DIOXIN LEVELS IN LIVESTOCK AND GRASSLAND AFTER AN ACCIDENTAL FIRE IN A SITE USED FOR DISPOSAL OF WASTE MATERIAL

Ceci R, Diletti G, Giovannini A, Scortichini G, Migliorati G.

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" National Reference Laboratory for Dioxins and PCBs in food and feed, Via Campo Boario, 64100 Teramo, Italy

Introduction

In July 2009 a fire broke up in a large warehouse used for disposal of waste material coming from industrial and farming activities, and plastic material from car wrecks. The warehouse was situated in the industrial area of Vascigliano, Umbria Region (middle of Italy). The fire lasted for a week and caused the precipitation of contaminated particles in the surrounding area. A total of six samples (milk, cheese, eggs, grass) were analysed for dioxins and their levels (except for cheese) exceeded the corresponding European Union (EU) maximum limits. Then the local government adopted several measures to limit human exposure to these contaminants. The consumption of food from the contaminated area was prohibited as well as animal pasture. In order to investigate the extent of contamination, the National Reference Laboratory (NRL) for dioxins and PCBs in food and feed in cooperation with the Local Health Authority developed a monitoring plan to investigate the farms and the grassland surrounding Vascigliano industrial area where several commercial livestock farming systems (cattle, sheep, poultry, pigs) are located. Both to reduce the large number of samples to be investigated and to protect the public health, a statistical approach to food and feed sampling was adopted. At the beginning the sampling area was delimited at a distance of 3 km from the fire and then was extended to 5 km. The sampling plan included milk, animal tissues, eggs, vegetables and feed samples.

This paper presents data on the PCDD/Fs and dl-PCBs contamination levels in food and feed samples collected between July 2009 and February 2010 and provides a statistical method as a tool in decision making in case of dioxin contamination incident.

Materials and methods

Sampling and monitoring plan design

Immediately after the fire 85 food and feed samples (including milk, eggs, animal tissues, vegetables, cereals, hay and grass) were collected near the fire site and analysed for PCDD/Fs and dl-PCBs (phase I).

Because of the high percentage of non-compliant samples detected, the local government decided to investigate livestock and grassland located at a distance of 3 km from the fire. In addition, the transport of animals and the pasture in this area were forbidden. Taking this into account, a statistical monitoring plan was developed in order to reduce the number of samples to be analysed (phase II).

Feed samples

A total of 16 forage samples grown in the sampling area were collected in November 2009 and analysed for PCDD/Fs and dl-PCBs. Livestock were allowed to graze in land where the mean levels of sample contamination plus two standard deviations did not exceed the EU maximum limits[1,2].

Food of animal origin

The number of farms was identified by means of georeferentiation provided by the National Data Bank for Livestock Registration. Considering the large number of samples to be investigated, livestock (cattle and sheep) were divided into "epidemiological units", having regard to breeding practices and production type (milk and meat production).

Cattle were divided into three "epidemiological units" according to forage management practices: i) animals free grazing in the area of study after the fire, ii) indoor farmed cattle fed forages of local production suspected of being contaminated, iii) indoor farmed cattle fed supposed uncontaminated feed.

Sheep were grouped into three epidemiological units: two units were livestock with more than 100 animals and third unit was constituted of farms with less than 100 animals (total of 742 animals). -

In each "epidemiological unit", the first 16 slaughtered animals were tested for PCDD/Fs and dl-PCBs levels in meat.

The whole "epidemiological unit" was considered compliant when no one sample was above the EU maximum limits, as well as the mean levels of sample contamination plus two standard deviations.

In dairy livestock, a bulk milk sample was analysed. The compliance of samples was evaluated according to EU legislation as well.
Under non-compliance conditions both milk and meat were withdrawn from the market and animal movement restrictions measures adopted.
In outdoor swine farms, a single animal was tested and a compliant value allowed the marketing of animals of the same age and breeding conditions group.
Poultry farming was exclusively represented by small productions for self-consumption and all animals were destroyed following several positive finding in eggs.
Due to non-compliant samples found during phase II, the sampling area was extended to 5 km from the fire and it was decided to collect and analyse 16 samples for each of the following groups: i) bovine muscle, ii) sheep muscle, iii) bovine milk and iv) sheep milk, taken in different farms uniformly distributed in the circle area between 3 and 5 km distance from the fire site.
PCDD/Fs and dl-PCBs analysis in food and feed samples were performed. A total of 264 samples were analysed: 31 milk and dairy products (bovine and ovine), 169 muscle samples (bovine, ovine, poultry and swine), 6 eggs, 24 vegetables and cereals samples for human consumption, and finally 34 animal feed products.

Analysis of food and animal feed samples
Samples were homogenized and tested by a validated method routinely used for PCDD/Fs and dl-PCBs analysis in food and feed and successfully tested in a number of inter-laboratory studies.
Before analysis all samples were spiked with the specific PCDD/Fs and dl-PCBs standard solution, a mixture of $^{13}C_{12}$-labelled congeners (Wellington Laboratories, Ontario, Canada).
The extraction and clean-up procedures as well as the analytical determination were carried out as previously reported[3].
PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.10 μm film thickness, J&W Scientific, California) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10,000 operating with electron ionisation (EI) at 40 eV in the selected ion monitoring (SIM) mode. The HRGC/HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XP (Thermo Fisher, Bremen, Germany). dl-PCBs were separated by HRGC on a VF-5 MS capillary column (60 m x 0.25 mm, 0.25 μm film thickness, Varian, California) and determined by HRMS, in the same operating conditions described for PCDD/Fs.
Toxic equivalent (TEQ) values were calculated using the World Health Organization Toxic Equivalency Factors established in 1998 (WHO-$TEFs_{1998}$). WHO-TEQs were expressed as upper bound concentrations assuming that all values of specific dioxins congeners below the limit of determination (LOD) are equal to their respective LOD.

Results and discussion
The analytical results are reported as pg WHO-TEQ/g fat for food samples and pg WHO-TEQ/g product for feeding stuffs referring to a moisture content of 12%, in conformity with the European legislation.
PHASE I
In the phase I, 40 food of animal origin, 27 vegetables and 18 feed samples were analysed.
The analytical results are synthesized as follows: i) low contamination levels in vegetable samples (under action levels given by EU recommendation[4]), ii) dioxins levels above EU maximum limits in food of animal origin as well as in forages, iii) uniform spreading of contamination around the fire site, according to the geographical distribution of non-compliant samples and related PCDD/Fs and dl-PCBs levels.
In detail, 17 food of animal origin samples (accounting for 43%) and 9 feed samples (accounting for 50%) exceeded the EU maximum levels for PCDD/Fs and/or PCDD/Fs + dl-PCBs.
PHASE II
As a consequence, the investigation was focused on food of animal origin and forages of local production.
Because of the high variability of PCDD/Fs and dl-PCBs concentrations found in different samples obtained from the same farm (e.g. PCDD/Fs + dl-PCBs levels ranging from 2.49 to 7.27 pg WHO-TEQ/g fat in bovine meat samples), the food safety could have been ensured by two approaches:

1) testing of all slaughtered animals
2) testing of a selected sample from the animal population by using a statistical method to make inferences about the population on the basis of PCDD/Fs and dl-PCBs sample results.

The first approach was quite difficult to be adopted in relation to relatively low economical value of sheep and high cost and long time of analysis of such a large number of samples (Table 1).
In the second approach, a sample size of 16 was a good compromise between statistical and economical issues.
In a normal distribution, 95% confidence limits of the mean are equal to:

$$\bar{x} \pm 1{,}96\frac{s}{\sqrt{n}}$$

where *s* is the standard deviation and *n* is the sample size.
When the sample size is 16, the corresponding 95% confidence limits of the mean are equal to:

$$\bar{x} \pm 0{,}49 \cdot s$$

which states that confidence interval is half standard deviation. This value represents an acceptable precision of the population mean estimation. Under these conditions, it is expected a maximum of 6.7% animals of the selected sample to exceed the EU maximum limits. Sample size should be increased to 238 to halve the above level of risk.
The most relevant results for food and feed samples are presented in Table 2 and 3.
Feed samples
In regard to feed materials, 16 forage samples of local production were collected after four months from the incident. All samples were compliant and the contamination ranged between 0.045 and 0.303 ng WHO-TEQ/kg product for PCDD/Fs + dl-PCBs.
As the mean levels of sample contamination plus two standard deviations did not exceed the EU maximum limits, the local government allowed animal grazing in the area 0-3 km from the fire site.
Bovine livestock
According to the defined rules for "epidemiological units" classification, three "epidemiological units" were identified for beef cattle and only one for dairy cattle.
In all beef cattle units, non-compliant samples were found. In each unit, the highest PCDD/Fs + dl-PCBs recorded values were 6.22, 5.61, 11.62 pg WHO-TEQ/g fat, respectively. The contaminated carcasses were destroyed and PCDD/Fs and dl-PCBs testing was extended to the remaining animals belonging to the "epidemiological unit", at the moment of slaughtering.
In the dairy cattle unit, three raw milk samples were collected between November 2009 and January 2010. All samples were compliant even though two samples showed PCDD/Fs and/or dl-PCBs concentrations above the action levels. The unit was then allowed to commercialise milk.
Sheep livestock
Among the three identified "epidemiological units", the first was compliant with contamination levels from 0.24 to 0.82 pg WHO-TEQ/g fat (sum for PCDD/Fs and dl-PCBs). As the mean levels of sample contamination plus two standard deviations were below the EU maximum limits, the unit was then allowed to commercialise milk and meat.
In both remaining units, non-compliant samples were found. In each unit, the highest PCDD/Fs + dl-PCBs values were 5.69 and 27.27 pg WHO-TEQ/g fat, respectively, and the same measures already described for bovine were taken.

As shown in Table 3, the relative contribution of PCDD/Fs and dl-PCBs to the total WHO-TEQ was about 50:50 % for all kind of samples except for muscle. In fact, the relative contribution of PCDD/Fs and dl-PCBs to WHO-TEQ was 40:60 % in ovine muscle and 30:70 % in bovine muscle.
It is important to underline that after four months from the fire accident PCDD/Fs and dl-PCBs levels decreased about 20 times in feed samples collected in the same area (from 3.75 to 0.176 ng WHO-TEQ/kg for PCDD/Fs + dl-PCBs).
Among congeners levels, a remarkable reduction was observed for some PCDDs (TCDD, PeCDD and HxCDDs) and non-orto dl-PCBs congeners (PCB-81, 126 and 169). In particular, the analytical concentration was reduced by a factor of 100 for the first group, and by a factor of 20 for the second.
After eight months from the accident, PCDD/Fs and dl-PCBs levels in food samples of animal origin, taken in the area 0-5 km from the fire, were still above EU maximum limits. Further research is needed to monitor PCDD/Fs and dl-PCBs levels in food and feed and to exactly identify the geographical extension of the contaminated area.

References
1. Commission Regulation (EC) N. 1881/2006.
2. Commission Directive 2006/13/EC.
3. Diletti G., Ceci R., Scortichini, G., Migliorati G. (2009); Organohalogen Compounds 71: 2344-2348.
4. Commission Recommendation 2006/88/EC.

Table 1. Number of animals and farms in the area 0-5 km

Species	N. farms	N. animals	N. farms	N. animals	Total farms	Total animals
	0-3 km		3-5 km		0-5 km	
Bovine	42	462	69	766	111	1228
Ovine	61	1766	19	233	80	1999

Table 2. Number of food/feed samples grouped for PCDD/Fs and dl-PCBs levels with respect to EU action levels (ALs) and maximum limits (MLs)

Matrix	Species	N. samples analysed	N. samples < AL_S	N. samples > AL_S and < ML_S	N. samples > ML_S
Sampling point distance 0-3 km from the fire site					
Feed materials		34	22	3	9
Milk	Bovine	6	1	2	3
	Ovine	4	3	1	-
Muscle	Bovine	54	4	28	22
	Ovine	51	25	18	8
	Poultry	3	3	-	-
	Swine	2	2	-	-
Eggs	Poultry	6	-	2	4
Vegetable		21	21	-	-
Sampling point distance 3-5 km from the fire site					
Milk	Bovine	1	-	1	-
	Ovine	20	9	8	3
Muscle	Bovine	40	5	25	10
	Ovine	19	2	9	8
Vegetable		3	3	-	-

Table 3. Mean PCDD/Fs and dl-PCBs concentrations in non-compliant samples

Matrix	PCDD/Fs (pg WHO-TEQ/g fat)	dl-PCBs (pg WHO-TEQ/g fat)	PCDD/Fs + dl-PCBs (pg WHO-TEQ/g fat)	Contribution of PCDD/Fs to WHO-TEQ Mean value (%)[b]	Contribution of dl-PCBs to WHO-TEQ Mean value (%)[b]
Sampling point distance 0-3 km from the fire site					
Feed materials	1.72[a]	1.74[a]	3.47	52	48
Bovine milk	4.9	6.1	11.0	44	56
Bovine muscle	3.3	5.9	9.2	33	67
Ovine muscle	5.9	8.1	14.1	42	58
Eggs	4.8	4.5	9.3	52	48
Sampling point distance 3-5 km from the fire site					
Ovine milk	7.4	6.5	13.9	51	49
Bovine muscle	2.4	5.3	7.7	30	70
Ovine muscle	3.8	6.3	10.1	37	63

[a] pg WHO-TEQ/g referred to a 12% moisture content
[b] Calculated as the mean of single sample contributions