

Dioxin Residues in Beef Carcasses in Beni-Suef City, Egypt

F. A. Khalafalla^{1*}, Fatma H. M. Ali¹, F. Schwägele², Mariem A. Abdel-Wahab¹

¹ Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt and ² Max Rubner Institute, Federal Center of Nutrition and Food, Kulmbach, Germany

A total of 100 fat samples were collected from slaughtered cattle at Beni-Suef slaughter house. Animals were randomly selected from the slaughter line. The age of the slaughtered cattle was normally less than three years (18-30 months). Samples were separately packed in plastic bags, identified and stored at -18°C until analysis. The analysis was conducted at Max Rubner Institute, Kulmbach, Germany for PCDDs, PCDFs and dl-PCBs. The average concentration sum for PCDD calculated as upper bound pg WHO-TEQ/g was 1.71 pg WHO-TEQ/g fat. While the average concentration sum for PCDFs was 4.93 pg WHO-TEQ/g fat. The average concentration sum of dl PCBs including non-ortho and mono-ortho PCBs was 5.091 pg WHO-TEQ/g fat. The total TEQ value for PCDD/Fs and dl-PCBs was 11.74 pg WHO-TEQ/g fat. The residual levels of PCDD/Fs and dl-PCBs in the examined beef fat samples were higher than the proposed maximum permitted levels set by the European Commission (2006).

Meat and meat products constitute an important part of human diet as well as an important source of a wide range of nutrients, but may also carry some toxic substances. Since this should be limited to an unavoidable minimum, much attention is paid to the occurrence of these substances in food. Monitoring programs have been carried out in many countries with the purpose of avoiding the distribution of food stuffs that could pose a risk to human health if consumed (López-Alonso *et al.*, 2000). Dioxins, which include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) are among the most hazardous chemicals that exhibit potential risks for human and environment due to their persistence and lipophilic character (De Voogt *et al.*, 1990; Fries, 1995), they tend to concentrate in the food chain. It has been concluded that ingestion of contaminated food of animal origin is the principal way of human exposure to these compounds, accounting for > 90% if compared to other ways such as inhalation and dermal contact. More

specifically high portion of exposure to human is expected from great consumption of animal fat (Toyoda *et al.*, 1999). The monitoring of raw meat for chemical residues is necessary to ascertain that approved compounds are not being misused and are not presenting a danger to consumers (Pullen, 1990). These contaminants mainly result from combustion processes of wastes, as chemical impurities in a range of manufactured organochlorine products, industrial processes, the manufacture of some pesticides, herbicides and fungicides, and pyrolysis processes (Olie and Buekens, 2007; Loutfy *et al.*, 2007). Levels of dioxins and dioxin like PCBs have been determined in different countries by many authors ranging from (0.03-13 pg WHO-TEQ/g fat) for PCDDs, (0.03-2.9 pg WHO-TEQ/g fat) for PCDFs and (0.05-3 pg WHO-TEQ/g fat) for dl-PCBs (Loutfy *et al.*, 2007; Kim *et al.*, 2007; Kim *et al.*, 2008). The aim of this study was to determine levels of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin like polychlorinated biphenyls (dl-PCBs) in beef fat samples collected from slaughtered carcasses of native cattle in Beni-Suef city, Egypt.

Materials and methods

Sample collection. A total of 100 fat samples were collected from slaughtered cattle at Beni-Suef slaughter house. Animals were randomly

* Corresponding author. Tel.: +20 082/2327982;
Fax: +20 082/2327982
E-mail address: fathykhalafalla@hotmail.com
(Fathy Khalafalla)

selected from the slaughter line. The age of the slaughtered cattle was normally less than three years (18-30 months). Samples were separately packed in plastic bags, identified and stored at -18°C until analysis.

Dioxins estimation

Extraction. Extraction of PCDD/Fs and PCBs was carried out using an Accelerated Solvent Extraction (ASE 200) device (Dionex, Sunny Vale, USA). The extraction cell was filled with dried sample material, drying substance and sea sand. For extraction n-hexane was used at a pressure 100 bar and a temperature of 100°C. The samples were extracted in two cycles with a static time of 10 min each. The solvent of the extract was evaporated in a nitrogen stream in a water bath at 40°C.

Gel permeation chromatography. For GPC, an LC glass column with an id of 25 mm was filled with 60 g bio-beads S-X3. The GPC (Abimed Gilson, Langenfeld, Germany) was carried out with a solvent mixture of cyclohexane: ethylacetate (50:50 v/v) at a flow rate of 5 ml/min (wasting time 0-28 and collecting time 28-38 minutes).

Clean-up with florisil. The samples were evaporated under pressure, carefully brought to dryness with nitrogen and dissolved in 1ml toluene. Florisil (calcined for 12 h at 55°C) was deactivated with 4% water. Three grams of deactivated florisil were filled into commercial disposable 8 ml solid phase extraction (SPE) columns (id 12 mm). After conditioning of the columns with 10 ml toluene, the samples were applied and eluted with 60 ml toluene to remove more polar compounds.

Clean-up with activated and endowed silica. After evaporation of the solvent to near dryness with rotation evaporation and nitrogen, the samples were dissolved in 1 ml n-hexane. Commercially available 8 ml SPE cartridges with an id of 12 mm were filled, in the following order with: 1- 0.3 g drying substance, 2- 1g 33% NaOH-Silica, 3- 0.25g activated silica, 4- 2.5g 44% H₂SO₄-Silica, 5- 0.5g 22% H₂SO₄-Silica (all together about 5g silica). After conditioning of the columns with 10 ml n-hexane, the sample extracts were put on the columns and eluted with 60 ml n-hexane.

Clean-up with activated charcoal. An LC glass column with an id of 12.5 mm was filled with 250

mg activated charcoal (Supelclean ENVI-Carb). For conditioning of the column, one blank run was made with a flow rate of 5 ml/min following program: 1) 20 ml solvent mixture 1(n-hexane/toluene; 99:1), 2) 20 ml solvent mixture 2 (n-hexane/toluene; 75:75), 3) 60 ml toluene (back-flush mode), 4) cleaning and conditioning of the column with toluene and solvent mixture 1 in both flow directions to use the column filling again for a new sample.

Samples were concentrated to a volume of 1 ml and fractionated with the mentioned program: In the first fraction (20 ml n-hexane/toluene; 99:1) the di- and mono-ortho-substituted PCBs were collected, and in the second fraction (20 ml n-hexane/toluene; 75:25) the non-ortho-substituted PCBs were collected. In the third fraction (60 ml toluene) the PCDDs/Fs were collected in back-flush mode because they have the characteristic to stick on activated charcoal. Cross-contamination was prevented by cleaning the activated charcoal column after each sample with solvent mixture 1 and with toluene in back-flush and in normal flow mode. Blank samples between normal samples could rule out possible cross-contamination.

GC-HRMS. Analysis of PCDD/Fs and dl-PCBs was performed with a gas chromatography (Hewlett-Packard 5860 series II gas chromatograph, Waldbronn, Germany) coupled with a VG Autospec Micromass (Manchester, UK) high-resolution mass spectrometer.

The quantification for PCDD/Fs and dl-PCBs was carried out by isotope dilution method USEPA 1613 (1994a) using relative response factors previously obtained from five standard solution injections. Two ¹³C-labeled PCDD and three ¹³C-labeled PCBs were added to the extracts before injection for the recovery calculations of PCDD/Fs and dl-PCBs, respectively.

Calculation. PCDD/Fs and DL-PCBs TEQ are calculated using the toxic equivalent factors (TEFs) according to WHO (Van den Berg *et al.*, 1998). The data below the detection limit (LOD) are calculated as lower, middle and upper bound values, assuming that all values of the different congeners are equal to zero, half the limit of detection or equal to their limit of detection, respectively. The numbering system of dl-PCBs congeners adapted by the International Union of Pure and Applied Chemistry (IUPAC) was used for confirmation and discussion of the results.

Results

Table (1): PCDDs concentrations (pg/g fat based on fresh weight) in beef fat samples and relative contribution of individual PCDDs congeners.

Congeners	No. of samples	Maximum	Mean	% contribution to sum PCDDs	% contribution to sum PCDDs/Fs
2,3,7,8-TeCDD	10	3.44	0.75	11.83	2.83
1,2,3,7,8-PeCDD	10	1.29	0.81	12.78	3.07
1,2,3,4,7,8-HxCDD	10	0.55	0.39	6.15	1.48
1,2,3,6,7,8-HxCDD	10	1.14	0.75	11.83	2.84
1,2,3,7,8,9-HxCDD	10	0.32	0.24	3.79	0.91
1,2,3,4,6,7,8-HpCDD	10	2.18	1.57	24.76	5.94
OCDD	10	2.63	1.83	28.86	6.92
Sum PCDDs	10	10.52	6.34	100.00	23.99

Table (2): PCDFs concentrations (pg/g fat based on fresh weight) in beef fat samples and relative contribution of individual PCDFs congeners.

Congeners	No. of samples	Maximum	Mean	% contribution to sum PCDFs	% contribution to sum PCDFs/Fs
2,3,7,8-TeCDF	10	7.55	1.06	5.28	4.01
1,2,3,7,8-PeCDF	10	1.35	0.43	2.14	1.63
2,3,4,7,8-PeCDF	10	9.51	7.72	38.45	29.22
1,2,3,4,7,8-HxCDF	10	5.59	4.33	21.56	16.39
1,2,3,6,7,8-HxCDF	10	4.46	3.59	17.88	13.59
2,3,4,6,7,8-HxCDF	10	1.99	1.34	6.67	5.07
1,2,3,7,8,9-HxCDF	10	0.16	0.10	0.50	0.38
1,2,3,4,6,7,8-HpCDF	10	1.19	0.93	4.63	3.53
1,2,3,4,7,8,9-HpCDF	10	0.51	0.35	1.74	1.32
OCDF	10	0.30	0.23	1.15	0.87
Sum PCDFs	10	29.22	20.08	100.00	76.01

Table (3): DL-PCBs concentrations (pg/g fat based on fresh weight) in beef fat samples and relative contribution of individual non and mono- ortho PCBs congeners.

Congeners	Maximum	Mean	% contribution to sum PCBs
3,3',4,4'-TCB (77)*	50.20	21.60	0.46
3,4,4',5'-TCB (81)	5.60	3.61	0.08
3,3',4,4',5'-PeCB (126)	55.00	42.76	0.91
3,3',4,4',5,5'-HxCB (169)	17.60	13.55	0.29
Sum non-ortho PCBs	115.5	81.52	1.74
2,3,3',4,4'-PeCB (105)*	1277.80	551.45	11.79
2,3,4,4',5'-PeCB (114)	165.80	105.81	2.26
2,3',4,4',5'-PeCB (118)	4727.50	2645.48	56.56
2',3,4,4',5'-PeCB (123)	326.50	139.18	2.98
2,3,3',4,4',5'-HxCB (156)	672.20	440.60	9.42
2,3,3',4,4',5'-HxCB (157)	148.80	115.39	2.47
2,3',4,4',5,5'-HxCB (167)	1009.00	519.46	11.11
2,3,3',4,4',5,5'-HpCB (189)	115.90	78.30	1.67
Sum mono-ortho PCBs	8403.1	4595.67	98.26

* IUPAC numbering system was used to classify PCBs congeners

Table (4): Average upper bound (pg WHO-TEQ/g fat) values for PCDD congeners, sum PCDDs and sum PCDDs/Fs.

Congeners	No. of samples	TEQ	% contribution to TEQ PCDDs/Fs
2,3,7,8-TeCDD	10	0.746	11.21
1,2,3,7,8-PeCDD	10	0.813	12.23
1,2,3,4,7,8-HxCDD	10	0.039	0.58
1,2,3,6,7,8-HxCDD	10	0.0754	1.13
1,2,3,7,8,9-HxCDD	10	0.024	0.36
1,2,3,4,6,7,8-HpCDD	10	0.01574	0.23
OCDD	10	0.0001828	0.01
Sum TEQ PCDDs	10	1.7133	25.75
Sum TEQ PCDDs/Fs	10	6.652	100

Table (5): Average upper bound (pg WHO-TEQ/g fat) values for PCDF congeners, sum PCDFs and sum PCDDs/Fs.

Congeners	No. of samples	TEQ	% contribution to TEQ PCDDs/Fs
2,3,7,8-TeCDF	10	0.1058	1.59
1,2,3,7,8-PeCDF	10	0.02125	0.32
2,3,4,7,8-PeCDF	10	3.8595	58.03
1,2,3,4,7,8-HxCDF	10	0.4329	6.52
1,2,3,6,7,8-HxCDF	10	0.3594	5.41
2,3,4,6,7,8-HxCDF	10	0.1346	2.02
1,2,3,7,8,9-HxCDF	10	0.0103	0.16
1,2,3,4,6,7,8-HpCDF	10	0.00928	0.14
1,2,3,4,7,8,9-HpCDF	10	0.0035	0.06
OCDF	10	0.000023	0.00
Sum TEQ PCDFs	10	4.936553	74.25
Sum TEQ PCDDs/Fs	10	6.652	100

Table (6): Average upper bound (pg WHO-TEQ/g fat) values for non-ortho, mono-ortho PCBs congeners and sum dl-PCBs.

Congeners	No. of samples	TEQ	% contribution to TEQ PCBs
Non-ortho PCBs	3,3',4,4'-TCB (PCB 77)	10	0.00216
	3,4,4',5'-TCB (PCB 81)	10	0.00036
	3,3',4,4',5'-PeCB (PCB 126)	10	4.276
	3,3',4,4',5,5'-HxCB (PCB 169)	10	0.1355
Sum TEQ non-ortho PCBs	10	4.41402	86.70
Mono-ortho PCBs	2,3,3',4,4'-PeCB (PCB 105)	10	0.055145
	2,3,4,4',5'-PeCB (PCB 114)	10	0.052905
	2,3',4,4',5'-PeCB (PCB 118)	10	0.264548
	2',3,4,4',5'-PeCB (PCB 123)	10	0.013918
	2,3,3',4,4',5'-HxCB (PCB 156)	10	0.2203
	2,3,3',4,4',5'-HxCB (PCB 157)	10	0.057695
	2,3',4,4',5,5'-HxCB (PCB 167)	10	0.0051946
	2,3,3',4,4',5,5'-HpCB (PCB 189)	10	0.00783
Sum TEQ mono-ortho PCBs	10	0.6775356	13.30
Sum TEQ PCBs	10	5.09155	100

Discussion

PCDD/Fs congener profiles. The results in Table (1) showed that the mean value of 7 PCDD congeners (sum PCDD) as upper bound values was 6.34 pg/g fat fresh weight with the maximum concentration of 10.52 pg/g fat fresh weight.

From the present data in Tables (1); 1, 2, 3, 4, 6, 7, 8-HpCDD and OCDD were the most abundant congeners in all samples with a mean value of 1.57 and 1.82 pg/g fat fresh weight, and contributed 24.76 and 28.86% respectively, to the total PCDDs concentration. The relative contribution of 1, 2, 3, 4, 7, 8-HxCDD and 1, 2, 3, 7, 8, 9-HxCDD is very low and constitute 6.15 and 3.79% respectively, to the total PCDDs concentration with a mean value of 0.39 and 0.24 pg/g fat fresh weight.

With regard to Table (2), it was found that the mean value of 10 PCDFs congeners (sum PCDFs) of 20.08 pg/g fat fresh weight with the maximum concentration of 29.22 pg/g fat fresh weight.

A significant contribution of 2, 3, 4, 7, 8-PeCDF, 1, 2, 3, 4, 7, 8-HxCDF, 1, 2, 3, 6, 7, 8-HxCDF and 2, 3, 4, 6, 7, 8-HxCDF has been observed in all samples represented 38.45%, 21.56%, 17.90 % and 6.67 % to the total PCDFs concentrations with a mean value of 7.72, 4.33, 3.59 and 1.34 pg/g fat fresh weight, respectively. The 1, 2, 3, 7, 8, 9-HxCDF contribution was not remarkable in all examined samples (0.50%) to the total PCDFs concentration with a mean value 0.10 pg/g fat fresh weight (Tables 2).

From the recorded data in Table (1 and 2) it is obvious that the pattern of PCDD/Fs in all analyzed samples is characterized generally by the dominance of PCDFs (76.01%), while PCDDs constitute (23.99%) in relation to the total PCDD/Fs concentrations.

Nearly similar results were recorded by Yu *et al.*, (2006); Loutfy *et al.*, (2007); Kim *et al.*, (2008).

Various toxic effects on immune, nervous, endocrine and reproductive systems, and potential carcinogenic effects have been reported for PCDD/Fs as reported by WHO, (1999).

PCDFs usually dominate the typical fingerprints combustion sources. This agrees with that reported by Huang and Buekens (1995); Loutfy *et al.*, (2007). The dominance of 2, 3, 4, 7, 8-PeCDF, 1, 2, 3, 4, 7, 8-HxCDF and 1, 2, 3, 6, 7, 8-HxCDF has been reported in the chimney soot

samples arising from wood burning reported by Bacher *et al.*, (1992), and the UK ambient air samples from an area where the domestic burning of wood and coal is widespread (Lohmann *et al.*, 2000). Moreover, Kim *et al.*, (2005) reported that PCDFs are generally produced from incineration processes. On the other hand, Ferrario *et al.*, (1996) found that beef fat samples from USA showed higher PCDDs contribution than PCDFs and suggested that pentachlorophenol-treated wood was one of the sources of PCDD contamination in USA and this was in agreement with Fries *et al.*, (2002) and Huwe *et al.*, (2004) as well as with Focant *et al.*, (2002) who found the same congener profiles in Belgium beef samples.

In general, it was found that the profiles obtained in this study are close to the profiles obtained from the combustion of household wastes (Wevers *et al.*, 2004). These results clearly reflect the situation in Egypt, where domestic and some agricultural solid waste open burning is quite common practice, and about one-fifth of the municipal solid waste is burned in open dump sites all over the country. Also no sludge of industrial waste is incinerated in Egypt. Incineration is very limited to some hospitals and some veterinary educational unit. However, some of the newly introduced industries such as pulp and paper bleaching and some metallurgic works constitute other sources of emission which agrees with Loutfy *et al.*, (2007).

DL-PCBs congener profiles. The average concentration values of 4 non-ortho and 8 mono-ortho PCBs are given in (Tables 3) along with maximum value for each congeners in pg/g lipid weight basis. This table illustrated that all non-ortho and mono-ortho PCBs congeners were observed in all investigated samples with a mean value of 81.52 and 4595.67 pg/g lipid weight basis for non-ortho and mono-ortho PCBs respectively. From Tables (3) it was obvious that the most abundant non-ortho PCBs congener was PCB 126 constituting 52.45% of the total amount of non-ortho PCBs with a mean value of 42.76 pg/g fat fresh weight, while PCB 81 constitutes 4.43% and represents the lowest non-ortho PCB concentration with a mean value of 3.61 pg/g fat fresh weight.

With regard to mono-ortho PCBs it was obvious that PCB 118 was the most abundant mono-ortho PCBs congener, comprising up to 57.57% of the total amount of mono-ortho PCBs

with a mean value of 2645.48 pg/g fat fresh weight. While PCBs 189 was the lowest mono-ortho PCBs concentration constituting 1.70% with a mean value of 78.30 pg/g fat fresh weight (Table 3).

In general, it was found that the pattern of dl-PCBs in all analyzed samples is characterized generally by the dominance of mono-ortho PCBs (98.26%) to the total dl-PCBs concentrations.

Exposure to polychlorinated biphenyls (PCBs) compounds resulted in various harmful effects including reproductive toxicity, immune suppression, birth defects, cancer and developmental and behavioural changes as mentioned by Safe, (1994).

Commercial PCB mixtures have been used in a wide variety of applications, including dielectric fluids in capacitors and transformers, heat transfer fluids, hydraulic fluids, lubricating and cutting oils and as additives in pesticides, paints, copying paper, carbonless copy paper, adhesives, sealants and plastics (Erickson, 1997).

On the contrary, Agency for Toxic Substances and Disease Registry, (2000) reported that although the manufacture, processing and distribution of polychlorinated biphenyls (PCBs) have been prohibited in almost all industrial countries since the late 1980s, their entry into the environment throughout the world still occurs. Improper waste disposal, both of house hold and industrial waste and leaks from electrical equipment and hydraulic systems still in use, are the major source of PCB emissions into the environment.

TEQs calculation and health implications. To assess the toxicity associated with presence of PCDD/Fs and dioxin like PCBs (non-ortho and mono-ortho PCBs) in foodstuffs, the WHO has proposed toxic equivalency factors (WHO-TEFs) for each of the toxic congeners, which relate the toxicity of the different congeners to that of 2, 3, 7, 8-TCDD, the most toxic one (Van den Berg *et al.*, 1998). Combining the concentration found for each congener with its corresponding WHO-TEF, a single WHO-TEQ value is obtained, suitable to compare levels between different studies and with the maximum content in food allowed by different international bodies.

PCDD/Fs TEQs. The average concentration values for individual PCDD/F congeners as well as the average concentration sums for PCDD/Fs

calculated as upper bound pg WHO-TEQ/g values are given in Tables (4 and 5).

From the present data it is clear that PCDF contribution to PCDD/Fs TEQ was predominated in all examined samples (74.25%), while PCDD constitute about 25.75 % of PCDD/Fs TEQ.

Nearly similar results were reported by Focant *et al.*, (2002); Kim *et al.*, (2008). On the contrary Ferrario *et al.*, (1996) reported that the total contribution of PCDD were higher than PCDFs in the USA beef fat.

Concerning the contribution of individual congeners to PCDD/Fs TEQ values, it is appear that 58.03 % of PCDD/Fs TEQ is actually due to 2, 3, 4, 7, 8-PeCDFs congener. With regard to PCDD, 1, 2, 3, 7, 8-PeCDD and 2, 3, 7, 8-TeCDD which have the highest toxic equivalent factors (TEFs) were the predominant congeners accounting for 12.23 and 11.21 % of the PCDD/Fs TEQ.

Generally, it can be concluded that the residual TEQ in the examined fat samples was mostly from PCDFs.

DL-PCBs TEQ. The average concentration values for 4 non-ortho PCBs congeners, 8 mono-ortho PCBs congeners and the average concentration sum for dl-PCBs expressed in picogram WHO-TEQ/g fat in upper bound limit of determination were given in Table (6).

It is obvious that non-ortho PCBs congeners constitute the largest portion of the total PCBs TEQ with a relative contribution of 86.7 % of the total PCBs TEQ. This agrees with Bordajandi *et al.*, (2004) who stated that in most cases the largest contribution to the TEQ value is due to dl-PCBs corresponded to non-ortho PCBs.

Concerning the contribution of individual congeners to the PCBs TEQ value, PCB 126 seemed to be the most important one represented 84 % of the total PCBs TEQ. This was in close agreement with Loutfy *et al.*, (2007) who reported that congener PCB 126 is the most toxic one, having the highest TEF and accounting for the larger contribution of PCBs to the TEQ. It contributes more or less 80 % of the total PCBs.

Regarding to mono-ortho PCBs congeners, it is worth noticing that although the congeners exhibiting the highest concentration in all samples were PCB 118, 167, 156, and 105, when data are expressed in WHO-TEQ, they represent a very low relative contribution to the total PCBs TEQ

value due to their low TEF value. In this respect, Loutfy *et al.*, (2007) found that mono-ortho PCBs congeners were detected in all samples with congener 118 as the most abundant, followed by congener 105.

Total TEQ. From the present data can be concluded that all examined fat samples are heavily contaminated (6.65 pg TEQ PCDD/Fs /g fat and 5.09 pg TEQ PCBs/g fat) in comparison with the European Union National Average Concentrations (3 pg TEQ/g fat) (European Commission, 2001), and the future EU limit (European Commission, 2006), if PCB, are included.

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بقايا الديوكسين في ذبائح الأبقار في محافظة بنى سويف

أجريت هذه الدراسة علي ١٠٠ عينة من دهون مذبوحات الأبقار في مجزر بنى سويف وقد تم اختيار الحيوانات عشوائيا من خطوط الذبح ، والتي تتراوح أعمارها من ١٨-٣٠ شهر وتم تغليف العينات في أكياس بلاستيكية وحفظها عند درجة -١٨ درجة مئوية حتي عملية الفحص. تم فحص العينات في معهد ماكس روبنر - كلومباخ المانيا لقياس مادة الديوكسين ، الفيوران ومتشابهات الديوكسين من البولي كلورينيتد بايفنيل ، وقد بلغ متوسط التركيز التراكمي لمادة الديوكسين ، الفيوران ومتشابهات الديوكسين من البولي كلورينيتد بايفنيل هو ١,٧١ ، ٤,٩٣ ، ٥,٠٩١ بيكو جرام لكل جرام من الدهن علي التوالي معبرا عنه ب WHO-TEQ. كما بلغ متوسط مجموع معدل السمية ١١,٧٤ بيكو جرام لكل كيلو جرام من الدهن معبرا عنه ب WHO-TEQ.