



Feeding Fats Safety

FEEDING FATS SAFETY

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PCDD/F and DL-PCB in fats, rate of transfer to animal tissues and repercussions on meat safety

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Toxic equivalency factors assigned by The World Health Organisation (WHO-TEFs) to PCDD/Fs and DL-PCBs

Compound	WHO-TEF	Compound	WHO-TEF
Dioxins		Non-ortho PCBs	
2378-TCDD	1	33'44'-TeCB (# 77)	0.0001
12378-PeCDD	1	344'5'-TeCB (# 81)	0.0001
123478-, 123678-, 123789-HxCDD	0.1	33'44'5'-PeCB (#126)	0.1
1234678-HpCDD	0.01	33'44'55'-HxCB (# 169)	0.01
OCDD	0.0001	Mono-ortho PCBs	
Furans		233'44'-PeCB (# 105)	0.0001
2378-TCDF	0.1	2344'5'-PeCB (# 114)	0.0005
12378-PeCDF	0.05	23'44'5-, 2344'5'- PeCB (#118, 123)	0.0001
23478-PeCDF	0.5	233'44'5-, 233'44'5'-HxCB (# 156, 157)	0.0005
123478-, 123678-, 123789-, 234678-HxCDF	0.1	23'44'55'-HxCB (# 167)	0.00001
1234678-, 1234789-HpCDF	0.01	233'44'55'-HpCB (# 189)	0.0001
OCDF	0.0001		

Expression of the results:

$$\text{WHO-TEQ} = \sum_{i=1}^N C_i \times \text{TEF}_i$$





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WP-1: Characterization and classification of feeding fats

Sample category	N° of samples	ng WHO-TEQ/ kg fat or oil	"upperbound levels"		
			Max-Min	Mean	Median
ANFA	22	PCDD/Fs	0.77-0.08	0.32	0.30
		PCDD/Fs + DL-PCBs	1.61-0.13	0.74	0.71
FISH	9	PCDD/Fs	12.65-0.40	3.32	1.98
		PCDD/Fs + DL-PCBs	42.47-1.87	13.61	9.40
AOCHE	15	PCDD/Fs	1.08-0.07	0.48	0.36
		PCDD/Fs + DL-PCBs	5.35-0.21	1.03	0.62
AOPHY	10	PCDD/Fs	2.25-0.13	1.06	0.97
		PCDD/Fs + DL-PCBs	6.94-0.25	2.36	1.68
LECI	8	PCDD/Fs	0.13-0.03	0.06	0.05
		PCDD/Fs + DL-PCBs	0.17-0.03	0.09	0.08
RECY	8	PCDD/Fs	0.52-0.11	0.30	0.31
		PCDD/Fs + DL-PCBs	0.86-0.16	0.55	0.58
HYBY	4	PCDD/Fs	1.74-0.26	0.98	0.96
		PCDD/Fs + DL-PCBs	2.14-0.54	1.32	1.30
EBE	2	PCDD/Fs	1.12-0.44	-	-
		PCDD/Fs + DL-PCBs	1.75-1.01	-	-
FACS	1 Pooled sample* (n=3)	PCDD/Fs	0.28*	-	-
		PCDD/Fs + DL-PCBs	0.37*	-	-
MIX	1 Pooled sample* (n=10)	PCDD/Fs	0.12*	-	-
		PCDD/Fs + DL-PCBs	0.31*	-	-

COMMISSION DIRECTIVE 2006/13/EC
of 3 February 2006

amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs

PCDD/Fs; PCDD/Fs + DL-PCBs

*Animal fat:
2.0 ng WHO-TEQ/kg
3.0 ng WHO-TEQ/kg

*Feed materials of plant origin with the exception of vegetable oils and their byproducts:
0.75 ng WHO-TEQ/ kg
1.25 ng WHO-TEQ/ kg

*Fish oil:
6 ng WHO-TEQ/kg
24 ng WHO-TEQ/kg

*Vegetable oils and their by-products:
0.75 ng WHO-TEQ/ kg
1.5 ng WHO-TEQ/ kg

Ábalos et al., Chemosphere 71, 1115-1126 (2008)



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WP-1: Characterization and classification of feeding fats

Main Conclusions

- ✓ Fish oil (FISH) samples showed the highest PCDD/F and DL-PCB concentrations. This was expected due to the animal origin of these products. In some particular cases, the concentrations were similar or above the maximum levels established for these pollutants at the Commission Directive 2006/13/EC.
- ✓ All the analyzed animal fat (ANFA) samples showed levels of PCDD/Fs and DL-PCBs below the maximum established at the Commission Directive 2006/13/EC.
- ✓ Different samples of vegetal origin (i.e. LECI) showed, in general, low concentrations of PCDD/Fs and DL-PCBs. However, special attention should be paid particularly to the category of acids oils from physical refining (AOPHY) since seven out of a total of nine of these samples would exceed the maximum level set at the Commission Directive 2006/13/EC for PCDD/Fs in by-products of vegetable oils. In addition, five of these seven samples would also exceed the maximum set for the sum of PCDD/Fs and DL-PCBs.





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WP-4: Levels of contamination in meat and other animal tissues, and rate of transfer from feed

(4.1. Effect of feeding selected fats (recycled ones) on the contamination of rabbit and poultry meat and other tissues)

pg WHO-TEQ/g oil "upperbound"	Fish oil A	Fish oil B "spiked"
PCDD/Fs	1,95	<u>9,78</u>
DL-PCBs	7,69	<u>19,02</u>
PCDD/Fs + DL-PCBs	9,64	<u>28,80</u>

Maximum levels for fish oil samples (COMMISSION DIRECTIVE 2006/13/EC 3 February 2006):

- 6 pg WHO-TEQ/g oil PCDD/Fs
- 23 pg WHO-TEQ/g oil PCDD/Fs + DL-PCBs



Maximum levels for compound feedingstuffs
(COMMISSION DIRECTIVE 2006/13/EC 3 February 2006):

- 0,75 pg WHO-TEQ/g PCDD/Fs
- 1,5 pg WHO-TEQ/g PCDD/Fs + DL-PCBs

Broilers - Treatment 1: 6% Fish oil A

Broilers - Treatment 2: 3% Fish oil A + 3% Fish oil B

Broilers - Treatment 3: 6% Fish oil B

pg WHO-TEQ/g feed "upperbound"	Treatment 1	Treatment 2	Treatment 3
PCDD/Fs	0,11	0,39	0,54
DL-PCBs	0,48	0,73	1,21
PCDD/Fs + DL-PCBs	0,59	1,11	<u>1,75</u>



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(COMMISSION DIRECTIVE 2006/13/EC 3 February 2006):

- 0,75 pg WHO-TEQ/g PCDD/Fs
- 1,5 pg WHO-TEQ/g PCDD/Fs + DL-PCBs

Rabbits - Treatment 1: 3% Fish oil A

Rabbits - Treatment 2: 1.5% Fish oil A + 1.5% Fish oil B

Rabbits - Treatment 3: 3% Fish oil B

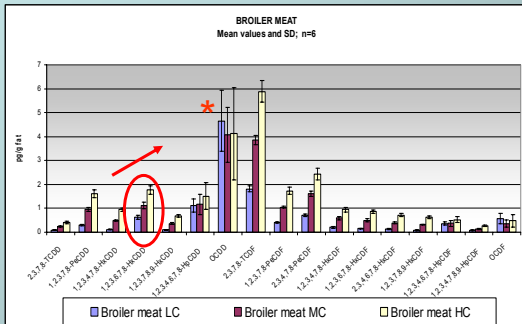
pg WHO-TEQ/g feed "upperbound"	Treatment 1	Treatment 2	Treatment 3
PCDD/Fs	0,10	0,15	0,27
DL-PCBs	0,23	0,38	0,59
PCDD/Fs + DL-PCBs	0,33	0,53	0,86





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☐ Poultry Meat:



Conc (pg/g fat)	MEAN	RSD(%)
HC		
n=6		
2,3,7,8-TCDD	0,407	12
1,2,3,7,8-PeCDD	1,617	10
1,2,3,4,7,8-HxCDD	0,945	7
1,2,3,6,7,8-HxCDD	1,765	10
1,2,3,7,8,9-HxCDD	0,690	8
1,2,3,4,6,7,8-HpCDD	1,510	37
OCDD	4,118	47
2,3,7,8-TCDF	5,892	8
1,2,3,7,8-PeCDF	1,733	9
2,3,4,7,8-PeCDF	2,436	10
1,2,3,4,7,8-HxCDF	0,948	9
1,2,3,6,7,8-HxCDF	0,863	7
2,3,4,6,7,8-HxCDF	0,719	9
1,2,3,7,8,9-HxCDF	0,628	8
1,2,3,4,6,7,8-HpCDF	0,530	23
1,2,3,4,7,8,9-HpCDF	0,285	9
OCDF	0,480	54

pg WHO-TEQ/g fat "upperbound" (n=6)	Broilers Treatment 1	Broilers Treatment 2	Broilers Treatment 3
PCDD/Fs	1,11 (RSD 6%)	2,85 (RSD 5%)	4,60 (RSD 8%)
DL-PCBs	4,92 (RSD 7%)	8,40 (RSD 5%)	12,11 (RSD 8%)
Total (PCDD/Fs + DL-PCBs)	6,03 (RSD 7%)	11,24 (RSD 4%)	16,71 (RSD 8%)

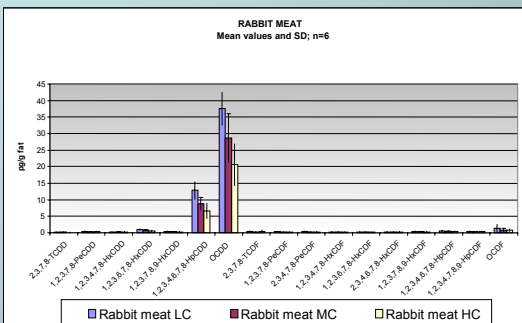
Maximum levels for meat and meat products of poultry and farmed game (COMMISSION REGULATION (EC) N° 199/2006 3 February 2006):

- 2 pg WHO-TEQ/g fat PCDD/Fs
- 4 pg WHO-TEQ/g fat PCDD/Fs + DL-PCBs



Feeding Fats Safety

☐ Rabbit Meat:



Conc (pg/g fat)	MEAN	RSD(%)
LC		
n=6		
2,3,7,8-TCDD	0,115	57
1,2,3,7,8-PeCDD	0,353	50
1,2,3,4,7,8-HxCDD	0,287	47
1,2,3,6,7,8-HxCDD	0,969	20
1,2,3,7,8,9-HxCDD	0,388	59
1,2,3,4,6,7,8-HpCDD	12,791	21
OCDD	37,501	13
2,3,7,8-TCDF	0,381	61
1,2,3,7,8-PeCDF	0,296	50
2,3,4,7,8-PeCDF	0,357	41
1,2,3,4,7,8-HxCDF	0,269	53
1,2,3,6,7,8-HxCDF	0,264	55
2,3,4,6,7,8-HxCDF	0,291	53
1,2,3,7,8,9-HxCDF	0,369	49
1,2,3,4,6,7,8-HpCDF	0,510	51
1,2,3,4,7,8,9-HpCDF	0,425	50
OCDF	1,277	89

Underlined values indicate 3 or more replicates below de LOD!!!

pg WHO-TEQ/g fat "upperbound" (n=6)	Rabbits Treatment 1	Rabbits Treatment 2	Rabbits Treatment 3
PCDD/Fs	1,12 (RSD 36%)	0,86 (RSD 15%)	0,75 (RSD 19%)
DL-PCBs	1,56 (RSD 18%)	1,69 (RSD 11%)	2,51 (RSD 15%)
Total (PCDD/Fs + DL-PCBs)	2,68 (RSD 22%)	2,55 (RSD 7%)	3,54 (RSD 22%)

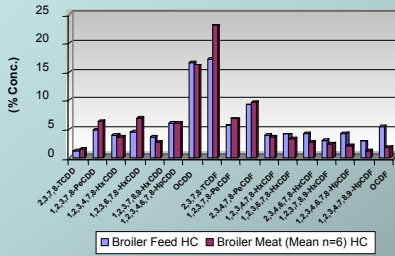
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- 2 pg WHO-TEQ/g fat PCDD/Fs
- 4 pg WHO-TEQ/g fat PCDD/Fs + DL-PCBs

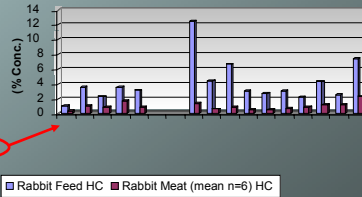
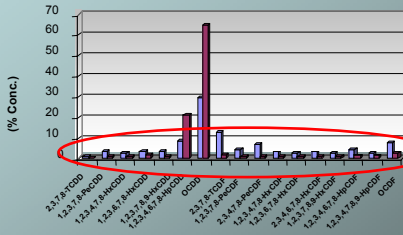




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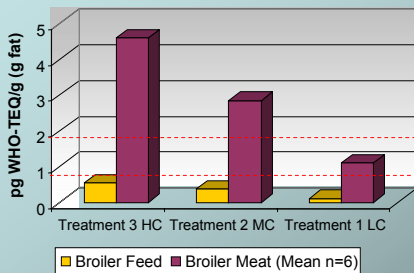


Comparison
PCDD/F congener contribution in feed
vs.
PCDD/F congener contribution in meat



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PCDD/Fs



Comparison
PCDD/F and DL-PCB levels in feed
vs.
PCDD/F and DL-PCB levels in broiler meat

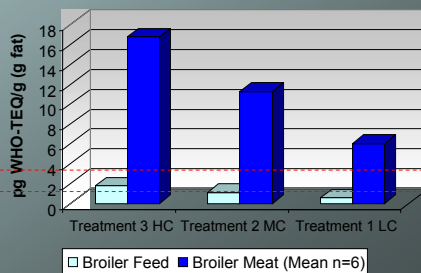
Maximum level for meat (2 pg WHO-TEQ/g fat)

Maximum level for feeds (0,75 pg WHO-TEQ/g)

Maximum level for meat (4 pg WHO-TEQ/g fat)

Maximum level for feeds (1,5 pg WHO-TEQ/g)

PCDD/Fs + DL-PCBs





Feeding Fats Safety

WP-4: Levels of contamination in meat and other animal tissues, and rate of transfer from feed

(4.1. Effect of feeding selected fats (recycled ones) on the contamination of rabbit and poultry meat and other tissues)

Main Conclusions (I)

- ✓ Remarkable differences in individual concentrations of PCDD/Fs in meat between broilers and rabbits.
- ✓ The experiment with broilers allowed us to achieve higher sensitivity and acceptable RSD(%) for PCDD/F and DL-PCB analysis.
- ✓ Good correlation between congener distribution profiles in the feeds and in the meat samples in the case of broilers.
- ✓ Meat broiler samples (including skin!!!) from some of the experiments would exceed the maximum PCDD/F and DL-PCB levels established at the European Commission Regulation (EC) N° 199/2006 3 February 2006.



Feeding Fats Safety

WP-4: Levels of contamination in meat and other animal tissues, and rate of transfer from feed

(4.1. Effect of feeding selected fats (recycled ones) on the contamination of rabbit and poultry meat and other tissues)

Main Conclusions (II)

- ✓ The differences in the results obtained between the two types of animals (chickens and rabbits) could be explained taking into account:
 - 1) The differences in the percentage of fat between chicken meat (aprox. 13% fat, including skin) and rabbit meat (aprox. 2,4% fat), which would explain lower levels of contaminants in the rabbit samples;
 - 2) Also, the lower levels of PCDD/Fs and DL-PCBs in the feeds for rabbits, due to the use of half of the percentage of fish oil in their preparation compared to the chicken feeds and;
 - 3) In addition, they could be differences in bioaccumulation processes between the two types of animals.





Feeding Fats Safety

WP-4: Levels of contamination in meat and other animal tissues, and rate of transfer from feed

(4.2. Rate of transfer of selected contaminants from spiked feed to poultry meat and other tissues)

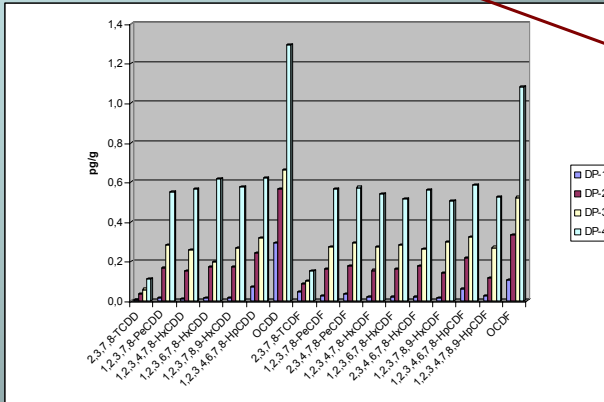
DP-1: Background

DP-2: ½ max: 0.38 pg WHO-TEQ/g (PCDD/Fs) and 0.38 pg WHO-TEQ/g (DL-PCBs)

DP-3: max: 0.75 pg WHO-TEQ/g (PCDD/Fs) and 0.75 pg WHO-TEQ/g (DL-PCBs)

DP-4: 2 max: 1.5 pg WHO-TEQ/g (PCDD/Fs) and 1.5 pg WHO-TEQ/g (DL-PCBs)

4 Experimental Feeds



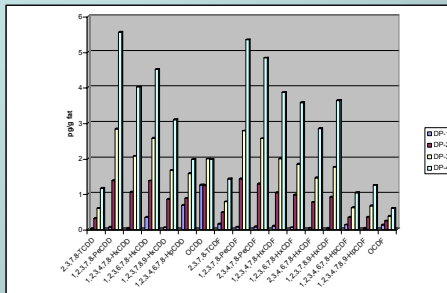
0,06 pg WHO-TEQ/g (PCDD/Fs)

0,03 pg WHO-TEQ/g (DL-PCBs)



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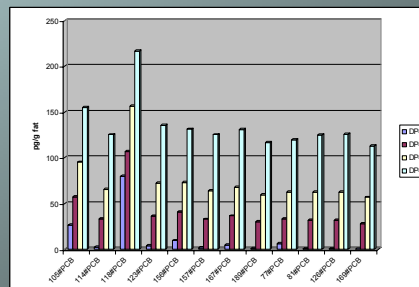
Poultry Meat:



PCDD/Fs

Mean values of
4 REPLICATES x TREATMENT

DL-PCBs





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Meat (mean values):

Maximum levels for meat and meat products of poultry and farmed game (COMMISSION REGULATION (EC) N° 199/2006 3 February 2006):

2 pg WHO-TEQ/g fat PCDD/Fs
4 pg WHO-TEQ/g fat PCDD/Fs + DL-PCBs

pg WHO-TEQ/g fat "upperbound" (n=4)	Meat DP-1	Meat DP-2	Meat DP-3	Meat DP-4
PCDD/Fs	0.21	3.16	6.50	12.57
Total (PCDD/Fs + DL-PCBs)	0.29	6.79	13.80	27.02

Liver (mean values):

Maximum levels for meat and meat products of poultry and farmed game (COMMISSION REGULATION (EC) N° 199/2006 3 February 2006):

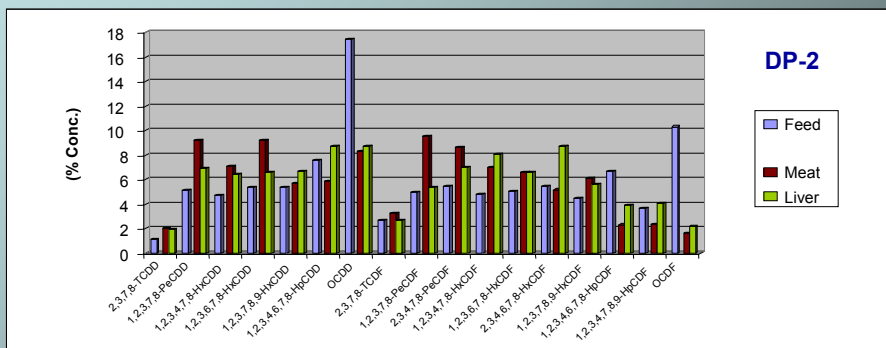
6 pg WHO-TEQ/g fat PCDD/Fs
12 pg WHO-TEQ/g fat PCDD/Fs + DL-PCBs

pg WHO-TEQ/g fat "upperbound" (n=4)	Liver DP-1	Liver DP-2	Liver DP-3	Liver DP-4
PCDD/Fs	0.72	7.24	12.99	26.53
Total (PCDD/Fs + DL-PCBs)	1.13	10.64	19.24	39.14



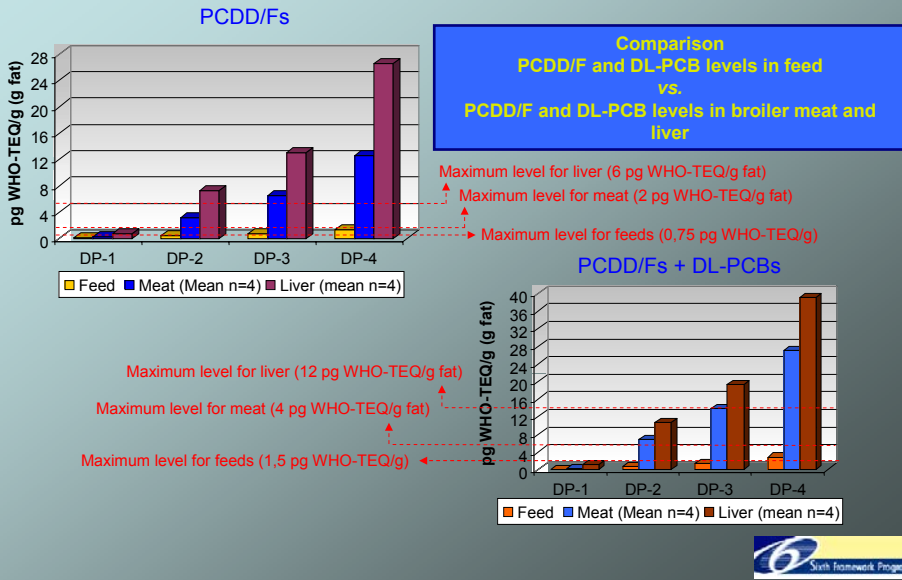
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Comparison
PCDD/F congener contribution in feed
vs.
PCDD/F congener contribution in meat and liver





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WP-4: Levels of contamination in meat and other animal tissues, and rate of transfer from feed
(4.2. Rate of transfer of selected contaminants from spiked feed to poultry meat and other tissues)

Main Conclusions

- ✓ Good correlation between congener distribution profiles in the feeds and in the meat and liver samples.
- ✓ Meat samples (including skin!!!) from DP-2 to DP-4 experiments exceed the maximum PCDD/F and DL-PCB levels established at the European Commission Regulation (EC) N° 199/2006 3 February 2006.
- ✓ Liver samples from DP-3 and DP-4 experiments exceed the maximum PCDD/F and DL-PCB levels established at the European Commission Regulation (EC) N° 199/2006 3 February 2006.
- ✓ Higher levels of PCDD/Fs, expressed in pg WHO-TEQ/g fat, were observed in liver compared to the meat samples, indicating differences in bioaccumulation between the two tissues.



Feeding Fats Safety

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