Thermostability of Organophosphate Pesticides and Some of Their Major Metabolites in Water and Beef Muscle[†]

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The thermostability of six organophosphate pesticides (OPs) and some of their major metabolites in water and beef muscle was investigated. Ten grams of lean (7.7% fat) beef and water samples, fortified with mixtures of OPs or their metabolites to 50 ppm concentration, were heated in a water bath at 70 or 80 °C. The compounds in water and those extracted from meat samples were analyzed by HPLC. Retrieval of parent OPs ranged between 64.5 and 98.4% from raw meat, between 30.0 and 87.4% from cooked meat, and between 10.6 and 107.2% from water. Recoveries of primary (oxon) and secondary (alcohol) metabolites varied between 56.0 and 103.0% from raw meat, between 23.9 and 81.0% from cooked meat, and between 72.7 and 105.0% from water. Alcohol metabolites from heat-degraded oxons in water and beef were identified by HPLC and GC–MS. The OPs and their metabolites were thermally degraded, but notable amounts were still present in the heated water and beef muscle.

INTRODUCTION

Organophosphates (OPs) have been used extensively as insecticides, fungicides, herbicides, and animal pesticides for more than four decades and currently account for over 50% of pesticide control agents for beef cattle. The use of these chemicals in place of organochlorine pesticides is attributed to their less persistent characteristics in the environment (Dikshith, 1991). While the degradation of organophosphate pesticides in the environment has been well documented, very little information exists on these compounds once they are in food products. In addition, there is no information that has evaluated the production of potentially toxic OP metabolites, even though their levels are regulated in many meat products (CFR, 1991).

To our knowledge only one study has been performed that has evaluated the disposition of OPs in a food product (Ishikura et al., 1984); the initial studies focused on the thermal decomposition of the parent compounds in water and during cooking in boiled rice. Additional experiments were carried out to attempt to remove the OPs from the rice by steam distillation. The results obtained from this research indicated that some OPs were heat-degraded, whereas others were readily removed by steam distillation from the aqueous medium. Furthermore, several of the OPs such as parathion, diazinon, and ronnel were thermally stable, while others were readily distilled from the residual aqueous solution. However, the fate of possible metabolites and the production of byproducts was not investigated.

Information on the thermal decomposition of OPs in meat and other food products is still very limited, though environmental studies do provide some insight into the chemical degradation mechanisms. A number of studies have reported on the mechanisms and the susceptibility of the OPs and their metabolites to be degraded in the environment. Abou-Assaf and Coats (1987) investigated the degradation of [¹⁴C]isofenphos in soil under different pHs, temperatures, and moisture. This research showed

that the OP was more degraded at 35 °C than at 25 or 15 °C. In addition, the above investigation revealed that the formation of isofenphos oxon, the primary metabolite, was greatly increased at 35 °C as compared to 25 and 15 °C. Similarly, other workers studied the hydrolysis of methyl parathion in a flooded soil and reported that the OP disappeared more rapidly at 35 °C than at 25 °C (Sharmila et al., 1989). Pusino et al. (1988) studied the catalytic decomposition of quinalphos on homoionic clays and concluded that hydrolysis activated by copper, sodium, and potassium montmorillonites was the principal pathway for its degradation in the environment. The results also indicated that the hydrolysis of the pesticide may involve two different mechanisms, depending on the nature of the exchangeable cations. Allmaier and Schmid (1985) studied the fate of bromophos and iodofenphos and their major metabolites in the environment and found that photodecomposition was a major contributor to their hydrolysis. The studies performed on the OPs and their metabolites in environmental and aqueous systems showed that the OPs can be oxidized and the primary metabolites hydrolyzed by several mechanisms.

There is little, if any, information on the effects of cooking/thermal processing on the stability of OP residues and many of the metabolites in beef. The purpose of this work was to investigate the thermostability of six parent OPs and some of their primary and secondary metabolites in water and beef muscle. The selected OPs are approved animal pesticides that are commonly used on beef. Parathion was included in our research because of its probable occurrence in animal tissues. In addition, there are a number of studies related to the oxidative and hydrolytic degradations of this compound in aqueous matrices (Gomaa and Faust, 1972). The stability of these compounds was studied under both normal cooking temperature and excessive heat treatments.

EXPERIMENTAL PROCEDURES

Chemicals. Ronnel, fenthion, parathion, paraoxon, and famphur standards were purchased from ChemService, Inc. (West Chester, PA). Ronnel oxon was supplied by Dow Elanco (Midland, MI), and fenthoxon was obtained from Mobay Corp. (Kansas City, MO). Stirofos was purchased from Supelco, Inc. (Bellefonte, PA), 3-methyl-4-(methylthio)phenol was obtained

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from Sigma Chemical Co. (St. Louis, MO), and both p-nitrophenol and 2,4,5-trichlorophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Optima grade ethyl acetate, methanol, and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA), and deionized water was obtained from a Sybron/Barnstead PCS unit (Barnstead/Thermolyne, Inc., Dubuque, IA).

Sample Preparation. Lean beef (7.7% fat) purchased from a local supermarket was ground in an electric food grinder to assure sample homogeneity. Then the meat sample was analyzed for moisture using the air oven method (AOAC, 1990) and for fat content by the Soxhlet extraction method procedure (AOAC, 1990) and frozen at −13 °C until used. To measure the pH of the meat samples, 10 g of sample was thoroughly mixed with 15 mL of deionized water in a 50-mL beaker. Then the pH was measured with a Markson Science Inc. (Phoenix, AZ) pH meter.

To test the thermostability of the OPs and their metabolites, 10-g samples of the ground tissue were weighed into 60-mL bottles. The samples were spiked with either the parent OPs or the primary or secondary metabolite mixtures using 1000 ppm stock solutions to reach a concentration of 50 ppm. Each sample was then thoroughly mixed for several minutes, and the bottles were capped and allowed to sit for 1 h at room temperature. The sample bottles were heated in a water bath at 70 °C for 1 or 2 h or at 80 °C for 1 h and then cooled to room temperature, and the compounds were extracted. The parent OPs and their primary and secondary metabolites were also extracted from raw meat samples prepared as previously described.

The thermostability of the parent OPs and their metabolites in water was tested by separately dissolving mixtures of parent OPs, oxon, and alcohol metabolites in deionized water in 5-mL vials to reach concentrations of 50 ppm. The high levels of the pesticide residues and metabolites (about 10-fold higher than the legal limits) for fortification of the meat samples were chosen to enable analysis and verification of the degradation products. Since the degradation process was probably a chemical process, it was assumed that the results would be applicable to lower levels usually found in commercial meat products. The vials were capped and heated in a water bath as previously described. Three replicates were performed for each heat treatment. Unheated water control samples were also prepared in triplicate with the parent OPs and their primary and secondary metabolites as in the previous sample preparation.

Extraction and Cleanup Procedure. The OPs and their metabolites were extracted from the meat matrix using a modified version of the procedure of Ioerger and Smith (1993). The modification consisted of washing the blender with an additional 5 mL of extraction solvent, ethyl acetate/methanol (90:10 v/v), in the final step. Next, the combined filtrates were brought to a final volume of 50 mL with the solvent. Finally, the total extract was frozen at −13 °C overnight and filtered through Whatman No. 2 paper to remove lipid material. The OPs and their metabolites were extracted from the raw meat samples according to the same modified procedure.

To have enough material for GC–MS analysis, three samples of the oxons, heated in water at 70 °C for 1 h, were combined and evaporated to dryness under nitrogen in a heat block. The same procedure was also performed with the oxon samples heated in water at 70 °C for 2 h and at 80 °C for 1 h. Similarly, the oxon samples in meat were combined according to each heat treatment as previously described. The residues obtained from the three heat treatments in both water and meat were dissolved in 1.0 mL of methanol, and 1.0 μL was injected into the GC–MS.

High-Performance Liquid Chromatography (HPLC). The separation and analysis of the parent OPs and their metabolites were achieved with a Hewlett-Packard Model 1090M Series II HPLC (Palo Alto, CA) equipped with a DR5 binary solvent delivery system and a scanning UV–visible photodiode array detector. Samples were manually injected using a Rheodyne Model 7125 valve fitted with a 20-μL loop. System control, data storage, and processing were achieved by a Hewlett-Packard ChemStation (Pascal series) using software versions HP79988A, rev. 5.22, and HP79997A, rev. 5.20. Separations were performed on a 250 mm × 4.6 mm Bio-Sil, C₁₈ HL-90 column (Bio-Rad Laboratories, Richmond, CA) with a particle size of 5 μm. The guard column consisted of a 10 mm × 4.6 mm cartridge packed with Econo-Sil C₁₈ 5 μm (Alltech Associates, Inc., Deerfield, IL).

Table I. UV Wavelengths at Which the Parent Organophosphates and Their Metabolites Were Monitored during HPLC Analysis

parent compound	metabolite		wavelength, nm
	oxon	alcohol	
chlorpyrifos			202
famphur			230
fenthion	fenthoxon	3-methyl-4-(methylthio)phenol	250
parathion	paraoxon	p-nitrophenol[a]	274
ronnel	ronnel oxon	2,4,5-trichlorophenol	202
stirofos[b]			207

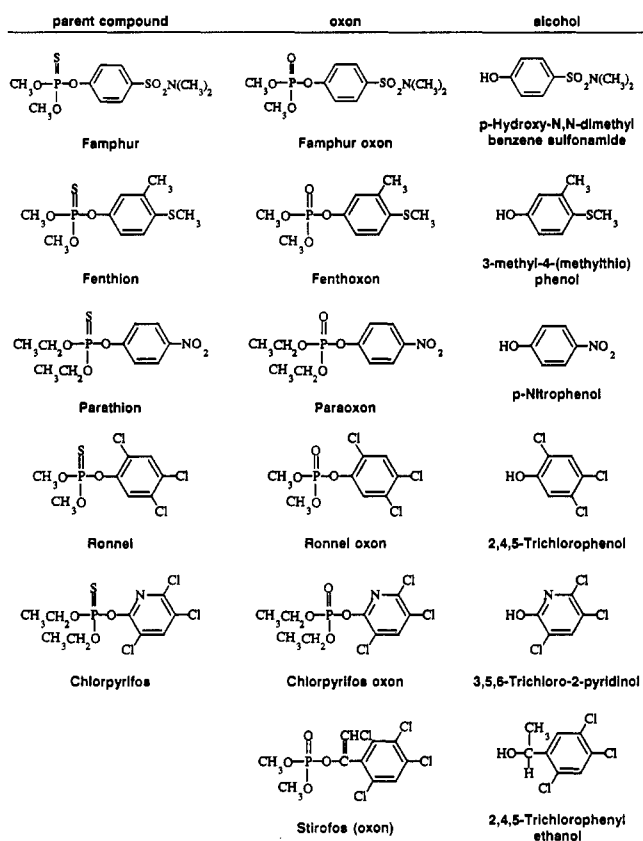[a] Monitored at 314 nm. [b] The parent compound is in the oxon form.

Figure 1. Chemical structures of the parent organophosphate pesticides and their primary (oxon) and secondary (alcohol) metabolites addressed in this study.

Each sample was filtered through a 0.45-μm nylon syringe filter (Alltech Associates) before injection.

Liquid Chromatographic Conditions. The parent OPs and their metabolites were separated using a step-gradient mobile phase according to the method of Ioerger and Smith (1993). The initial mobile-phase composition was acetonitrile/water (55:45 v/v), at a flow rate of 1.0 mL/min, which was held constant from 0.00 to 11.00 min. At 11.10 min, acetonitrile was increased to 65%. From 11.10 min, the gradient was programmed linearly to a final composition of acetonitrile/water (70:30 v/v) at 32.00 min. Each sample run was followed by a postrun of 10 min (acetonitrile/water, 55:45 v/v), and 100% of acetonitrile was run through the column at a flow rate of 1.0 mL/min for 20 min. Absorption spectra of the OPs and their metabolites were obtained by scanning wavelengths between 190 and 350 nm at 2-nm increments. Individual wavelengths were monitored on the basis of spectral library sensitivity and/or interference considerations. Table I lists the parent OPs, their metabolites, and the UV absorbance wavelengths at which these compounds were monitored. The chemical structures of the parent compounds and metabolites are presented in Figure 1.

Gas Chromatography–Mass Spectrometry (GC–MS). The GC–MS system was a Hewlett-Packard Model 5890A GC

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interfaced with a HP 5970 mass-selective detector. The compounds were separated on a Hewlett-Packard 5% phenyl methyl silicone cross-linked fused silica capillary column (12 m × 0.2 mm i.d. × 0.33 μm film thickness, HP-5) using helium carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 40 to 175 °C at a rate of 10 °C/min and held for 2 min. Then the temperature was raised to 250 °C at a rate of 15 °C/min and held for 5 min. The transfer line was maintained at 280 °C, while the source temperature was 200 °C, and the injection temperature was set at 250 °C. Positive ion spectra were obtained by electron ionization at 70 eV with an electron multiplier setting of 2200 eV. Data were acquired with a HP 59970C ChemStation. The GC–MS analysis was carried out to identify the alcohol metabolites resulting from the thermal degradation of the oxons in water and beef muscle. Tentative identification of the heat-induced secondary metabolites was achieved using Probability-Based Matching (PBM) software of the spectra with the NBS spectral library.

Recovery Analysis and Identification. Recovery studies were performed at the 50 ppm fortification level. Three replicate samples were prepared for the parent OPs and the primary and secondary metabolites. Each replicate was injected twice into the HPLC. Similarly, two injections were made from each replicate of triplicate standard solutions containing a concentration of 50 ppm of all the OPs and their metabolites. Average integrated peak areas of standards and samples were used to calculate the concentrations of each compound. Compound identification by retention time and spectral absorbance profile was accomplished via a system spectral matching algorithm based on a user-generated library. This user library was created from injection of standards into the HPLC. Identification involved matching the results of the HPLC analysis with those of the GC–MS or comparing the UV spectra and the retention times of the standards with those of the unknown compounds.

Statistical Analysis. A completely randomized design was used to compare the means of the recoveries for each compound from the meat and water systems with and without heat treatment. The means of the recoveries of the OPs and the metabolites were separated according to a Fisher least significant difference test (with $p < 0.05$) using Statistical Analysis System (SAS), version 6.07 (SAS Institute Inc., Cary, NC). The objective of this statistical analysis was to compare the stability of the OPs and the primary and secondary metabolites in water and beef muscle under all of the heat treatments.

RESULTS AND DISCUSSION

Preliminary studies showed that all of the OPs and their metabolites possessed some degree of volatility. Thus, it was decided to perform all treatments in closed containers to minimize the volatile losses. Volatility of the OPs has been reported by other researchers who investigated the dissipation and persistence of chlorpyrifos in littoral enclosures (Knuth and Heinis, 1992). Similarly, other co-workers took advantage of the volatility of parathion and ronnel for their removal from an aqueous solution by steam distillation (Ishikura et al., 1984).

As part of the preliminary experiments, the meat and water samples used in this investigation were analyzed and did not show the presence of any residue of the parent OPs or their metabolites. The pH of the raw meat was 5.5, which typically increased to about 5.8 following the heat treatments. This slight increase probably did not affect the stability of the compounds. The stability of the OPs under acidic conditions, in the pH range of 1–6, has been previously reported (Eto, 1974). The samples that were dissolved in water and subjected to the same heat treatments maintained a pH (6.2) similar to that of the meat samples.

The thermostability of the OPs and the metabolites was evaluated at 70 °C, which corresponds to a well-cooked temperature for most meat and meat products. While the cooking time depends on the type of meat product, we

Table II. Effect of Heat on the Recoveries of Parent Organophosphates from Water Spiked with 50 ppm and Heated in a Closed Vial at 70 and 80 °C[a]

organophosphate	unheated water %	CV	70 °C/1 h %	CV	70 °C/2 h %	CV	80 °C/1 h %	CV
famphur	105.0[a]	6.3	107.2[a]	6.0	106.4[a]	1.7	105.0[a]	4.4
fenthion	44.6[a]	6.5	40.0[b]	21.1	42.7[c]	5.7	41.2[c]	8.5
parathion	46.8[a]	5.1	38.6[b]	20.8	43.0[c]	5.2	42.4[c]	7.5
stirofos	96.7[a]	3.7	95.7[a]	6.4	94.5[a]	3.0	95.6[a]	1.2
chlorpyrifos	20.0[a]	12.0	10.6[b]	18.7	15.2[c]	7.8	13.0[d]	14.1
ronnel	25.0[a]	9.3	24.5[a]	16.7	21.0[b]	12.0	14.1[c]	16.5

[a] Means of three replicates in the same row marked by different letters are significantly different ($p < 0.05$). CV, coefficient of variation.

Table III. Effect of Heat on the Recoveries of Parent Organophosphates from Meat Samples Spiked to 50 ppm and Heated in a Closed Vial at 70 and 80 °C[a]

organophosphate	uncooked meat %	CV	70 °C/1 h %	CV	70 °C/2 h %	CV	80 °C/1 h %	CV
famphur	95.6[a]	2.0	87.4[b]	4.4	66.4[c]	6.3	69.5[d]	3.6
fenthion	98.4[a]	2.1	73.5[b]	2.4	54.4[c]	1.5	68.6[d]	3.2
parathion	91.1[a]	1.7	75.5[b]	2.3	59.3[c]	1.3	53.7[d]	4.0
stirofos	93.2[a]	2.9	45.6[b]	3.3	30.0[c]	9.6	40.6[d]	7.2
chlorpyrifos	78.0[a]	2.9	69.7[b]	3.8	64.2[c]	5.2	71.1[b]	2.7
ronnel	64.5[a]	5.3	55.0[b]	4.0	45.4[c]	9.7	50.3[d]	1.7

[a] Means of three replicates in the same row marked by different letters are significantly different ($p < 0.05$). CV, coefficient of variation.

chose 1 h as the general cooking time. The temperature of 80 °C was chosen to evaluate the stability of the OPs and the metabolites during excessive heat treatment.

Table II summarizes the recoveries of the parent OPs in unheated and heated water samples. Only chlorpyrifos and ronnel exhibited any type of major degradation. There were small, but significant, losses of fenthion and parathion at all treatment times and temperatures. Overall, the recoveries were lower with the mildest treatments. Why this would occur is unknown, though experimental variation is a possibility. Generally, it appears that most of the OPs studied showed considerable stability even when heated for 2 h at 70 °C.

The results presented in Table III summarize the thermal degradation of the parent OPs in the meat system. When these compounds were heated in meat at 70 °C for 1 h, their recoveries, ranging between 45.6 and 87.4%, were significantly lower than those of the raw meat. As the heating time was increased to 2 h at the same temperature, the recoveries of the parent OPs dropped further.

These observations indicate that the meat system was enhancing the thermal breakdown of famphur and stirofos. These two parent compounds (famphur and stirofos) were heat stable in the water system, with recoveries greater than 94%. The data in Table III show that heating the parent OPs in meat at 70 °C for 2 h induced greater decomposition than heating them for 1 h at the same temperature. Similar observations have been reported by Abou-Assaf and Coats (1987), who studied the degradation of [14C]isofenphos, a broad-spectrum organophosphate soil insecticide, under different pHs, temperatures, and moisture contents. These authors demonstrated that 35 °C was more efficient than 25 °C in inducing the degradation of the compound. Another investigation carried out on several OPs and some of their metabolites reported that parathion and ronnel were thermally stable when cooked in a mixture of rice and water for 18 min

Table IV. Effect of Heat on the Recoveries of Organophosphate Metabolites from Water Spiked to 50 ppm and Heated in a Closed Vial at 70 and 80 °C[a]

metabolite	unheated water		70 °C/1 h		70 °C/2 h		80 °C/1 h	
	%	CV	%	CV	%	CV	%	CV
paraoxon	105.0[a]	3.3	104.0[a]	1.5	105.0[a]	2.2	101.1[b]	5.9
fenthoxon	104.7[a]	2.5	104.0[a]	7.2	104.0[a]	3.0	103.0[a]	2.9
ronnel oxon	105.0[a]	6.3	88.4[b]	4.2	80.9[c]	5.3	89.0[b]	0.6
stirofos	100.4[a]	3.7	88.6[b]	6.4	81.0[c]	2.6	88.7[b]	5.7
p-nitrophenol	105.0[a]	2.5	99.5[b]	2.9	96.3[c]	2.7	99.8[b]	1.7
2,4,5-trichlorophenol	103.4[a]	2.6	94.2[b]	9.5	72.7[c]	9.2	73.1[c]	8.0
3-methyl-4-(methylthio)phenol	94.0[a]	4.3	94.3[a]	4.0	92.2[a]	3.0	85.4[b]	2.4

[a] Means of three replicates in the same row marked by different letters are significantly different ($p < 0.05$). CV, coefficient of variation.

Table V. Effect of Heat on the Recoveries of Organophosphate Metabolites from Meat Samples Spiked to 50 ppm and Heated in a Closed Vial at 70 and 80 °C[a]

metabolite	uncooked meat		70 °C/1 h		70 °C/2 h		80 °C/1 h	
	%	CV	%	CV	%	CV	%	CV
paraoxon	103.0[a]	3.3	73.9[b]	2.0	67.0[c]	2.1	75.3[b]	7.3
fenthoxon	88.0[a]	4.3	67.1[b]	2.6	55.0[c]	2.0	49.0[d]	12.8
ronnel oxon	90.8[a]	4.2	46.1[b]	3.2	40.6[c]	3.5	29.1[d]	18.0
stirofos	93.7[a]	2.9	45.6[b]	3.3	30.0[c]	9.6	40.6[d]	7.2
p-nitrophenol	93.6[a]	4.4	80.0[b]	5.4	81.0[b]	2.8	74.3[c]	6.3
2,4,5-trichlorophenol	56.0[a]	9.2	49.3[b]	5.7	49.6[b]	4.0	23.9[c]	6.5
3-methyl-4-(methylthio)phenol	70.0[a]	10.0	58.5[b]	1.4	60.1[b]	1.3	39.2[c]	20.9

[a] Means of three replicates in the same row marked by different letters are significantly different ($p < 0.05$). CV, coefficient of variation.

(Ishikura et al., 1984). However, our results did not show these two compounds to be heat stable in the water or meat system. The differences between our findings could be attributed to the longer times of exposure (1 and 2 h) used in our investigation.

The results in Table IV illustrate the effect of heat and time on the recoveries of the OP metabolites heated in water. While most of the corresponding parent compounds were poorly recovered from the unheated water samples, all of the metabolites had recoveries above 94% except for 3-methyl-4-(methylthio)phenol. When the oxon mixtures were heated in water, we noted the presence of some secondary (alcohol) metabolites such as 2,4,5-trichlorophenol, derived from the hydrolysis of ronnel oxon. In addition, (2,4,5-trichlorophenyl)ethanol, the alcohol metabolite of stirofos, was tentatively detected in the heated oxon samples. The formation of these secondary metabolites was slightly greater when ronnel oxon and stirofos were heated in water for 2 h at 70 °C than for 1 h at the same temperature.

From a chemical point of view, the heat stability of paraoxon and fenthoxon noted in the water system at 70 °C was expected. The oxons are already in the oxidative state and will rarely undergo further oxidative reactions. Therefore, the only compounds susceptible to be oxidized are the parent OPs with a sulfur atom bound to the phosphate group. Replacement of this sulfur atom by an oxygen is obtained through oxidation, and this leads to the formation of the oxons. The resistance of paraoxon to oxidative degradation was previously reported (Gomaa and Faust, 1972). From a chemical structural standpoint, we would also expect the oxons such as ronnel oxon, fenthoxon, and stirofos to resist oxidative decomposition due to the fact that they all exist in the oxidative state.

The alcohol metabolites 2,4,5-trichlorophenol and 3-methyl-4-(methylthio)phenol were sensitive to heat in the water medium. However, 3-methyl-4-(methylthio)phenol did not undergo any remarkable degradation until it was heated at 80 °C for 1 h. Despite the significant heat degradation of these alcohol metabolites, appreciable quantities of their residues were still found intact in the water system.

Table V shows the effect of heat on the overall recoveries of the OP metabolites from meat samples at 70 and 80 °C. A significant degradation of all the metabolites occurred when they were heated in meat at 70 °C for 1 h. Heating for 2 h produced additional significant losses for the oxons. On the basis of the chemical structures (Figure 1), the oxons can be dealkylated by nucleophilic attack of the hydroxide ion (OH⁻) and the hydrogen peroxide ion (HO₂⁻). The formation of the hydrogen peroxide ion in the meat system is believed to be induced by peroxidases. However,

both ions are likely to hydrolyze the oxons and the parent OPs by nucleophilic substitution on the organophosphorus ester followed by removal of the phosphate group (Qian et al., 1985). On the other hand, the alcohol metabolites are structurally different from the oxons and will not undergo further hydrolytic reactions. On the basis of the chemical properties of the metabolites, the oxons were expected to be much more heat sensitive than the alcohol metabolites in the meat system.

The presence of some alcohol metabolites was observed subsequent to the heat degradation of the oxon metabolites in the meat system. These secondary metabolites were p-nitrophenol, 3-methyl-4-(methylthio)phenol, (2,4,5-trichlorophenyl)ethanol, and 2,4,5-trichlorophenol obtained from the hydrolysis of paraoxon, fenthoxon, stirofos, and ronnel oxon, respectively. When the alcohol metabolites were heated in the meat matrix, their recoveries were significantly lower than those of the raw meat samples. In addition, the lowest recoveries of these metabolites were obtained at 80 °C for 1 h. The recoveries of the alcohol metabolites from the meat system were much lower than those from the water medium. The degradation of 2,4,5-trichlorophenol and (2,4,5-trichlorophenyl)ethanol in the meat system can be explained by nucleophilic substitution of one of the chlorine groups by an hydroxide ion. However, (2,4,5-trichlorophenyl)ethanol (see Figure 1) is susceptible to further oxidation to form 2,4,5-trichlorobenzoic acid. Also during cooking of the meat system, the proteins unfold and become denatured. These denatured proteins could react with the alcohol metabolites by ionic, hydrophobic, or hydrophilic interactions. Consequently, the alcohol metabolites bound to the proteins may not be extracted from the meat matrix, resulting in lower recoveries.

Identification of Compounds. The compounds identified in our investigation were the alcohol metabolites, p-nitrophenol, 2,4,5-trichlorophenol, 3-methyl-4-(methylthio)phenol, and (2,4,5-trichlorophenyl)ethanol, obtained from the thermal degradation of paraoxon, ronnel oxon, fenthoxon, and stirofos, respectively. Retention times of unknowns relative to standards were used for preliminary identification of the first three alcohol metabolites. This tentative identification was then confirmed by computer matching of the compounds with the library of stored UV absorbance profiles of standards. Subsequent to the heat treatment of the oxons in water at 70 °C for 1 h, only 2,4,5-trichlorophenol was identified by retention time and spectrum matching via HPLC analysis and by GC–MS. After the oxon primary metabolites were heated at 70 °C for 2 h, the same alcohol metabolite was identified in the system at much higher concentration than 1 h of heating at 70 °C produced. An intermediate compound

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resulting from the degradation of stirofos was observed by the HPLC spectra matching, which was similar to that of the parent compound. However, the retention time was much shorter, indicating an oxygenated species. Akhtar and Foster (1977) investigated the metabolism of stirofos using chicken liver homogenates and found that 2,4,5-trichloroacetophenone was the major metabolite. We did tentatively identify, by GC–MS, appreciable amounts of (2,4,5-trichlorophenyl)ethanol, a likely breakdown product of the acetophenone derivative.

In the oxon and meat mixtures heated at 70 °C for 1 and 2 h, we identified p-nitrophenol, 3-methyl-4-(methylthio)phenol, and 2,4,5-trichlorophenol by UV spectra and retention time matching and by mass spectral matching with the NBS library. In addition, the alcohol metabolite of stirofos, (2,4,5-trichlorophenyl)ethanol, was tentatively identified by GC–MS spectrum matching. When the oxons and meat mixtures were cooked at 80 °C, all of the heat-induced alcohol metabolites were observed but the levels were not as high as those obtained at 70 °C. These observations imply that there was a good correlation between the decomposition of the primary metabolites and the formation of the alcohol metabolites in beef and water.

In view of the findings of this investigation, we believe that cooking beef muscle at an internal temperature of 70 °C for about 1 h could induce a significant thermal decomposition of the OPs and their metabolites. However, despite the heat-induced degradation of the OPs and the metabolites, over 50% (and more) of many of the residues still existed in both the water and beef muscle. Thus, heating (cooking) could not be counted on to totally eliminate most residues of OP parent compound and their metabolites.

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