number of methoxy groups present. Our tests with myristicin (one methoxy group) and apiol (two methoxy groups) seem to confirm this principle with fruit flies, since apiol was more toxic to these insects than was myristicin and also had a more pronounced synergistic activity with parathion (Figure 8) than did myristicin.

Toxicity to Mammals. Acute intravenous toxicity tests with mice and synthetic *d*-carvone were conducted by the Wisconsin Alumni Research Foundation. Adult male mice of the Swiss-Webster strain, weighing 25-35 g, were used. *d*-Carvone was diluted to a 5% concentration in distilled water and a drop of Tween 80 was added to facilitate the dispersion of the *d*-carvone in water. The test sample was administered *via* a tail vein and the treated animals were observed for a 2-week period. Under these test conditions synthetic *d*-carvone had an approximate intravenous LD_{50} value of 1500 mg/kg.

Table II summarizes the available information pertaining to the intravenous toxicity of the insecticidal dill components. No figures for dill-apiol were obtained because of insufficient amounts of this compound at our disposal.

In summary it can be stated that of the four insecticidal and synergistic components isolated from dill plants, *d*-carvone was the least active. However, at relatively high concentrations it exhibited insecticidal properties by both contact and fumigant action. Moreover, at sublethal insecticidal dosages by itself it exhibited pronounced synergistic effects with the carbamate and organophosphorus insecticides tested. Myristicin, apiol, and dill-apiol from dill roots were considerably more toxic to insects than *d*-carvone and synergized the insecticidal activity at much smaller dosages. Based on the data reported above with standard amounts of carbaryl and parathion, the ratio of insecticide to synergist (apiol or myristicin) at the LD_{50} value was 1:0.005 with carbaryl, 1:2.8 with parathion plus apiol, and 1:4.1 with parathion plus myristicin. Apiol, having one additional methoxy group in comparison to myristicin, appeared to be somewhat more toxic to fruit flies than myristicin, and was slightly more effective as a synergist with parathion, but not with carbaryl.

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Effect of Urea, Diethylstilbestrol, and Type of Diet on the Distribution of Aldrin and Dieldrin Residues in Finished Beef Heifers

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The effect of nutritional regimen on aldrin and dieldrin residue concentrations and distribution was studied in 16 Angus heifers. All heifers were on experiment from 14 days of age to slaughter (18 months) and were fed 1.0 mg of aldrin/kg of body weight daily from 42 days. The heifers were assigned to a factorial arrangement of nutritional regimens to compare urea *vs.* soybean meal, a concentrate *vs.* a forage diet, and diethylstilbestrol (DES) implants *vs.* no DES. The average concentration of aldrin was 7 times greater and that of dieldrin 14 times greater in fat tissue than in organ and muscle tissues. The average tissue concentration of dieldrin was more than 100 times greater than that of aldrin. Among the fat tissues, the concentration of residues was highest in heart and kidney fats, intermediate in external rib and caul fats, and lowest in internal rib, brisket, perianal, and ruffle fats. Nutritional regimens did not affect the distribution of residues among fat tissues but did influence the average residue concentration in beef heifers.

Because of the persistence of chlorinated hydrocarbons in our environment, our food chain will be subject to low levels of residual contamination for many years after more

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biologically labile substitutes are used (Fries, 1970). The select use of persistent pesticides for which there are no substitutes is also a source of low level environmental contamination. A meaningful monitoring system for persistent pesticides depends on knowledge of the distribution of these chemicals in biological systems.

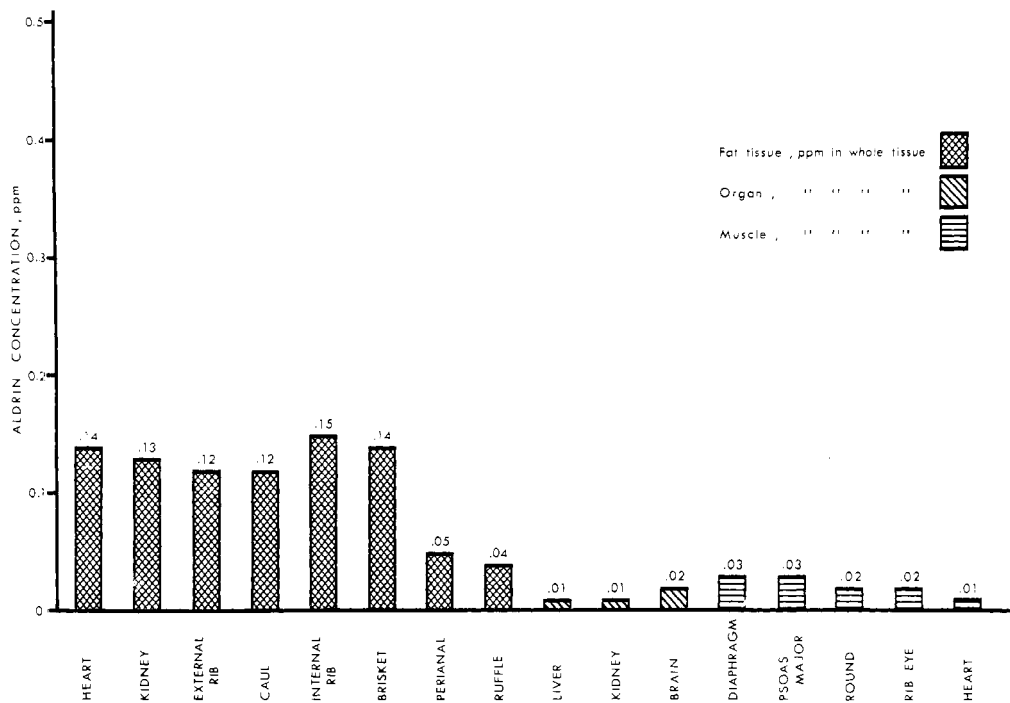Previous research has established that aldrin

Figure 1. Distribution of aldrin in depot fat, organ, and muscle tissues of finished beef heifers after long-term daily exposure to 1.0 mg of aldrin/kg body weight.

(1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo,-exo*-5,8-dimethanonaphthalene) is biologically oxidized to dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene), which, like DDT, accumulates in adipose tissue (Ivey *et al.*, 1961). In sheep, dieldrin was found to accumulate equally in the fat of bone, carcass, adipose, and heart tissues after long-term exposure (Davison, 1970); however, distribution of residues among adipose tissues was not compared. In a previous trial with high levels of oral DDT contamination, Rumsey *et al.* (1967) concluded that residues were evenly distributed among depot fat tissues of beef cattle, although residue concentrations ranged from 664 ppm in heart fat to 456 ppm in external rib fat. In more recent trials, the DDT residue content among depot fat tissues differed significantly (T. S. Rumsey, unpublished data). Small differences in DDT content of depot fat tissues have been suggested in trials with sheep (Harrison and Shanks, 1965) and swine (Carter *et al.*, 1948). There are little data reported concerning the influence that nutritional regimen may have on the concentration of aldrin and dieldrin residues in finished cattle, although Stoewsand and Bourke (1968) have shown that the level of dietary protein influences the storage of dieldrin in rats.

This report describes the effects of DES (diethylstilbestrol), urea, and a concentrate *vs.* a forage diet on the tissue concentrations of aldrin and dieldrin in finished beef heifers after a long-term exposure to aldrin.

EXPERIMENTAL PROCEDURE

Sixteen female Angus calves, 3–10 days of age, were placed in individual pens and bucket-fed whole milk. At 14 days of age the calves were placed on experiment in a 2 × 2 × 2 factorial arrangement of treatments. From 0 to 84 days on experiment, half the calves were given access to a corn-based starter diet containing soybean meal as the supplemental protein and half were given access to the starter diet containing urea as the supplemental nitrogen source. The calves were weaned at 84 days on experiment and at this time half of the calves on each diet were switched to an 87.8% forage diet. The forage diet was sup-

plemented with either urea or soybean meal consistent with the starter treatment. All diets were fed on an isonitrogenous basis. At 168 days and again at 346 days on experiment, half of the heifers in each diet-supplement group were implanted with 12 mg of DES/heifer. Details of the diets, management, and performance (Bond *et al.*, 1971) and physiological changes (Rumsey and Bond, 1972) have been reported.

Starting at 42 days on experiment, all calves were fed aldrin at the rate of 1.0 mg/kg of body weight, daily, for the duration of the experiment. The level of aldrin fed was intermediate between the contamination level of both dieldrin and aldrin expected on treated crops and a level that would be toxic. The pesticide was fed by dissolving aldrin in a mixture of acetone and ethanol and spreading the daily allotment of pesticide over a small portion of diet for each heifer at the morning feeding. After the pesticide was completely consumed (approximately 2 hr), *ad libitum* feeding was resumed.

All tissue samples were collected at slaughter. The fat depots sampled were pericardial (heart), perirenal (kidney), subcutaneous (external rib), omental (caul), internal rib, brisket, perianal, and misenteric (ruffle). Organs sampled were liver, kidney, and brain; muscles sampled were diaphragm, psoas major, semitendinosus (round), longissimus dorsi (rib eye), and heart. Samples were immediately frozen and stored at −10° until analyzed for dieldrin and aldrin.

After the extraction of samples with petroleum ether, the extracts were prepared and analyzed by using a hexane–acetonitrile partitioning and florisil column cleanup as described by Mills (1961) and the gas chromatographic conditions described by Rumsey *et al.* (1969). The percentage of ether extract was determined for all depot fat tissues based on the above petroleum ether extraction.

The data were analyzed statistically by using analysis of variance techniques and Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The distribution of aldrin in body tissues of finished beef heifers is shown in Figure 1. The average concentra-
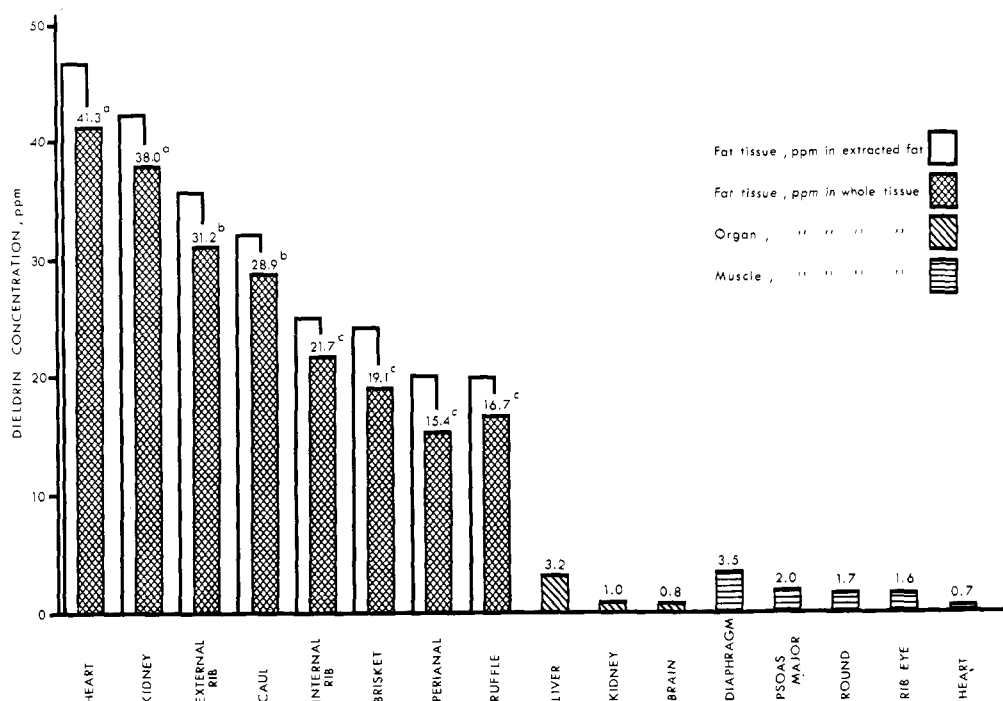
Figure 2. Distribution of dieldrin in depot fat, organ, and muscle tissues of finished beef heifers after long-term daily exposure to 1.0 mg of aldrin/kg body weight. Open bars represent concentration in extracted fat and shaded bars with numerical values represent concentration in whole tissue. Among the fat tissues, numerical values with different superscripts are different ($P < 0.05$).

tion of aldrin in fat tissues was approximately seven times greater than in organ and muscle tissues ($P < 0.01$). In general, the concentration of aldrin was quite low in all tissues and there were no statistical differences for aldrin concentration among tissues within depot fats, organs, or muscles.

The distribution of dieldrin in body tissues is shown in Figure 2. The open bars represent concentration in extracted fat and the shaded bars represent concentration in the whole tissue (numerical values). There was more than a 100-fold higher concentration of dieldrin than aldrin. This represents a marked conversion of aldrin to dieldrin, which is consistent with current knowledge on the metabolism of aldrin (Bann et al., 1956; Ivey et al., 1961).

As is typical of chlorinated hydrocarbon residues, dieldrin accumulated to a greater extent in fat tissue ($P < 0.01$) than in tissues of organs and muscles. Dieldrin concentration was approximately 14 times greater in fat tissue than in organ and muscle tissues. The concentrations of dieldrin within the different organ and muscle tissues were not statistically different ($P < 0.05$), although there were trends. The concentration in liver tissue tended to be higher than in kidney tissue and brain tissue, which agrees with results of Davison (1970). The diaphragm muscle tissue tended to contain higher concentrations of dieldrin than the other muscle tissues. Although there are no other studies that compare dieldrin concentrations in these muscle tissues, a similar trend was noted with DDT residues (Rumsey et al., 1967).

The concentration of dieldrin differed among the fat tissues. The relative differences were similar when calculated on both a whole tissue basis and an extracted sample basis. Heart fat tissue contained the highest concentration of dieldrin, but was not significantly different from kidney fat tissue. Both heart and kidney fat tissues contained a greater concentration ($P < 0.05$) than the other fat tissues, and external rib and caul fat tissues contained higher concentrations ($P < 0.05$) than internal rib, brisket, perianal, and ruffle fat tissues. In previous DDT trials, heart fat tissue contained the highest concentration of residues (Rumsey et al., 1967; T. S. Rumsey, unpublished data). The reasons for the differences among depot

fat tissues are not understood but could be related to blood supply of fat depots and the dynamic state of each tissue.

The distribution of residues was not affected by the dietary treatments or the use of DES in this study. Treatment did not affect the percentage of ether extract in the fat tissue; however, the average concentration of residues for all fat tissues was influenced by treatment (Table I). Because the concentration of aldrin was low, the total residue concentrations reflect the differences in dieldrin concentrations. The average total residue concentration was greater in the concentrate-fed heifers than in the forage-fed heifers (37.0 vs. 25.0, $P < 0.01$), and this difference was more pronounced in the urea groups (32.8 vs. 24.4 for soybean meal and 41.1 vs. 25.5 for urea, $P < 0.05$). The slightly higher residue concentrations in the urea heifers may partly have been a dilution effect. Less fat cover in the urea heifers (Bond et al., 1971) would suggest less total carcass fat into which the residues were partitioned. The greater concentration of residues in the concentrate-fed heifers than in the forage-fed heifers cannot be ex-

Table I. Effect of Type of Diet, DES, and Nitrogen Supplements on the Dieldrin and Aldrin Residue Content of Depot Fat in Beef Heifers

	Residue content of depot fat, ppm[a]		
Treatment[b]	Dieldrin	Aldrin	Total
C – U	43.76[c]	0.26[c]	44.02[c]
C – S	35.13[cd]	0.18[cde]	35.31[cd]
C + U	38.02[cd]	0.20[cd]	38.22[cd]
C + S	30.04[d]	0.06[de]	30.10[d]
F – U	31.81[d]	0.08[de]	31.89[d]
F – S	29.09[de]	0.05[e]	29.14[de]
F + U	18.97[e]	0.11[de]	19.08[e]
F + S	19.64[e]	0.10[de]	19.74[e]

[a] Each mean is an average of 16 samples and represents concentration in extracted fat; means within each column are different ($P < 0.05$) if they do not contain a common letter (c, d, e) in the superscript. [b] Concentrate diet (C), forage diet (F), no DES (–), DES implanted (+), urea supplement (U), soybean meal supplement (S).

plained on a dilution basis and is not understood at this time. Possibly the dietary effect was a result of differences in ruminal metabolism which concurrently influenced pesticide absorption from the gastrointestinal tract. Sink et al. (1972) reported that DDT metabolism was different in the rumen of concentrate vs. forage-fed sheep and Rumsey et al. (1970) reported that the concentration of total DDT residues was different between concentrate vs. forage-fed steers.

The average residue concentration of fat tissue was lower in DES-implanted heifers (26.8 vs. 35.1 ppm, P < 0.01) than in heifers receiving no DES. The difference was primarily due to the low concentrations found in the implanted heifers that were fed forage (19.4 vs. 30.6 for forage plus or minus DES, respectively, and 34.1 vs. 39.7 for concentrate plus or minus DES, respectively, P < 0.01). These differences may reflect an apparent increase in metabolic rate of DES-implanted cattle. A consistently higher heart rate was found in the present trial for DES-implanted heifers than for heifers that received no implants (Rumsey and Bond, 1972) and also in a separate trial with steers (Rumsey et al., 1973). DES may also have a direct effect on certain hepatic enzyme systems (Bitman and Cecil, 1970; Street et al., 1966).

Davison (1970) and Murphy and Korschgen (1970) indicated that dieldrin accumulation plateaus after about 28 weeks of exposure, the plateau concentration being dependent on the dosage level. Assuming that a plateau was reached in the present study (approximately 16 months exposure), the data suggest that the plateau concentration is influenced by type of diet and the use of DES and that both the use of DES and urea interact with type of diet to change the plateau concentration. Apparently the effect

on residue concentration operates across all fat depots, possibly related to overall changes in absorption or metabolic balance, or both, within the animal.

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Effect of Wet Heating on the Physicochemical Properties of Groundnut Proteins[1]

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Defatted groundnut meal was heated with steam at atmospheric pressure and under 1 and 2 kg/cm² pressure. The extractability of the proteins in aqueous solution and DEAE-cellulose chromatographic, gel filtration, gel electrophoresis, and spectral characteristics of the extracted proteins were studied and compared with those of the untreated sample. The sample heated at atmospheric pressure did not differ much from the untreated sample in the measured properties. Samples heated under pressure showed considerable differences. The changes were such as to suggest that pressure heating depolymerized the proteins. The phosphorus and carbohydrate contents of the protein increased due to wet heating.

Heat treatment of oilseed and oilseed meal is an inherent feature of their processing. Application of heat is involved at various stages such as decuticling of kernel, mechanical removal of oil, and desolventization of solvent-extracted meal. Dry roasting (Lee et al., 1969) and wet heating (Coomes et al., 1966) have been reported to reduce the aflatoxin content of groundnut. Several studies have been made on the nutritive value of heat-treated groundnut proteins (Mcosker, 1962; Dunn and Goddard, 1948; Fournier et al., 1949; Balasundaram et al., 1958; Dawson, 1968; Neucere et al., 1972). In general, drastic heat treatment reduces the nutritive value. The solubility of the protein in 10% NaCl and buffer solutions decreases

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(Woodham and Dawson, 1966; Neucere, 1972). Differences in the amino acid composition of the conarachin fraction from heated oilseed meals have been observed (Dawson and Woodham, 1966). Moving boundary electrophoretic patterns also show differences (Cama et al., 1958). Neucere (1972) has reported differences in disc electrophoretic and immunoelectrophoretic patterns.

In the processing of edible groundnut meal, where extrusion cooking is involved, the protein is subjected to heat under steam pressure. Therefore, it was of interest to study the effect of wet heating on groundnut proteins. In this investigation the solubility, DEAE-cellulose chromatographic, gel filtration, polyacrylamide gel electrophoresis, and spectral characteristics of proteins extracted from wet heated groundnut meal have been studied. The phosphorus and carbohydrate contents of the protein were also estimated. The nutritive value of such a groundnut meal