a tissue more amenable to large population surveys, and developed a reliable and reproducible procedure which permitted the more representative stratification of population samples. Using a more rapid and simpler modification[7] of this method, and selecting only p,p'-DDT* and p,p'-DDE† as examples for epidemiologic studies, preliminary information on the prevalence of these pesticides in different general population groups of south Florida is described and the potential of using blood rather than adipose tissue for surveys is explored. Significant differences due to person, place, and time were observed in both fat and blood surveys and possible explanations of these differences are presented.

Materials and Methods

The medical examiner of Dade County, Fla., collected adipose tissue samples from 159 persons accidentally or violently killed. These specimens were tested for pesticide content by the method of Radomski and Fiserova[9] at the Florida State Board of Health labora-

* All references to DDT and DDE are to the p,p'-isomers. DDT: 2,2-bis(p-chlorophenyl)1,1,1-trichloroethane.

† DDE: 2,2-bis(p-chlorophenyl)1,1-dichloroethylene.

tory in Jacksonville (104 specimens), and at the Department of Pharmacology of the University of Miami School of Medicine (55 specimens).[10] Additional fat and blood samples were obtained at caesarean section from 29 mothers at Jackson Memorial Hospital in Miami. The fat specimens were examined in the Jacksonville laboratory.

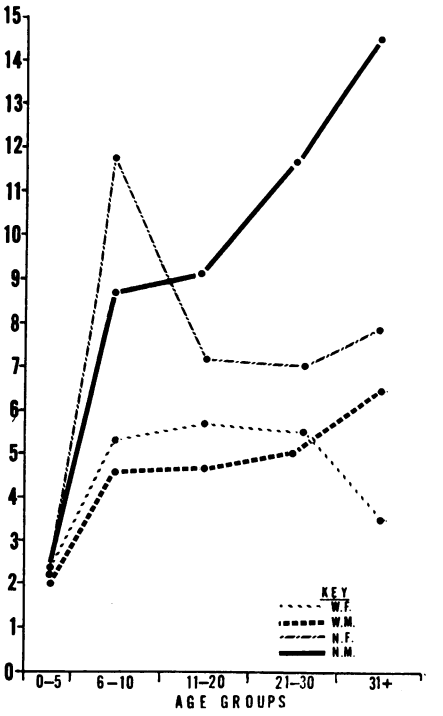
All blood samples were examined for pesticide residues by a modification of the Dale, Curley, and Cueto method[6] at the Miami laboratory of the Florida State Board of Health; this modification had previously been determined as comparable to the original method.[7] These whole blood specimens included those from 29 mothers at caesarean section *(supra)* and 119 persons consisting of a group of employees of the Dade County Health Department, plus another group in a correctional institution in south central Florida. These 119 persons were designated group A (74 of these 119 persons were sampled 30-60 days apart to determine their serial blood levels for DDT and DDE). Blood levels from another group of 100 persons, food handlers, attending a Dade County Health Department clinic for serologic examinations, are reported (group B). Fifty-six children between the ages of one and seven, under care of Children and Youth Project of the Dade County Health Department, were sampled (group D) and 26 cord bloods, obtained at delivery from normal infants at Jackson Memorial Hospital, Miami, completed the survey reported here (group C).

The homes of 13 families were visited to investigate possible reasons for various DDE levels found in the children from these households. The family members were questioned regarding their pesticide usage or other possible exposure to DDT. Note was made of the location of homes relative to possible agricultural exposure and the general physical condition of the housing.

Results

The effect of age, race, and sex on DDE levels observed in the necropsy adipose survey of 159 persons accidentally or violently killed in Dade County is shown in Figure 1. An almost identical figure was obtained when total DDT-derived materials were plotted.[8] Medians from the 0-5, 6-10, 11-20, 21-30, and 31+ year-old age groups are plotted in Figure 1. Age values beyond 31 years were not separately grouped because 1946 is taken as the first year of the widespread commercial distribution and use of DDT, and the DDT age of individuals in 1968 cannot be more than 22 DDT years. In the youngest age group, although stillbirths were also included, no zero values were observed,

Figure 1—Median levels of p,p'-DDE residues (ppm) in necropsy adipose tissues from 159 persons killed in Dade County, Fla., 1965-1967.



confirming the contribution of prenatal exposure of these insecticides to the pesticide body burdens of these children. In the six to ten group and subsequent age groups, diverse stratification trends—primarily due to race differences—were apparent, and all were leveling off in the older age groups except the Negro male category.

When levels of DDE in whole blood from the living general population (119) were determined and compared with DDE values obtained in the necropsy adipose survey (125), the same differences due to race and sex were recognized (Table 1). Tissue and blood samples from children under six years of age were excluded to better compare the data from these sources. When DDE levels in adipose tissue of the 29 mothers undergoing caesarean section were compared with DDE values in their blood, the correlation was significant at the 0.01 level.

The mean. median, and ranges of DDE in blood by sex and race from 119 persons in a general population group (group A) are presented in Table 2. Individuals were excluded from study if they had a history of occupational exposure to pesticides. In order to insure randomization. a second survey of blood was drawn from 100 adult persons attending the food handlers' clinic in the Dade County Health Department (group B). No specific interrogation as to past occupational exposure to pesticides was carried out in this group. Although medians were similar in both surveys, the upper ranges of DDE were greater in the food handlers' group due to differences of the males in the population. Later, specific interrogation of the two individuals with high values did, indeed, confirm that their high values were probably due to exposure in the pest control industry or had been heavily exposed through their own enthusiastic domestic use of insecticides (Table 3).

From these two surveys (groups A and B), DDE in blood was found in measurable concentrations (>1 ppb) in all but one observation in 219 adult persons. In contrast, DDT in measurable concentrations (>4 ppb) was observed to occur more sporadically. From separate studies[11] of the occupationally exposed. DDT blood levels indicated only very recent exposure in contrast to DDE which reflected chronicity of DDT exposure. In a study of paired bloods, obtained one month apart from 74 persons of the general population (group A). the correlation coefficient (r) of

Table 1—Comparison of necropsy adipose tissue data (125) of DDE (ppm) with whole blood data of DDE (ppb) from the living general population (119), Dade County 1967-1968

	Necropsy adipose tissue data DD (ppm)				Whole blood data DDE (ppb)			
Group	Number† persons	$\bar{X}$	Median	p*	Number† persons	$\bar{X}$	Median	p*
White total	90	5.5	4.9	<0.001	68	8	8	<0.001
Negro total	35	10.8	9.6		51	16	14	
White males	52	5.6	5.0	<0.001	23	8	9	<0.001
Negro males	19	12.5	12.9		12	21	19	
White females	38	5.4	4.7	0.01>p>0.001	45	8	7	<0.001
Negro females	16	8.8	7.2		39	14	13	

* Rank Test.
† Data from 6 years+only.

Table 2—Comparison of sex and race associated differences of DDE in blood from a general population group of adults in south Florida, Dade County, 1967

Groups	Group A. Number persons	Number observ.	$\bar{X}$ age (yr)	$\bar{X}$ and range DDE (ppb)	Median	p Value*
Sex						
Negro males	12	24	32	21.4 (9–54)	19	0.02>p>0.01
Negro females	39	51	24	14.4 (4–38)	13	
White males	23	57	37	8.3 (3–16)	9	N.S.†
White females	45	66	31	7.9 (2–19)	7	
Race						
White total	68	123	33	8.0 (2–19)	8	<0.001
Negro total	51	75	26	16.0 (4–54)	14	
White males	23	57	37	8.3 (3–16)	9	<0.001
Negro males	12	24	32	21.4 (9–54)	19	
White females	45	66	31	7.9 (2–19)	7	<0.001
Negro females	39	51	24	14.4 (4–38)	13	

* By Rank Test.
† N.S. Not significant (considered p>0.02).

DDE within individuals was 0.84 ($p<0.001$). Thus, the constancy of this material under conditions of general population exposure was confirmed, and its potential as a measure of chronic long-term exposure to DDT was emphasized.

Effects of age on the level of DDE were explored through analyses of blood specimens from 56 children between the ages of one and seven years (group D). These levels were compared with adult values and with levels obtained in study of 26 cord bloods from newborn infants (group C, Table 4). Most surprising was the observation that by seven years of age children reached levels of the same order of magnitude as those expected in adults. It was only when the newborns were studied that significantly lower levels, due to age, were encountered.

Thirteen homes of siblings in the study of 56 children (from ages one to seven) were visited. No unusual exposure to pesticides was admitted by these families, though the observation was made that children who lived in housing proj-

Table 3—Comparison of DDE levels in blood in a general population group (group A) with a selected group of food handlers (group B), Dade County, 1967

Group A				Group B			
Groups	Number persons	DDE (ppb) $\bar{X}$ and range	Median	Groups	Number persons	DDE (ppb) $\bar{X}$ and range	Median
White total	68	8.0 (2–19)	8	White total	50	10.6 (<1–55)	9
Negro total	51	16.0 (4–54)	14	Negro total	50	16.0 (4–78)	12
White males	23	8.3 (3–16)	9	White males	25	12.0 (3–55)	9
White females	45	7.9 (2–19)	7	White females	25	9.0 (<1–16)	8
Negro male	12	21.4 (9–54)	19	Negro males	25	21.0 (4–78)	16
Negro female	39	14.4 (4–38)	13	Negro females	25	11.0 (4–22)	11

Table 4—Comparison of DDE concentrations (ppb) in whole blood of various age groups in the general population, Dade County, 1967-1968

Age: Groups	(Group C) Birth			(Group D) 1-7 yr			(Groups A and B) 18+ yr		
	Number persons	$\bar{X}$ and range	Median	Number persons	$\bar{X}$ and range	Median	Number persons	$\bar{X}$ and range	Median
White total	10	5.9 (2–13)	6	26	8.4 (2–17)	7	118	9.0 (<1–55)	8
Negro total	16	4.9 (3–9)	5	30	22.1 (3–69)	14	101	16.1 (4–78)	14
White males				12	6.7 (2–13)	6	48	10.1 (3–55)	9
White females				14	10.0 (2–17)	8	70	8.2 (<1–19)	8
Negro males				16	27.5 (4–69)	15	37	21.4 (4–78)	17
Negro females				14	16.4 (3–43)	13	64	13.0 (4–36)	12
Grand total	26	5.3 (2–13)		56	15.7 (2–69)		219	12.6 (<1–78)	

ects, located near agricultural areas and in dwellings in poor condition, tended to have higher DDE levels than those children who lived under more favorable conditions. Clustering of DDE levels occurred, so that children of these families could be said to have high (>24 ppb), medium (12-24 ppb), or low (<12 ppb) levels. No remarkable differences in dietary habits among these families could be ascertained although diet sampling was not carried out.

Discussion

Because organochlorine insecticides had been isolated in erythrocytes, as well as serum and plasma, Kadis and Jonasson[12] limited their studies to whole blood. Dale, et al.,[6] however, claimed that the six insecticides they routinely identified in the general population were so predominantly in plasma that serum or plasma values were satisfactory for toxicological studies. The practicality of their recommendation of doubling whole blood values to compare with serum values was confirmed during these studies, so long as a severe anemia did not co-exist.[7] Since they demonstrated in vitro binding of insecticide, they postulated in vivo binding and suggest that n-hexane extract studies measured only the free or unbound moiety. Whether this concept is valid or not, the absolute amount of insecticide will depend not only upon the special physical and chemical characteristics of the method of determination, such as the type or vigor of shaking or the specific solvent, but also upon the biochemical and hemodynamic state of the individual.

The principal purpose of these studies was to identify differences in the concentrations of DDT and DDE within healthy persons of the general population, so that special sampling and stratification needs for a true prevalence would be realized. This emphasis on

difference, rather than absolute quantitation of the entire DDT load in blood, called for an accurate and rapid method to identify differences in a large number of people. Using n-hexane as solvent, this selective clean-up method was found to be sensitive enough to consistently identify demographic differences of DDE in blood which were due to person, place, and time and which suggested subtle variations in DDT exposure within the general population.

Earlier pilot studies of DDT and DDE in the blood of persons occupationally exposed[11] not only separated this universe from that of the general population, but serial studies provided useful insight into the different epidemiologic interpretations of DDT and DDE levels in blood. Numerous observations suggested unpredictable levels of DDT, whereas DDE values appeared to remain approximately the same on repeated samplings from the individual worker.[11] Analysis of blood samples from five pesticide formulators 3 and 20 days after exposure to a DDT dust formulation demonstrated the evanescent nature of blood DDT, reflective of recent exposure, in significant contrast to DDE which was more indicative of chronicity of DDT exposure.[11] The results obtained in studying 74 pairs of blood samples from persons in the general population (group A) demonstrated that intrapersonal variations of the DDE level are relatively slight over a period of one month.

The age, sex, and race association observed both in fat and blood surveys begged for further explanation. Insofar as age was concerned, adipose tissue and blood data provided clues. Data from study of adipose tissue for four separate age and sex categories suggested a leveling off of levels in the older age groups, except for the Negro male. The continued rise for him may have reflected the inadvertent inclusion of persons previously exposed to pesticides through occupation. In any case, the plateau appeared at the 10-year-age category. In contrast to other studies,[3] these data were not supportive of a general decline of DDT levels in the general population thought to have occurred during the past decade. The argument that each age group of the necropsy adipose data reflected a lowered cohort was untenable in view of the DDE in blood data, wherein levels in children were of the same order of magnitude as the older age groups. Sex-associated differences of DDT have been previously described.[8] Significantly higher values were found in the Negro male than in the Negro female, whereas no sex differences were apparent in white persons. One explanation was the probable inclusion of Negro males with past, though forgotten, occupational exposure to pesticides. This, however, did not explain the higher values in Negro boys than in Negro girls.

The striking race-associated differences of DDE, seen both in adipose and blood surveys, had been previously observed by Hoffman, et al.[3] in necropsy studies, thus indicating that the findings were not peculiar to south Florida. We have not observed these differences in comparisons of groups of adults of both races when they were composed of medically indigent persons who were receiving welfare assistance. This suggested that the difference was due to factors associated with socioeconomic conditions, such as housing and general sanitation.

In support of this hypothesis, we observed that children in the same household tended to have similar DDE values. Families could be characterized as having children with mean DDE levels of a low (<12 ppb), medium (12 to 24 ppb), or high (>24 ppb) value. In the homes of 13 families, which we visited to investigate possible reasons for this clustering, it was observed that high DDE values were associated with the

economically disadvantaged; often, too, homes were in close proximity to available sources of pesticides, such as rural agricultural areas. Negro children whose home conditions were more favorable had low or medium values. All the families engaged in some measure of pest control, the less advantaged tending to use liberally the less expensive pesticides containing DDT. In urban areas of the community, only 9 per cent of the Negro children had DDE values greater than 24 ppb, whereas in the rural districts 35 per cent had levels of this order of magnitude. Differences such as these cannot entirely be explained on the basis of dietetic differences of DDT ingestion. In this tropical climate, our impression is that environmental factors largely contribute to these residues in blood and explain the differences in DDE levels associated with race.

Summary

The prevalence of DDE in blood and adipose tissue of demographically stratified samples of a population was reported. The effects of age, race, and sex on DDE levels in both tissues were found comparable.

DDT in blood was found in measurable quantities only sporadically in the general population and was considered to reflect recent DDT exposure. DDE was found regularly and in relatively constant amounts when measured intrapersonally over periods of time. Blood DDE levels were considered measures of chronic DDT exposure.

Demographic differences in DDE levels were discussed and considered to be principally associated with environmental factors to which individuals are exposed.

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