Determinants of Serum DDT and DDE Concentrations

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Since chlorinated hydrocarbon pesticides have been looked upon as potential human health hazards, objective assessment of their threat to man has relied increasingly on measurement of these chemicals in human blood specimens. Techniques for qualitative detection have reached a high degree of sophistication through gas-liquid chromatography. Quantitative measurement in body fluids remains difficult, due largely to the uncertainties implicit in extraction of minute amounts of chemical from a highly complex fluid such as blood. Least explored are the physiologic determinants of blood pesticide levels.

We began an inquiry into the last question by evaluating day to day variation in serum levels of DDT and DDE in three male and three female adults from our own office and laboratory, using standardized analytical techniques, carried out by a single analyst. In this way, we expected to achieve consistency of technique, if not quantitative precision. Analytical, temporal, and between-subject sources of variation were evaluated, as was the effect of a meal prior to blood sampling.

Certain findings then led us to assess the impact of deliberate DDT dosing on blood pesticide levels measured over 1) a 24 hour interval after a single dose, and 2) a 5 month period of daily dosing.

Methods and Materials

Serum DDT and DDE Measurements

Extraction Method I: (Dale, Curley and Cueto (1)): This technique was used consistently in measuring serum DDT and DDE levels in six subjects on six successive days. Briefly, serum (2 ml) separated by centrifugation in chemically clean containers was shaken for 3 minutes with redistilled hexane (5 ml). A 4 ml aliquot of the hexane extract was evaporated to 1 ml volume for analysis.

Extraction Method II: Aiming for a higher degree of absolute accuracy in following pesticide levels during DDT feeding, the above method was modified to the extent of carrying out three serial hexane extractions (10 minutes each, using 2 ml serum and 6 ml hexane) in place of the single 3 minute extraction.

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TABLE I

Serum DDT in Six Subjects
on Six Successive Days

Subjects	Days 1 PP+	2 F*	3 PP	4 F	5 PP	6 F	Subject Means
M ♀	9.1	4.3	4.2	3.9	6.9	4.3	5.5
S ♀	3.3	2.9	2.0	3.3	3.3	2.9	3.0
T ♀	6.0	4.5	4.1	4.0	4.4	4.0	4.5
L ♀	3.9	4.8	3.5	4.1	3.9	2.9	3.8
P ♀	4.2	5.8	3.2	2.5	3.5	2.7	3.6
R ♀	2.7	3.1	1.8	3.2	2.1	2.4	2.5
Day Means	4.9	4.2	3.1	3.5	4.0	3.2	

+PP = post prandial

* F = fasting

TABLE II

Serum DDE in Six Subjects
on Six Successive Days

Subjects	Days 1 PP+	2 F*	3 PP	4 F	5 PP	6 F	Subject Means
M ♀	41	22	20	16	28	18	24
S ♀	13	9	8	9	7	9	9
T ♀	28	21	16	17	16	19	19
L ♀	34	35	22	27	27	21	27
P ♀	19	26	11	10	14	9	15
R ♀	10	11	6	9	5	9	9
Day Means	24	20	14	15	16	14	

+PP = post prandial

*F = fasting

Extraction Method III: To confirm pesticide levels on serial time course samples, sera (2 ml) were extracted with 10 ml 1:1 hexane-acetone mixture, using moderate shaking for 5 minutes. After three washes with distilled water, the hexane phase was passed through a column of activated florisil. The extract was followed through the column by 20 ml of pentane-ethyl ether-alcohol mixture (17:2.94:0.06). Solvent volume was reduced by evaporation to a quantity suitable for chromatographic analysis.

Detection: Samples were analyzed on a Micro Tek 220 gas chromatograph with tritium foil electron capture detector. Operating parameters for the instrument were:

Column:	6' length, 1/8" interval diameter
Column packing:	Chromasorb W 60/80 mesh
Column coating:	10% Dow 11, 15% QF-1
Inlet temperature: 230°C	Column temperature: 205°C
Detector temperature: 195°C	Polarizing voltage: 12
Nitrogen flow:	90 ml per minute

Total Serum Lipids: The method of Folch, et al. (2) was used. A filtered methanol-chloroform extract of serum was equilibrated against water, then a chloroform-methanol-water mixture to remove non-lipid solutes. Material mreaining after evaporation and desiccation was weighed.

DDT Feeding: Technical DDT (77.2% pp' isomer, 22.8% op' isomer) was fed daily to two subjects for a 5 month period. The chemical was given in the form of an emulsion, essentially identical to that administered in a previous DDT feeding study (3). The DDT was first dissolved in vegetable oil, then an emulsion was generated by vigorous mixing with an aqueous suspension of gum arabic.

Results. Tables I and II present serum DDT values in six subjects on six successive days, measured by single hexane extraction.

Each pesticide value shown represents a mean of duplicate measurements on each sample.[1] From the replicates, analytical variation for DDT was found to be 24% of the mean (coefficient of variation), the same as day to day variation. For DDE, day to day variation (30%) somewhat exceeded analytical variation (20%). Most impressive, however, is the variation between subjects: in the case of DDE 49%, and for DDT 32%, of the respective means.

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[1]Actually, one part of each serum sample was run immediately, while the remainder was stored frozen for 1-6 days corresponding to the day of drawing. No consistent differences were found in the stored frozen samples. Random differences were attributed to measurement error, and treated as analytical variation.

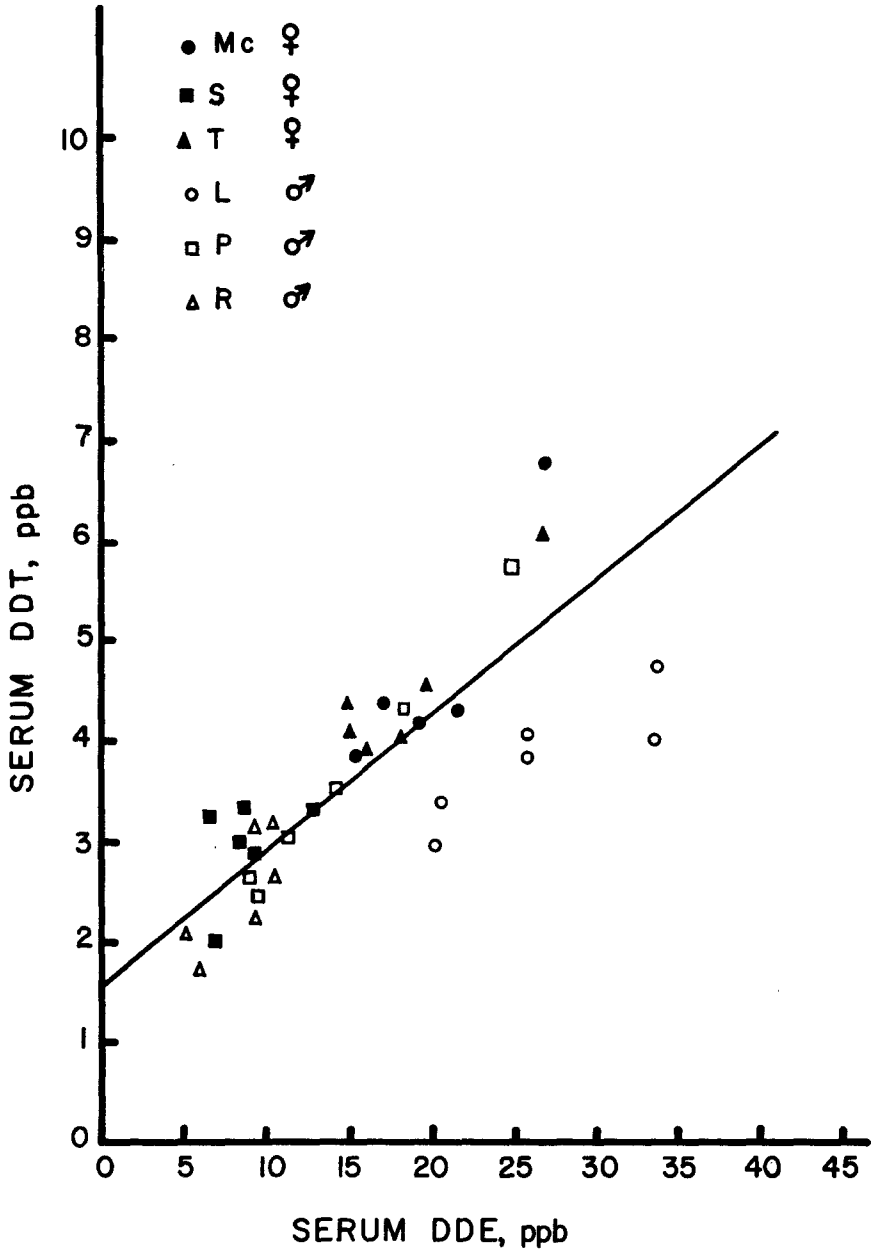

Fig.1 Correlation Between Serum DDE and DDT in Day to Day Samples in Six Subjects (Single Hexane Extracts)

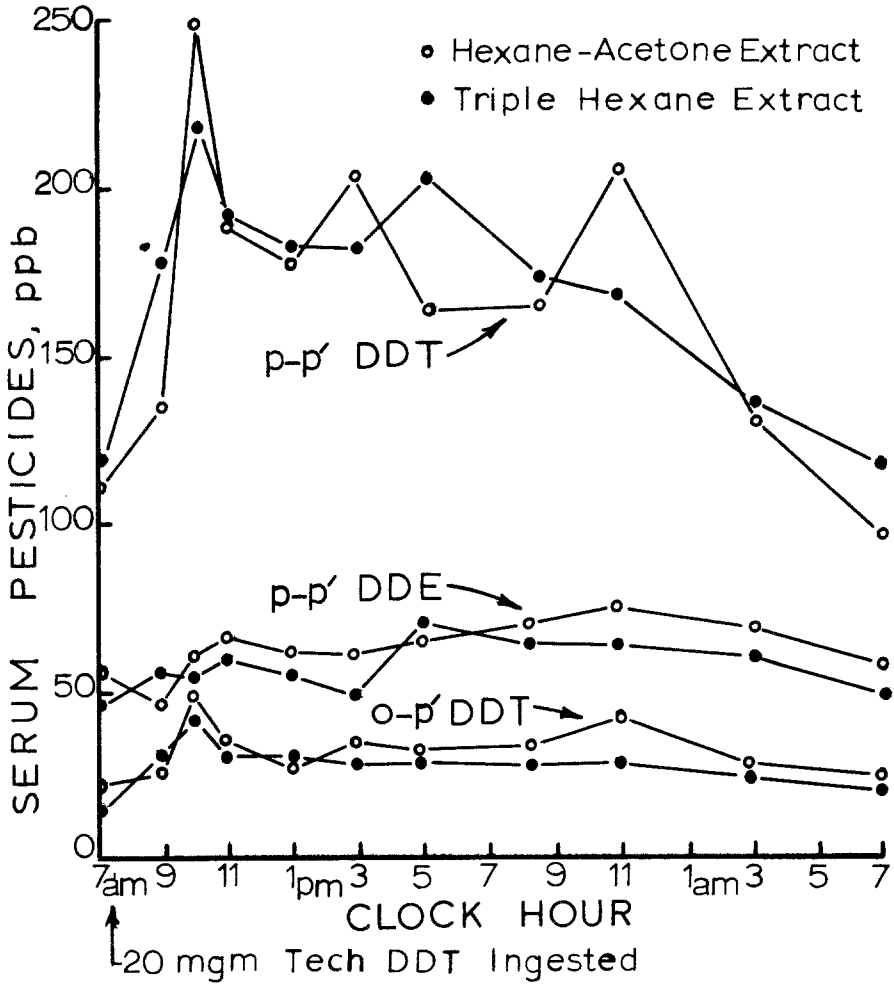

Fig.2 24-Hour Time Course of Serum DDT and DDE in One Subject Following Ingestion of 20 mgm Tech DDT

The tendency of DDE in particular to maintain a level unique for the individual is readily appreciated by inspection of Table II. The F ratio comparing individual variation to all other variability was 11.2 for DDE, 5.3 for DDT.

No consistent effect on pesticide level could be found from the taking of an ordinary breakfast prior to sampling. Total serum lipid averaged 6.2% higher in the post prandial samples than in the fasting. No correlation between total serum lipid values and pesticide concentrations appeared.

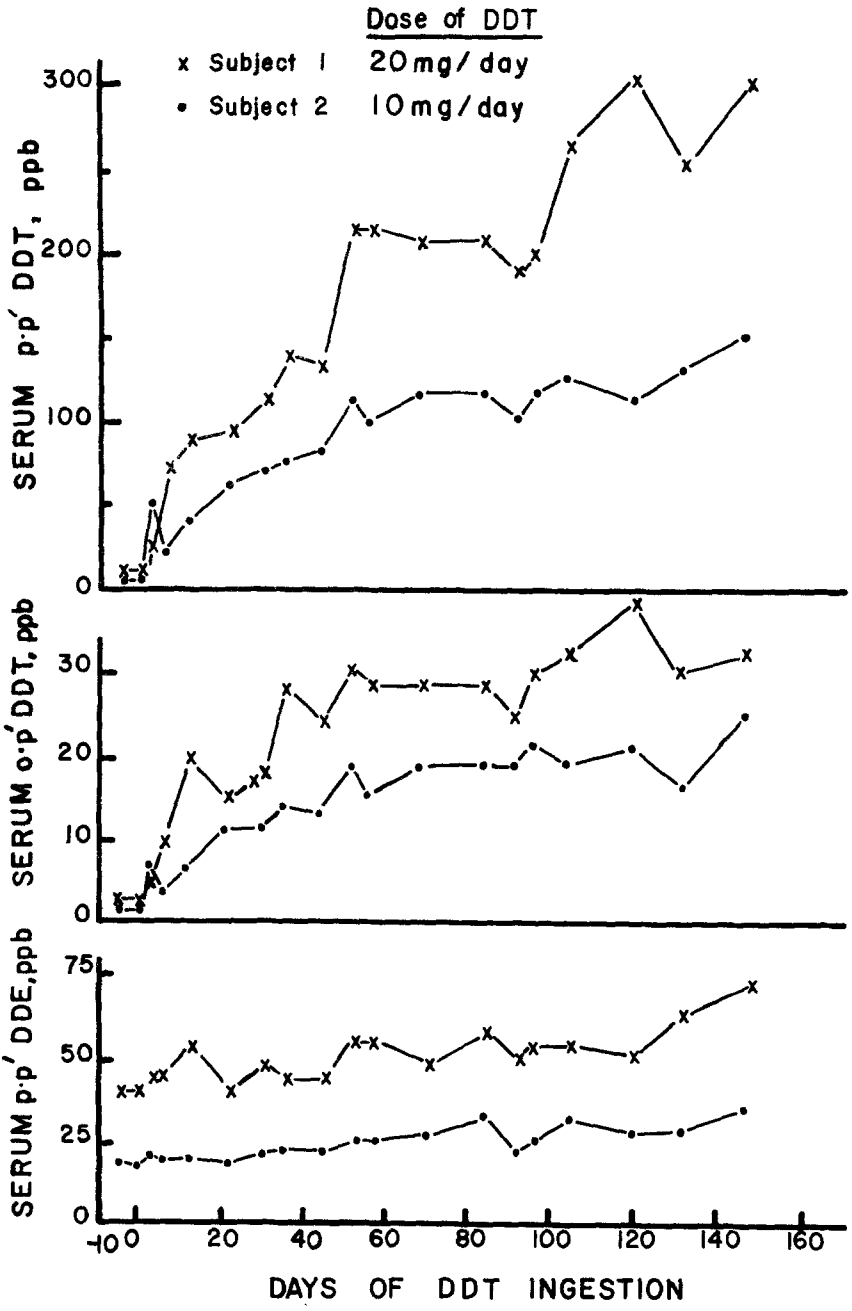

Fig.3 Time Course of Serum DDT and DDE Concentrations During 5 Months of DDT Ingestion by Two Human Subjects

In addition to the relative prominence of intersubject variation in these data, another feature is clear from Figure 1: strong correlation between DDT and DDE among serum samples from a given subject. The covariance correlation coefficient representing day to day correlation between DDT and DDE within subjects is 0.91. The counterpart correlation based on subject variation within days is weaker: 0.78.

The simplest interpretation of these findings would be that differences in serum levels are due primarily to fluctuating intakes of DDT itself, the metabolite (DDE) concentration changing in response to varying tissue concentration of the parent chemical (DDT).

Such an interpretation is tenable if serum levels fluctuate promptly in response to varying intakes of DDT. We tested the time relations involved in serum changes by measuring serum DDT and DDE at brief intervals for 24 hours following a single oral dose of 20 mgm of DDT in a subject who had been taking this amount daily.

The time course is shown in Figure 2.

The prompt rise in serum DDT, followed by steady decline back to initial values certainly suggests rapid absorption followed by conversion, excretion, and/or storage. It is tempting to interpret the later hump in DDE level as a temporary imbalance of synthesis in excess of degration, conversion and/or storage of this metabolite.

So far, our findings are entirely consistent with the reliability of serum DDT and DDE as indicators of recent DDT intake. Can both measures be counted on to reflect a step change in DDT intake over a long period of time? If so, both serum pesticide levels should rise progressively following an increased DDT intake, roughly in proportion to the dose level.

Time courses of serum DDT and DDE following such step increases in DDT intake are shown for two subjects in Figure 3. They reveal an extraordinary disparity between behaviors of the two serum concentrations. DDT rises progressively, approaching a steady state asymptote in about 5 months, while DDE in both subjects changes very slowly. To the extent that serum DDE is a function of DDT intake in any given subject, the relationship must represent a cumulative effect extending over several months.

If oral DDT intake is a weak and sluggish determinant of serum DDE level, inquiry must be turned to identification of those factors that are more direct. In addition, our other findings (wide individual variation, and covariation in serum DDT and DDE) require explanations not based on differences in DDT intake.

We propose the following interpretations of our findings:

1) Tissue DDE is derived primarily from preformed DDE, rather than DDT. Prompt response of adipose DDE to DDE feeding has been well documented in the monkey, as has the inability of the monkey to synthesize DDE from DDT (4). To the extent that tissue DDE is derived from dietary DDT in man, the conversion mechanism must be saturated at very low tissue levels, and extraordinarily slow to adapt to increased intake.

2) The day to day covariation of DDT and DDE in samples subjected to single hexane extraction may well be due to analytical rather than physiologic factors. For example, day to day differences in plasma composition and physical properties may influence similarly the extractability of DDT and DDE from this complex fluid.

3) The wide between-individual differences found in serum DDE could also be referable to the extractability factors mentioned in 2). However, more rigorous extraction methods have confirmed the existence of widely different and relatively stable serum DDE levels in human subjects (see Figure 3 for an example). It seems unlikely that dietary intakes of DDE-containing materials are as divergent as these differences suggest. These considerations lead us to suspect the importance of highly individual mechanisms for excretion and/or chemical conversion of DDE. This might take the form of individual patterns of enzymatic conversion, or of balance between excretion and reabsorption in an enterohepatic circulation of the chemical.

Conclusions

1) Measurements of serum DDT concentration are capable of relfecting changes in oral DDT intake accurately and promptly.

2) Serum DDE level is only slightly increased by a 300-600 fold increase in DDT intake over a period of 5 months. It is therefore an essentially worthless measure of recent change in oral DDT intake. Stable individual differences in serum DDE levels, seemingly much wider than differences in dietary patterns (i.e. DDE intake), suggest the importance of individual metabolic factors in determining this level.

3) A high degree of correlation between serum DDT and DDE in day to day samples extracted once by hexane, probably reflects changes in extractability influencing the two measurements similarly.

References

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