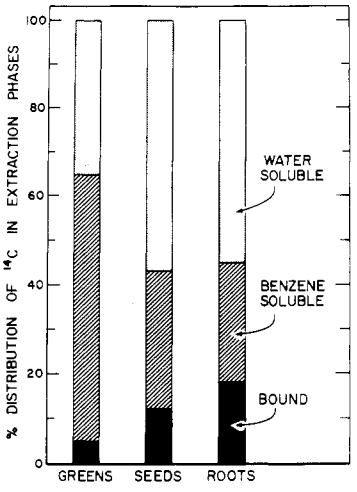


**Figure 2.** Distribution of extractable and bound residues in oat plants, after having grown in soils containing primarily bound residues of methyl [$^{14}$C]parathion.

thus, after the 2-week growing periods, a greater proportion of the residues in the greens were benzene soluble, while a greater proportion of the residues in the roots and seeds were water soluble.

It is interesting to note that the majority of soil-bound residues taken up by earthworms had again become bound in these worms, while most of the residues in the oat plants were extractable.

Data presented herein indicate that soil-bound insecticide residues are not excluded from environmental interaction. These residues could in fact be released from soils and were absorbed by both earthworms and roots of oat plants. Once they had penetrated into the animal or plant tissue, they were translocated and found partially in a bound form or as benzene and water-soluble $^{14}$C compounds. It is, therefore, important to determine how soil-bound pesticide residues can be released and interact in the environment, thus potentially affecting biological systems.

LITERATURE CITED

Bartha, R., *J. Agric. Food Chem.* **19**, 385 (1971).
Flashinski, S. J., Lichtenstein, E. P., *Can. J. Microbiol.* **20**, 399 (1974).
Katan, J., Fuhremann, T. W., Lichtenstein, E. P., *Science* **193**, 891 (1976).
Katan, J., Lichtenstein, E. P., *J. Agric. Food Chem.* **25**, 1404 (1977).
Kükenthal, W., Matthes, E., Renner, M., "Leitfaden fur das Zoologishe Praktikum" 15 ed, Gustav Fischer Verlag, Stuttgart, 1967, p 194.
Lichtenstein, E. P., Fuhremann, T. W., Hochberg, A. A., Zahlten, R. N., Stratman, F. W., *J. Agric. Food Chem.* **21**, 416 (1973b).
Lichtenstein, E. P., Fuhremann, T. W., Schulz, K. R., Liang, T. T., *J. Econ. Entomol.* **66**, 863 (1973a).
Lichtenstein, E. P., Katan, J., Anderegg, B. N., *J. Agric. Food Chem.* **25**, 43 (1977).
Süss, A., Grampp, B., *Weed Res.* **13**, 254 (1973).
Van Alfen, N. K., Kosuge, T., *J. Agric. Food Chem.*, **24**, 584 (1976).

Received for review October 31, 1977. Accepted February 6, 1978. Part of a dissertation submitted by T. W. Fuhremann in partial fulfillment of the requirements of the Ph.D. degree. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by a grant from the Environmental Protection Agency (R 804920). Contribution by Project 1387 from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project 96, entitled "Environmental Implications of Pesticide Usage".

# Fate of Dichlorodiphenyltrichloroethane and Its Metabolites during the Preparation of Fish Protein Concentrate

Virginia F. Stout

The partitioning of dichlorodiphenyltrichloroethane and its metabolites, dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene (ΣDDT = the sum of the three compounds), between protein and lipid fractions during the laboratory production of fish protein concentrate (FPC) from Pacific herring (*Clupea harengus pallasi*) by isopropyl alcohol solvent extraction (IPA) and aqueous phosphate fractionation (APF) was determined. Analysis of the protein fractions of the raw herring flesh showed that myofibrillar proteins contained 0.06 ppm ΣDDT and sarcoplasmic proteins ~0.014 ppm. The FPC was essentially free of ΣDDT (<0.01 ppm) in both cases. The oil from the APF process contained more than three times as much ΣDDT (1.12 ppm) as the IPA oil (0.33 ppm).

The presence of organochlorine pesticides and industrial materials in fishery products (Stout, 1968; Henderson et al., 1969; Jensen, 1966; Risebrough et al., 1968; Jensen et al., 1969) suggested the need for information about the ultimate fate of these substances during the production of fish protein concentrate (FPC), a high-quality, nonperishable food prepared by separation of the water and lipids from the proteinaceous matter of fish. Species proposed for FPC production include many of the pelagic fatty species such as the herrings, menhaden, and anchovies utilized primarily for fishmeal and animal feed production. Chlorinated hydrocarbons are retained in fish to a variable extent during food preparation, that is, baking, poaching, frying, broiling, smoking, and canning (Smith et al., 1973; Reinert et al., 1972; Stout et al., 1970). In general, the concentration of chlorinated hydrocarbons in fish tissue changes in conjunction with the separation of lipids and the denaturation of the proteins. The degree of this change is related to the concentration and distribution of lipids in the animal, the size and the species of

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Utilization Research Division, Seattle, Washington 98112.

fish, and the nutritional and breeding status of the fish (Reinert et al., 1972; Smith et al., 1973).

This paper reports the results of a study on the fate of the insecticide dichlorodiphenyltrichloroethane (DDT) and its metabolites, dichlorodiphenyldichloroethane (TDE) and dichlorodiphenyldichloroethylene (DDE) (ΣDDT = the sum of the three compounds), during the production of FPC from Pacific herring (*Clupea harengus pallasi*) by two methods, isopropyl alcohol solvent extraction (IPA) (Bureau of Commercial Fisheries, 1966) and aqueous phosphate fractionation (APF) (Spinelli, 1971). Also the ΣDDT content of certain protein fractions, sarcoplasmic and myofibrillar proteins (Spinelli and Koury, 1970), was determined.

## EXPERIMENTAL SECTION

Pacific herring (*Clupea harengus pallasi*) caught at Eliza Island in Bellingham Bay, Puget Sound, Wash., were frozen until use. The herring were passed through a Yanagiya flesh separator that mechanically removed skin and bone. The flesh, 91% of the initial weight of whole fish, was divided into two lots.

One lot was extracted (Bureau of Commercial Fisheries, 1966) with azeotropic isopropyl alcohol (IPA) (91% isopropyl alcohol, 9% $H_2O$ v/v) (1.9 kg of herring to 1500 mL of IPA) at 70 °C. The liquid called the first miscella was removed by filtration through a stainless steel screen. Repetition of the process twice yielded the second and third miscellas. The solid fraction, i.e., the protein, was dried in air and then in a vacuum oven at 60 °C to yield IPA FPC. The oil was recovered from the individual miscellas by separation of the oil phase after cooling to room temperature in a separatory funnel. The oil was shaken twice with an equal volume of water and centrifuged to separate the phases.

The second lot of fish was suspended (Spinelli, 1971) in $H_2O$, heated to 80 °C, acidified with $H_2SO_4$ to pH 4.5, treated with sodium hexametaphosphate (1.0–1.5% based on weight of wet fish), acidified with $H_2SO_4$ to pH 3.8, and centrifuged. Twice, the solids were suspended in $H_2O$ at 80 °C and centrifuged. The combined liquids from the three centrifugations were centrifuged to separate oil from water. Twice the solids were suspended in IPA and centrifuged to remove residual water and oil. The solids were then dried to give APF FPC.

In a related experiment, two fractions of proteins were obtained (Spinelli and Koury, 1970). Minced fish tissue was extracted three times with 0.1 M NaCl. The insoluble fraction constituted the myofibrillar proteins. The sarcoplasmic proteins were recovered from the 0.1 M NaCl extracting solution as phosphate complexes using sodium hexametaphosphate as the condensed phosphate and 0.1 M $H_2SO_4$ as the acidulent.

Whole raw fish, separated flesh (without skin and bone), FPC, protein fractions, and oil were analyzed for DDE, TDE, and DDT residues. The AOAC method for determination of chlorinated pesticides in nonfatty foods (AOAC, 1970a) was used for fish, flesh, protein fractions, and FPC by adjusting the sample size to limit the oil content to 2 g and adding $H_2O$ to make a total $H_2O$ volume of 80 mL. The oil was analyzed by extending the backwash procedure of Porter et al. (1970) of the AOAC method for fatty foods (AOAC, 1970b). Oil (3 g), diluted to 15 mL with petroleum ether, was extracted with 30 mL of acetonitrile saturated with petroleum ether. The acetonitrile fraction was back-washed with 15 mL of petroleum ether. Five additional 30-mL portions of acetonitrile were shaken successively with the two petroleum ether solutions. This extensive partitioning process is necessary for fish oils to obtain quantitative extraction of the chlorinated hydrocarbons and to remove interfering substances. The combined acetonitrile fractions were treated with 2% (wt/v) aqueous NaCl and 100 mL of petroleum ether in the usual manner.

Table I. Fate of DDT during Production of Fish Protein Concentrate

| Sample | Process | DDE, ppm | TDE, ppm | DDT, ppm | Σ-DDT,[a] ppm |
|---|---|---|---|---|---|
| Whole raw herring | | 0.044 | 0.012 | 0.041 | 0.097 |
| Separated raw herring[b] | | 0.036 | 0.011 | 0.037 | 0.084 |
| Myofibrillar proteins | APF[c] | 0.034 | *d* | 0.026 | 0.060 |
| Sarcoplasmic proteins | APF | <0.002 | *d* | 0.014 | 0.014 |
| Fish protein concentrate | APF | <0.002 | *d* | <0.004 | <0.006 |
| Fish protein concentrate | IPA[e] | <0.002 | *d* | *d* | <0.002 |
| Oil | APF | 0.497 | 0.144 | 0.481 | 1.12 |
| Oil: miscella 1[f] | IPA | 0.764 | 0.227 | 0.757 | 1.75 |
| Oil: miscella 2 + 3[g] | IPA | 0.151 | *d* | 0.175 | 0.33 |
| Oil: miscella 2 + 3, washed with $H_2O$ | IPA | 0.133 | *d* | 0.151 | 0.28 |

[a] The sum of DDE, TDE, and DDT. [b] Skin and bones removed. [c] Aqueous phosphate fractionation. [d] No peak in GLC; therefore, <0.002 ppm. Values shown as < indicate a peak was present, but below quantifiable level. [e] Isopropyl alcohol process. [f] Mainly phospholipids, not usually considered edible. [g] Triglycerides, potentially acceptable for food use.

The purified extracts were quantitated with a Varian 600-B gas chromatograph with a tritium detector and a 5 ft × 0.125 in. glass column containing a mixture of equal parts of 15% QF-1 on 80–100 mesh Gas-Chrom Q and 10% DC-200 on the same support (Burke and Holswade, 1966). Standards of *p,p'*-DDE, *p,p'*-TDE, and *p,p'*-DDT were obtained from the Environmental Protection Agency, Quality Assurance Section, Research Triangle Park, N.C.; they were at least 99% pure. Standard curves of peak height vs. concentration were used to determine the concentrations of components in the extracts. The sensitivity throughout each run was assured by frequent injections of standard solutions. The quantifiable limit was 0.004 ppm. The average recovery of spiked standards was 85%. The values reported were not corrected for recovery.

## RESULTS AND DISCUSSION

The FPC from both processes was essentially free of ΣDDT [<0.002 (IPA) vs. 0.006 ppm (APF)]. The concentrations of DDE, TDE, DDT, and ΣDDT are shown in Table I. Since the proportions of the major components, DDE and DDT, remained essentially constant throughout processing except in the sarcoplasmic proteins, for simplicity the results are discussed in terms of ΣDDT only. The solvent washings remove the residual water and lipids and, simultaneously, the pesticides to produce an essentially oil- and pesticide-free protein. The myofibrillar proteins contained 0.060 ppm and the sarcoplasmic proteins 0.014 ppm ΣDDT. Although the FPC from the two processes contained such small amounts of ΣDDT that the difference between methods was insignificant, the oil from the IPA process contained only one quarter as much ΣDDT (0.33 ppm) as that from the APF process (1.12

ppm). In the IPA process, a small amount of oil separates from the first miscella. This oil consists predominantly of phospholipids, which are not generally considered edible. This fraction contains a much higher concentration of ΣDDT (1.75 ppm) than the subsequent triglyceride-laden second and third miscellas (0.33 ppm). Apparently pesticides partition preferentially into the first miscella, even though it contains a high proportion of water (roughly 45%), because of the presence of phospholipids, which are potent solubilizing agents. In the APF process, the oil is separated from the protein without benefit of a partial fractionation and, consequently, the edible oil contains essentially all of the pesticides originally present in the fish.

Although this study was conducted with fish containing substantially less DDT than the current FDA interim tolerance of 5 ppm, the production of FPC from fish containing excessive residues of DDT would probably still contain low levels of residues because most of the lipophilic pesticides are partitioned into the oil. Support for this generalization comes from data from our laboratory on a commercial fish meal produced from Atlantic menhaden (*Brevoortia tyrannus*) containing substantially higher residues than the fish used in this study. Fresh menhaden containing 1.1 ppm ΣDDT yielded meal containing 0.36 ppm ΣDDT. That the meal contains even 0.36 ppm ΣDDT is attributable to the residual oil. Menhaden meal may contain more than 10% oil and the fat-soluble pesticides would remain in the meal in direct proportion to the residual fat content. Since FPC produced by the processes described in this paper contains only about 0.1% oil, the ultimate chlorinated hydrocarbon concentration is predicted to be minimal, as was, indeed, found in this study.

Although DDT and its metabolites TDE and DDE were the only chlorinated hydrocarbons studied in this investigation, the behavior of other known environmental pollutants, such as polychlorinated biphenyls and dieldrin, would be similar. FPC production would allow utilization of protein resources from highly polluted waters such as Lake Michigan, where interstate shipment of several species is currently limited by U.S. Food and Drug Administration intervention (Healton, 1975).

ACKNOWLEDGMENT

John Spinelli and Dave Wieg made the APF FPC; Clifford Houle made the IPA FPC. F. Lee Beezhold assisted in the pesticide analyses.

LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis," 11th ed, Washington, D.C., No. 29.002 and 29.009, 1970a, p 475.

Association of Official Agricultural Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., No. 29.010, 1970b, p 480.

Bureau of Commercial Fisheries, Technological Laboratory, College Park, Maryland, U.S. Fisheries Leaflet No. 584, Government Printing Office, Washington, D.C., 1966.

Burke, J. A., Holswade, W., *J. Assoc. Off. Anal. Chem.* **49**, 374 (1966).

Healton, D. C., "A Review of PCB's in the Great Lakes Area", Governors' Great Lakes Regional Interdisciplinary Pesticide Council, Chicago, Ill., Jan 30, 1975, p 19.

Henderson, C., Johnson, W. L., Inglis, A., *Pestic. Monit. J.* **3**, 145 (1969).

Jensen, S., *New Sci.* **32**, 612 (1966).

Jensen, S., Johnels, A. G., Olsson, M., Otterlind, G., *Nature (London)* **224**, 247 (1969).

Porter, M. L., Young, S. J. V., Burke, J. A., *J. Assoc. Off. Anal. Chem.* **53**, 1300 (1970).

Reinert, R. E., Stewart, D., Seagran, H. L., *J. Fish. Res. Board Can.* **29**, 525 (1972).

Risebrough, R. W., Rieche, P., Herman, S. G., Peakall, D. B., Kirven, M. N., *Nature (London)* **220**, 1098 (1968).

Smith, W. E., Funk, K., Zabik, M. E., *J. Fish. Res. Board Can.* **30**, 702 (1973).

Spinelli, J., United States Patent 3 598 606 (1971).

Spinelli, J., Koury, B., *J. Agric. Food Chem.* **18**, 284 (1970).

Stout, V. F., *Bull. Environ. Contam. Toxicol.* **3**, 240 (1968).

Stout, V., Beezhold, F. L., Houle, C. R., Technical Conference on Marine Pollution and its Effect on Living Resources and Fishing, Rome, Italy, 1970, E-106; in abbreviated form in "Marine Pollution and Sea Life", Fishing News (Books) Ltd., Surrey, England, 1972, p 550.

Received for review October 17, 1977. Accepted November 29, 1977. Reference to trade name(s) in this publication does not imply endorsement of commercial product(s) by the National Marine Fisheries Service, NOAA.