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# **ENVIRONMENTAL QUALITY AND FOOD SAFETY: BIOINDICATORS OF PERSISTENT ORGANIC POLLUTANTS EXPOSURE**

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## Potential bioindicators of environmental contaminants

### Environmental quality and food safety relationship (1)

Food of animal origin represents the main source of exposure for the general population to persistent organic pollutants (POPs).

The relationship between environmental quality and POPs bioaccumulation and bioconcentration is consolidated, as well as the use of animal species as bioindicators of contaminant exposure<sup>1</sup>.

In particular, sheep flocks are very sensitive to the top soil intake, via their grazing activity and the ingestion of soil particles incorporated into the hay especially during harvesting operations.

1. Scortichini G. et al. *Small Rum Res*, 135, 66-74 (2016)





## Potential bioindicators of environmental contaminants

### Environmental quality and food safety relationship (2)

Eggs of free-ranging hens are also particularly prone to contamination through soil ingestion even though the intake of pollutants can be due to a lack of application of good farm management practices or to the administered feed materials<sup>2,3</sup>.



In addition, the honeybee is considered a good biological indicator reflecting chemical impairment of the environment by its high mortality and the presence of pollutants in its body or in beehive products, such as honey<sup>4,5</sup>.



2. Menotta S. et al. *Anal Chim Acta*, 672, 50-54 (2010)

3. Hoogenboom R.L.A.P. et al. *Chemosphere*, 150, 311-319 (2016)

4. Porrini C. et al. *B Insectol*, 67 (1) 137-146 (2014)

5. Perugini M. et al. *J Agric Food Chem*, 57, 7440-7444 (2009)



# CAMPANIA REGION DIOXIN CRISIS



## Campania region dioxin crisis

### **Milk and feed monitoring plan 2002 – 2004 (1)**

November 2001: two sheep milk samples non-compliant for PCDD/Fs (taken in the framework of the National Residue Surveillance Plan).

April 2002 - November 2004: 595 samples of sheep/goat, cow, buffalo milk, and 434 samples of feedingstuffs from Campania region were analysed<sup>6</sup>.

**Milk samples:** 27% non-compliant, 45% of which sheep milk (median and maximum values 2.66 and 30.4 pg WHO<sub>1998</sub>-TEQ/g fat, maximum level 3.0 pg WHO<sub>1998</sub>-TEQ/g fat).

**Feed samples:** 13% non-compliant out of 267 samples of grass, silage and hay grown in the area of study.

6. Diletti G. et al. In: E. Mehmetli, B. Koumanova (Eds.), The fate of persistent organic pollutants in the environment. Springer, 301-314 (2008)

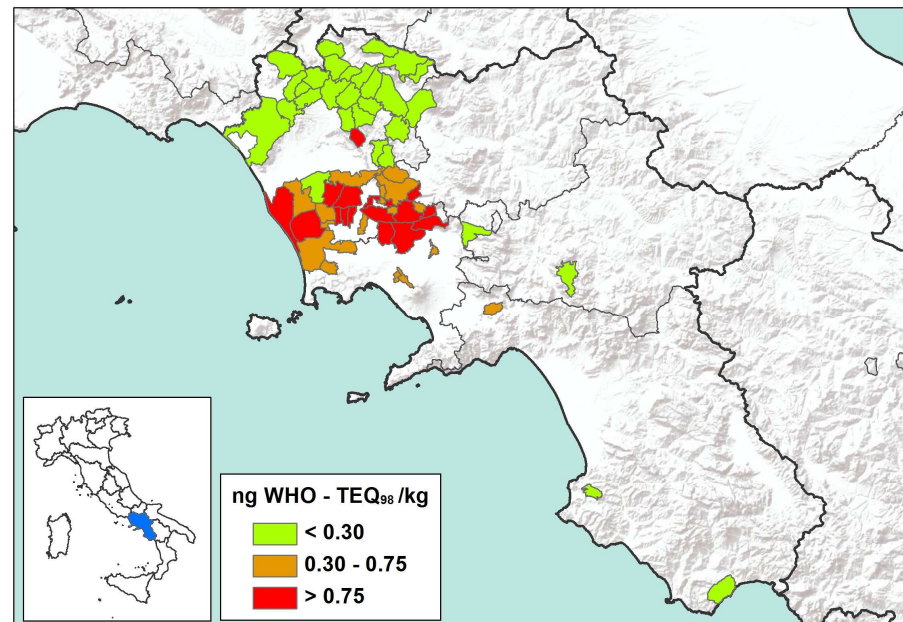
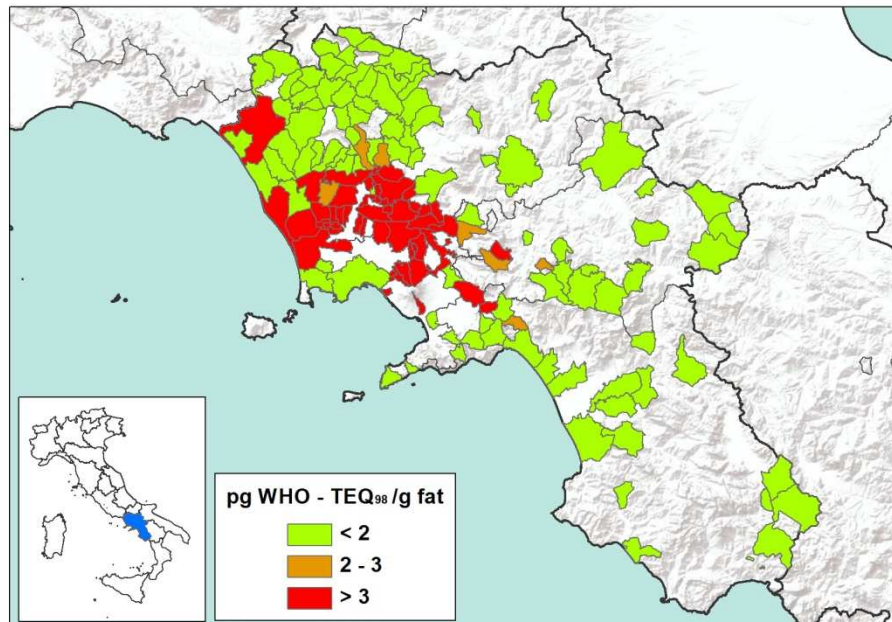


## Campania region dioxin crisis

### Milk and feed monitoring plan 2002 – 2004 (2)

PCDD/F levels in milk

PCDD/F levels in feed



The spatial analysis results highlighted an area in Caserta and Naples districts characterized by a widespread practice of illegal waste dumping and burning.



## Campania region dioxin crisis

### Buffalo milk crisis

At the end of 2007, a surveillance plan revealed 19% of non-compliance for mozzarella cheese samples. The EC required a monitoring plan for buffalo milk in Campania region<sup>7</sup>.

Phase I: 39 out of 271 samples (14%) from Avellino, Caserta and Naples were not compliant.

Phase II: 116 samples from Benevento and Salerno were all compliant.



|         | Phase I                           | Phase II                          |
|---------|-----------------------------------|-----------------------------------|
| PCDD/Fs | pg WHO <sub>1998</sub> -TEQ/g fat | pg WHO <sub>1998</sub> -TEQ/g fat |
| Min     | 2.54                              | 0.13                              |
| Mean    | 4.32                              | 0.44                              |
| Median  | 3.13                              | 0.40                              |
| Max     | 14.80                             | 2.05                              |



7. Scortichini G. et al. *Organohalogen Compd*, 71, 2349-2352 (2009)



## Campania region dioxin crisis

### Buffalo milk crisis follow-up

After the crisis of buffalo dairy fields in Campania, a re-assessment of the contamination was also necessary for other animal species bred in the region<sup>8</sup>.

The concentration of PCDD/Fs and DL-PCBs were determined in 69 sheep and goat milk samples from 63 farms. In 11 samples from 6 sheep farms the PCDD/F levels exceeded the maximum level (range 3.89 – 12.90 pg WHO<sub>1998</sub>-TEQ/g fat).

These farms were located in the same spot highlighted by previous surveys where plastic materials were illegally burnt near sheep and goats grazing areas.

A surveillance plan was performed in 2011–2014: all dairy products from 50 farms (bovine buffalo and ovine origin) resulted compliant to the maximum levels for PCDD/Fs and DL-PCBs<sup>9</sup>.

8. Esposito et al. *Food Addit Contam Part A*, 3, 58-63 (2010)

9. Serpe F.P. et al. *Int J Anal Mass Spectrom Chromatogr*, 3, 32-36 (2015)





## Campania region dioxin crisis

### **Eggs contamination in the “Land of Fire” (1)**

PCDD/F, DL-PCB and NDL-PCB concentrations were measured in eggs from free-range hens in Campania region (Caserta and Naples provinces)<sup>10</sup>. A total of 56 egg samples were collected between April 2014 and December 2015.

Free-range hens were living in close contact with bare soil contaminated by waste from civil, industrial and hospital activities.

Aim of the study: to establish the contamination levels and to assess the risk to the health of consumers.



10. Lambiase S. et al. *Food Addit Contam Part A*, 34 (1) 56-64 (2017)



## Campania region dioxin crisis

### Eggs contamination in the “Land of Fire” (2)

- **PCDD/Fs:** min 0.01 – max 6.18, median 0.41 pg WHO<sub>2005</sub>-TEQ/g fat (ML = 2.5 pg WHO-TEQ/g fat).
- **DL-PCBs:** min 0.01 – max 14.60, median 0.70 pg WHO<sub>2005</sub>-TEQ/g fat.
- **PCDD/Fs + DL-PCBs:** min 0.08 – max 17.15, median 1.15 pg WHO<sub>2005</sub>-TEQ/g fat (ML = 5.0 pg WHO-TEQ/g fat).
- **NDL-PCBs:** min 0.48 – max 67.55, median 7.35 ng/g fat (ML = 40 ng/g fat).

Six samples exceeded the maximum levels for PCDD/Fs and/or PCDD/Fs + DL-PCBs. Two samples exceeded the maximum level for NDL-PCBs.

Eleven samples exceed the action level for PCDD/Fs (1.75 pg WHO<sub>2005</sub>-TEQ/g fat). Eleven samples exceed the action level for DL-PCBs (1.75 pg WHO<sub>2005</sub>-TEQ/g fat).



## Campania region dioxin crisis

### Eggs contamination in the “Land of Fire” (3)

**Tolerable weekly intake:** 14 pg WHO-TEQ/kg body weight.

Egg fat weekly intake: 19.6 g adult, 17.5 g children

|                | PCDD/Fs<br>+ DL-PCBs (pg<br>WHO-TEQ/g fat) | Estimated weekly intake<br>(pg WHO-TEQ/kg body weight) |      |
|----------------|--|--|------|
| <b>Minimum</b> | 0.08                                       | Adult (70 kg)  | 0.02 |
|                |  | Children (9 y, 32 kg)                                  | 0.04 |
| <b>Maximum</b> | 17.15                                      | Adult (70 kg)  | 4.80 |
|                |  | Children (9 y, 32 kg)                                  | 9.19 |
| <b>Mean</b>    | 2.60                                       | Adult (70 kg)  | 0.73 |
|                |  | Children (9 y, 32 kg)                                  | 1.42 |



# CONTAMINATION OF THE FOOD CHAIN IN TARANTO



## Contamination of the food chain in Taranto

### First evidence of the contamination

March 2008: PCDD/Fs and PCDD/Fs + DL-PCBs above the EU maximum levels in two milk samples (from sheep and goat), collected in a farm near the industrial area of Taranto.



The Local Health Authority implemented multiple plans over the years to monitor the farms of Taranto district including mussel farming located in Mar Grande and in the two shelves of Mar Piccolo (First and Second Basins)<sup>11,12,13</sup>.

11. Diletti G. et al. *Organohalogen Compd*, 71, 2344-2348 (2009)

12. Esposito V. et al. *Sci Total Environ*, 492, 118-122 (2014)

13. Di Leo A. et al. *Environ Sci Pollut Res Int*, 21(23), 13196-13207 (2014)



## Contamination of the food chain in Taranto

### Monitoring plan for food/feed 2008-2010 (1)

Food (milk and dairy products, eggs, animal tissues, olive oil) and feed samples.

Feed samples were taken in livestock and grassland sited within 10 km from the industrial area.

The sampling area was progressively extended to 15 and 20 km to account for the potential medium-range airborne transport of pollutants.





## Contamination of the food chain in Taranto

### Monitoring plan for food/feed 2008-2010 (2)

Total samples and non-compliant samples analysed for PCDD/Fs and DL-PCBs

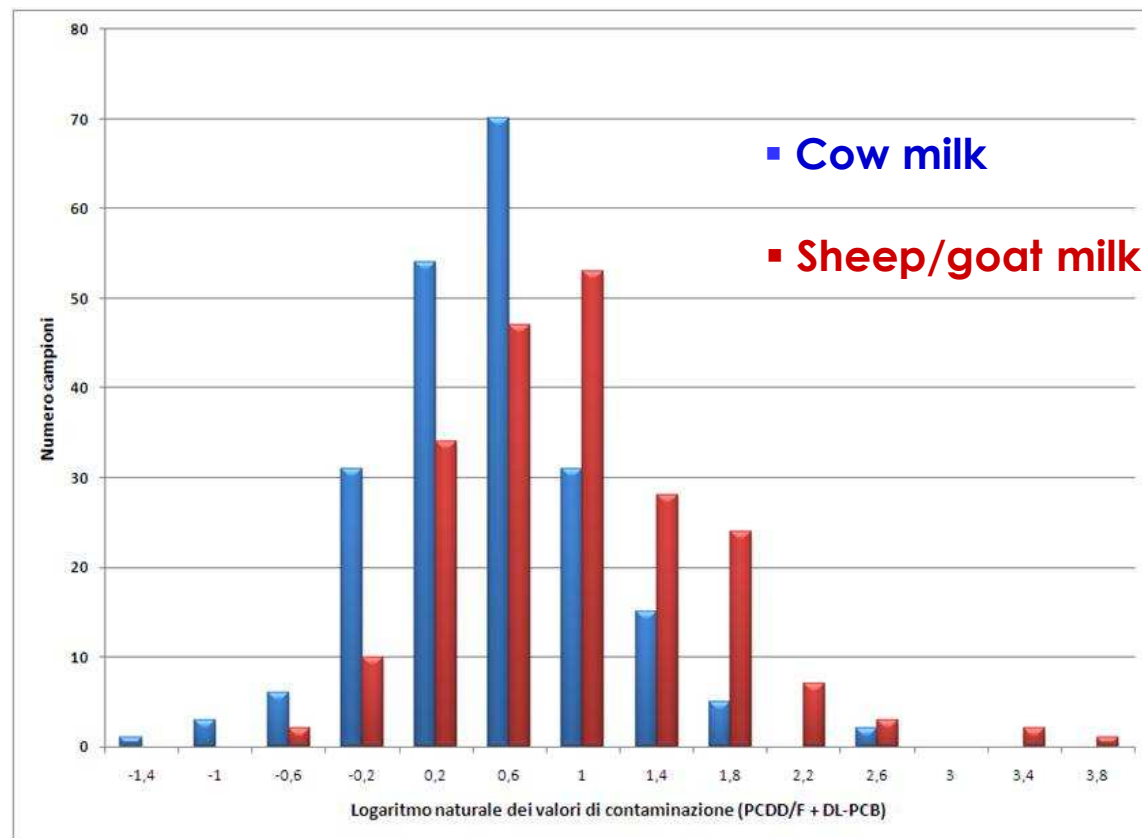
| <b>MATRIX</b>        | <b>N. SAMPLES</b> | <b>NON-COMPLIANT SAMPLES</b> |
|----------------------|-------------------|------------------------------|
| MILK (bovine)        | 215               | 1                            |
| MILK (sheep/ goat)   | 210               | 9                            |
| CHEESE (sheep/ goat) | 3                 | 0                            |
| LIVER (sheep/ goat)  | 51                | 51                           |
| FAT (sheep/ goat)    | 21                | 11                           |
| MUSCLE (sheep/ goat) | 38                | 16                           |
| EGGS                 | 9                 | 2                            |
| FEED                 | 30                | 0                            |
| <b>TOTAL</b>         | <b>577</b>        | <b>90</b>                    |



## Contamination of the food chain in Taranto

### Monitoring plan for food/feed 2008-2010 (3)

Distribution of PCDD/Fs + DL-PCBs levels in milk samples



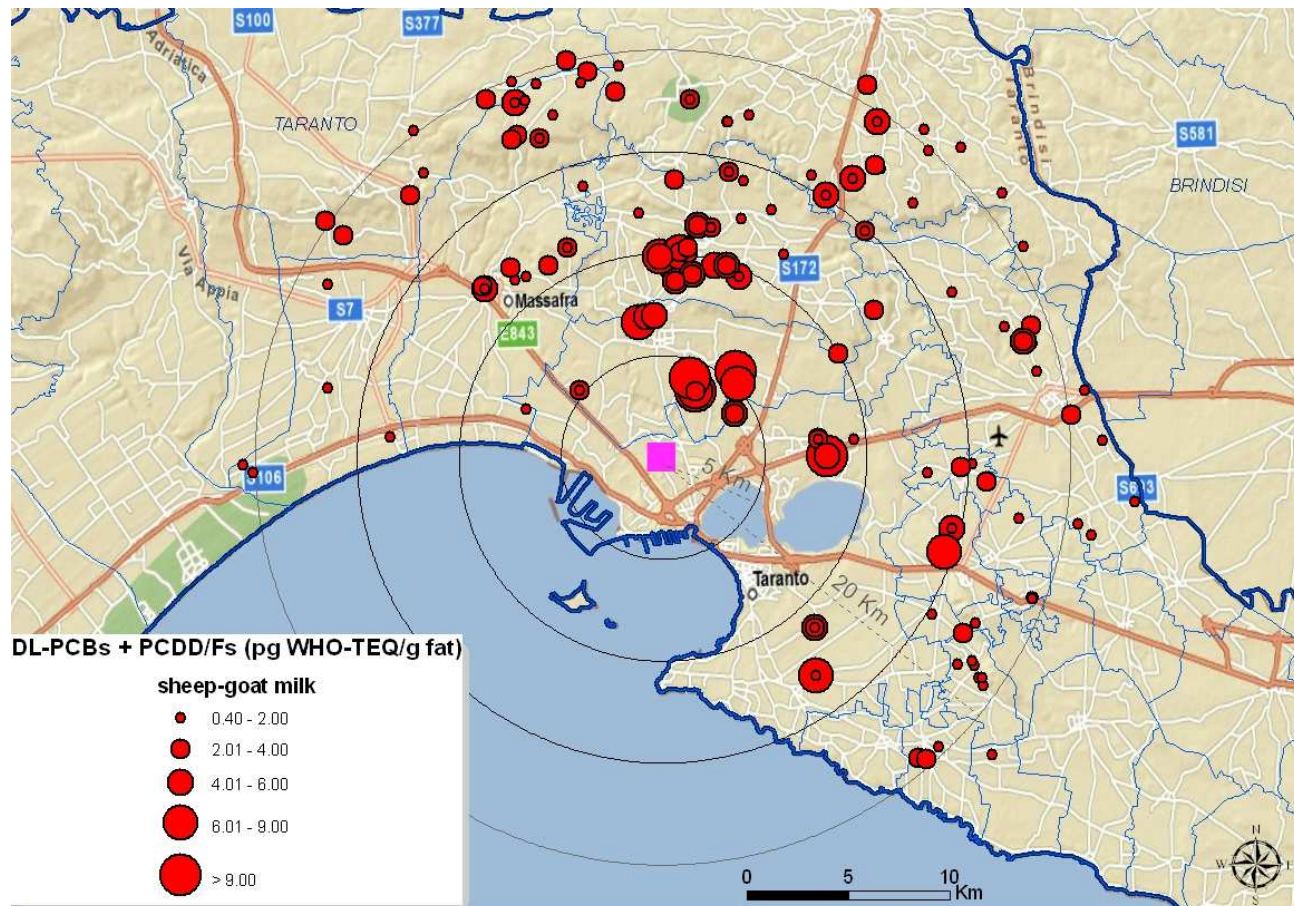




## Contamination of the food chain in Taranto

### Monitoring plan for food/feed 2008-2010 (4)

PCDD/Fs + DL-PCBs levels in sheep/goat milk

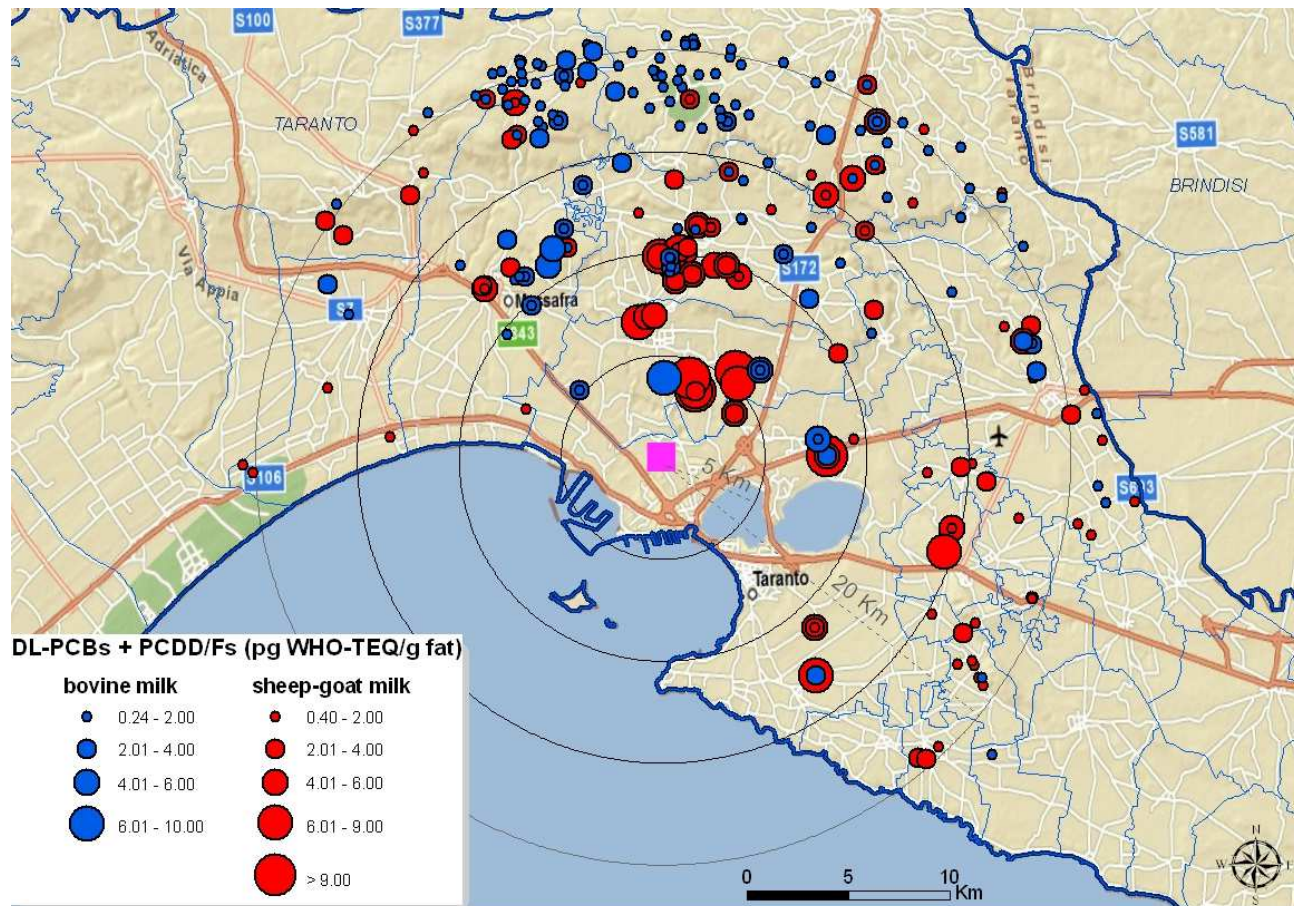




## Contamination of the food chain in Taranto

### Monitoring plan for food/feed 2008-2010 (5)

PCDD/Fs + DL-PCBs levels in cow and sheep/goat milk





## Contamination of the food chain in Taranto

### Monitoring plan for mussels 2010-2012 (1)

September 2010: PCDD/F and DL-PCB levels in fish and mussel samples above the EU action level (Recommendation 2006/88/EC). A monitoring plan on mussels was implemented in Mar Grande and Mar Piccolo sub-basins.





## Contamination of the food chain in Taranto

### Monitoring plan for mussels 2010-2012 (2)

Compliant and non compliant samples for PCDD/Fs and DL-PCBs grouped by years and sampling area

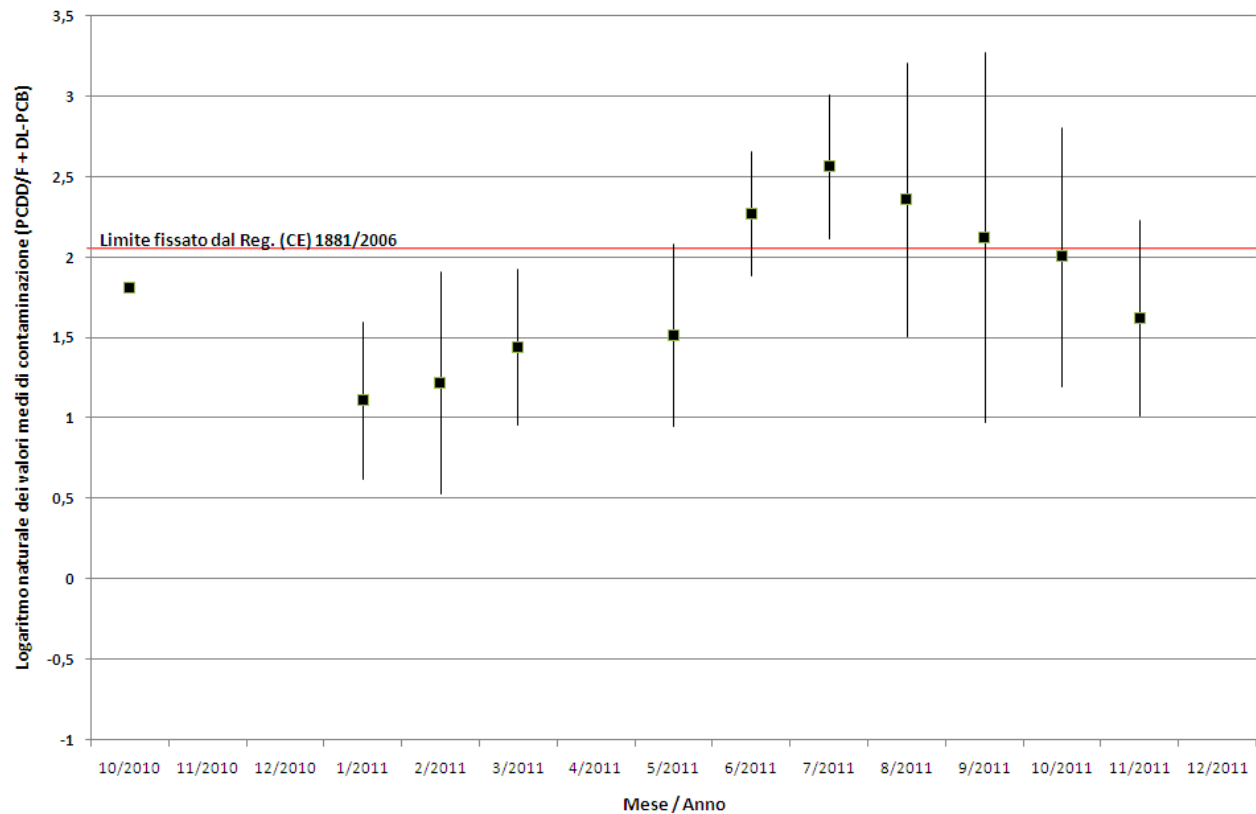
| Sampling Area                     | Sampling Year | Compliant Samples | Non Compliant Samples |
|-----------------------------------|---------------|-------------------|-----------------------|
| MAR GRANDE                        | 2010          | 4                 | ---                   |
|                                   | 2011          | 46                | ---                   |
|                                   | 2012          | 12                | ---                   |
| MAR PICCOLO 2 <sup>nd</sup> BASIN | 2010          | 4                 | ---                   |
|                                   | 2011          | 55                | ---                   |
|                                   | 2012          | 14                | ---                   |
| MAR PICCOLO 1 <sup>st</sup> BASIN | 2010          | 5                 | ---                   |
|                                   | 2011          | 61                | 23                    |
|                                   | 2012          | 12                | 4                     |



## Contamination of the food chain in Taranto

### Monitoring plan for mussels 2010-2012 (3)

Mean values ( $\pm$  2 standard deviation) for PCDD/Fs + DL-PCBs in mussels sampled in Mar Piccolo first basin (2010-2011)

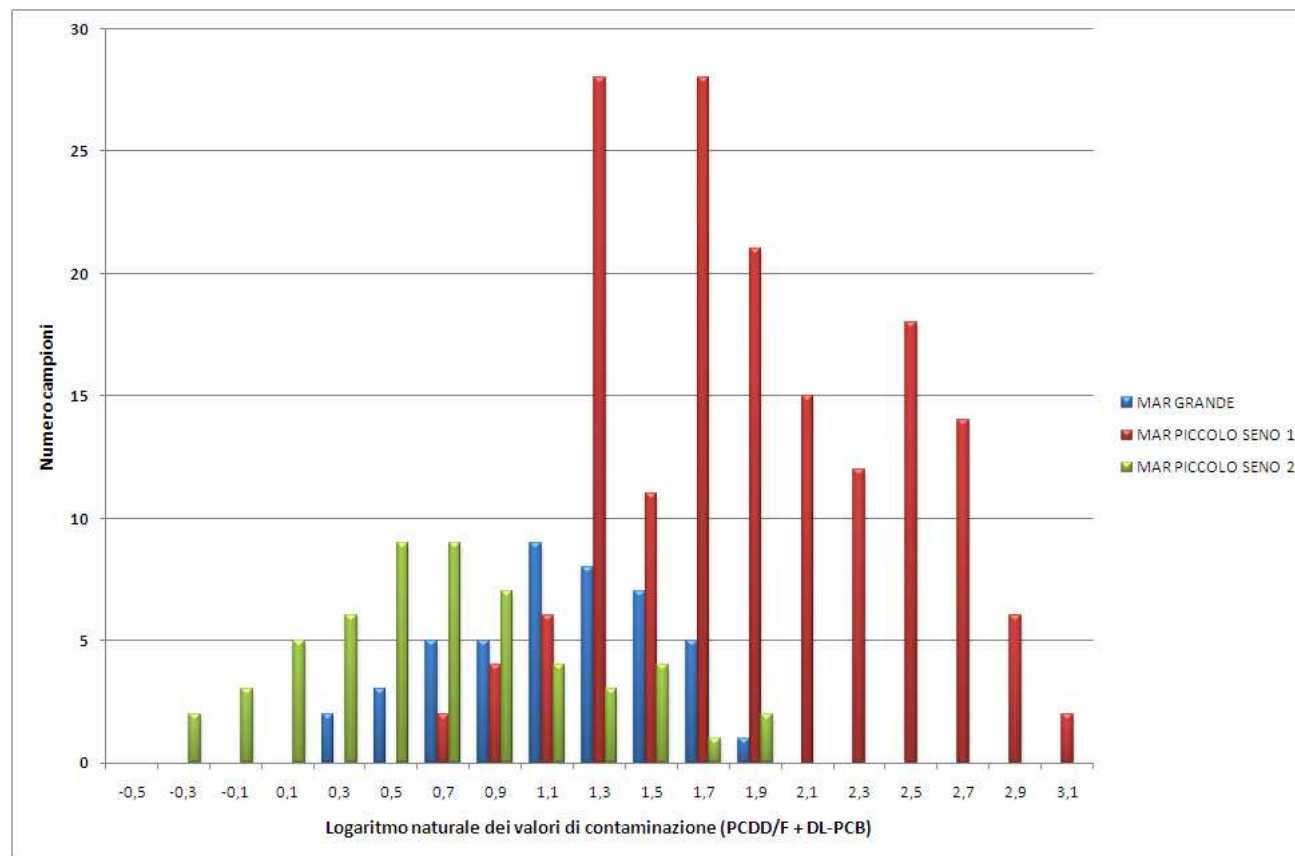




## Contamination of the food chain in Taranto

### Monitoring plan for mussels 2010-2012 (4)

Distribution of PCDD/F + DL-PCB levels in mussels sampled in Mar Grande and Mar Piccolo sub-basins (2010-2011)





# MILK CONTAMINATION BY $\beta$ -HEXACHLOROCYCLOHEXANE



## Milk contamination by $\beta$ -hexachlorocyclohexane

### Detection of a not compliant sample from monitoring

March 2005: bovine milk sample from Gavignano (Roma) was not compliant for  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH): 0.062 mg/kg 4% fat (maximum level 0.003 mg/kg, 4% fat).  $\beta$ -HCH was detected in a maize silage from the same farm<sup>14</sup>.

$\beta$ -HCH was also found in sheep milk at levels above 0.003 mg/kg, 4% fat.

It is a by-product of Lindane ( $\gamma$ -HCH), an organochlorine insecticide.



14. Ubaldi A. Unpublished data (2005)





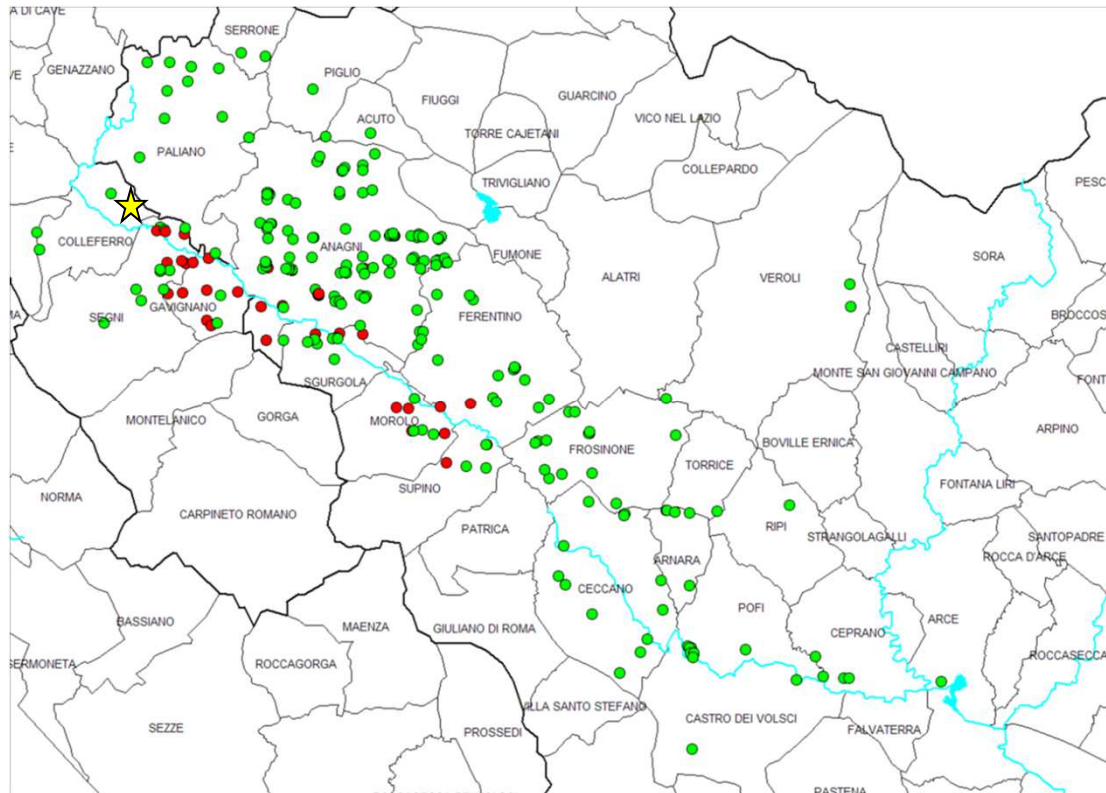
## Milk contamination by $\beta$ -hexachlorocyclohexane

### Valle del Sacco monitoring plan

All the 244 farms for milk production were tested (milk and locally produced grass, silage, hay): 34 farms were not compliant for  $\beta$ -HCH in milk.

Forages have been irrigated with river water or have been grown in flooded fields.

Most likely contamination source: old pesticides production plant in Colleferro (Roma).





# **EGGS CONTAMINATION BY ALUMINUM SMELTER EMISSIONS**



## Eggs contamination by aluminum smelter emissions

### The investigated area

High PCDD/F concentrations were found in the soil near a secondary aluminum smelter (AS) in Piedmont region<sup>15</sup>. It is well known that secondary AS emissions may contain PCDD/Fs and PCBs probably due to impurities in the raw materials used in the process.

Free-range eggs from small rural farms within a distance of 4.5 km from secondary AS were investigated in the period 2012-2013<sup>16</sup>.

Sampling zone 1: 0.1 km (7 eggs)

Sampling zone 2: 0.7-1.9 km (8 eggs)

Sampling zone 3: 3.3-4.5 km (6 eggs)



15. Colombo et al. *Chemosphere*, 85, 1719-1724 (2011)

16. Squadrone S. et al. *Environ Pollut*, 206, 429-436 (2015)



## Eggs contamination by aluminum smelter emissions

### Results from the investigation

- **Sampling zone 1:** all 7 samples not compliant (median PCDD/Fs 13.5 pg WHO<sub>2005</sub>-TEQ/g fat, maximum level 2.5 pg WHO<sub>2005</sub>-TEQ/g; median PCDD/Fs + DL-PCBs 62.0 pg WHO<sub>2005</sub>-TEQ/g fat, maximum level 5.0 pg WHO<sub>2005</sub>-TEQ/g; median NDL-PCBs 146 ng/g fat, maximum level 40 ng/g fat).
- **Sampling zone 2:** 1 sample not compliant for PCDD/Fs (3.39 pg WHO<sub>2005</sub>-TEQ/g fat and for PCDD/Fs + DL-PCBs (12.1 pg WHO<sub>2005</sub>-TEQ/g fat, 1 sample not compliant for PCDD/Fs + DL-PCBs (10.70 pg WHO<sub>2005</sub>-TEQ/g fat).
- **Sampling zone 3:** all samples compliant.





## Eggs contamination by aluminum smelter emissions

### Estimation of dietary intake

**Tolerable weekly intake:** 14 pg WHO-TEQ/kg body weight.

Egg fat weekly intake: 19.8 g adult, 17.5 g children, 7.7 g infant.

|               | Median PCDD/Fs<br>+ DL-PCBs (pg<br>WHO-TEQ/g fat) | Estimated weekly intake<br>(pg WHO-TEQ/kg body weight) |      |
|---------------|---|--|------|
| <b>Zone 1</b> | 62.0  | Adult (70 kg)  | 17.5 |
|               |   | Children (9 y, 32 kg)                                  | 33.9 |
|               |   | Infant (3 y, 15 kg)                                    | 31.8 |
| <b>Zone 2</b> | 5.1   | Adult (70 kg)  | 1.4  |
|               |   | Children (9 y, 32 kg)                                  | 2.8  |
|               |   | Infant (3 y, 15 kg)                                    | 2.6  |
| <b>Zone 3</b> | 2.6   | Adult (70 kg)  | 0.74 |
|               |   | Children (9 y, 32 kg)                                  | 1.42 |
|               |   | Infant (3 y, 15 kg)                                    | 1.33 |



# BIOINDICATORS OF EXPOSURE: A PILOT STUDY



## Bioindicators of exposure: a pilot study

### **Selected bioindicators and POPs**

In 2009-2010, a pilot study was conducted in Italy to evaluate sheep/goat and honeybee (*Apis Mellifera* L.) as potential bioindicators of environmental contamination<sup>17</sup>.

#### **POPs**

Dioxins and furans (PCDD/Fs), dioxin-like and non dioxin-like polychlorinated biphenyls (DL-PCBs/NDL-PCBs)

Polybrominated diphenyl ethers (PBDEs)

Perfluoroalkyl acids (PFAAs)

Pentachlorobenzene (PeCB)

Hexachlorobenzene (HCB)

Pentachlorophenol (PCP)

**Bioindicators:** sheep/goat milk and honey



The samples were collected from areas affected by the presence of POP emission sources, and from control areas.

17. Diletti G. et al. *Organohalogen Compd*, 78, 325-328 (2016)



## Bioindicators of exposure: a pilot study

### Sampling plan (1)

Choice of samples to provide representative bioindicators:

- milk from sedentary sheep or goat flocks (better reflect the environmental quality)
- wildflower honey (produced in a longer period in comparison to monofloral ones)

Samples were taken:

- nearby pollutants emission sources or contaminated areas already identified, marked as exposed (E) zones
- in control sites with expected background contamination, such as national or regional natural parks, marked as non-exposed (NE) zones







## Bioindicators of exposure: a pilot study

### Sampling plan (2)

Samples were collected from 16 Italian regions:

- for milk, 15 samples were from E-zones, 13 from NE-zones
- for honey, 15 sample were from E-zones, 12 from NE-zones





## Bioindicators of exposure: a pilot study

### PCDD/Fs and DL-PCBs in milk samples

Four samples from E-zones were above the action levels (ALs) for PCDD/Fs (1.75 pg WHO<sub>2005</sub>-TEQ/g fat) and DL-PCBs (2.0 pg WHO<sub>2005</sub>-TEQ/g fat) designed to prompt competent authorities and operators to identify a source of contamination and to take measures for its reduction or elimination.

ALs were exceeded for PCDD/Fs in Colleferro (Lazio), and for DL-PCBs in Val Trompia (Lombardy), Milazzo (Sicily) and Crotona (Calabria).

These sites are characterized by the presence of industrial plants.





## Bioindicators of exposure: a pilot study

### **NDL-PCBs, PBDEs, and PFAAs in milk samples**

NDL-PCBs sum was between 2.4 and 13.3 ng/g fat (ML = 40 ng/g fat).

PBDE sum was in the range 146-920 pg/g fat with predominant congeners in the order 47 > 99 > 100 > 153 > 154 reflecting past use of technical mixtures constituted of tetra- and hexa-bromodiphenylethers as flame retardants<sup>18</sup>.

Regarding PFAAs, the maximum values for PFOS (0.26 ng/g, Sardinia E-zone) and PFOA (0.02 ng/g, Umbria NE-zone) were below the mean values reported in the literature<sup>19</sup>.

18. Schechter, A. et al. *Environmental Health Perspectives*, 118, 357-362 (2010)

19. Hlouskova V et al. *Food Additives and Contaminants Part A*, 30 (11), 1918-1932 (2013)



## Bioindicators of exposure: a pilot study

### **HCB, PeCB and PCP in milk samples**

For HCB, the obtained values were in the range 1.69 – 9.27 ng/g fat (EU maximum level 0.01 mg/kg fat). The highest level was recorded in Trentino (E-zone, presence of a steel plant).

For PeCB concentrations between 0.05 and 0.57 ng/g fat were recorded.

All samples were negative for PCP (< 5 ng/g).





## Bioindicators of exposure: a pilot study

### Honey samples

The Mann-Whitney U test could not be performed for honey samples because most of results were below the LOQ values.

PBDEs  $\leq 38$  pg/g

PCDD/Fs  $\leq 0.080$  pg WHO<sub>2005</sub>-TEQ/g

DL-PCBs  $\leq 0.078$  pg WHO<sub>2005</sub>-TEQ/g

NDL-PCBs  $\leq 0.50$  ng/g

PFOS, PFOA  $\leq 0.010$  ng/g

PeCB, HCB  $< 0.03$  ng/g

PCP  $< 0.15$  ng/g



This matrix resulted not suitable for monitoring POPs considered in this study, nevertheless useful data were obtained for trace elements as a part of the study not discussed here.



## Concluding remarks

**Grazing sheep** flocks represents sentinels of the overall quality of the environment.

**Mussels** are good biological indicators of the presence of POPs in marine environment.

**Home-produced eggs** may be contaminated due to the pecking of soil by the animals but it is generally difficult to point out specific sources.

**Honey** contamination appears mainly attributable to beekeeping practices (e.g. acaricides and antibiotics use) rather than environmental contaminants, with some exceptions.

Sentinel animals are of relevance for both food security and food safety issues for the potential human health risks associated with contaminants dietary intake.





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