Bull. Environ. Contam. Toxicol. (1985) 35:411–417

Environmental Contamination and Toxicology

Serum Organochlorine Residues in Florida Citrus Workers Compared to the National Health and Nutrition Examination Survey Sample

J. Griffith[1] and R. C. Duncan[2]

[1]U.S. Environmental Protection Agency, Health Effects Research Laboratory, MD-55A, Research Triangle Park, NC 27711 and [2]The University of Miami School of Medicine, Department of Oncology (D8-4), P.O. Box 016960, Miami, FL 33101

The monitoring of organochlorine residues is important because they accumulate in the tissues of man and animals. All chlorinated hydrocarbon insecticides can be absorbed through dermal, oral, and respiratory routes. Storage is determined primarily by metabolic and excretion rates. Interactions among the compounds have complex effects on metabolism which appear to be species dependent. An exhaustive review of research findings with respect to these chemicals has been provided by Hayes (1982). Organochlorine pesticides absorbed in sufficient doses may result in severe dysfunction of the central nervous system through interference with axonic transmission of nerve impluses (Morgan, 1977). Symptoms include behavioral changes, sensory and equilibrium disturbances, involuntary muscle activity, and depression of vital centers, especially those controlling respiration. Sufficient dosage may also result in tissue damage of the liver and irritability of the myocardium of the heart muscle (Morgan, 1977).

MATERIALS AND METHODS

In a 1981 health survey of 1,811 Florida citrus fieldworkers, 567 serum samples were collected and analyzed for organochlorine residues. The fieldworker values were collected on a random sub-sample of fieldworkers from randomly selected citrus growers.

Serum samples were collected in vacutainer tubes, placed in a test tube rack, kept at a temperature not exceeding 85 degress Fahrenheit for 30-45 minutes to allow for plasma separation, and then placed in a styrofoam container with wet ice and kept in this manner until the day's field work was completed. Upon return to the field coordinating facility the blood was routinely spun down and the serum was collected in a separate tube and frozen with dry ice. The frozen tubes were placed in a test tube rack and wrapped with foam rubber to prevent breakage during transport to the laboratory.

On each subject, individually wrapped isopropyl alcohol (70%) swabs were used to clean the skin thoroughly before venipuncture. Sterile disposable hypodermic needles, non-toxic, non-pyrogenic, non-reactive to tissue, with needle size 20 or 21G x 1 or 1 1/2 inches were used to collect the samples into pre-labelled 10ml red stopper sterile vacutainer tubes with no additive. Supplies such as tourniquets, test tube racks, needle cutters, etc. were cleaned thoroughly with soap and water between farm samples. Contaminated needles and syringes were placed in the Destruclip hypodermic safety device and the contaminated box for used equipment. Betadine was used for handwashing.

Samples sent from the field via courier were received by staff of the University of Miami National Pesticide Hazard Assessment Project (NPHAP). Laboratory number, fieldworker name, identification number, field location of sample collection, responsible field coordinator, date and time of receipt, and pertinent remarks were entered in a bound journal which also had columns for the results of the chemical analyses. The residue analyses were performed by staff of the Division of Chemical Epidemiology, University of Miami School of Medicine.

Serum samples were maintained in a frozen state at -12 to -18 degrees Celsius pending analysis. Storage was never in a freezer containing standard solutions. For analysis the serum was defrosted, a 2-cc aliquot was removed with a clean pipette syringe, and the stock solution quickly refrozen and returned to storage. An aliquot of every twenty-fifth sample was shipped frozen to a cooperating NPHAP laboratory for quality control analysis. Detectors were calibrated daily using approved methods (U.S. EPA, 1979). The Miami laboratory participates in the EPA interlaboratory quality control program, which involves the analysis of uniform samples, and also in the intralaboratory quality control program which assists individual laboratories to maintain the accuracy and precision of analyses by providing guidelines for top quality analytical methodology and technique.

The statistical treatment of pesticide residue values presents problems because of the question of how to handle trace or non-detected values and because of the (possible) non-normality of the measurable values. This often results in the simple reporting of mean values, functions of transformed values, and ranges of variation. Also, frequency distributions of observed values are sometimes tabulated. However,there often is a reluctance to use standard normal theory procedures to make inferences about population means. Because of the utility of the population mean (the expected value for any randomly selected subject from the population) sample mean values should be utilized whenever possible as should straightforward statistical tests. Normal theory tests (e.g. the Student-t test) depend on the sample estimates of mean values being approximately normally distributed. This will always be satisfied when sampling from normal distribution. When not sampling from a normal distribution, the sample size needs to be large enough to ensure that sample estimates of the population mean are distributed approximately normally.

In this report mean values of observations above detection limits are compared using the Student-t test when it can reasonably be assumed that the combination of the sample mean value, the sample standard deviation, and the sample size indicate approximate normality. Such an assumption is made when the sample mean is equal to twice the sample standard error. This ensures that the approximate 95% confidence interval excludes zero, since the true mean residue value for any population having positive values must be positive. It should be noted that since the HANES data did not include standard errors, the appropriate FCFW sample variance was used to calculate the standard error for comparison with HANES mean values.

RESULTS AND DISCUSSION

A total of 567 serum samples were analyzed for the presence of HCB, Lindane, beta-BHC, oxychlordane, heptachlor-epoxide, trans-nonachlor, p,p'-DDE, dieldrin and p,p'-DDT. Samples were collected from fieldworkers during the spraying season when mostly permanent and semi-permanent workers are employed in grove maintenance (including pesticide applications) and also during the harvest season when pickers, including migrant workers, are the most numerous workers in the groves. The sample numbers, percent above detection limits, means, and standard errors for the FCFW and the HANES population are shown in Table 1. Time constraints did not allow the completion of analyses on all subjects for HCB residues.

Statistical comparisons among the FCFW spray and harvest fieldworkers and the HANES population are shown in Table 2. The spray season FCFW are found to have residue levels above detection limits more frequently than either the harvest season FCFW or HANES, or both, for beta-BHC, oxychlordane, and trans-nonachlor. The HANES population

Table 1. Serum organochlorine residues (ppb) among Florida citrus fieldworkers and the HANES national sample.

	Spray Season	Harvest Season	Total FCFW	HANES
HCB				
n	15	47	62	3,441
% Above Trace	20.0	0	4.8	4.4
Mean (SE)	1.2(.09)*	- (-)	1.2(.09)	2.1
Lindane				
n	311	256	567	4,577
% Above Trace	0.6	0.4	0.5	0.2
Mean (SE)	1.9(.20)	1.4(-)	1.7(.20)	2.6
Beta-BHC				
n	311	255	566	4,517
% Above Trace	39.5	14.1	28.1	15.0
Mean (SE)	2.2(.13)	1.7(.12)	2.1(.10)	2.4
Oxychlordane				
n	311	256	567	4,582
% Above Trace	7.7	2.7	5.5	3.5
Mean (SE)	2.2(.50)	1.4(.15)	2.0(.39)	1.9
Heptachlor-epoxide				
n	311	256	567	4,585
% Above Trace	1.6	0.4	1.1	3.9
Mean (SE)	2.4(.92)	1.2(-)	2.2(.78)	2.4
Trans-nonachlor				
n	311	256	567	4,584
% Above Trace	32.5	10.9	22.8	6.6
Mean (SE)	2.9(.79)	1.9(20)	2.7(.62)	1.8
p,p'-DDE				
n	311	256	567	4,590
% Above Trace	95.8	95.3	95.6	98.7
Mean (SE)	23.2(1.42)	31.4(2.53)	26.9(1.39)	18.1
Dieldrin				
n	311	256	567	4,584
% Above Trace	3.2	2.7	3.0	9.1
Mean (SE)	1.9(.57)	1.8(.27)	1.8(.34)	1.9
p,p'-DDT				
n	303	256	559	4,573
% Above Trace	26.1	22.7	24.5	33.3
Mean (SE)	3.9(.25)	5.6(.69)	4.6(.33)	4.4

*Non-detectable and trace values excluded from calculations of means and standard errors.

Table 2. Statistical analysis of serum organochlorine residues among Florida citrus fieldworkers and the HANES national sample.

A. Comparison[a] of frequency of observations above detection limits

	Spray Season vs HANES	Harvest Season vs HANES	Spray Season vs Harvest Season
HCB	n.s.	n.s.	n.s.
Lindane	n.s.	n.s.	n.s.
Beta-BHC	*	n.s.	*
Oxychlordane	*	n.s.	n.s.
Heptachlor-epoxide	n.s.	n.s.	n.s.
Trans-nonachlor	*	n.s.	*
p,p'-DDE	*	*	n.s.
Dieldrin	*	*	n.s.
p,p'-DDT	n.s	*	n.s.

B. Comparison[b] of mean values for observation above detection limits

	Spray Season vs HANES	Harvest Season vs HANES	Spray Season vs Harvest Season
HCB	n.s.	–	–
Lindane	n.s.	–	–
Beta-BHC	n.s.	*	n.s.
Oxychlordane	n.s.	n.s.	n.s.
Heptachlor-epoxide	n.s.	–	–
Trans-nonachlor	n.s.	n.s.	n.s.
p,p'-DDE	*	*	*
Dieldrin	n.s.	n.s.	n.s.
p,p'-DDT	n.s.	n.s.	n.s.

a – Chi-square with continuity correction (or Fisher's Exact Probability Test). Each comparison at alpha/27 = .002 using the Bonferroni procedure.

b – Student-t test. Each comparison at alpha/21 = .0025 using the Bonferroni procedure.

* – Statistically significant at the stated probability level.

is found to have detectable residues more frequently than one or both of the FCFW groups for heptachlor-epoxide, dieldrin, p,p'-DDE, and p,p'-DDT (Table 2A). When comparing levels of the residues however, it is seen in Table 2B that the major difference among the three groups is that both the spray season and the harvest season workers have significantly higher mean p,p'-DDE serum values than does the HANES population. Further, between the FCFW fieldworkers, it is the harvest season cohort which has the higher p,p'-DDE levels.

The serum residue levels of the organochlorines found in this study reflect very well several previous findings. Milby, et al., (1968) found mean serum lindane levels of 0.9 ppb among 21 non-exposed workers and 2.2 ppb among 8 subjects exposed only to home vaporizers. The mean serum lindane level for the total FCFW sample in this study is 1.7 ppb, while that for HANES is 2.6 ppb. The reported mean serum level of 3 ppb for beta-BHC by Hayes (1982) compares favorably with 2.1 ppb for the FCFW and 2.4 ppb for HANES. Serum oxychlordane and trans-nonachlor levels were reported by Failing, et al., (1976) following an investigation of a possible contamination of a municipal water supply with chlordane. Their findings of individual serum levels as high as 0.4 ppb oxychlordane and 1.3 ppb trans-nonachlor are of the same order of magnitude as the levels reported in Table 1. Hayes and Curley (1968) reported serum dieldrin levels of 2 ppb for occupationally unexposed subjects which is quite close to the 1.8 ppb found in this study among the FCFW and the 1.9 ppb reported by HANES.

The most intriguing finding in this report is the serum p,p'-DDT and p,p'-DDE levels among the FCFW. Apple, et al., (1970) found serum DDT and DDE levels averaged over six subjects for six days to be 3.8 ppb and 17.3 ppb respectively, The DDT levels shown in Table 1 are 4.6 ppb for the FCFW and 4.4 ppb for HANES. The DDE value for the HANES population is 18.1 ppb. However the serum DDE values for the FCFW are significantly higher, especially for the harvest season workers, which is seen to be 31.4 ppb. It may well be that these levels are related to the heavy use of dicofol in the Florida citrus industry which was documented during the course of this study.

In summary, although there are variations in the frequency of finding serum residues of the organochlorines above trace levels among Florida citrus fieldworkers when compared to the national population represented by the HANES sample, the actual residue levels are quite similar between the two populations and both are similar to the findings of other investigators discussed above. An exception is that the FCFW appear to have higher serum p,p'-DDE levels than the HANES sample or levels reported in the literature for unexposed subjects (Morgan and Roan, 1974; Clifford and Weil, 1972).

Acknowledgements. This study was funded by the National Pesticide Hazard Assessment Program, OPP, HED, HEB, Environmental Protection Agency, Washington, DC, (CR80705101). The views expressed herein are those of the investigators and do not necessarily reflect those of the agency.

REFERENCES

Apple G, Morgan DP, Roan CC (1970) Determinants of serum DDT and DDE concentrations. Bull Environ Contam Toxicol 5:16-23

Clifford NJ, Weil J (1972) Cortisol metabolism in persons occupationally exposed to DDT. Arch Environ Health 24:145-147

Failing F, Rimer C, Wooley R, Sandifer SH, Hutcheson RHJ, Saucier JW, Ward C, Kutz FW (1976) Chlordane contamination of a municipal water system-Tennessee. MMWR 25:117

Hayes WJ Jr. (1982) Pesticides studied in man. Williams and Wilkins, Baltimore, pp 172-283

Hayes WJ Jr., Curley A (1968) Storage and excretion of dieldrin and related compounds. Arch Environ Health 16:155-162

Milby TH, Samuels AJ, Ottoboni F (1968) Human exposure to lindane I: Blood lindane levels as a function of exposure. J Occ Med 10:584-587

Morgan DP (1977) Recognition and management of pesticide poisonings. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C.

Morgan DP, Roan, CC (1974) Liver function in workers having high tissue stores of chlorinated hydrocarbon pesticides. Arch Environ Health 29:14-17

US Environmental Protection Agency (1979) Manual of analytic methods for the analysis of pesticide residues in human and environmental samples. Health Effects Research Laboratory, Research Triangle Park, North Carolina

Received September 4, 1984; Accepted October 30, 1984