Reduction in Polychlorinated Dibenzodioxin and Dibenzofuran Residues in Hamburger Meat during Cooking

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Human exposure to the environmental pollutants in the polychlorinated dibenzodioxin and dibenzofuran classes is mainly through bioaccumulation of residues in animal food products. Estimates of risk require detailed knowledge of diet, consumption patterns, and the effects of food processing and handling on residues. Cooking (pan-frying) hamburger patties was found to reduce the amount of polychlorinated dibenzodioxins and dibenzofurans actually consumed by 40–50% if the pan fats and juices were discarded. Preparation of the hamburger patties from high residue-level tissues obtained from steers used in a dioxin metabolism experiment and exposed to pentachlorophenol treated wood allowed for improved analytical precision and accuracy in this study.

Keywords: *Dioxins; furans; residues; cooking; 2,3,7,8-TCDD*

INTRODUCTION

The polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are tricyclic, planar, aromatic compounds produced as unwanted byproducts in many industrial and natural processes. They are lipophilic, poorly water-soluble, stable, and environmentally persistent. The 2,3,7,8-substituted isomers are particularly resistant to metabolic and abiotic degradation and tend to bioaccumulate in animals. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin isomer (TCDD, dioxin) has been claimed to be one of the most toxic of all known anthropogenic compounds and one of the most carcinogenic chemicals yet tested in animals by the United States Environmental Protection Agency (U.S. EPA) (Zook and Rappe, 1994); however, issues involving natural versus anthropogenic sources, toxic effects, carcinogenicity, and species differences remain controversial. Our studies on dioxins in beef show a strong correlation of dioxin levels with animal exposure to an anthropogenic source, pentachlorophenol treated wood, in production facilities (Feil et al., 1995; 1997). Recently, attention has been focused on the immunosuppressive and endocrine disruptive effects of dioxins and related compounds (polychlorinated biphenyls, diphenyl ethers, and naphthalenes).

The most important route of exposure to PCDDs/PCDFs in humans is through food, particularly from the consumption of animal products such as beef, dairy and fish. Different maximum daily intakes of dioxins have been proposed. The World Health Organization has recommended a maximum daily intake of all dioxin-like compounds not to exceed 10 pg/kg of body wt/day (World Health Organization, 1989). The Netherlands has set 4 pg/kg/day and the U.S. EPA has recommended 0.006 pg/kg/day as maximum daily intakes (Webster and Commoner, 1994). It is believed that 95% of the possible exposure to dioxins in humans comes from the diet

(Theelen, 1991). Estimates of actual human dioxin intake to date have been based on random surveys of market-basket food items (Schecter et al., 1994), models based on atmospheric deposition to forage and bioaccumulation in food animals (Lorber et al., 1994), and mass-balance dietary analysis in humans (Schrey et al., 1996). The latter experiments should have provided an estimate of actual absorption of dioxins from food in the intestinal tract. It was noted instead that the mean daily PCDD/F-excretion exceeded intake by 2.4-fold (range: 0.74–5.3-fold). To improve our understanding of actual human exposures, more detailed information is needed about the fate of dioxins in all steps of food production, processing, and consumption.

Broiling of ground beef has been reported to reduce dioxin levels in the meat to be consumed (Schecter et al., 1996). The effect of broiling on dioxin content in beefsteak, bacon, chicken, trout, and catfish has also been reported (Schecter et al., 1997). Other experiments involving pork and salt fish claimed pan-frying had no effect on PCDD/F levels and some cooking methods led to possible *de novo* formation of dioxins (Korner and Hagenmaier, 1990). For most PCDD/F isomers, however, the very low commonly occurring residue levels are subject to the uncertainty involved in current method detection limits and analytical uncertainty due to the ubiquity of low levels of dioxin contamination. In the case of fish that had dioxin levels 2 orders of magnitude higher than is usually found in meat, various types of cooking clearly led to significant (41.4–70.6%) reductions in levels of 2,3,7,8-TCDD (Stachiw et al., 1988).

We report here the results of experiments involving the effects of cooking (pan-frying) on hamburger patties containing levels of some dioxin congeners high enough to allow robust interpretation of the results. The tissues used in these experiments came from steers involved in a PCCD/PCDF feeding study (Feil et al., 1996). The treated animals were dosed with the compounds listed in Table 1. The control animals were not deliberately fed any dioxins, but both groups developed similar high

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residue levels of certain PCDDs/PCDFs typical of pentachlorophenol treated wood from exposure to barn structures while confined during the course of the feeding experiment. This ground beef is therefore representative of the high levels of dioxin residues that might occur sporadically under current animal husbandry practices. Rather than using "marketbasket" hamburger purchased at a supermarket, the use of tissues from these control steers allowed us to focus on a more realistic situation involving the behavior during cooking of those dioxin congeners likely to be involved in actual worst-case contamination incidents. The spiking of tissues containing low levels of dioxins to mimic high levels of contamination might generate analytical results different from tissues with naturally incurred high levels. The use of tissues with levels of dioxins well above the normal background levels usually found in beef improved the accuracy and reproducibility of the results by minimizing the uncertainty involved in making quantitative measurements at or near the method detection limits. Analysis of these tissues also provided information on the effect of cooking tissues with large differences in dioxin contamination levels.

MATERIALS AND METHODS

Safety. *Caution: All extracts and analytical standard materials containing dioxins should be considered extremely toxic and hazardous and appropriate precautions taken to prevent human exposure or workplace contamination.*

Reagents and Reference Materials. All procedures were performed with analytical or pesticide grade chemicals from the following suppliers: Aldrich, Milwaukee, WI, dodecane; Burdick and Jackson, Muskegon, MI, acetone, ethyl acetate, hexane, methanol, methylene chloride, toluene, and water; Curtin Matheson Scientific, Houston, TX, potassium hydroxide; E M Science, Gibbstown, NJ, benzene and sodium sulfate; J. T. Baker, Phillipsburg, NJ, silica gel (60–200 mesh), sulfuric acid. Fluid Management Systems, Watertown, MA (FMS), columns were used for the automated cleanup step. Solid supports and glassware were prepared and cleaned as recommended by U.S. EPA Method 1613 (U.S. Environmental Protection Agency, 1994). All analytical standards were obtained from Isotec Inc. (Wellington Laboratories; Miamisburg, OH)

Cooking Methods. Muscle tissue (rib eye) and subcutaneous back fat, stored at −60 °C, were thawed and mixed to generate ground beef with a fat content of 20%. The samples were ground 3 times with a Hobart mixer equipped with a grinder attachment. The mixture was shaped into uniform patties (9.5 × 1.0 cm, surface area 171 cm^2, approximately 110 g, weighed to the nearest 0.1 g) by pressing into a glass Petri dish fitted with a Teflon disk liner to facilitate patty removal. The procedures were conducted in a chemical fume hood isolated from the sample extraction and analytical areas. Patties were placed into a 15-cm diameter stainless steel frying pan on a hot plate, both at room temperature. The hot plate was turned on to a setting that resulted in a cooking time of approximately 20 min (the final hot plate temperature was approximately 210 °C). The pan was covered with an inverted glass funnel to imitate common kitchen practices to control spattering and to collect volatiles. Analysis of volatiles collected on polyurethane foam filters (Supelco, Inc., Bellefonte, PA) attached to the funnels in one set of experiments showed no residues above the method quantitation limits (see Dioxin Analysis below) so the filters were omitted from the protocol. The patties were turned once during the cooking process with stainless steel spatulas and were heated to an internal temperature of 74 °C (165 °F) as measured by a 30-cm digital thermometer probe bent to conform to the pan and inserted horizontally 4.5 cm into the patty. Fats and juices were collected from the pan with disposable polyethylene pipets and

transferred to Teflon bottles for extractions. Equipment was cleaned between each use according to the EPA Method 1613 glassware protocol.

Sample Extraction and Cleanup. Raw and cooked patties were mixed and ground with sufficient anhydrous sodium sulfate to yield a free flowing dry mixture. Portions of the ground beef/sodium sulfate mixture (approximately one-third of the uncooked patty and one-half of the cooked patty) were weighed into thimbles containing 0.5-cm silica gel, spiked with ^{13}C quantitation standards, covered with 1-cm glass wool, placed into 500-mL Soxhlet apparatuses wrapped with aluminum foil and extracted for 12–16 h with hexane/methylene chloride (1:1). Certain PCDD/PCDF isomers have been shown to be photodegraded during prolonged Soxhlet extraction; the procedure described gave method spike recoveries ranging from 80 to 110% for all isomers and met U.S. EPA Method 1613 specifications. Soxhlet extracts were concentrated and extracted with 20% aqueous potassium hydroxide, HPLC grade water, and concentrated sulfuric acid. Each basic wash step was repeated once, each acid step was repeated until the sulfuric acid phases were colorless (3× minimum), and after a final water extraction the organic phase was dried over sodium sulfate and reduced on a TurboVap system (Zymark Corporation, Hopkinton, MA) to 0.5 mL for chromatographic cleanup on the FMS system. Duplicate analyses of ground beef/sodium sulfate mixtures from the same patty showed good agreement (% relative standard deviation = 0.10–9.8%, depending on congener) so means of two or more extractions were used to calculate total parts per trillion (ppt) in the ground beef samples.

The fats and juices collected from the pans were spiked with ^{13}C standards, mixed with methylene chloride and sodium sulfate, and filtered through sodium sulfate. This was then reduced, stirred with acid-washed silica, filtered again, and concentrated as above for chromatographic cleanup as above.

The FMS system uses solvents and columns equivalent to those described in U.S. EPA 1613. Briefly, the sample is spiked with a ^{37}Cl recovery standard and loaded onto a layered column consisting of neutral silica, sodium hydroxide impregnated silica, neutral silica, concentrated sulfuric acid impregnated silica, and neutral silica. The column is eluted with hexane, and the eluate is directed to an alumina column and eluted first with hexane then 2% methylene chloride/hexane. The dioxin fraction is transported to an activated carbon column with methylene chloride/hexane (1:1). The carbon column is eluted with ethyl acetate/benzene (1:1) then with hexane. Final isolation is achieved by reversing the flow through the carbon column and eluting the dioxins with toluene.

Dioxin Analysis. Samples were analyzed according to the specifications of U.S. EPA Method 1613. Analyses were performed on an Autospec-Ultima (Micromass, Manchester, U.K.) high-resolution mass spectrometer operating in the electron impact ionization mode with selected ion monitoring. Data reduction was done with the manufacturer's software OPUSquan. The mass spectrometer was run at a resolution of 10 000–11 000, at an accelerating voltage of 8000 V. The data were taken by selected ion monitoring of two masses for each congener by using perfluoroalkane as the reference mass; five distinct sampling settings were used as the run progressed. The chromatographic separations were achieved on a Carlo Erba 8000 chromatograph using the splitless mode of the injector. A 0.5-m deactivated precolumn (0.530-mm i.d.) was joined with a press fit union to a DB-5MS column (60 m × 0.32-mm i.d., 0.25-μm film), all J&W Scientific, Inc., Folsom, CA. Total split flow was 13 mL/min; column head pressure was adjusted to achieve a transit time of about 3 min for butane at a column temperature of 180 °C. The initial column temperature was 140 °C, the final temperature, 300 °C, programmed to meet the chromatographic resolution required by U.S. EPA Method 1613. The injector was held at 240 °C, the three segments of the transfer line were 160, 250, and 240 °C, and the spectrometer source was 240 °C. The injection solvent was dodecane. Prepared sample volumes were 20 μL, and 2 μL (10% of the sample) was injected. Peak identification

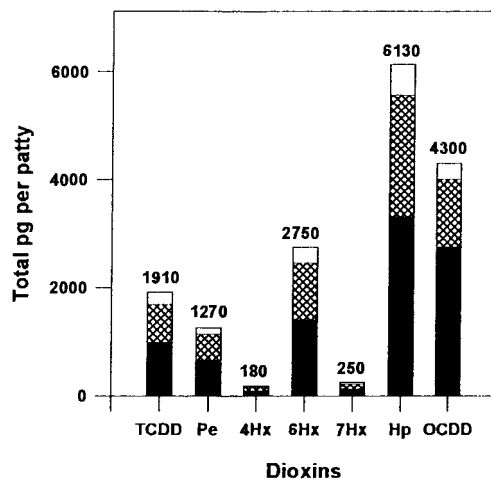**3282** *J. Agric. Food Chem.,* Vol. 46, No. 8, 1998
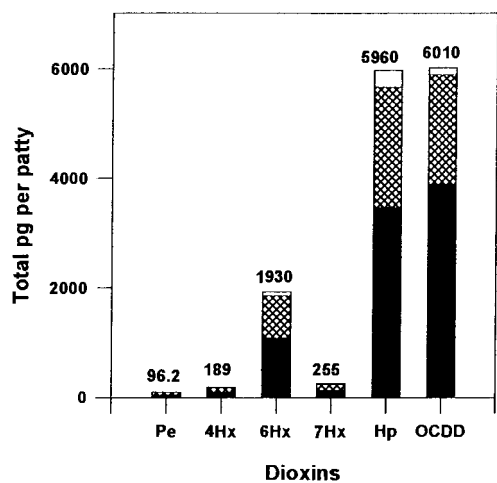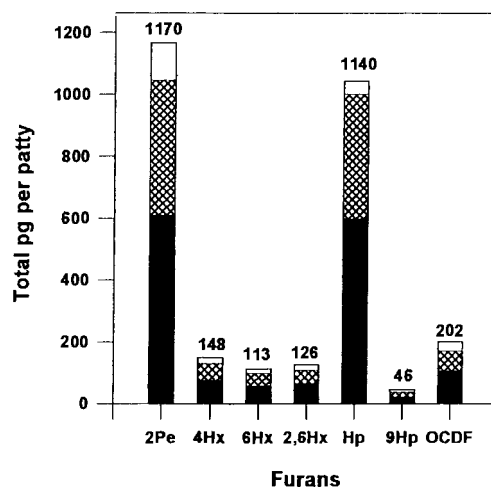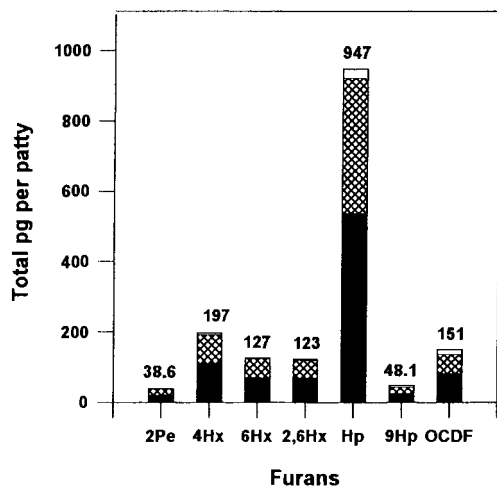
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Figure 1. Reduction in dioxin/furan levels after cooking in control steers hamburger. Black bars, cooked; crosshatched bars, fats and juices; white bars, loss.

criteria were set as S/N > 3, isotope ratio within 15% of theoretical value, peak maxima of molecular cluster ions within 5 s of the retention time standards, and native ^{12}C analytes eluting within 2 s of corresponding ^{13}C analogues.

Response factors (RFs) were calculated from a five-point calibration curve (2–400 ppt tetra-, 5–1000 ppt penta-, hexa-, and hepta-, 10–2000 octacongeners) containing all of the 2,3,7,8-PCDD/PCDF congeners of interest and two internal standards. Sample concentrations were determined by the isotope dilution method, in which peak areas from the characteristic ions of the ^{12}C native analytes and their ^{13}C labeled analogues were used in conjunction with the RFs from the internal calibration data. Each sample set included a method blank, laboratory control spike, and a midrange calibration check sample. QA/QC records were reviewed by a QA/QC officer from outside the analytical project staff. The percent difference between the check sample RFs and the calibration curve RFs had to be <20% for the native analytes and <35% for the recovery surrogates. Throughout the analytical period recoveries of the ^{13}C analogues were within 40–110% and recoveries of the laboratory control spikes were between 40 and 110%, well within the specifications of US EPA Method 1613. Quantitation limits were calculated as 5 times background interferences; mean method blank values for the most important toxic isomers were 0.275 ± 0.042 ppt for TCDD and 0.156 ± 0.064 ppt for PeCDD, well below the 1.0 ppt detection limit specified in EPA Method 1613.

RESULTS AND DISCUSSION

The effects of pan frying on the specific PCDD/PCDF congener concentrations in ground beef patties having

Figure 2. Reduction in dioxin/furan levels after cooking in treated steers hamburger. Black bars, cooked; crosshatched bars, fats and juices; white bars, loss.

a fat content of approximately 20% are shown in Figures 1 and 2. The figures show the amounts (in picograms) of dioxins and furans found in 110-g patties before cooking (numbers above bars), the amounts remaining in the cooked patties, the amounts in the fats and juices, and by difference, the amounts lost. The ground beef was from animals involved in a feeding study where dosed animals received the dioxins and furans shown in Table 1. Dosed animals and control animals were fed in a typical production facility. It should be noted that some of the more highly chlorinated congeners were found in control animals at concentrations equal to or greater than in dosed animals. The production facility was subsequently shown to contain pentachlorophenol treated wood components. The quantities of some congeners consumed by the animals from the treated wood (by licking, chewing and rubbing) exceeded the quantities consumed from the dose (Feil et al., 1996; 1997). The dioxin concentrations found in the tissues used in this cooking study were similar to concentrations found in tissues of some animals from a geographical survey of domestic beef (Feil et al., 1995), indicating that meat with these high levels of contamination could on occasion be found in grocery stores. All congeners that were substantially above the detection limits were reduced significantly in the edible portions of the ground beef patty. Results for the isomers present at close to the method detection limit (1,2,3,7,8-PeCDF and

Table 1. Dose Components and Levels of the Feeding Study

congener	TEF[a]	daily dose per animal, ng	total dose per animal, μg
1,2,7,8-TCDD	0	750	90
1,3,7,8-TCDD	0	750	90
1,4,7,8-TCDD	0	750	90
2,3,7,8-TCDD	1	83.3	10
1,2,3,7,8-PeCDD	0.5[b]	83.3	10
1,2,3,6,7,8-HxCDD	0.1	150	18
1,2,3,4,6,7,8-HpCDD	0.01	750	90
OCDD	0.001[b]	750	90
2,3,7,8-TCDF	0.1	150	18
2,3,4,7,8-PeCDF	0.5	83.3	10
1,2,3,4,6,7,8-HpCDF	0.1	150	18
OCDF	0.001	750	90

[a] NATO/CCMS, 1988. [b] Proposed changes for TEFs in humans/mammals by a WHO Working Group in Stockholm (June, 1997): 1,2,3,7,8-PeCDD, 1.0; OCDD, 0.0001.
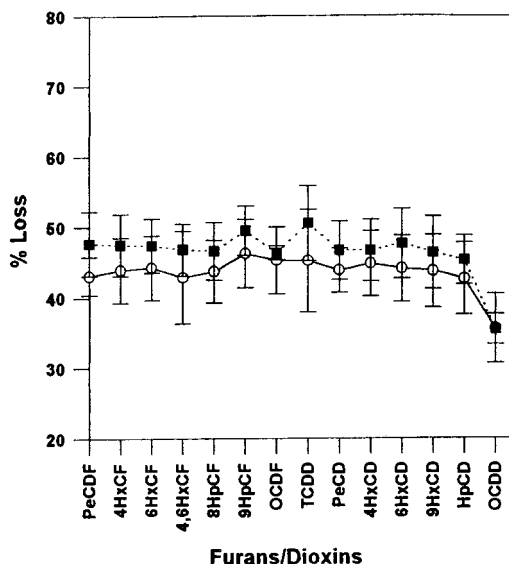
Figure 3. Percent loss by congener during cooking hamburger, mean ± SD. ○, controls; ■, treated.

1,2,3,7,8,9-HxCDF in all steers; 2,3,7,8-TCDD and 2,3,7,8-TCDF in control animals) were highly variable and difficult to interpret and were omitted from the graphs. Assuming that the fats and pan juices are not eaten, pan frying ground beef patties reduces the amount of PCDDs/PCDFs actually consumed by 40–50% depending on the isomer (Figure 3). The loss of lipids correlated well with the loss of dioxins during cooking, ranging from 42.0 to 53.3% with a mean of 47.6%. The slightly greater losses in the treated animals were not statistically significant (Student's *t*-test, *P* < 0.3–0.46, normality, equal variance passed).

These results are in good agreement with previous studies showing loss of dioxin residues during food preparation steps and confirm the importance of including cooking as a factor in adjusting exposure estimates in modeling human TEQ intakes. An estimated daily intake based only on dioxin residues in grocery store or slaughterhouse meat samples could be off by a factor of almost 2 if the fats and juices are discarded or have little effect if most of the fats and juices are retained in the prepared food. Preparations made by cooking loosely broken up ground beef in the pan may show even greater reduction of total TEQs (if fats and juices are discarded), since loss of lipophilic and volatile residues generally increases with increases in surface area.

Table 2. Mean Dioxin/Furan Levels (*n* = 4) in Raw Hamburger before Cooking (pg/g, ppt)

congener	control steers mean	control steers ±SD	treated steers mean	treated steers ±SD
2,3,7,8-TCDF[b]	ND[a] (0.024)		0.134	0.036
1,2,3,7,8-PeCDF	ND (0.090)		ND	
2,3,4,7,8-PeCDF[b]	0.098	0.026	3.026	0.225
1,2,3,4,7,8-HxCDF	0.499	0.139	0.385	0.129
1,2,3,6,7,8-HxCDF	0.321	0.061	0.294	0.118
2,3,4,6,7,8-HxCDF	0.311	0.065	0.326	0.112
1,2,3,7,8,9-HxCDF	ND (0.228)		ND	
1,2,3,4,6,7,8-HpCDF[b]	2.40	0.540	2.95	0.748
1,2,3,4,7,8,9-HpCDF	0.121	0.035	0.119	0.028
OCDF[b]	0.382	0.081	0.523	0.077
2,3,7,8-TCDD[b]	ND (0.275)		4.96	0.555
1,2,3,7,8-PeCDD[b]	0.241	0.027	3.28	0.303
1,2,3,4,7,8-HxCDD	0.474	0.083	0.469	0.175
1,2,3,6,7,8-HxCDD[b]	4.87	1.45	7.13	1.83
1,2,3,7,8,9-HxCDD	0.641	0.105	0.650	0.026
1,2,3,4,6,7,8-HpCDD[b]	15.2	4.53	15.9	3.65
OCDD[b]	15.6	4.75	11.2	3.18

[a] ND, not determined; below limit of quantitation. Values in parentheses are mean method blanks (*n* = 10). [b] Dose component fed to treated steers.

Table 3. Average Recoveries from Cooked Hamburger (Cooked Patty + Pan Fat and Juices/Raw Patty)

congener	control steers mean, %	control steers ±SD	treated steers mean, %	treated steers ±SD
2,3,7,8-TCDF[b]	ND[a]		94.7	3.7
1,2,3,7,8-PeCDF	ND		ND	
2,3,4,7,8-PeCDF[b]	97.6	8.2	89.8	1.6
1,2,3,4,7,8-HxCDF	99.1	4.4	88.3	1.7
1,2,3,6,7,8-HxCDF	97.8	5.6	88.0	1.8
2,3,4,6,7,8-HxCDF	97.8	3.7	87.8	1.7
1,2,3,7,8,9-HxCDF	ND		ND	
1,2,3,4,6,7,8-HpCDF[b]	97.8	4.1	88.4	2.6
1,2,3,4,7,8,9-HpCDF	91.8	3.9	81.7	7.1
OCDF[b]	91.6	4.9	85.1	3.1
2,3,7,8-TCDD[b]	ND		89.0	4.3
1,2,3,7,8-PeCDD[b]	98.8	6.8	91.1	1.8
1,2,3,4,7,8-HxCDD	95.3	3.7	89.6	2.4
1,2,3,6,7,8-HxCDD[b]	97.2	6.5	90.0	3.0
1,2,3,7,8,9-HxCDD	94.9	4.4	91.4	4.4
1,2,3,4,6,7,8-HpCDD[b]	96.0	7.5	91.1	1.5
OCDD[b]	98.3	1.5	93.4	0.7

[a] ND, not determined; below limit of quantitation. [b] Dose component fed to treated steers.

The relative precision of the data from such naturally high level tissues shown in Table 2 also indicates that there is probably no significant degradation or conversion of any one dioxin/furan congener to a differently substituted isomer during the cooking process. Recoveries in Table 3 range from 82 to 99% for different congeners and show no evidence of unexplained increases of any congeners. Mass balance estimates of global dioxin fluxes and animal or human intake/excretion mass balance experiments have shown 2–4-fold higher levels of octa-, hexa- and heptadioxins than can be accounted for theoretically (Brzuzy and Hites, 1996; Schrey et al., 1996; Moser et al., 1996; Fries et al., 1997). There is no evidence from these experiments that the effects of cooking can be contributing to these anomalously higher levels by congener interconversions or possible formation from predioxins present in food.

Examination of the unaccounted-for losses (6–16%) in the highest residue level treated steers in Figure 4 shows an exact opposite trend in the dioxin isomer series compared to the furan isomers. The unaccounted for material could be due to mechanical losses (equal
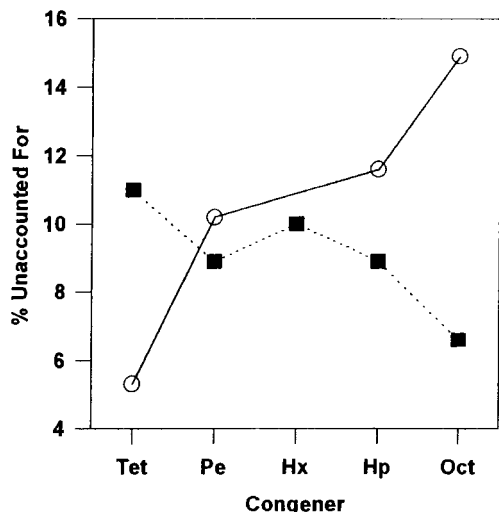
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Figure 4. Percent loss unaccounted for according to chlorine substitution. ○, furans; ■, dioxins.

for all congeners), degradation, and volatility. The losses were greater for the lower chlorinated dioxin congeners than for the more highly chlorinated congeners, but the opposite effect is observed with the furans. These results could be explained if volatility is a dominant source of loss in the dioxin series and degradation is a dominant source of loss in the furan series. In conclusion, pan frying of ground beef patties significantly reduces the amounts of PCDDs/PCDFs consumed provided that the fats and juices are not consumed, while congeners released as volatiles may pose a secondary mode of human exposure.

ABBREVIATIONS USED

For Figures 1 and 2: TCDD, 2,3,7,8-TCDD; Pe, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF; 4HX, 1,2,3,4,7,8-HxCDF and 1,2,3,4,7,8-HxCDD; 6Hx, 1,2,3,6,7,8-HxCDF and 1,2,3,6,7,8-HxCDD; 2,6Hx, 2,3,4,6,7,8-HxCDF; 7Hx, 1,2,3,7,8,9-HxCDD; Hp, 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,6,7,8-HpCDD; 9Hp, 1,2,3,6,7,8,9-HpCDF. For Figure 3: PeCDF, 2,3,4,7,8-PeCDF; 4HxCF, 1,2,3,4,7,8-HxCDF; 6HxCF, 1,2,3,6,7,8-HxCDF; 4,6HxCF, 2,3,4,6,7,8-HxCDF; 8HpCF, 1,2,3,4,6,7,8-HpCDF; 9HpCF, 1,2,3,4,7,8,9-HpCDF; PeCD, 1,2,3,7,8-PeCDD; 4HxCD, 1,2,3,4,7,8-HxCDD; 6HxCD, 1,2,3,6,7,8-HxCDD; 9HxCD, 1,2,3,7,8,9-HxCDD; HpCD, 1,2,3,4,6,7,8-HpCDD. For Figure 4: Tet, 2,3,7,8-TCDF and 2,3,7,8-TCDD; Pe, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD; Hx, 1,2,3,6,7,8-HxCDD; Hp, 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,6,7,8-HpCDD; Oct, OCDF and OCDD.

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