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A Comparison of the Influence of Thermal Processing and Broiling on Naturally Occurring and Spiked Residues of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane and Its Metabolites in Ground Beef

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Raw and processed samples of ground beef containing naturally occurring residues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites were compared with the same ground beef to which an ethanol–water solution of *p,p'*-DDT had been added. Two treatments, a 104 °C steritort process for 342 min and broiling patties for 4 min on each side, were found to cause different losses of pesticides. No difference existed between method of heating with respect to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) in nonspiked or spiked samples. Nonspiked samples decreased in DDT content ($P < 0.01$) on steritort processing and broiling. Spiked samples, however, lost DDT on steritorting ($P < 0.05$) but not during broiling. Total residues (DDE + DDD + DDT) also differed between spiked and nonspiked samples. Results indicated that research with pesticide residues should be conducted on naturally contaminated rather than artificially spiked matrices.

There exist numerous reports with respect to degradation of pesticide residues resulting from thermal processing and preparation of foods. Studies involving heat treatment of foods containing naturally occurring residues as well as foods to which pesticides have been added at residue levels (i.e., "spiked") have been reported.

Initial studies on the influence of heat treatment on pesticide residues were performed with artificially contaminated foods. Tressler (1947) and Britten and Fairing (1950) added various pesticides in solution to batches of fruits and vegetables for thermal processing. Losses after canning ranged from 20 to 95%. Farrow et al. (1966) studied the dehydrohalogenation of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) during processing of spinach. Retail cans of spinach were spiked with an acetone solution of *p,p'*-DDT. Recanning and processing revealed no *p,p'*-DDT but substantial amounts of *p,p'*-DDD were found, indicating a conversion of DDT to DDD.

In an effort to determine which food components were responsible for conversion of DDT to other compounds during thermal processing, Ralls and Cortes (1972) investigated the changes of DDT in aqueous solution during heating with vitamins, amino acids, peptides, casein, yeast nucleic acid, and diphosphopyridine nucleotide. Results demonstrated that pH was not a factor in DDT transformations but amino acids containing a sulfhydryl group promoted hydrogenolysis of DDT to DDD.

Stemp and Liska (1966) and McCaskey and Liska (1967) attributed the reduction of artificial residue contaminates in milk during processing to codistillation with water vapor during thermal processing. Langlois et al. (1964) determined the effects of processing and storage of dairy products on natural and artificial residues of DDT and lindane. Generally, the manufactured products contained the same amount of pesticides as raw milk when expressed

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on a fat basis. The only substantial loss of DDT occurred in manufacture of dry whole milk during spray drying while appreciable amounts of lindane were lost during drum drying. Sterilization produced some degradation of DDT to DDD.

The influence of various methods of preparation on naturally occurring residues in meat and poultry have been reported (Carter et al., 1948; Liska et al., 1967; Ritchey et al., 1967, 1969). More recently Lane et al. (1978) studied the effects of two equivalent thermal processes and two cooking methods on natural residues of DDT and its metabolites in ground beef. A 66-min steritort process at 127 °C was found to cause a smaller decrease (12–17%) of pesticide levels than processing for 342 min at 104 °C (26–27%), cooking by microwave energy (25–27%), or cooking by broiling (28–29%). Both steritort processes and cooking methods resulted in increased levels of DDD and a loss in 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE).

The purpose of this study was to determine the validity of comparing pesticide residue data obtained from thermal treatment of naturally contaminated samples to that of spiked samples. An investigation was made of the effects of two treatments, a low-temperature steritort process and broiling, on samples of ground beef containing natural residues of DDE, DDD, and DDT, to which an ethanol–water solution of *p,p'*-DDT had been added.

EXPERIMENTAL SECTION

Materials. Experimental material for this study was obtained from beef cattle which were fed a daily ration, for approximately 7 months, consisting of 0.9 kg of cottonseed meal, 8.4 kg of corn, and 3 kg of gin trash per head per day (Martin, 1974). The gin trash contained 1.59 ppm DDE, 0 ppm DDD, and 7.25 ppm DDT. After the animals were slaughtered, the total edible portion from each animal consisting of fat and lean tissue was deboned, composited, and ground. Two lots of the naturally contaminated beef were selected for study based on results from pesticide residue analyses of fat from each composite. These two lots were identical with those previously studied by Lane et al. (1978). The selected levels were identified as low (5.02 ppm) and high (8.11 ppm), representing total residues (DDE + DDD + DDT) on a fat basis.

Spiked ground beef samples were obtained by the addition of a *p,p'*-DDT in ethanol–water solution to the low and high samples previously described which contained natural residues. The spiked samples (low-spike and high-spike) were then stored in high density polyethylene bags at −18 °C for 18 months until pesticide residue analysis was performed. Total residue levels (DDE + DDD + DDT) for the spiked samples after freezer storage were 7.88 ppm (low-spike) and 12.81 ppm (high-spike) expressed on a fat basis. To achieve homogeneity, the spiked samples were reground three times, producing a product which exhibited physical characteristics different from those of the once ground beef.

Analytical Methods. Raw, steritort-processed, and broiled samples were lyophilized for 72 h and ground with grandular anhydrous sodium sulfate in a mortar with pestle. Samples were placed in 25 × 100 Whatman cellulose extraction thimbles and refluxed with petroleum ether by Soxhlet extraction for 24 h. The petroleum ether extract was dried to constant weight at ambient temperature. Three grams of extracted fat was weighed into a 10-mL graduated cylinder fitted with a ground glass stopper and made to volume with petroleum ether (distilled in glass). A 1-mL aliquot was pipetted directly onto a 5-g bed of deactivated aluminum oxide (Wolm, neutral grade for column chromatography, activity grade IV) contained in a chromatographic column (Kontes 37.1 × 1.3 cm). Deactivation was attained by addition of 10% water by weight to dried aluminum oxide and shaken until free flowing. The column was eluted with 50 mL of petroleum ether into a 100-mL beaker and then transfered to a Kuderna-Danish concentrator for evaporation to the appropriate volume.

A Barber Coleman Model 5360 pesticide analyzer equipped with a ^{63}Ni concentric-type electron-capture detector was employed for all pesticide residue analyses. A column (6 ft × $^1/_4$ in.) containing a 1:1 mixture of 10% DC-200 and 15% QF-1 on Gas-Chrom Q was maintained at 200 °C for separation and identification of pesticides. Confirmation was established by gas chromatographic analysis on a column containing 10% DC-200. Pesticide standards (DDE, DDD, and DDT) were obtained from the Environmental Toxicology Division, Environmental Protection Agency, Research Triangle Park, NC. All were 99% purity.

Psychophilic Plate Count. Eleven grams of the raw ground beef was blended for 2 min in 99 mL of sterile phosphate buffer from which appropriate dilutions were made for a standard plate count. Pour plates were made containing the diluted sample and hydrated plate count agar (Difco Laboratories, Detroit, MI). Plates were incubated for 10 days at 5 °C prior to counting.

Statistical Analysis. The experiment was performed as a 3 × 2 × 2 factorial arrangement of treatments in a completely randomized design with three methods of cooking (raw, 104 °C Steritort, and broiled), two levels of pesticide residue (low and high), and two levels of spiking (nonspiked and spiked). Four determinations of DDE, DDD, and DDT were made for each treatment combination. The analysis was based on the mean of the determination with the three-way interaction used as the error term (Snedecor and Cochran, 1967).

Steritort Processing Study. Heat penetration data were obtained as previously reported (Lane et al., 1978). A calculated process time at 104 °C (F_0 = 6) of 342 min for once-ground beef did not differ from that of the spiked beef which was ground three times. Four 303 × 406 cans were filled, closed, and processed simultaneously in a steritort for each of the four levels of pesticide: Low, low-spike, high, high-spike. After processing, pesticide residues were determined for all levels as described in the Analytical Methods section.

Broiling Study. Four 25-g patties were broiled simultaneously in a conventional electric oven for each of the four levels of pesticide: low, low-spike, high, and high-spike. Cooking, sampling, and extraction were performed as described in a previous study (Lane et al., 1978).

RESULTS AND DISCUSSION

Means from pesticide residue analyses are contained in Table I as well as means from treatment combinations. The analysis of the treatment means is presented in Table II.

The analysis of DDE residues indicated that there was a significant ($P < 0.05$) difference resulting from methods of cooking with raw ground beef being significantly higher than steritorting or broiling (4.38 ± 0.15, 3.22 ± 0.15, and 3.13 ± 0.15, respectively). The nonsignificant interactions indicated that these effects were consistent for all treatment combinations for DDE.

Analysis of DDD demonstrated that selected residue levels (high and low) were significantly ($P < 0.05$) different. The mean DDD from spiked samples, 3.70 ± 0.16, was

Table I. Pesticide Levels in Raw and Cooked Ground Beef[a]

		pesticide residue means, ppm fat basis						
		nonspiked			spiked			
residue	method of cooking	low	high	marginal means	low	high	marginal means	method means
DDE	raw	3.21	5.56	4.39	3.28	5.47	4.38	4.38
	104 °C steritort	2.93	3.86	3.39	2.37	3.73	3.05	3.22
	broil	2.18	3.71	2.95	2.50	4.13	3.32	3.13
	treatment mean	2.77	4.38	3.58	2.72	4.44	3.58	3.58
DDD	raw	0.27	0.74	0.51	2.53	5.57	4.05	2.28
	104 °C steritort	0.85	1.30	1.08	2.86	4.97	3.92	2.50
	broil	0.76	1.39	1.08	2.34	3.94	3.14	2.11
	treatment mean	0.63	1.14	0.89	2.58	4.83	3.70	2.30
DDT	raw	1.54	1.81	1.65	2.06	1.76	1.91	1.79
	104 °C steritort	0.05	0.07	0.06	0.04	0.09	0.07	0.06
	broil	0.30	0.21	0.26	1.71	1.66	1.69	0.97
	treatment mean	0.63	0.70	0.66	1.27	1.17	1.22	0.94
total	raw	5.03	8.11	6.57	7.88	12.81	10.35	8.46
	104 °C steritort	3.82	5.23	4.52	5.27	8.78	7.03	5.78
	broil	3.42	5.31	4.36	6.54	9.73	8.14	6.21
	treatment mean	4.09	6.21	5.15	6.56	10.44	8.51	6.82

[a] $s\bar{x}$ = 0.15 (DDE), 0.40 (DDD), 0.18 (DDT), 0.25 (total).

Table II. Analysis of Pesticide Residues[a]

	degrees of	mean square for residue			
source	freedom	DDE	DDD	DDT	total
level (low vs. high)	1	8.300**	5.741*	0.001	27.573**
spike (spiked vs. nonspiked)	1	0.000	23.801**	0.930*	34.239**
method of cooking	2	1.944*	0.151	2.996**	8.302**
level * spike	1	0.012	2.253	0.021	2.142*
level * method	2	0.324	0.111	0.003	0.718
spike * method	2	0.126	0.548	0.586*	0.581
error	2	0.023	0.161	0.031	0.064

[a] (*) Significant at the 0.05 level; (**) significant at the 0.01 level.

significantly higher ($P < 0.01$) than those from nonspiked samples, 0.89 ± 0.16, indicating that the major portion of the *p,p'*-DDT–ethanol solution used in spiking the ground beef had been converted to *p,p'*-DDD during frozen storage for 18 months.

Similarly, Ecobichon and Saschenbucker (1967), Castro (1964), and Deloach and Hemphill (1971) have reported that DDT was reduced to DDD in the presence of metal porphyrin complexes which were rapidly oxidized. Also, Plummer et al. (1968) and Kallman and Andrews (1963) observed the reductive dechlorination of DDT to DDD by action of microorganisms. Conversion of DDT to DDD in this study must have been the result of a chemical redox reaction in the presence of hemoglobin in blood or myoglobin in tissue since a psychrophilic plate count (10 days at 5 °C) on the raw ground beef indicated no microbial activity.

Method of cooking did not significantly ($P > 0.25$) reduce DDD residue levels. The nonsignificant interactions for DDD indicated that the above effects were consistent in all treatment combinations. The level by spike interaction, however, approached significance ($P < 0.10$), resulting from a larger difference between high-spike and low-spike (4.83 ± 0.23 vs. 2.58 ± 0.23) than between high-nonspike and low-nonspike samples (1.14 ± 0.23 vs. 0.63 ± 0.23).

The analysis of DDT residues indicated that no difference ($P > 0.25$) existed between pesticide levels (high vs. low); however, a significant ($P < 0.05$) difference resulted from spiking and method of cooking. Since the spike by method interaction was significant ($P < 0.05$), the effect of method of cooking was not the same in spiked and nonspiked samples. In the nonspiked samples, both heat treatments significantly reduced DDT levels while only the steritort treatment significantly reduced the DDT level in spiked samples. The observations for nonspiked samples were consistent with those reported earlier by Lane et al. (1978). Means from the spiked samples, however, indicated that DDT was not rendered with fat from tissues during broiling as were naturally occurring residues (Liska et al., 1967; Young et al., 1969; Lane et al., 1978).

Analyses for total residues showed that a significant reduction ($P < 0.01$) occurred for cooked samples of ground beef which was consistent for all treatment combinations. Although level and spiking resulted in significantly ($P < 0.01$) higher residue contents, there was a significant interaction between level and spiking. Treatment means for high and low levels differed by 2.21 ± 0.25 in the nonspiked samples. The difference between spiked samples was significantly ($P < 0.05$) greater than for nonspiked samples. The difference between the logs of the means, however, was not significantly different ($P < 0.07$). This observation indicated that the samples were degrading at an exponential rate.

The extremely small mean square for the level effect on DDT and correspondingly high mean squares for DDD and DDE indicated that the major portion of the original *p,p'*-DDT was converted to *p,p'*-DDD and *p,p'*-DDE during storage at −18 °C for 18 months. The significant spike effect on DDT and DDD, while not on DDE, indicated that added DDT did not degrade at the same rate as naturally contaminated samples.

Similarities were demonstrated between naturally occurring and spiked residues in ground beef with respect to the influence of heating methods on DDE and DDD (see marginal means, Table I). DDT residues, however, were

influenced differently by cooking method depending on whether the samples were spiked or nonspiked. Naturally occurring residues of DDT were reduced significantly ($P < 0.01$) by the steritort process and by broiling with a concomitant increase in DDD as a result of dechlorination of DDT. These results are in agreement with earlier reports by Thronburg (1963), Langlois et al. (1964), Ott and Gunther (1964), Richey et al. (1967, 1969), Elkins et al. (1972), and Lane et al. (1978). Spiked samples, on the other hand, decreased in DDT content during the steritort process, but not during broiling. Moreover, there was no increase of DDD levels in spiked samples (low and high) when subjected to heat treatment. These findings may be a result of a greater tendency in spiked samples for further degradation of DDD to compounds which are not detected by electron capture gas chromatography.

Results for total residues also differed between spiked and nonspiked samples with respect to the 104 °C steritort process and broiling. There was no difference in total residue content between the two heat treatments with the ground beef containing naturally occurring residues, but there was a significant difference ($P < 0.05$) in the material which contained the spiked DDT when subjected to the steritort method and broiling. These data point up the necessity for studying samples containing residues from natural contamination such as feeding, rather than from artificially contaminated materials.

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Fate of Selected Fungicides in a Terrestrial Laboratory Ecosystem

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The disposition of ^{14}C-labeled pentachloronitrobenzene (PCNB), two of its analogues pentachlorophenol (PCP) and hexachlorobenzene (HCB), and captan was examined as seed-protectant coatings in a terrestrial microcosm chamber (TMC) in comparison to a reference compound, dieldrin (HEOD). The TMC contained a synthetic soil medium, agricultural crops, numerous invertebrates, and a gravid gray-tailed vole (*Microtus canicaudus*). Captan and PCP degraded more rapidly in soil and plants than did PCNB, which was degraded somewhat more quickly than HCB and HEOD. By 45 days postplanting, total soil residues (parent + metabolites + bound residues) had declined from a nominal 3 ppm to about 1 ppm for all chemicals but captan (0.26 ppm), while parent residues became undetectable for PCP and captan. Residues in invertebrates and the vole were low for all chemicals, but HCB and HEOD showed ecological magnification indices (EMs) in the vole of 17.7 and 2.1, respectively, as compared to 1.2 for PCNB. None of the chemicals adversely affected vole survival, although the PCNB-exposed vole had no surviving pups. Only HEOD greatly decreased cricket survival.

As part of a continuing effort to enhance man's knowledge of the fate and effects of chemicals introduced into the environment, the U.S. Environmental Protection Agency has embarked upon a program which uses simulated ecosystems as an intermediate test between bench and field studies. The data presented herein reflect the second in a series of studies in which representatives from broad categories of pesticides were examined in a system developed at the U.S. EPA's Corvallis Environmental Research Laboratory (CERL). The system used in this study is almost identical with one described earlier (Gillett and Gile, 1975, 1976).

The chemicals examined were or are commonly used as fungicides: pentachloronitrobenzene (PCNB), two of its analogues [pentachlorophenol (PCP) and hexachlorobenzene (HCB)] and captan [*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide]. These were compared with the insecticide dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-*endo*,*exo*-dimethanonaphthalene) which serves as a reference compound in our system.

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