

APPENDIX 9

CARSO-LABORATOIRE SANTE ENVIRONNEMENT HYGIENE DE LYON

Laboratoire Agréé pour les analyses d'eaux par le Ministère de la Santé

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ASSAY REPORT	PCDD/F ANALYSES in samples of 2 phytosterols
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ACCREDITATION
N°1-1531
FORTEE
COMMUNIQUEE
SURDEMANDE



Date : 20/03/06

The assay "LSE06-2022" was carried out on request of :

VITAE CAPS

C/ Gutemberg 356 Poligono Torrehierro
45600 TALAVERA DE LA REINA-TOLEDO

ESPAGNE

To **GARCIA LOZANO**

LSE references : **LSE06-2022**

Customer references : Analysis of 17/02/06

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It includes 8 pages.

The report deals with the samples submitted to the assays only.

The skills of the laboratory are recognized by the COFRAC accreditation only for the assays covered by the accreditation.

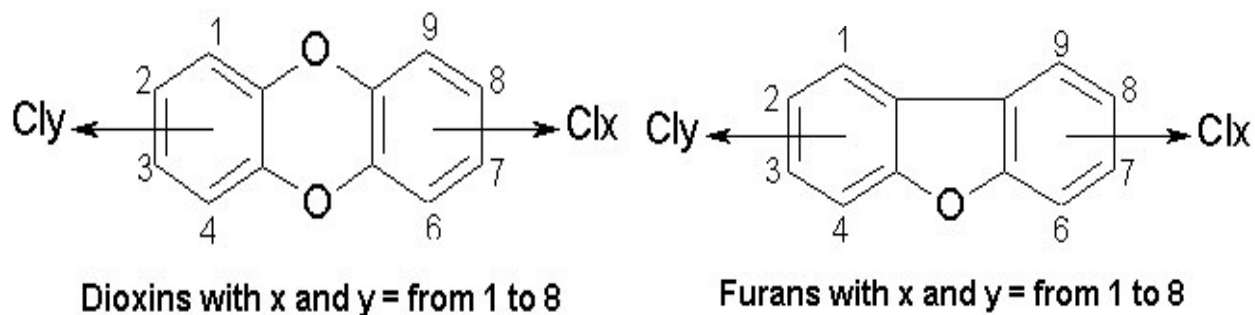
Dr Daniel Fraisse
Deputy Scientific Director

I.1 SUBJECT OF THE ASSAY

The subject of the assay report referenced under LSE06-2022 deals with PCDD/F analysis in a sample of 2 phytosterols.

I.1.1 COMPOUNDS TO BE ANALYSED

Polychloro-dibenzo-p-dioxins(PCDD) and polychloro-dibenzofurans (PCDF) are represented in the following drawing (The numerated positions can be occupied by some hydrogen or chloride atoms).



Among 210 dioxins and furans, only 17 congeners are considered as toxic, listed in paragraph III. These 17 dioxins and furans have a toxicity from a factor 1 for 2,3,7,8 TCDD to 0.0001 one for OCDD and OCDFs.

The toxicity of a sample is performed through the quantitative measurement of all these 17 toxic congeners, to which a specific toxic equivalency factor (I-TEF) is applied. Thus, the concentration of a sample in « Toxic Equivalent Dioxins and Furans or I-TE » is given.

The two standards for toxic equivalency factors applied were set up by the NATO/CCMS in 1998 and the WHO in 1997 (see II).

As regards food matrices, the WHO standard is recommended.

I.1.2 SAMPLING

Sampling was carried out by : the customer.

CARSO subcontracted sampling activities : No

I.1.3 RESULTS

Results are presented globally in the chapter II of the report.

The following chart lets appear some information about the analysed sample (customer's references, CARSO references, etc.), the quantities found out for each identic sery (tetra-aux octa-chlorinated) for PCDDs and PCDFs, the results for each toxic congener and the result of the global toxicity of the sample calculated from the 17 toxic congeners and expressed in toxic equivalent 2,3,7,8-TCDD, given by I-TEQ.

The results of the toxic quantities expressed in equivalent 2,3,7,8-TCDD, I-TEQ detected on the samples submitted to analysis, are summed up in the following chart :

Chart 1-1 : Summary of the results in I-TEQ

Customer references	LSE References		I TEQ WHO 1997	Unit
Vitasterol S-80 non GMO muestra fitosteroles – Batch ST (181-183)	0601-7251-2	medium bound (nd=0)	0.18	pg/g initial matter
Vitasterol S-80 Esterified non GMO muestra esters de fitosterol Batch ST (181- 183)E1	0603-7252-2	medium bound (nd=0)	0.069	pg/g initial matter

(n.c. : data not communicated by the customer)

When a congener is below the limit of detection, the I-TEQ results indicated above are calculated with this congener, equal to 0.

I.1.4 INFORMATION ABOUT THE ASSAY

Description	Information
Reception of samples	17/02/06
Storage methods	Ambiant temperature
Internal methods of analysis	MET-009
Reference standard	EPA1613
Elaboration of the sample before extraction	None
Measurement instrument HRGC/HRMS	Micromass : Autospec, seria ULTIMA
Injected volume, in μL	1 à 3 μL
Final volume	20 - 50 μl

1.2 SPECIFIC PARAMETERS FOR THE ASSAYS

Many parameters comply with each analytical sequence such as the spiking file, analyses of the blank of solvents or the HRGC/HRMS instrumentation (Possibly blank(s) of processus and control analyses). The results of the analyses can be consulted at the lab on request.

1.3 UNCERTAINTY IN ANALYTICAL MEASUREMENT

The uncertainty in analytical measurement is estimated by analyses made several times for a sample from the same nature than unknown samples ; or by the analysis of a certified or internal material in the lab or during ring tests.

The uncertainty is estimated by 15%.

1.4 SPECIFIC OBSERVATIONS ABOUT THE ASSAY

Samples were analysed in compliance with the internal method listed in paragraph I.1.4

NB. : No particular remark compared to the analytical protocol used.

1.5 QUALITY ASSURANCE – QUALITY CONTROL

The assay report was audited by the CARSO-LSEHL quality department.

II. DETAILS OF RESULTS

Project LSE06-2022 : Sample : LSE0601-7251-2							
							Print
Analysis of PHYTOSTEROL for PCDD/Fs							
							Date: 09/03/2006
CLIENT ID:	VITAE CAPS					Water content, %:	3,27
CLIENT SAMPLE ID:	VITASTEROL S-80 NON GMO : MUESTRA FITOSTEROLES - BATCH ST (181-183)						
						Extracted initial mass, g:	11,62
CARSO PROJECT ID :	LSE06-2022						
CARSO SAMPLE ID:	LSE0601-7251-2						
Sample Matrix:	PHYTOSTEROL						
Analysis start time:	17/02/2006						
HRGC/HRMS File Name:	3MARS						
	I-TEQ WHO 1998	pg/extract	pg/g of initial matter		LoD in pg/extract	LoD I-TEQ pg, WHO	Recovery %
Total TCDD		8,308	0,715				
Total PeCDD		5,073	0,437				
Total HxCDD		7,209	0,620				
Total HpCDD		14,394	1,239				
Sum from tetra- to octa-CDD		55,3	4,759				
Total TCDF		25,908	2,230				
Total PeCDF		11,579	0,996				
Total HxCDF		5,353	0,461				
Total HpCDF		4	0,344				
Sum from tetra- to octa-CDF		53,422	4,597				
Polychlorodibenzo-p-dioxins							
2,3,7,8-TCDD	1,00				0,25	0,2450	71
1,2,3,7,8-PeCDD	1,00				0,66	0,6610	66
1,2,3,4,7,8-HxCDD	0,10				0,93	0,0929	100
1,2,3,6,7,8-HxCDD	0,10				1,06	0,1056	91
1,2,3,7,8,9-HxCDD	0,10				0,99	0,0990	
1,2,3,4,6,7,8-HpCDD	0,010	4,839	0,416				63
OCDD	0,0001	20,316	1,748				50
Polychlorodibenzofurans							
2,3,7,8-TCDF	0,100	2,723	0,234				66
1,2,3,7,8-PeCDF	0,050	0,842	0,072				78
2,3,4,7,8-PeCDF	0,500	1,779	0,153				74
1,2,3,4,7,8-HxCDF	0,100	0,874	0,075				88
1,2,3,6,7,8-HxCDF	0,100				0,79	0,0793	93
2,3,4,6,7,8-HxCDF	0,100				0,85	0,0854	96
1,2,3,7,8,9-HxCDF	0,100				1,92	0,1917	80
1,2,3,4,6,7,8-HpCDF	0,010	2,176	0,187				77
1,2,3,4,7,8,9-HpCDF	0,010				0,77	0,0077	64
OCDF	0,0001	6,582	0,566				50
I-TEQ Results							
		pg/extract	pg/g of initial matter				
TOTAL I-TEQ nd=0 (lower bound):		1,4	0,12				
TOTAL I-TEQ nd=LoD/2 (medium bound) :		2,1	0,18				
TOTAL I-TEQ nd=LoD (upper bound):		2,9	0,25				
n. c. = unavailable information							
LoD = Limit of detection							
VNC = not communicated volume							

Project LSE06-2022 : Sample : LSE0601-7252-2							Print
Analysis of PHYTOSTEROL for PCDD/Fs							
						Date:	17/02/2006
CLIENT ID:	VITAE CAPS						
CLIENT SAMPLE ID:	VITASTEROL S-80 ESTERIFIED NON GMO : MUESTRA ESTERES DE FITOSTEROL - BATCH ST (181-183) E1					Extracted initial mass, g:	8,12
CARSO PROJECT ID :	LSE06-2022						
CARSO SAMPLE ID:	LSE0601-7252-2						
Sample Matrix:	PHYTOSTEROL						
Analysis start time:	17/02/2006						
HRGC/HRMS File Name:	10FEB						
	I-TEQ WHO 1998	pg/extract	pg/g of initial matter		LoD in pg/extract	LoD I-TEQ pg, WHO	Recovery %
Total TCDD		0,556	0,068				
Total PeCDD		1,125	0,139				
Total HxCDD		0,641	0,079				
Total HpCDD		2,888	0,356				
Sum from tetra- to octa-CDD		14,686	1,809				
Total TCDF		2,584	0,318				
Total PeCDF		1,225	0,151				
Total HxCDF		1,324	0,163				
Total HpCDF		1,551	0,191				
Sum from tetra- to octa-CDF		8,812	1,085				
Polychlorodibenzo-p-dioxins							
2,3,7,8-TCDD	1,00				0,12	0,1160	78
1,2,3,7,8-PeCDD	1,00				0,13	0,1300	84
1,2,3,4,7,8-HxCDD	0,10				0,26	0,0255	80
1,2,3,6,7,8-HxCDD	0,10	0,641	0,079				75
1,2,3,7,8,9-HxCDD	0,10				0,24	0,0238	
1,2,3,4,6,7,8-HpCDD	0,010	1,758	0,217				69
OCDD	0,0001	9,476	1,167				54
Polychlorodibenzofurans							
2,3,7,8-TCDF	0,100	0,516	0,064				73
1,2,3,7,8-PeCDF	0,050	0,203	0,025				99
2,3,4,7,8-PeCDF	0,500	0,382	0,047				74
1,2,3,4,7,8-HxCDF	0,100	0,346	0,043				59
1,2,3,6,7,8-HxCDF	0,100	0,164	0,020				67
2,3,4,6,7,8-HxCDF	0,100				0,21	0,0209	87
1,2,3,7,8,9-HxCDF	0,100				0,11	0,0113	98
1,2,3,4,6,7,8-HpCDF	0,010	1,042	0,128				57
1,2,3,4,7,8,9-HpCDF	0,010				0,12	0,0012	75
OCDF	0,0001	2,128	0,262				50
I-TEQ Results							
		pg/extract	pg/g of initial matter				
TOTAL I-TEQ nd=0 (lower bound):		0,40	0,049				
TOTAL I-TEQ nd=LoD/2 (medium bound) :		0,56	0,069				
TOTAL I-TEQ nd=LoD (upper bound):		0,73	0,089				
n.c. = unavailable information							
LoD = Limit of detection							
VNC = not communicated volume							

III – CAS references

List of polychlorodibenzo-p-dioxins and polychlorodibenzofurans determined by isotopic dilution and internal spikes by High resolution gaz chromatography (HRGC) and High resolution mass spectrometry (HRMS).

CDD/CDF	CAS reference	Congener ¹³ C	CAS reference
2,3,7,8-TCDD	1746-01-6	¹³ C-2,3,7,8-TCDD	76523-40-5
Sum TCDD	41903-57-5	-	-
2,3,7,8-TCDF	51207-31-9	¹³ C-2,3,7,8-TCDF	89059-46-1
Sum TCDF	55722-27-5	-	-
1,2,3,7,8-PeCDD	40321-76-4	¹³ C-1,2,3,7,8-PeCDD	109719-79-1
Sum PeCDD	36088-22-9	-	-
1,2,3,7,8-PeCDF	57117-41-6	¹³ C-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	¹³ C-2,3,4,7,8-PeCDF	116843-02-8
Sum PeCDF	30402-15-4	-	-
1,2,3,4,7,8-HxCDD	39227-28-6	¹³ C-1,2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	¹³ C-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	¹³ C-1,2,3,7,8,9-HxCDD	109719-82-6
Sum HxCDD	34465-46-8	-	-
1,2,3,4,7,8-HxCDF	70648-26-9	¹³ C-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	¹³ C-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	¹³ C-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	¹³ C-2,3,4,6,7,8-HxCDF	116843-05-1
Sum HxCDF	55684-94-1	-	-
1,2,3,4,6,7,8-HpCDD	35822-46-9	¹³ C-1,2,3,4,6,7,8-HpCDD	109719-83-7
Sum HpCDD	37871-00-4	-	-
1,2,3,4,6,7,8-HxCDF	67562-39-4	¹³ C-1,2,3,4,6,7,8-HxCDF	109719-84-8
1,2,3,4,7,8,9-HxCDF	55673-89-7	¹³ C-1,2,3,4,7,8,9-HxCDF	109719-94-0
Sum HpCDF	38998-75-3	-	-
OCDD	3268-87-9	¹³ C-OCDD	114423-97-1
OCDF	39001-02-0	¹³ C-OCDF	

***** The end *****

CARSO - LABORATOIRE SANTÉ ENVIRONNEMENT HYGIÈNE DE LYON

Laboratoire agréé pour les analyses d'eaux par le Ministère de la Santé

ASSAY REPORT	« Dioxin like » PCB analysis in 2 phytosterols
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ACCREDITATION
N°1-1531
PCB/PC
COMMISSION
SURVEILLANCE



Date : 20/03/06

The assay «LSE06-2022» was carried out on behalf of :

VITAE CAPS
C/Gutenberg 356 Poligono Torrehierro
45600 TALAVERA DE LA REINA-TOLEDO

ESPAGNE

To Mr GARCIA LOZANO

CARSO code assay : **LSE06-2022 PCB**

Customer code : Analysis of 17/02/06

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It includes 8 pages.

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Dr Daniel FRAISSE
Deputy Scientific Director

I.1 SUBJECT OF THE ASSAY

The subject of the assay report referenced under LSE06-2022 PCB deals with dioxin-like PCB analysis in 2 phytosterols.

I.1.1 COMPOUNDS TO BE ANALYSED

Polychloro-chloro-biphenyls (PCBs) are complex mixtures of various congeners got from chloration of the biphenyl. They can be ranked in the following chart :

Not ortho coplanar PCBs	
3,4,4',5-Tetra chlorobiphenyl	PCB 81
3,3',4,4'-Tetra chlorobiphenyl	PCB 77
3,3',4,4',5-Penta chlorobiphenyl	PCB 126
3,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 169
Mono- and di-ortho coplanar PCBs	
2',3,4,4',5-Penta chlorobiphenyl	PCB 123
2,3',4,4',5-Penta chlorobiphenyl	PCB 118
2,3,4,4',5-Penta chlorobiphenyl	PCB 114
2,3,3',4,4'-Penta chlorobiphenyl	PCB 105
2,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 167
2,3,3',4,4',5-Hexa chlorobiphenyl	PCB 156
2,3,3',4,4',5'-Hexa chlorobiphenyl	PCB157
2,3,3',4,4',5,5'-Hepta chlorobiphenyl	PCB 189
Indicator PCBs	
2,4,4'-Tri chlorobiphenyl	PCB 28
2,2',5,5'-Tetra chlorobiphenyl	PCB 52
2,2',4,5,5'-Penta chlorobiphenyl	PCB 101
2,3,4,4',5-Penta chlorobiphenyl	PCB 118
2,2',4,4',5,5'-Hexa chlorobiphenyl	PCB 153
2,2',3,4,4',5'-Hexa chlorobiphenyl	PCB 138
2,2',3,4,4',5,5'-Hepta chlorobiphenyl	PCB 180

The toxicity of a sample is performed through the quantitative measurement of these 12 toxic congeners, to which a specific toxic equivalency factor (I-TEF) is applied. Thus, the concentration of a sample in « Toxic equivalent factor » is given.

The two standards for toxic equivalent factors applied were set up by that of Ahlborg in 1994, and that of the WHO in 1997 (cf II). As regards food matrices, the WHO standard is recommended.

I.1.2 SAMPLING

Sampling was carried out by : the Customer

I.1.3 RESULTS

Results are presented globally in the chapter II of the report.

The following charts lets appear some information about the analysed sample (customer's references, CARSO references, etc.), the quantities found out for each congener and the result of the global toxicity of the sample calculated from the 13 toxic congeners and expressed in toxin equivalent 2,3,7,8-TCDD, given by I-TEQ.

The results of the toxic quantities expressed in equivalent 2,3,7,8 TCDD, I-TEQ detected on the samples submitted to analysis are summed up in the following chart :

Customer references	LSE references	I-TEQ Results « diolix in like » PCBs	Unit
Vitasterol S-80 non GMO muestra fitosteroles – Batch ST (181-183)	0601-7251-2 PCB	0.17	pg/g of initial matter
Vitasterol S-80 Esterified non GMO muestra esters de fitosterol Batch ST (181-183)E1	0603-7252-2 PCB	0.0080	pg/g of initial matter

I.1.4 INFORMATION ABOUT THE ASSAY

Description	Information
Reception of samples	17/02/06
Storage methods	ambiente temperature
Internal method(s) of analysis	MET-038
Other standard method(s)	EPA 1668
Elaboration of the sample before extraction	none
Measurement instrument HRGC/HRMS	Micromass : Autospec, ULTIMA sery
Injected volume, in µL	From 1 to 3 µL
Final volume :	20 - 50 µl
Notes :	

I.3 UNCERTAINTY IN ANALYTICAL MEASUREMENT

The uncertainty in analytical measurement is estimated by analyses made several times for a sample from the same nature than unknown samples ; or by the analysis of a certified or internal material in the lab or during ring tests. The uncertainty is estimated around 15 %.

I.4 SPECIFIC OBSERVATIONS ABOUT THE ASSAY

Sample were analysed in compliance with the internal method listed in § I.1.4.

NB : no particular remark compared to the analytical protocol used.

I.5 QUALITY ASSURANCE – QUALITY CONTROL

The assay report was audited by the CARSO quality department.

II. DETAILS OF RESULTS

Assay LSE06-2022 : Sample : LSE0601-7252-PCB							
							Print
Analysis of DL PCBs (non ortho, mono ortho)							
							Date 23/02/2006
Customer:	VITAE CAPS					Water content, %:	
Customer sample code:	VITASTEROL S-80 ESTERIFIED NON GMO : MUESTRA ESTERES DE FITOSTEROL - BATCH ST (181-183) E1					Initial extracted mass, g:	8,12
CARSO Assay:	LSE06-2022						
CARSO Sample No:	LSE0601-7252-PCB						
Matrix	17/02/2006						
Analysis start time:	PHYTOSTEROL						
HRGC/HRMS file:	20FEB79 , 1MAR25						
		I-TEF OMS 1998	pg/extract	pg/g initial product	LoD pg/extract	LoD I- TEQ pg, WHO	Recover y %
PCB "dioxin-like" = DL, Markers = M							
2,4,4'-TriCB (CB 28)	M		537,115				
2,2',5,5'-TeCB (CB 52)	M						
3,3',4,4'-TeCB (CB 77)	DL	0,00010	39,684	4,887			73
3,4,4',5'-TeCB (CB 81)	DL	0,00010	1,758	0,217			75
2,2',4,5,5'-PeCB (CB 101)	M						
2,3,3',4,4'-PeCB (CB 105)	DL	0,00010	97,644	12,025			67
2,3,4,4',5'-PeCB (CB 114)	DL	0,00050	3,291	0,405			85
2,3',4,4',5'-PeCB (CB 118)	DL,M	0,00010	221,618	27,293			87
2',3,4,4',5'-PeCB (CB 123)	DL	0,00010	1,857	0,229			93
3,3',4,4',5'-PeCB (CB126)	DL	0,10000			2,006	0,201	84
2,2',3,4,4',5'-HxCB (CB 138)	M						
2,2',4,4',5,5'-HxCB (CB 153)	M						
2,3,3',4,4',5'-HxCB (CB 156)	DL	0,00050	46,333	5,706			94
2,3,3',4,4',5'-HxCB (CB 157)	DL	0,00050	5,348	0,659			98
2,3',4,4',5,5'-HpCB (CB 167)	DL	0,00001	17,653	2,174			92
3,3',4,4',5,5'-HxCB (CB 169)	DL	0,01000			1,416	0,014	93
2,2',3,4,4',5,5'-HpCB (CB 180)	M						
2,3,3',4,4',5,5'-HpCB (CB-189)	DL	0,00010	8,266	1,018			95
TCDD-Equiv. (I-EQT)	non ortho		0,0041	0,00		0,215	
TCDD-Equiv. (I-EQT)	mono ortho		0,06	0,01			
Totals Marker PCBs:							
I-TEQ for "dioxin-like" PCBs:			pg/extract	pg/g initial product			
TOTAL I-TEQ nd=0 :			0,065	0,0080			
TOTAL I-TEQ nd=LoD :			0,280	0,034			
n.c. = unavailable information							
DL = "Dioxin-like" PCBs							
M = "Marker" PCBs							
LoD : Limit of detection							

CAS references of analysed compounds

Not-ortho coplanar PCBs		N° CAS
3,4,4',5-Tetra chlorobiphenyl	PCB 81	70362-50-4
3,3',4,4'-Tetra chlorobiphenyl	PCB 77	32598-13-3
3,3',4,4',5-Penta chlorobiphenyl	PCB 126	57465-28-8
3,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 169	32774-16-6
Mono and di-ortho coplanar PCBs		N° CAS
2',3,4,4',5-Penta chlorobiphenyl	PCB 123	65510-44-3
2,3',4,4',5-Penta chlorobiphenyl	PCB 118	31508-00-6
2,3,4,4',5-Penta chlorobiphenyl	PCB 114	74472-37-0
2,3,3',4,4'-Penta chlorobiphenyl	PCB 105	32598-14-4
2,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 167	52663-72-6
2,3,3',4,4',5-Hexa chlorobiphenyl	PCB 156	38380-08-4
2,3,3',4,4',5'-Hexa chlorobiphenyl	PCB157	69782-90-7
2,3,3',4,4',5,5'-Hepta chlorobiphenyl	PCB 189	39635-31-9
Not ortho marked coplanar PCBs		N° CAS
¹³ C ₁₂ -3,4,4',5-Tetra chlorobiphenyl	PCB 81*	208461-24-9
¹³ C ₁₂ -3,3',4,4'-Tetra chlorobiphenyl	PCB 77*	105600-23-5
¹³ C ₁₂ -3,3',4,4',5-Penta chlorobiphenyl	PCB 126*	208263-65-4
¹³ C ₁₂ -3,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 169*	208263-70-1
PCB coplanaires mono- et di-ortho marqués		N° CAS
¹³ C ₁₂ -2',3,4,4',5-Penta chlorobiphenyl	PCB 123*	208263-64-3
¹³ C ₁₂ -2,3',4,4',5-Penta chlorobiphenyl	PCB 118*	104130-40-7
¹³ C ₁₂ -2,3,4,4',5-Penta chlorobiphenyl	PCB 114*	208263-63-2
¹³ C ₁₂ -2,3,3',4,4'-Penta chlorobiphenyl	PCB 105*	208263-62-1
¹³ C ₁₂ -2,3',4,4',5,5'-Hexa chlorobiphenyl	PCB 167*	208263-69-8
¹³ C ₁₂ -2,3,3',4,4',5-Hexa chlorobiphenyl	PCB 156*	208263-68-7
¹³ C ₁₂ -2,3,3',4,4',5'-Hexa chlorobiphenyl	PCB157*	235416-30-5
¹³ C ₁₂ -2,3,3',4,4',5,5'-Hepta chlorobiphenyl	PCB 189*	208263-73-4

***** **End of the report** *****