Table III.	Distribution o	f Radiocar	bon af	ter	
Fluorochlo	ridone Photoly	sis at pH	7 at 25	°C after	7.5 Days

compd ^a	R_{f}^{b}	radioact, dpm	rec,° %	
1	0.656	94967	19.55	
8	0.640	14918	3.06	
7 (trans + cis)	0.475	189588	39.03	
unknown A	0.205	4765	0.97	
3	0.188	27157	5.57	
4 + 5	0.150	2898	0.59	
6	0.088	10751	2.21	
unknown B	0.000	58294	12.00	
total		403338	82.98	

^aRefer to Figure 3 for structures. ^bSolvent system: dichloromethane-ethyl acetate (40:60, v/v). ^cCalculated from initial count of 485528 dpm and background count of 39 dpm.

Figure 3 and two remain unidentified. Compounds 3–6 were identified as products of hydrolysis (above) and also occurred as photolysis products. The major photolytic degradation product, which contained 39% of the radio-activity, is 4-(chloromethyl)-3-hydroxy-1-[3-(trifluoromethyl)phenyl]-2-pyrrolidinone (7). Compound 7 is a mixture of cis and trans isomers. The position of the hydroxy group of 7 was established by observing spin-spin coupling between the hydroxy and the adjacent methine protons in the NMR spectrum run in DMSO- d_6 . Compound 8, 4-(chloromethyl)-1,5-dihydro-1-[3-(trifluoromethyl)phenyl]pyrrol-2H-one was identified by GC/MS analysis. Authentic compounds of 7 and 8 were not available for comparison.

The formation of product 7 suggests the major photolytic pathway involves homolytic cleavage of the carbonchlorine bond at the 3-position of the pyrrolidinone ring of flurochloridone. A homolytic C-Cl cleavage has been reported to account for the photodegradation products of propachlor (2-chloro-*N*-isopropylacetanilide) (Rejto et al., 1984). The free-radical intermediate can react with water to give 7 or eliminate a hydrogen radical to form 8.

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Tetrachlorodibenzo-*p*-dioxin Residue Reduction through Cooking/Processing of Restructured Carp Fillets¹

Nancy C. Stachiw, Mary E. Zabik,* Alden M. Booren, and Matthew J. Zabik

Restructured carp fillets were used to study the potential of reducing dioxin levels during cooking. Carp from Saginaw Bay were mechanically deboned and washed. Samples were analyzed for 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin (TCDD) by selected ion monitoring GC-MS. Half was spiked to obtain levels of approximately 100 pptr. Fillets (7.5 cm in diameter) were roasted covered and uncovered at 177 °C to end internal temperatures of 60, 70, and 80 °C. To study the effect of surface area, fillets (10 cm in diameter) were roasted uncovered and charbroiled to 80 °C only. Fillets (7.5 cm in diameter) were also charbroiled to 60 and 80 °C. Spiked and unspiked (control) samples were cooked for each method/end temperature. Cooking resulted in reductions of TCDD in restructured carp surimi fillets. The magnitude of reduction was similar for unspiked and spiked fillets. Charbroiling resulted in greater TCDD reduction than roasting uncovered. Increasing internal end point temperature or surface area increased TCDD loss.

Bottom-feeding fish, i.e., carp and suckers, collected from Michigan rivers and streams in 1979 have been found to contain nondetectable to 120 pptr body weight of dioxins (Kaczmar, 1983). Fehringer et al. (1985) found fish collected in 1983 from Saginaw Bay and corresponding rivers to have the highest TCDD levels with values ranging from nondetectable to 102 pptr using HRGC-EC analyses. Fish from other Michigan rivers contained <10 pptr TCDD. Thus, every effort must be made to reduce the level of dioxins going into the environment and to reduce the levels of residues already in the environment. In preliminary studies using charbroiling of three carp fillets from Michigan rivers, extremely variable but substantial losses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) ranging from 30 to 70% were recorded (Kaczmar, 1983). This

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Food Science and Human Nutrition (N.C.S., M.E.Z., A.M.B.) and Pesticide Research Center (M.J.Z.), Michigan State University, East Lansing, Michigan 48824.

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TCDD Losses during Cooking/Processing

contrasts to the less than 10% loss of polychlorinated biphenyls (PCBs) and chlorinated hydrocarbons during the cooking of carp fillets and salmon steaks from the Great Lakes (Zabik et al., 1982; Smith et al. 1973). Nevertheless, Puffer and Gossett (1983) reported that panfrying white croaker reduced DDT compounds by 74% and 39% for fish caught from Santa Monica Bay and in Orange County, respectively. Losses of PCBs were 65% and 28%, respectively. Reinert et al. (1972) also reported broiling and frying reduced DDT concentrations in lake trout by 64-72% while Zabik et al. (1979) reported statistically significant losses of PCBs during the cooking of fat lake trout from Lake Superior. Cooking and processing also appear to have real promise in reducing TCDD residues in fish and other food sources.

As a continuation of initial studies by Kaczmar (1983), it was proposed to use a restructured carp fillet from surimi to increase sample homogeneity to study the potential of cooking/processing to reduce TCDD levels. Mechanically deboned carp and sucker are currently manufactured in Michigan. Surimi is a Japanese term for mechanically deboned fish, which is used as a base for preparing fabricated seafood products. Surimi type restructured fish parts are widely accepted products in Japan and the United States using ocean fish surimi (Lanier, 1984; Lee, 1984; Buck and Faford, 1985). Use of underutilized fish from the Great Lakes in surimi products is of interest to Michigan firms unless environmental contaminants are prohibitive.

The specific objectives of this study were to determine whether the degree of heating and/or the rate of heating affects the level of TCDD reduction, to establish whether moist or dry heat was more effective in TCDD reduction, and to determine whether increased surface area promoted TCDD loss.

MATERIALS AND METHODS

Carp (Cyprinus carpio), caught commercially from Saginaw Bay, Lake Huron, using trap nets were procured from Bay Port Fish Co., Bay Port, MI. All carp were trapped on June 30, 1985, and ranged in size from 4 to 7 kg. The fish were dressed, deheaded, and evicerated by hand within 4 h of harvest as outlined by Rippen (1981). A Bibun Model SDX13 (Bibun Co., Fukuyama Kiroshima, Japan) belt type machine with a 5-mm perforated drum was employed for deboning. Carp were loaded so that they passed flesh side down to the drum where they were subsequently recovered from the deboner. The fish flesh was packaged in polyethylene bags and held at 0 °C. Twenty-four hours after harvest, 72.7 kg of the deboned fish flesh was packed in ice and transported to Michigan State University, East Lansing, MI. Prior to processing, the temperature of the minced flesh was recorded at 0 °C.

Three water wash treatments were performed on the carp meat (Figure 1). The first wash was in a ratio of 3 parts ice water to 1 part fish. The flesh and ice water were gently mixed with a stainless-steel paddle for 3 min and then allowed to settle for 5-8 min after which the water, lipid, and water-soluble protein fraction were decanted, leaving residual water and flesh. This same procedure was repeated during the second wash using a 2:1 weight ratio of ice water to fish. The third treatment used 0.125% NaCl added to the ice water, which was again used at a 2:1 weight ratio to fish. The 0.125% NaCl was included in the final wash to aid in dewatering and to leach color from the fish flesh.

The slurry was dewatered over fiberglass window screens attached to plastic lugs for the collection of wash water. To facilitate the dewatering process, the slurry was gently



Figure 1. Flow diagram of surimi-making process.

agitated manually. The lug systems were then covered and refrigerated overnight at 0 °C. A final dewatering treatment was performed with the aid of centrifugation. The carp flesh was placed in net bags and put into a commercial laundry-type centrifuge. After 20 min of spinning, the bags were removed.

The deboned, washed, and dewatered flesh was then placed into a Hobart Vertical Chopper (Model VCM40E, Hobart Manufacturing Co., Troy, OH). A mixture of 4% sorbitol, 4% sucrose, and 0.5% Freez-gard (FP-19 sodium tripolyphosphate; Stauffer Chemical Co., Westport, CT) was added to the mixture by sprinkling onto the flesh to form surimi. This addition occurred in the first 30 s of chopping. The flesh was chopped for 3 min. The processed flesh was removed from the chopper and weighed. The flesh weighed approximately 38.6 kg for a yield of about 53% from the deboned, unprocessed flesh. The surimi was wrapped in aluminum foil and vacuum-packaged in 4.5-kg packages. The surimi was held frozen at -20 °C in a blast freezer until further analysis.

For preparation of the spiked sample, 60 pptr of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was added to the surimi to bring the total amount of TCDD to approximately 100 pptr, based on preliminary analysis of the raw surimi. Addition was accomplished by mixing the dioxin (solubilized in acetone) with 2% salt. With a stainless-steel laminar flow hood, the salt and dioxin were blended in a Sorvall Omni-Mixer FF 3163, at speed 5 for 30 s (Sorvall Inc., Newtown, CT). The mixture was allowed to rest for 10 min and then mixed again for 1 min at speed 3. Following evaporation of the acetone for 30 min, the salt-dioxin mixture was added to the surimi during the first minute of chopping with the Silent Cutter. All equipment and materials were rinsed thoroughly in hexane after exposure to dioxins. Swipes were taken from all cleaned equipment and analyzed, showing TCDD to be below limits of detection before equipment was returned to general use.

In preparation for being stuffed into casings to form the restructured carp fillets, the surimi was tempered at 0.6 °C to an internal temperature of -1.1 °C. The surimi was chopped in a Hobart Silent Cutter (Model 84181D) to 5 °C. A temperature range of 2.8-5 °C was recorded at various locations in the bowl. Salt (2%) was sprinkled onto

the surimi as outlined by Lanier (1984) during the first minutes of chopping.

The surimi was stuffed through a piston-type sausage stuffer into 10.0- and 7.5-cm-diameter moisture-proof casings. The surimi cylinders were frozen at -20 °C. Forty-eight hours after processing and stuffing, the frozen cylinders were sliced to a uniform 1-cm thickness by a power meat saw to form restructured carp fillets. The casings were peeled off the surimi steaks, which were then wrapped in aluminum foil, vacuum-packaged, and stored frozen at -20 °C.

Cooking Methods. Using the laminar flow hood, the restructured carp fillets were charbroiled with an electric John Deere charbroiler, Model 57 E (Deere & Co., Moline, IL). Four 7.5-cm-diameter fillets of both the spiked and control were charbroiled at the hi temperature setting to internal temperatures of 60 and 80 °C. Four 10-cm-diameter fillets of the spiked and control were also charbroiled to internal temperatures of 80 °C. The restructured surimi fillets were flipped upon reaching half their respective end point temperature. Internal temperatures were monitored with an Omega potentiometer, Model 660 (Omega Engineering, Inc.).

Restructured surimi fillets were roasted uncovered in a Farberware convection turbo oven. Individual surimi steaks were tempered to approximately -6 °C, placed on a cooking rack, and placed into an aluminum pan. Four fillets of each of the 7.5-cm control and TCDD-spiked samples were roasted at 177 °C to internal temperatures of 60, 70, and 80 °C. Four samples of the 10-cm-diameter restructured fillets (control and spiked) were roasted to an internal temperature of 80 °C.

Covered roasting was accomplished by placing individual fillets at -6 °C on racks and covering the pans with aluminum foil. The surimi steaks were roasted at 177 °C; 7.5-cm controls and TCDD-spiked samples were roasted to internal temperatures of 60, 70, and 80 °C.

All samples were weighed before and after cooking to determine total cooking losses. Upon completion of cooking, all samples were wrapped in aluminum foil and put into polyethylene bags. All cooked surimi steaks were held frozen at -20 °C until time of dioxin analyses.

Swipe samples were performed on equipment used before and after processing and cooking. All equipment was rinsed in hexane after use. Dioxin analyses revealed undetectable amounts of TCDD following handling.

Proximate Analyses. Moisture determinations were performed on processed fish flesh in triplicate, according to AOAC Method 7.003 (1980). Ash content of surimi were determined in triplicate according to AOAC Methods 18.025 and 31.013 (1980). Fat content were determined by Bligh and Dyer's rapid method of total lipid extraction and purification (1959).

Analyses of protein in the surimi were performed by the micro Kjeldahl method for total nitrogen determination. Duplicate 1-g samples were digested with sulfuric acid, sodium sulfate, and copper sulfate at 400–500 °C until completion. Samples were transferred to a distillation apparatus, (Buchii Kjeldahl Machine, Brinkman Instruments) and distilled according to AOAC Methods 2.057 and 7.015. Total percent protein was calculated on the basis of percent nitrogen in the samples multiplied by a factor of 6.25.

TCDD Analysis. The procedure of Kaczmar (1983), which is adapted from procedure ML-AM-78-63, Dow Chemical Co., Michigan Division, Midland, MI, which is similar to the procedure of Lamparski et al. (1979), was used to quantify TCDD levels. The following reagents were employed:

Solvents including benzene, hexane, methylene chloride, carbon tetrachloride, methanol, chloroform, and isooctane were all distilled-in-glass quality. Chromatographic grade silicic acid as 100/120-mesh Bio-Sil A and aluminum oxide as 100/200-mesh Bio-Rad Basic alumina AG-10 were used. Reagent-grade silver nitrate, sodium hydroxide, sodium bicarbonate, and phosphorus pentoxide were used; 1 N aqueous NaOH and 10% NaHCO₃ solutions were prepared with use of organic-free distilled water. [¹³C]-2,3,7,8-TCDD was supplied by Dow Chemical Co.

To a 20-g sample of homogenized raw or cooked restructured carp fillet, or a blank, i.e., an empty flask, was added 5 ng of [13 C]-2,3,7,8-TCDD tracer. Reversed-phase high-performance liquid chromatographic sample cleanup was conducted with the following instrumentation: Altex microprocessor-controlled HPLC system 420 equipped with a Hitachi ultraviolet detector operating at a wavelength of 235 nm and a sensitivity of 0.01 AUFS; Altex Model 110 pump, Du Pont column compartment oven to control the temperature of the two Du Pont Zorbax-ODS RP-18 columns (6.2 × 250 mm) connected in series at 50 °C; Rheodyne Model 7120 high-pressure injector, equipped with a 50- μ L sample loop.

The GC-MS system included a Delsi Model DI 700 capillary gas chromatograph equipped with a split/splitless injector interfaced to a Nermag Model R10-10^C quadrupole mass spectrometer. A 60 m × 0.25 μ m (i.d.) DB-1 0.25- μ m-thick capillary column was used. Oven temperature was programmed from 50 to 250 °C at 20 °C/min and held isothermally at 250 °C. Temperatures for the injector port, transfer line, and source were all 250 °C. The column was positioned directly onto the source area. Helium served as the carrier gas with a column head pressure of 0.5 bar. Ions monitored included m/z 257, 320, 322, 332, and 334.

The concentration of 2,3,7,8-TCDD present in the fish sample was calculated by

pptr (ng/kg) 2,3,7,8-TCDD =
$$\frac{AE}{CH} \frac{DG}{BF}$$

where A = peak area of native 2,3,7,8-TCDD in the sample, B = peak area of added [¹³C]-2,3,7,8-TCDD in the sample, C = peak area of [¹²C]-2,3,7,8-TCDD in the standard, D = peak area of [¹³C]-2,3,7,8-TCDD in the standard, E =weight of [¹²C]-2,3,7,8-TCDD in the standard (ng), F =weight of [¹³C]-2,3,7,8-TCDD in the standard (ng), G =weight of [¹³C]-2,3,7,8-TCDD added to the sample (5 ng), and H = weight of the fish sample (kg).

The data were analyzed for variance, and Duncan's (1957) multiple-range test was used to sort any significant differences established by the analyses.

RESULTS AND DISCUSSION

Analyses indicated the surimi had $11.86 \pm 0.14\%$ protein, $1.91 \pm 0.11\%$ fat, $0.74 \pm 0.04\%$ ash, and $75.35 \pm$ 0.27% moisture. Initial TCDD analyses indicated that the carp surimi contained 49 pptr TCDD (limit of detection 7 pptr) while carp fat had 63-85 pptr TCDD (limit of detection 7-12 pptr) and carp flesh had 24-33 pptr TCDD (limit of detection 2-8 pptr). Further analyses of the restructured raw carp fillets for TCDD established the average range of TCDD to be from 37 to 46 pptr. Data for each individual sample as well as its m/z ratio for 320/322 and 332/334, the limit of detection, and percentage recovery are included in the supplementary material; m/z ratios were in the expected ratio of 0.8 ranging from 0.75 to 0.81. All samples showed $(M - COCl)^+$ [(M - 63)⁺] at mass 257. Limits of detection at $2^{1}/_{2} \times$ noise ranged from 6 to 23 pptr and were generally 10-15 pptr.

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Table I. Cooking Losses of Restructured Fillets Prepared Using Carp Surimi Roasted at 177 °C Either Covered or Uncovered

fillet diameter.	int end	total cookin	ng losses, %
cm	temp, °C	covered	uncovered
<u></u>	Control Sa	amples	
7.5	60	5.2 ± 0.7^{a}	15.1 ± 1.5
	70	6.4 ± 1.0	16.0 ± 1.6
	80	8.9 ± 1.1	17.1 ± 2.8
10.0	80		15.3 ± 0.4
	Spiked Sa	mples ^b	
7.5	60	5.4 ± 0.5	14.3 ± 1.0
	70	6.2 单 0.7	15.5 ± 1.2
	80	8.6 ± 0.8	17.8 ± 1.8
10.0	80		15.1 ± 1.3

 ${}^{a}n = 4$. b Samples were spiked to approximately 100 pptr TCDD.

Table II. Cooking Losses from Charbroiled Restructured Fillets Prepared Using Carp Surimi

fillet diameter.	int temp.	total cooking losses, %		
cm	°C	control	spiked ^a	
7.5	60	18.7 ± 3.2	15.3 ± 4.3	
	80	22.5 ± 1.5	18.3 ± 2.1	
10.0	80	22.2 ± 0.8	16.7 ± 2.4	

^aSamples were spiked to approximately 100 pptr TCDD. ^bn = 4.

Percentage recoveries ranged from 76 to 99% and were generally 80-85%.

Xenobiotic reduction in meat, fish, and poultry has been attributed to fat rendering and moisture evaporation during cooking (Puffer and Gossett, 1983; Zabik et al., 1979, 1982). Therefore, total cooking losses, e.g. both moisture and fat losses, were calculated for each sample and are presented in Tables I and II. Analyses of variance were performed to determine statistically significant differences related to roasting covered or uncovered, internal temperature, and spiked versus control samples using data from 7.5-cm diameter fillets and related to roasting uncovered versus charbroiled, 60 versus 80 °C internal temperature, and control versus spiked using data from 7.5cm-diameter samples. The effect of surface area, roasting uncovered versus charbroiling, and control versus spiked samples using data from both diameter fillets cooked to 80 °C were analyzed.

Samples roasted covered had statistically significantly lower cooking losses (p < 0.001) than did samples roasted uncovered. Increasing the internal end point temperature statistically significantly increased cooking losses (p < 0.01). Use of Duncan's multiple-range test (1957) indicated the cooking losses of the roasted samples were each statistically significantly different (p < 0.01); i.e., losses when cooked to 80 °C were greater than those of 70 °C, which were greater than those of 60 °C. Charbroiling resulted in statistically significantly greater cooking losses (p < 0.001) than did roasting uncovered.

TCDD levels on individual cooked samples are available as supplementary material. Average pptr values on a wet-weight basis of raw and cooked samples are presented in Table III. Cooking resulted in a significant reduction (p < 0.001) in pptr TCDD concentration in the restructured carp fillets. Since expressing TCDD data in pptr on a wet-tissue basis does not take into account differences in cooking losses, the weight of each sample was multiplied by its pptr TCDD to calculate picograms of TCDD in each raw and cooked sample. These data were used to calculate a percentage reduction summarized in Table IV. Analyses of variance indicated the loss of TCDD from samples roasted uncovered was not significantly different from those roasted covered even though the total cooking losses had been statistically significantly greater. Percentage TCDD reduction was 2–8 times greater than total cooking losses.

Percentage TCDD reduction was not statistically significantly affected at $p \leq 0.05$ by the concentration of TCDD in the raw samples, i.e., control versus spiked for any of the analyses of variance. Puffer and Gossett (1983) had attributed the greater losses of PCBs and DDT compounds from white croaker caught in Santa Monica Bay to the higher residue levels than those found in croaker from Orange County. These investigators were looking at levels in the ppm range, however. In the current study, TCDD residues were in the pptr range and percentage reduction from levels of approximately 40 and 100 pptr were similar. Thus, reductions similar to those obtained in the current study could be expected for fish found in the environment.

Increasing the end point cooking temperature statistically significantly increased the percentage of TCDD loss (p < 0.01) in all roasted and charbroiled samples. This might be anticipated from the roasted samples as cooking losses were significantly increased. Nevertheless, the variability in xenobiotic residue analyses often negates a significant difference. Again, the magnitude of the percentage of TCDD reduction was sufficient to make this difference significant so consumers can be advised that increasing the degree of doneness will significantly reduce level of exposure to TCDD.

Increasing the surface area of the restructured fillet also statistically significantly increased the percentage of TCDD loss (p < 0.01) for both charbroiled and samples roasted uncovered. Increasing the diameter from 7.5 to 10.0 cm increased the surface area by 166%. The increased loss of TCDD occurred in the charbroiled samples even though the cooking losses were reduced, but the magnitude

Table III.	TCDD Levels in	Raw and Coo	ked Restructured	l Fillets Prer	ared Using	Cart	n Surimi
Tanto III.	TODD Levels in	Itaw and Coo	Ver Theset recenter	. T. III ČÍS I I Čľ	area Osing	Jair	, curim

	fillet diameter, cm	int temp, °C	pptr TCDD			
cooking method			control		spiked ^d	
			raw	cooked	raw	cooked
roasted covered	7.5	60	46 ± 3^{b}	29 ± 1	90 ± 5	53 ± 3
		70	41 ± 3	22 ± 2	91 ± 5	51 ± 2
		80	45 ± 3	18 ± 2	92 ± 6	45 ± 1
roasted uncovered	7.5	60	37 ± 4	28 ± 3	87 ± 2	54 ± 5
		70	39 ± 3	23 ± 1	89 ± 3	51 ± 2
		80	39 ± 6	20 ± 2	89 ± 4	46 ± 1
roasted uncovered	10.0	80	45 ± 3	18 ± 1	90 ± 7	43 ± 2
charbroiled	7.5	60	39 ± 3	21 ± 2	90 ± 8	43 ± 5
		80	38 ± 5	19 ± 2	92 ± 10	41 ± 3
charbroiled	10.0	80	38 ± 4	16 ± 3	97 ± 6	34 ± 8

^aSamples were spiked to approximately 100 pptr TCDD. bn = 4.

Table IV. Percentage Reduction in TCDD Residue during Roasting or Charbroiling Restructured Fillets Prepared Using Carp Surimi

	fillet diameter.	end temp.	TCDD reduction, %		
cooking method	cm	°C	control	spiked	
roasted covered	7.5	60	41.4 ± 4.0^{b}	44.2 ± 3.9	
		70	50.5 ± 7.9	47.7 ± 2.1	
		80	63.4 ± 4.3	55.0 ± 3.9	
roasted	7.5	60	34.2 ± 8.7	47.0 ± 4.4	
uncovered		70	49.2 ± 6.3	51.3 ± 2.4	
		80	56.6 ± 5.9	57.5 ± 2.0	
roasted uncovered	10.0	80	65.9 ± 2.6	59.2 ± 2.4	
charbroiled	7.5	60 80	55.3 ± 6.5 62.0 ± 4.3	59.3 ± 5.2 63.6 ± 3.7	
charbroiled	10.0	80	67.5 ± 7.8	70.6 ± 7.7	

^aSamples were spiked to approximately 100 pptr TCDD. ^bn = 4.

of TCDD loss was much greater than fat and volatile losses alone. Previous work had suggested dieldrin losses were enhanced in pork round roasts with the greatest surface area (Yadrick et al., 1972).

Thus, cooking results in a substantial reduction of TCDD in fish fillets and cooking methods that promote loss are those applying high heat and/or cooking to a well-done stage. Increasing surface area also enhanced TCDD reduction. These factors may be important in evaluating the risk to human health of eating fish contaminated with TCDD.

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Registry No. TCDD, 1746-01-6.

Supplementary Material Available: Tables of individual TCDD analyses for the restructured fillets made from carp surimi (6 pages). Ordering information is given on any current masthead page.

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