CONTROL OF PCB LEVELS IN FOOD OF ANIMAL ORIGIN IN ITALY: ANALYTICAL QUALITY CONTROL, ORGANIZATION AND RESULTS

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Introduction

The National Reference Laboratory (NRL) for residues in food of animal origin[1] is located in the Istituto Superiore di Sanità (National Institute of Health). The NRL has different units for the different molecules included in the Italian Residue Control Plan (RCP). The unit dealing with PCBs, PCDDs and PCDFs was established in 1999.

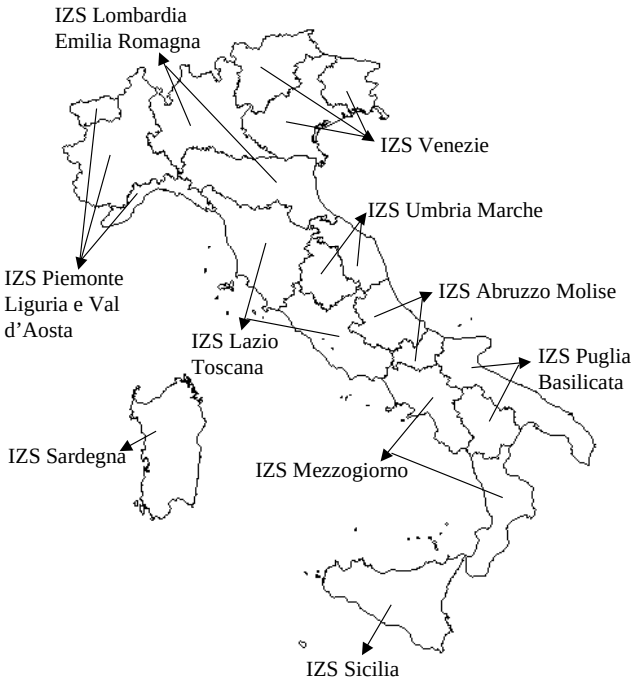
The work accomplished in the past years includes:

for PCDDs and PCDFs: organization of one intercalibration exercise with 11 participating laboratories (1999); organization of a quality control program together with the one regional laboratory (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise) in charge, since the year 2000, for controls in the whole Italian territory;

for PCBs: organization of three interlaboratory exercises with the 10 Italian regional control laboratories (Istituti Zooprofilattici Sperimentali) to achieve uniform analytical performance in the whole national territory; selection of a list of 18 congeners to approximate total PCB content; setting up of a system to collect the results obtained by the regional laboratories; creation of a data base; elaboration of the data acquired in the last three years.

This paper describes the work accomplished with PCBs, explains the criteria underlying technical choices and presents the results obtained, including the relevance of the elaboration of the collected data to establish background levels for the matrices of interest.

Figure 1. Partition of the Istituti Zooprofilattici Sperimentali (IZS) in the Italian territory.



Experimental

Control network: The control of food of animal origin in Italy is carried out by ten regional laboratories (Istituti Zooprofilattici Sperimentali, IZS).

Two Regions of Italy are generally associated in the management of a single IZS, except for Sicily and Sardinia that have each its own IZS. Each IZS has sections in the main cities of its area and cooperate with the veterinary offices of the Italian National Service of Public Health. Veterinary officers perform the sampling of food of animal origin whilst IZSs perform the analyses. This is the backbone of the Italian control network. In Figure 1 is reported the distribution of the IZSs with their area of pertinence in the Italian territory.

Interlaboratory exercises: Three interlaboratory exercises were organized, with the following criteria: participation was open to public laboratories operating controls, both in the food (IZSs) and environment sectors; each lab used its own analytical method and the efficiency of the extraction technique was evaluated by weighing the extracted fat; quality objectives were progressively more demanding in terms of analytical performances from one exercise to the next; the target

analytes were the 7 indicator congeners in the first exercise, whilst in the following ones 18 congeners were determined; analytical reference values for the materials used were set by the NRL in the first two exercises by analyzing the material with different analytical methods; in the third exercise, consensus values were to be established by statistical analysis of the data of all participants.

Results and discussion

The scheme of the three interlaboratory exercises will be briefly resumed here.

The first exercise (March-July 2000) was attended by 21 laboratories (including 11 IZSs and their sections), 20 of which reported results; the analysed material was a seasoned homogenate of pork meat (salame filling); two different materials were distributed: one with about 15 ng/g fat contamination, the second with about 150 ng/g fat; the seven indicator congeners (28, 52, 101,118, 138,153,180) were determined.

The second exercise (October 2000-June 2001) was attended by 26 laboratories (including 12 IZSs and their sections), 18 of which reported data; the analysed material was lyophilzed egg yolk, with analyte content of about 20 ng/g fat; eighteen congeners (28, 52, 95, 99, 101, 105, 110, 118, 138, 146, 149, 151, 170, 180, 183, 187) were determined. The rationale for the choice of the congeners to be determined is that different animal matrices display different congener distribution profiles: the selection of the eighteen congeners was made in order to account for a portion of total PCBs in the approximate range 70-90% for the various matrices included in the RCP. Toxicological criteria were not considered in the selection.

The first two exercises on PCBs, together with the 1999 exercise on dioxins, and the results therein obtained are fully described in a report[2] also available on the web at the following address http://www.iss.it/publ/rapp/2002/0238.pdf. Although it is written in Italian, it contains tables, graphs and z-score graphs easily readable.

The third exercise (September 2002-February 2003) was attended by 23 laboratories, 21 of which reported data; the analysed material was a fish oil with a contamination level of about 300 ng/g; the same eighteen congeners of exercise 2 were determined: the raw results obtained in the third exercise are reported in Table 1. In the last line is reported the reference value for each congener, defined as the mean value of all labs after elimination of outliers.

A detailed explanation of the results obtained in the exercises is however beyond the purpose of this presentation: what really matters is that the IZSs providing PCB data in the frame of the RCP yield data that can be considered homogeneous in terms of accuracy and use methods adequate to the limit (100 ng/g fat of 18 PCB congeners for non fish matrices) of the Italian RCP.

The NRL was then charged by the Italian Ministry of Health of collecting and elaborating PCB data acquired nationwide by the regional laboratories in the frame of RCP.

Table 2. Number and matrices analyzed for PCBs in Italian Residue Control Plan.

MATRICES	RCP 2001	RCP 2002	RCP 2003*
Bovine meat	89	123	57
Swine meat	168	133	116
Cow's milk	67	146	81
Milk from goat or sheep	16	33	7
Poultry	88	109	76
Turkey	13	22	13
Egg	102	100	58
Honey	12	22	8
Bass	19	33	28
Trout	37	55	18
Eel	7	10	6
Feed for lactating cows	29	60	22
Feed for pigs	15	24	23
Feed for fish	18	31	25
Other	63	175	72
OVERALL SAMPLES	743	1076	610

*****the data collection is still in progress

The data processing allowed since 2001 the creation of a data base. The data can be accessed by matrix, region, single congener, sum of congeners and allow a detailed representation of the situation emerging from RCPs for PCBs.
At this moment the collection of data for the years 2001 and 2002 has been completed, whilst for the year 2003 it is still in progress. Table 2 reports the number of samples, ordered by type of matrix, collected in the data base. It is impossible to summarise here the outcome. A complete report on these data is in preparation and will soon be published.
One of the most interesting uses that can be done with these data is the estimate of the background contamination levels for the different matrices. To this purpose the limit of determination (LOD) of the analyses it is a limiting factor, especially if LOD is high. The LOD of the methods used by the various IZSs are different for the different laboratories, varying generally between 1 and 5 ng/g fat for each

congener. Most of the laboratories have a 2 ng/g fat LOD for each congener. This limit is adequate for legal compliance (100 ng/g for non-fish matrices) but insufficient to detect background contamination in many matrices. As a consequence, most of the data reported by IZSs are LOD, as it is shown in Table 3. Table 3 reports the number of analyses and the number of determined (above LOD) values for two different congeners in the RCP of year 2001. In Table 3 also the mean values of the determined data are reported: it is relevant to notice that these do not represent the mean contamination levels, but the mean of the data above LOD, then the mean of the more contaminated samples. To obtain the real mean contamination it is necessary to consider the contribution of samples exhibiting background contamination; these are generally contaminated at levels under LOD, and are the great majority of samples, as evidenced by Table 3. This introduces the problems connected to the LOD.

As mentioned, different laboratories have different LODs. So, for PCB 153 in cow milk there are (see Table 3) 53 values under LOD; but 32 of these are lower than 2 ng/g fat, 17 are lower than 5 ng/g fat, four have other LODs: a 2 ng/g LOD means, on 18 congeners, an overall, upperbound content of 36 ng/g, whilst a 5 ng/g LOD means an overall of 90 ng/g (the upperbound concentration is calculated assuming that all values less than the LOD are equal to the LOD[4]) the concentration of an analyte . These levels are higher, for most matrices, than the background contamination: this suggests that the upperbound it is not a good approach to estimate the background contamination level; to reach this goal it is then necessary to try other approaches.

Table 3. Number of analyses, number of determined (above LOD) values, and mean value of determined data for PCB congener 28 and 153. The analyses were carried out in the 2001 Italian Residues Control Plan.

Matrices	T_3CB 28			H_6CB 153		
	Number of analyses	Above LOD	Mean, ng/g fat	Number of analyses	Above LOD	Mean, ng/g fat
Bovine meat	89	1	2,60	89	13	4,42
Swine meat	168	0		168	4	3,22
Chicken	88	1	2,23	88	9	6,38
Turkey	13	0		13	6	5,97
Trout	37	9	4,90	37	25	25,8
Eel	7	3	4,70	7	7	38,9
Bass	19	7	5,84	19	18	53,3
Cow milk	67	0		67	14	4,28
Milk from goat or sheep	16	0		16	0	
Eggs	102	0		102	7	8,15
Feed for lactating cows	29	1	3,40	29	4	5,68
Feed for pigs	15	0		15	0	
Feed for fish	18	3	2,13	18	16	12,6
Honey	12	0		12	0	
Other	63	4	3,65	63	8	615
Overall analyses	743	29		743	131	

We are now working on the 2001-2003 RCP data to extract, through profile analysis, the background contamination for each matrix. For profile analysis we mean the determination, for each matrix, of the mean profile (ratio of each congener to the PCB 153, generally the most abundant). Once obtained the profile becomes possible the calculation of the approximate, realistic content or limit for each congener with respect to the content or limit of PCB 153. For instance, if a determination on milk has a LOD of 2 ng/g for PCB 153, it will probably have the same analytical LOD on PCB 28, and the two congeners will contribute 2 ng/g each, through the medium bound approach, to the background contamination. On the contrary, it is known and it is apparent from Table 3 that levels of PCB 28 are

lower than those of PCB 153. The mean profile of milk could permit to say that PCB 28, at a certain confidence level, does not exceed a certain percentage of PCB 153, and allow to calculate a more realistic contribution of PCB 28 as a function of the concentration (or LOD) of PCB 153.
The same would apply to all other congeners. In this way we could calculate a more realistic estimate of total PCB content in a sample under LOD, and get a better estimate of the background level.
To obtain the mean profile for each matrix, we need a certain number of samples with all congeners determined. The limited number of determined values available (see Table 3) for each year suggests to aggregate all the 2001-2003 data to obtain significant mean profiles for each matrix. This analysis is now in progress.

References

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2. **C. La Rocca, N. Iacovella, W. Quattrocchi, L. Turrio Baldassarri - Circuito d'intercalibrazione nazionale per il rilevamento di PCB e PCDD/PCDF in matrici alimentari. ISTISAN 02/38, 2002, ISSN 1223-3117, 60 p.**
3. **L. Turrio Baldassarri, M.L. Casella, A. di Domenico, N. Iacovella, C. La Rocca: Analysis of 60 PCB congeners in drinkable water samples at 10-50 pg/l level, Microchemical Journal, submitted.**
4. **Official Journal of the European Communities, Council Regulation (EC) N° 2375/2001, L321, 1-5; 29 November 2001.**

Congener	28	52	95	99	101	105	110	118	138	146	149	151	153	170	177	180	183	187
Lab 02			8,0	26	20	8,0		25	60	16	12		82	10		24		12
Lab 03	4,7	13	6,5	28	17	3,8	4,2	17	43	10	5,6	2,5	71	6,6	3,3	17	3,8	9,5
Lab 04		46	9,8	40	23		14	31	79	139		15	104	19	30	32		25
Lab 05	7,5	22	12	114	26	7,0	4,5	26	67	18	10	9,5	104	12	5,0	24	14	15
Lab 06	LIM	16	8,0	38	25	3,0	4,4	17	72	18	18	2,0	69	3,6	3,5	25	5,0	12
Lab 07	3,7	12	5,0	25	13	9,5	3,5	14	37	8,5	4,0	1,9	62	5,0	2,3	12	2,9	7,2
Lab 08	3,8	12	5,9	25	17	4,5	4,0	20	59	12	5,3	2,4	102	9,3	3,0	25	4,4	12
Lab 10	10	7,8	6,4	41	13	5,4	3,3	35	78	7,3	6,2	1,8	67	2,6	3,1	13	7,0	14
Lab 12	2,0	11	17	47	22	12	6,5	23	65	23	26	2,0	80	10	5,5	22	6,0	15
Lab 14	LIM	15	6,7	37	17	2,8	4,9	16	81	9,6	2,9	13	92	4,2	2,5	10	3,4	7,3
Lab 15	LIM	15	7,5	12	12	0,60	2,8	17	39	LIM	7,4	1,2	39	3,8	1,4	7,7	1,6	5,2
Lab 17	3,0	10	2,7	26	20	3,6	3,2	12	50	10	6,1	29	78	5,7	3,0	18	2,5	7,7
Lab 18	2,6	9,9	5,8	26	16	4,2	4,2	18	44	5,4	5,5	2,5	47	7,0	3,4	18	2,4	6,1
Lab 19	1,3	13	7,4	28	16	4,3	4,5	22	55	9,3	6,6	3,1	78	5,2	3,2	14	3,2	6,5
Lab 20	1,3	7,2	3,7	22	12	3,5	3,0	16	44	10	4,1	2,0	72	6,7	2,9	19	3,4	10
Lab 21	3,5	8,5	17	20	6,0	21	5,5	16	34	9,5	21	1,5	66	6,5	4,5	15	3,0	10
Lab 22	4,0	12	11	72	18		19	18	80	15	6,2	32	117	11	5,0	38	8,0	16
Lab 23	3,3	18	8,3	50	20	6,3	5,8	27	94	16	7,7	4,1	138	13	5,2	32	5,9	17
Lab 36	LIM	12	14	36	19	5,7	4,0	21	66	13	5,7	3,5	80	8,0	3,4	20	13	5,6
Lab 37	17	8,0	3,0	9,5	6,5	1,0	2,0	11	37	6,0	3,0	1,5	41	4,0	2,0	12	1,0	4,5
Lab 38	6,6	21	12	45	29	6,3	6,4	38	96	18	9,1	4,5	134	16	11	31	7,2	18
Ref. Mean	3,6	12	7,6	31	17	4,7	4,2	19	61	12	6,3	2,9	76	7,0	3,4	19	4,2	11

Table 1. Raw results of the laboratories in the third exercise (September 2002-February 2003). Concentrations of congeners are in ng/g fat. LIM=Value under LOD. Ref. Mean= mean value after elimination of outliers. The blank cell indicates a value not reported.