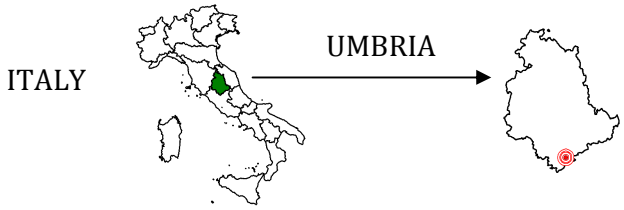
EVALUATION OF PCDD/Fs AND DL-PCBs CONCENTRATION IN ANIMALS AND FEED IN CENTRAL ITALY FOLLOWING A FIRE CONTAMINATION

Maresca C[1], Menotta S[2], Scoccia E[1], Biasini G[1], D'Antonio M[2], Accurso D[2], Cenci T[1], Fedrizzi G[2]
[1]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, 1, via G. Salvemini, Perugia, Italy;
[2]Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna, Chemical Department, 5, via P. Fiorini, Bologna, Italy;
Corresponding author: simonetta.menotta@izsler.it

Introduction.
Polychlorinated dibenzo-p-dioxins (PCDDs), Polychlorinated dibenzofurans (PCDFs) and Dioxin like polychlorinated biphenyls (PCBs) are persistent organic pollutants, that are formed during the incomplete combustion of organic materials in the presence of chlorine. Their characteristics, high lipophilicity and long biological half-lives cause their accumulation[1]. When automobiles are recycled[2], the carcasses are typically shredded, and the metallic components are separated from the non metallic components using a combination of magnetic or gravimetric methods. The non-metallic residues (fluff) are usually baled and landfilled. Occasionally the bales of shredder fluff can catch fire. According to some authors[3], also where small quantities of shredder fluff were combusted, emissions of PCDD/Fs are exceedingly high. When PCDD/Fs homologues were analyzed, there is a prevalence of PCDFs rather than PCDDs and the most abundant congeners are TCDFs and PeCDFs. In the summer of 2009 in Umbria, central Italy, there was a fire at a car scrap recycling site that caused massive contamination of Dioxin on and around the site. There was serious concern about possible contamination of the affected areas and especially about the danger of contamination of animal and agricultural food products from the local production. The area was rich of farms raising sheep, cattle, goats and dairy cattle: a study was conducted around the site to monitor the levels of PCDD/Fs and DL-PCBs in animals and animal feed in the farms for 1 year, in order to determine the concentration levels and to control the possible contamination of the local food product.

Materials and methods
The car scrap recycling site was situated in Umbria Region in the middle of Italy, between latitudes 42° 29' 35'' N and longitudes 12° 37' 40''E, elevation 170 meters MLS (Figure 1).

Figure 1: study area



From the fire site, five sampling circle buffer zones were established (3 kilometers radius buffer; 3-5 kilometers radius buffer; 5-6 kilometers radius buffer; 6-8 kilometers radius buffer; over 8 kilometers radius buffer). The circle buffer areas were mostly in rural area but Terni town was in the last circle buffer (more than 8 kilometers radius buffer). The animals were reared and fed in the farms in circle buffer zones with forage from the same area. From the different buffer areas, animals (cattle, sheep, goats) were slaughtered in the local slaughter house: samples of tissues and dairy milk were collected and analyzed. From the same areas were sampled and analyzed forage samples, that had been collected from the ground or were ready to feed the animals. The samples distribution is represented in Table 1. The analysis were performed according to US EPA 1613/B (1994)[4] for determination of 17 congeners 2,3,7,8 substituted of PCDD/Fs and US EPA 1668/B (2008)[5] for determination of 12 congeners of DL-PCBs. All homogenized samples were freeze-dried, spiked with labeled internal standard (Dioxins and PCBs)[6] and extracted by ASE 300 (Dionex Sunnyvale CA) with toluene at 135 °C. All the extracts were transferred to *n*-exane before the clean-up step. The lipid fraction was subjected to acid purification using chromatographic columns packed with Extrelut powder soaked with sulphuric acid[7]. Then, the

extracts were cleaned up using automatic three-column system with pre-packed disposable columns containing multilayer silica, alumina and carbon. Two fractions were eluted, one with PCDD/Fs and one with PCBs. The analysis were conducted using an HRGC HP 6890 Plus (Agilent Technologies USA) coupled to an high resolution Mass Spectrometer (Micromass Autospec Ultima Waters USA) operating in EI mode with resolution of 10.000 (5% valley). The statistical analysis was conducted using Pearson correlations: significant probabilities (p-values) were calculated for the respective number of analyzed samples. A p-value less or equal than 0.01 was considered significant. All analyses were performed using the software package (STATA 11.1 Copyright 2009 StataCorp LP). The case definition was every meat or animal feed sample contained PCDD/Fs concentration that exceeded the maximum levels permitted by the European Union[8-10] .

Results and discussion:

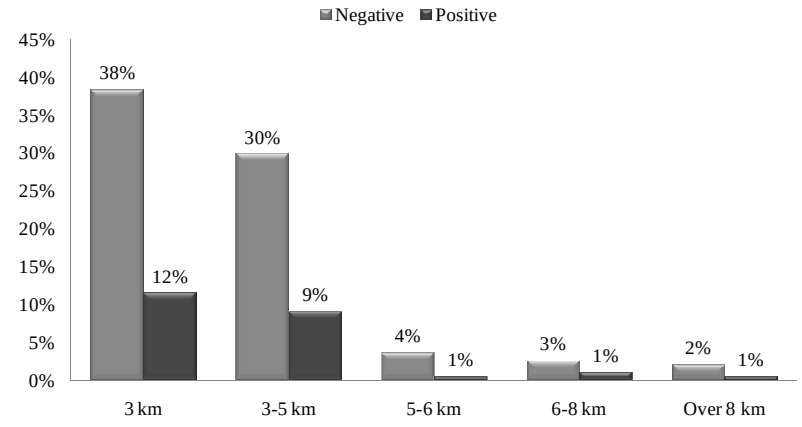
A total of 563 samples were analyzed (438 animals, 48 dairy products, 38 forage samples, 11 eggs, 17 feed and 10 vegetables for food) within a 1 year time-frame (July 17th 2009 - July 1th 2010). No positive samples were found among vegetables and feed. One hundred and one animals, 9 dairy products, 5 eggs from rural holdings and 13 forage samples were classified as not-compliant according to EU legislation[10]. Locations of the sampling are shown in Table 1.

Table 1: circle buffer zones and samples

Buffer	Animals tissues		Dairy products		Forage		Eggs	
	Compliant	Not compliant	Compliant	Not compliant	Compliant	Not compliant	Compliant	Not compliant
3 km	163	47	16	4	18	10	2	4
3-5 km	139	43	17	5	5	3	1	0
5-6 km	14	3	3	0	1	0	3	0
6-8 km	10	5	2	0	1	0	0	1
> 8 km	11	3	0	0	0	0	0	0
Total	**337**	**101**	**39**	**9**	**26**	**13**	**6**	**5**

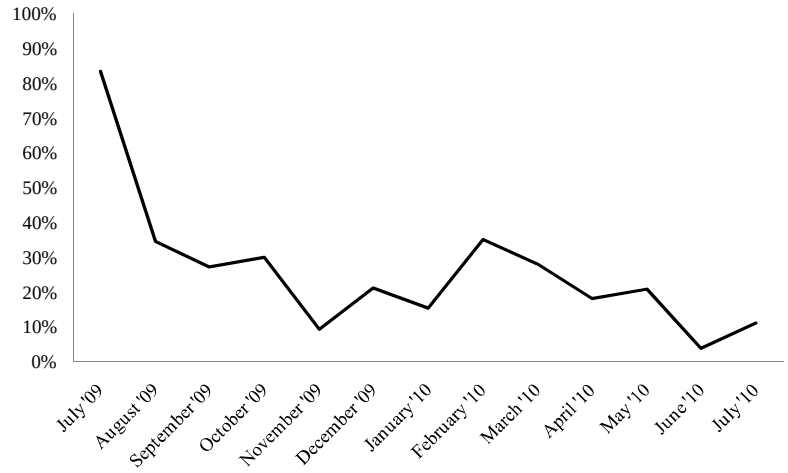
The percentage of not compliant analyzed bovine tissues was about 50% of total irregular samples. The percentage of farms that had irregular samples was about the same in the 3-5 km buffer area (35% of farms) and the 3km buffer area (37% of farms). The percentage of irregular farms was less than 10% in the 5-6 km buffer area. Similarly the not compliant samples decreased as you move away from the fire: the nearest area had the higher percentage of non-compliant samples (12% of the all analyzed samples). According to Authors[3] the largest contribution to not-compliance derived from PCDD/Fs TEQ values rather than DL-PCBs TEQ values. The percentage distribution of not-compliant samples is represented in Figure 2.

Figure 2: Percentage distributions of non-compliant samples



During the monitoring year there was a significant decrease of PCDD/Fs PCBs concentrations in collected samples: the reduction was from about 90% to less than 10%. The decrease in monthly is represented in Figure 3.

Figure 3: percentage decrease of non-compliant samples during the monitoring year.



The statistical analysis was performed comparing the Dioxins (PCDF and PCDD) congeners concentrations grouped by number of chlorine atoms in non compliant animal tissues and forage: as can be seen in Table 2 and Figure 4 there was a positive correlation (from moderate to strong) among congeners groups, especially between TCDD/F, PeCDD/F, HxCDD/F and HpCDD/F (0,35<p<0,90). There was a very weak correlation between the OCDD/F and the other congeners but the origins of the contamination for OCDD/F could be very different.

Table 2: the Pearson correlated coefficient between congeners concentration grouped by number of chlorine atoms in not-compliant animal samples at level 99% (*positive correlation)

Congeners	**TCDD/F**	**PeCDD/F**	**HxCDD/F**	**HpCDD/F**
TCDD/F	-			
PeCDD/F	0,79*	-		
HxCDD/F	0,60*	0,90*	-	
HpCDD/F	0,35*	0,44*	0,54*	-
ODD/F	0,04	0,09	0,04	0,62*

Figure 4: scatter plot between congeners concentration grouped by number of chlorine atoms in not-compliant animal samples at level 99%

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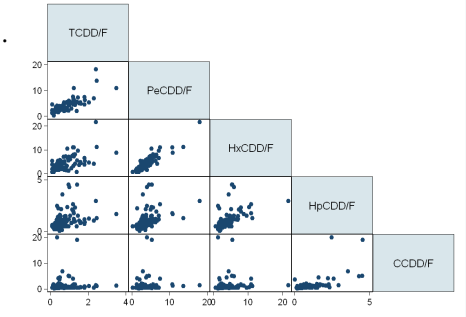
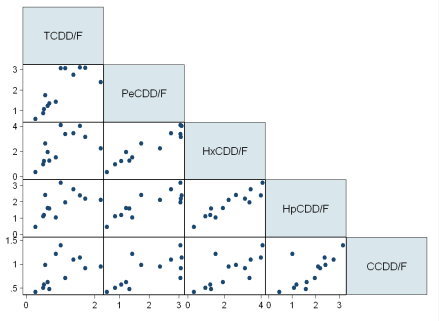


The same correlation was present in not compliant forage samples as can be seen in Table 3 and Figure 5: in this case almost all congeners presented a strong correlation also with Octa group. Probably the principal origin of contamination for the animal tissues and the forage could be the same and the different correlations of the OCDD/F could be probably due to more and different origins.

Table 3: the Pearson correlated coefficient between congeners concentration grouped by number of chlorine atoms in not-compliant forage at level 99% (*positive correlation)

Congeners	TCDD/F	PeCDD/F	HxCDD/F	HpCDD/F
TCDD/F	-			
PeCDD/F	0,78*	-		
HxCDD/F	0,62	0,95*	-	
HpCDD/F	0,57	0,84*	0,91	-
ODD/F	0,52	0,68	0,75	0,72*

Figure 5: scatter plot between between congeners concentration grouped by number of chlorine atoms in not-compliant forage at level 99%



No one correlation was found among DL-PCBs congeners grouped by number of chlorine atoms in animal tissues while a weak correlation was found for forage samples. Probably in the first case the contribution of the fire to DL-PCBs levels was very low: on the contrary for the forage the contribution of the fire was quite more. Three animals tissues collected in the buffer area over 8 km exceed the EU limits[10]. The congeners profile of these samples was quite different from most of the other. In fact the concentration of DL-PCBs was about triple than the Dioxins concentration. The sample collections and analysis are still continuing and some not compliant samples are still finding: it's not known if the origin is the fire of 2009 or there are some different punctual causes: the Terni town is highly industrialized but there were only a few monitoring samples before the fire, so it isn't possible today to know if there was a previous issue. Perhaps, if the monitoring system will be continue it will know the causes.

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