Effects of Dietary Levels of Protein and Fat on DDT 
(1,1,1-Trichloro-2,2-bis(p-Chlorophenyl)Ethane) 
and Liver Lipid Metabolism

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Summary  In order to examine the effect of dietary protein and fat on 
DDT metabolism and liver lipid concentration, rats were supplied with 
calory adjusted diets consisting of various amounts of protein and fat. The 
results suggested that dietary protein and fat changed the liver lipid 
concentration. They also showed that dietary protein and fat affected the 
residual concentration of DDT and its metabolites in the liver and adipose 
tissue.
The change of the concentration of lipids in liver accompanied a change of 
the residual concentration of DDT in liver. This fact indicates that one 
effect of dietary protein and fat on the metabolism of DDT is attributable 
to the metabolic change of lipids in liver. Dietary protein accelerated the 
metabolism of DDT and reduced its residual concentration in liver. 
The results suggest that regression equations exist between the residual 
concentration of DDT in liver and (1) dietary factors and (2) lipid 
concentration in liver:

\[ \ln(DDT) = a \cdot \ln(x_1) + b \cdot \ln(x_2) + c \cdot \ln(TL) + d \] 
\[ \ln(DDD \text{ or } DDE) = a' \cdot \ln(TL) + b' \cdot \ln(PL) + c' \]

where \( x_1, x_2, TL, \) and \( PL \) are the dietary protein, dietary fat content, total 
lipid, and phospholipid concentration in liver, respectively. \( a, a', b, b', c, 
c', \) and \( d \) are constants. The concentrations of DDT and its metabolites 
estimated from equations (1) and (2) agrees well with the measured 
concentrations in liver.

Key Words  DDT, pesticide, chlorinated hydrocarbon, P-450, drug meta-
bolism, lipid metabolism, liver function, protein, fat, nutrition

It has been established by many investigators that the fat-soluble chlorinated 
hydrocarbons such as DDT (1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane) and
PCB (polychlorinated biphenyls), spread through the environment and accumulate in human tissues, especially lipid-rich tissue (1-5). In an earlier investigation (6), it was shown that dietary protein and fat content modified DDT metabolism in the liver. Since the fat-soluble chlorinated hydrocarbons are metabolized by the hepatic microsomal enzyme systems, the metabolism and excretion of these chemicals may be closely related to enzyme activity (7).

In an earlier experiment on DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), one of the major metabolites of DDT, it was shown that the excretion of DDE was modified by its dose level (8). It was also clarified that the excretion pattern of DDT and DDE fits the two-compartment model well (9). Investigation of the factors affecting the compartment relating metabolism and excretion which is represented by DDT concentration in liver is important.

Because they are fat-soluble chemicals, most chlorinated hydrocarbons which contaminate the human body may combine with lipids (10). Therefore, the metabolism of these chemicals in the body should be explained by taking the metabolism of lipids into account. Dietary protein together with dietary fat also affect the lipid concentration in the liver. It is necessary to investigate the relation between the metabolism of the fat-soluble chlorinated hydrocarbons such as DDT and lipid concentration in the liver, since DDT metabolism may be related to the concentration of some of the lipids present in the liver.

It is obvious from many previous reports (11, 12) that dietary protein and fat influences DDT metabolism and also the concentration of lipids in the liver, but the relation between DDT metabolism and dietary factors was found to be non-linear, so that a more detailed experimental design is necessary. In this experiment, the author applied a $5 \times 5$ factorial experimental design to analyze the interaction between dietary protein and fat, and a multiple regression analysis was carried out.

MATERIALS AND METHODS

One hundred and twenty five six-week-old male Wistar-strain rats, weighing from 104 to 135 g, were used in this experiment. A light-dark cycle of 9-15 hr was used in the animal quarter, which was maintained at 23.8°C. Animals were divided into 25 groups, five rats to each group. They took water ad libitum and were supplied with 25 different kinds of experimental diets, as shown in Table 1. The caloric content was adjusted with carbohydrate while the volume was adjusted with cellulose powder. The weights of rats and the food consumed were measured every 3 to 4 days.

After supplying the rats with the experimental food for 7 days, they were injected intraperitoneally with p,p'DDT (analytical reagent grade 99.9%) dissolved in olive oil at the dose level of 7.2 mg/kg body weight. The other five rats were injected with only olive oil for normal control. Two weeks later, all rats were sacrificed by heart puncture while under ether anesthesia. Blood hematocrit (%) was determined by centrifugation immediately after the heart puncture. The liver,
Table 1. Composition of experimental diets. 5 x 5 factorial experimental design.

| Ingredients            | %          | Protein (%) | Fat (%) |
|------------------------|------------|-------------|---------|
| Linoleic acid          | 0.5        | i=0.5       | j=5.0   |
| Cellulose              | 3.0        | 2.5         | 10.0    |
| Salt mixture*          | 6.2        | 20.0        | 5.0     |
| Vitamin mixture*       | 1.0        | 10.0        | 20.0    |
| Casein*                |            | 1            |         |
| Corn oilb              |            | 2            |         |
| Carbohydratec          |            | 4            |         |
| Cellulose powderd      |            | 10           |         |

* Nihon Clea Co., Ltd. a 5, 10, 20, 30 and 40% protein as shown right table. b 0, 2.0, 4.5, 9.5, and 19.5% lipid as shown right table. c Corn starch: sucrose (3 : 1) was added to make isocalory. d Cellulose powder was added to make equal capacity. e xij is group number of experimental rats.

kidney and testes were removed, rinsed with physiological saline and weighed. After adding an equal volume of physiological saline to the liver, the latter was homogenized in a 30 ml Potter-Elvehjem glass homogenizer in an ice bath, placed in screw-capped test tubes, and stored at -20°C.

Two grams of liver homogenate and experimental food were mixed with 2 ml of formic acid (analytical reagent grade 99%), cleaned by n-hexane (reagent grade for pesticide analysis). Ten ml of n-hexane were added to this and then mixed. After standing for 24 hr, the mixture was centrifuged and n-hexane was then extracted. More n-hexane was added and also extracted. The precipitate was rinsed with a small amount of n-hexane. The extracted n-hexane layers were combined and then washed with 2 ml of 5% potassium carbonate (analytical reagent grade 99.9%) according to the method described by Dale et al. (13).

The n-hexane layer was cleaned by florisil (reagent grade for pesticide analysis) column chromatography. n-Hexane–ethyl ether (96:4) was used for the elution solvent. p,p'DDT and its metabolites were determined by use of a gas-liquid chromatograph equipped with a 63Ni electron-capture detector. A glass column (4ft x 1/8 in i.d.) packed with 1% OV-17 and 1% SE-30 (1 : 1) on 80–100 mesh Chromosorb W (AW-DMCS) was used. The column temperature was 200°C and the flow rate of pure nitrogen as a carrier gas was 30 ml/min. The recovery of p,p'DDT, p,p'DDD and p,p'DDE has been described in a previous report (Ando) (9).

Lipids were extracted from the liver homogenate with a chloroform–methanol mixture (2 : 1) using the method of Folch et al. (14). The lipid extract was evaporated, weighed to determine total lipids (TL) and then dissolved in ethanol. Phospholipid (PL) was determined by use of the method of Takayama et al. (15). Triglyceride (TG) was determined using the method of Eggstein and Kreutz (16).
Total cholesterol (TCHO) and free cholesterol (FCHO) were also determined by using method of Allain et al. (17). Cholesterol ester (ECHO) was calculated by subtracting free cholesterol from total cholesterol. Free fatty acid (FFA) was determined by use of the method of Itaya and Ui (18).

The effect of dietary factors (protein and fat) on the concentration of various lipids in the liver was assessed by an analysis of variance of a $5 \times 5$ factorial experimental design. The design allows for analysis of possible interactions between both factors. Statistical analysis was carried out using the $F$ and $t$-test of Snedecor and Cochran (19). Multiple regression analysis was carried out using the FORTRAN program of Hirosaki and Kobayashi (20).

RESULTS

Trace contamination by DDT and its metabolites was observed in the experimental foods. The concentration of DDT and DDD in these foods was less than 0.2 ng/g foods. The concentration of DDE in the foods with 0.5, 5, and 20% fat was $0.51 \pm 0.15$, $0.79 \pm 0.28$, and $0.91 \pm 0.14$ ng/g foods, respectively. Trace contamination by DDT and its metabolites was also observed in the liver of normal control rats. The concentration of DDT and DDD in the liver was less than 2 ng/g liver. The concentration of DDE in the liver was $13.8 \pm 5.8$ ng/g liver.

The effect of dietary protein and fat on the residual concentration of DDT and its metabolites in the liver and adipose tissue was investigated. Dietary protein and

![Graph](image-url)  

**Fig. 1.** The concentration change of DDT and DDD in the liver and adipose tissue in rats fed on 20% dietary fat.
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fat affected the residual concentration of DDT and its metabolites in the liver and adipose tissue.

Figure 1 shows the concentration change of DDT and its metabolite, DDD, in the liver and adipose tissue in rats fed on high fat diet (20%). When dietary protein content increased, the residual concentration of DDT in the liver and adipose tissue decreased. The concentration of DDD in the liver and adipose tissue increased according to the decrease of DDT in the liver and adipose tissue.

The distribution of the concentration of DDT and its metabolites was

| Variables | Normal value | Logarithmic value |
|-----------|--------------|------------------|
|           | Skewness     | Kuratosis        | Skewness     | Kuratosis |
| TL        | 1.394*       | 4.252*           | 0.917*       | 3.156     |
| FFA       | 1.483*       | 4.509*           | 0.530*       | 2.416     |
| FCHO      | 0.687*       | 5.289*           | -0.216       | 3.687     |
| TCHO      | 1.739*       | 8.058*           | 0.805*       | 4.061     |
| TG        | 1.092*       | 3.976            | 0.512*       | 2.711     |
| PL        | 0.586*       | 4.147            | -0.006       | 3.196     |
| ECHO      | 1.116*       | 5.366*           | -1.084*      | 3.838     |
| DDT       | 2.275*       | 9.921*           | 0.162        | 3.000     |
| DDD       | 1.274*       | 4.196            | -0.082       | 3.388     |
| DDE       | 1.391*       | 5.200*           | 0.170        | 2.878     |

* Significantly different from normality (p<0.01).

Fig. 2. The relation between the concentration of total lipids in liver and dietary protein content. Each point represents the mean of five rats (mean ± SD). *, significant difference from 2.5% fat diet group (p<0.05).

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Fig. 3. The relation between the concentration of total lipids in liver and dietary fat content. Each point represents the mean of five rats (mean ± SD). *, significant difference from 5% protein diet group (p < 0.05).

Table 3. Correlation coefficients among the concentration of lipids and DDT in the liver.

| Variables | ln (TL) | ln (FFA) | ln (FCHO) | ln (TCHO) | ln (TG) | ln (PL) | ln (ECHO) |
|-----------|---------|----------|-----------|-----------|---------|---------|-----------|
| ln (TL)   | 0.672** |          |           |           |         |         |           |
| ln (FFA)  |         | 0.513** | 0.353**   |           |         |         |           |
| ln (FCHO) | 0.757** | 0.559** | 0.721**   |           |         |         |           |
| ln (TCHO) | 0.692** | 0.395** | 0.634**   | 0.752**   |         |         |           |
| ln (TG)   | 0.481** | 0.284** | 0.476**   | 0.598**   | 0.485** |         |           |
| ln (PL)   | 0.478** | 0.380** | -0.024    | 0.624**   | 0.382** | 0.255** |           |
| ln (ECHO) | 0.340** | 0.258** | 0.101     | 0.269**   | 0.302** | -0.005 | 0.284**   |
| ln (DDT)  | 0.500** | 0.319** | 0.286**   | 0.457**   | 0.345** | 0.432** | 0.284**   |
| ln (DDE)  | 0.400** | 0.223** | 0.167     | 0.339**   | 0.296** | 0.351** | 0.276**   |

* Significant level of correlation coefficient (p < 0.05). ** Significant level of correlation coefficient (p < 0.01).

examined in all groups. Table 2 shows the skew and kurtosis of the distribution. The data show that the concentration of DDT and its metabolites follow a log-normal distribution. It is considered that the logarithmic value of the concentration of lipids in liver fits normal distribution better than the original one. Therefore, to normalize the distribution, the logarithmic value of each measurement was calculated.

Figures 2 and 3 show the relation between the concentration of total lipids in the liver and dietary factors. From more than 10% dietary fat, the total lipid concentration was highest in rats fed on the diet with 20% protein. The concentration of total lipids in the liver increased when dietary fat increased.

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Table 4. Coefficients of the regression equation between DDT, DDD, DDE (ng/g) and the dietary factors, and lipids concentration in the liver.

| Variables      | ln (DDT) | ln (DDD) | ln (DDE) |
|----------------|----------|----------|----------|
| ln (TL)        | 0.7726*  | 0.7762*  | 0.5435*  |
| ln (PL)        | 0.7576*  |          | 0.5537*  |
| ln (protein)   | −0.4925* |          |          |
| ln (fat)       | 0.1027*  |          |          |
| Constant       | 4.5810   | 1.1406   | 0.7105   |

\[ R^2 = 46.3 \quad 29.7 \quad 19.3 \]
\[ F = 34.8** \quad 25.8** \quad 14.6** \]

*Significant level of correlation coefficient \((p<0.05)\). **Significant level of F-value \((p<0.01)\). \(R^2\), coefficient of determination; \(F\), F-value.

Table 3 summarizes the correlation coefficients between DDT and various lipid concentrations in the liver. A significant positive correlation was found to exist. It was recognized that a non-linear relationship existed between the concentration of DDT and the dietary protein or fat content. In order to analyze the interaction of dietary protein and fat with the DDT concentration in the liver, an analysis of the variance was carried out. The results showed that dietary protein and fat both affected DDT concentration in the liver.

From the results described above, multiple regression analysis was carried out to confirm the effect of dietary protein and fat on DDT metabolism concerned with the lipid concentration in the liver. The most suitable equation was determined from calculating the predictive sum of squares, as follows;

\[
\ln (DDT) = a \cdot \ln (x_1) + b \cdot \ln (x_2) + c \cdot \ln (TL) + d
\]
\[
\ln (DDD or DDE) = a' \cdot \ln (TL) + b' \cdot \ln (PL) + c'
\]

where, \(x_1, x_2, TL, \) and \(PL\) are the dietary protein, dietary fat content, total lipid, and phospholipid concentration in liver, respectively. \(a, a', b, b', c, c',\) and \(d\) are constants.

Table 4 summarizes the partial correlation coefficients of the regression equations (1) and (2). From the equations, the concentration of DDT and its metabolites were functions of lipid concentration, dietary protein, and fat content. The estimated concentration of DDT and its metabolites from the equations agreed well with their measured concentration in liver.

**DISCUSSION**

In earlier experiments, it was evident that the concentration of lipids, especially of triglyceride, in the liver decreased according to the administration of DDT. The minimum dose of DDT which has an effect on the liver triglyceride content is more...
than 13 mg/kg body weight. A dose level of DDT of under 10 mg/kg body weight has no effect on the lipid concentration in the liver (21). In this experiment, the dose level of DDT is 7.2 mg/kg body weight, therefore, the concentration change of lipids in the liver is due to the effect of dietary protein and fat.

On the other hand, the liver is a vital organ which metabolizes and excretes the fat-soluble chlorinated hydrocarbons such as DDT and PCB. The time pattern of DDT excretion was explained by a compartment model. In early investigations, the excretion of DDT and DDE could be adequately described by a two-compartment model (8, 9).

The concentration change of DDT and its metabolites in the liver and adipose tissue in rats fed on high-fat diet is also clarified. When dietary protein increases, the residual concentration of DDT decreases in the liver and adipose tissue, whereas that of DDD increases. DDT is metabolized to DDE and DDD by the dehydrochlorinase and the hepatic microsomal drug-metabolizing enzymes which contain two independent systems; one requiring cytochrome P-450 and the other requiring FAD (22, 23). It is well known that the activity of hepatic microsomal drug-metabolizing enzymes is influenced by dietary protein (24). Therefore, the metabolism of DDT is influenced by the consumption of protein.

Recently, it has been reported that poly-unsaturated fatty acids have an effect on the hepatic microsomal drug-metabolizing enzyme systems (25). Dietary fat also increases the lipid concentration in the liver because fatty acid incorporation increases.

Because they are fat-soluble chemicals, chlorinated hydrocarbons accumulated in the human body may be combined with lipids (10). Therefore, it is assumed that the metabolism of DDT may be explained by taking the metabolism of lipids in the liver into account.

The results suggest that regression equations exist between the concentration of DDT in the liver and dietary factors (protein and fat), and lipid concentration in the liver. The concentrations of DDT and its metabolites estimated from the equations (1) and (2) agree well with the measured concentrations in the liver. In the equations, it is decided that the concentration of DDT and its metabolites is connected with the lipid concentration in the liver. DDT metabolism is also modified by the dietary protein and dietary fat content.

From the results described above, the activity of the drug-metabolizing enzymes is influenced by the dietary protein and fat content. Therefore, it is necessary to investigate the relation between the fat-soluble chemicals metabolized by the hepatic drug-metabolizing enzymes and the dietary factors, and to confirm their metabolism with respect to lipids and drug metabolism in the body.

The first target organ of fat-soluble chlorinated hydrocarbons at low concentration was the liver (25), and their biological effect and toxicity changed in proportion to the residual concentration in the liver (26). Therefore, we may say that increasing the dietary consumption of protein increases the metabolism and excretion of chlorinated hydrocarbons that remain in the body. On the other hand,
increasing dietary fat raises the residual concentration of the chemicals in the liver and may modify their toxicity.

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REFERENCES

1) Masuda, Y., Kagawa, R., and Kuratsune, M. (1974): Comparison of polychlorinated biphenyls in Yusho patients and ordinary persons. Bull. Environ. Contam. Toxicol., 11, 213–216.
2) Jensen, S. (1972): The PCB story. AMBIO, 1, 123–131.
3) Jasson, B., Jensen, S., Olsson, M., Renberg, L., Sundström, G., and Vaz, R. (1975): Identification by GC-MS of phenolic metabolites of PCB and p,p'DDE isolated from Baltic guillemot and seal. AMBIO, 4, 93–97.
4) Brown, J. R., and Chow, L. I. (1975): Comparative study of DDT and its derivatives in human blood samples in Norfolk Country and Holland Marsh, Ontario. Bull. Environ. Contam. Toxicol., 13, 483–488.
5) Kuwabara, K., Yakushiji, T., Watanabe, I., Yoshida, S., Koyama, K., and Kunita, N. (1979): Increase in the human blood PCB levels promptly following ingestion of fish containing PCBs. Bull. Environ. Contam. Toxicol., 21, 273–278.
6) Ando, M. (1982): Studies on the effect of dietary protein and fat content upon DDT metabolism in rat liver. accepted in J. Toxicol. Environ. Health.
7) Sato, R., and Omura, T. (1978): Cytochrome P-450. Tokyo, Kodansha and Academic Press, New York.
8) Ando, M. (1982): Dose-dependent excretion of DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) in rats. Arch. Toxicol., 49, 139–147.
9) Ando, M. (1979): Effects of cadmium on the metabolism of DDT (2,2-bis-(p-chlorophenyl), 1,1,1-trichloroethane) in rats. Environ. Res., 19, 70–78.
10) Leighty, E. G., Fentiman, A. F., and Thompson, R. M. (1980): Conjugation of fatty acids to DDT in the rat: Possible mechanism for retention. Toxicology, 15, 77–82.
11) Mitjavila, S., Carrera, G., and Fernandez, Y. (1981): II. Evaluation of the toxic risk of accumulated DDT in the rat: during fat mobilization. Arch. Environ. Contam. Toxicol., 10, 471–481.
12) Tsujikawa, M., and Kimura, S. (1980): Changes in lipid synthesis in rat adipose tissue during development. J. Nutr. Sci. Vitaminol., 26, 367–374.
13) Dale, W. E., Miles, J. W., and Gaines, T. B. (1970): Quantitative method for determination of DDT and DDT metabolites in blood serum. J. Assoc. Off. Anal. Chem., 53, 1287–1292.
14) Folch, J., Lees, M., and Sloane-Stanley, G. H. (1951): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497–509.
15) Takayama, M., Itoh, S., Nagasaki, T., and Tanimizu, I. (1977): A new enzymatic method for determination of serum choline-containing phospholipids. Clin. Chim. Acta, 79, 93–98.
16) Eggstein, M., and Kreutz, F. H. (1966): Eine neue Bestimmung der Neutralfette in Blutserum und Gewebe. Klin. Wschr., 44, 262.
17) Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., and Fu, P. C. (1974): Enzymatic determination of total serum cholesterol. *Clin. Chem.*, 20, 470-475.

18) Itaya, K., and Ui, M. (1965): Colorimetric determination of free fatty acids in biological fluids. *J. Lipids Res.*, 6, 16-20.

19) Snedecor, W., and Cochran, W. G. (1967): Statistical Methods, 6th Ed. The Iowa State University Press, Ames.

20) Hirosaki, S., and Kobayashi, F. (1979): Some selection methods of criterion variables by prediction sum of squares (PSS) in regression analysis. *Bull. Comput. Cent. Res. Agric. Forest. Fish.*, 15, 45–104.

21) Ando, M. (1974): DDT residue and its effect on the level of liver triglyceride. *Acta Med. Univ. Kagoshima*, 16, 101–106.

22) Esaac, E. G., and Matsumura, F. (1980): Mechanisms of reductive dechlorination of DDT by rat liver microsomes. *Pestic. Biochem. Physiol.*, 13, 81–93.

23) Dinamarca, M. L., Saavedra, I., and Valdes, E. (1969): DDT dehydrochlorinase. 1. Purification and characterization. *Comp. Biochem. Physiol.*, 31, 269–282.

24) Weatherholtz, W. M., Campbell, T. C., and Webb, R. E. (1968): Effect of dietary protein level on the toxicity and metabolism of heptachlor. *J. Nutr.*, 98, 90–94.

25) Hart, L. G., and Fouts, J. R. (1965): Further studies on the stimulation of hepatic microsomal drug metabolism in the rats. *Proc. Soc. Exp. Biol. Med.*, 114, 388–392.

26) Hayes, K. J., Jr. (1975): Toxicology of Pesticides, Williams and Wilkins, Baltimore.