

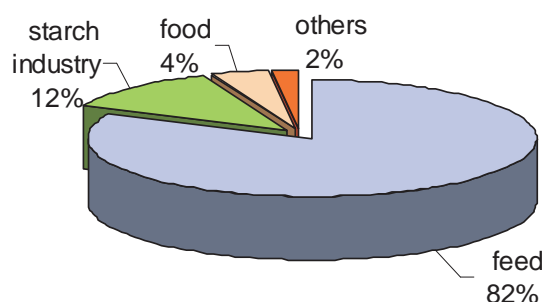
## FUMONISINS IN ITALIAN MAIZE: ANALYSIS AND CONSIDERATIONS

### 1 The maize food chain in Italy

About 1,1 million hectares of maize are grown in Italy, yielding more than 10.6 million tons of grain (Tab. 1) 85% of which is for feed, approx. 10% is for human consumption (food and drugs) and 5% is used for industry (paper) (Fig. 1)

**Table 1. Cultivation of Maize in Italy, year 2005 (source ISTAT)**

|              | <i>Surface</i><br>(ha) | <i>Yield</i><br>(t/ha) | <i>Total<br/>production</i><br>(t) |
|--------------|------------------------|------------------------|------------------------------------|
| <b>Italy</b> |                        |                        |                                    |
| North        | 984.827                | 9.8                    | 9.706.729                          |
| Central      | 87.523                 | 7.6                    | 662.467                            |
| South        | 45.506                 | 6.4                    | 291.513                            |
| <b>Total</b> | <b>1.117.856</b>       | <b>9.5</b>             | <b>10.660.709</b>                  |



**Fig. 1. Uses of Maize in Italy.**

Maize is mainly grown in Northern Italy (Piemonte, Lombardia, Veneto, Friuli V. G., Emilia Romagna). In this area a portion of the arable land ranging from 13 to 61% is cropped with maize, and 9.7 millions tons of grains, corresponding to 91% of the Italian production, are produced here (Tab. 2).

**Table 2. Cultivation of Maize in Northern Italy, year 2005 ( source: ISTAT)**

| <b>Regions</b>        | <i>Surface</i><br>( ha ) | <i>Yield</i><br>(t/ha) | <i>Total yield</i><br>(t) | <i>Total cultivated<br/>area</i><br>(ha) | <i>Total arable<br/>land</i><br>(ha) | <i>maize area / arable<br/>land area</i> |
|-----------------------|--------------------------|------------------------|---------------------------|--|--------------------------------------|--|
| <b>Piemonte</b>       | 185.570                  | 9.4                    | 1.736.230                 | 1.068.298,73                             | 577.277,70                           | 32,15%                                   |
| <b>Lombardia</b>      | 274.776                  | 11.3                   | 3.104.498                 | 1.035.791,51                             | 727.351,85                           | 37,78%                                   |
| <b>Veneto</b>         | 302.983                  | 9.9                    | 3.008.482                 | 852.743,88                               | 582.819,24                           | 51,99%                                   |
| <b>Friuli V. G.</b>   | 107.804                  | 7.6                    | 814.928                   | 238.806,97                               | 175.532,31                           | 61,42%                                   |
| <b>Emilia Romagna</b> | 112.930                  | 9.2                    | 1.038.643                 | 1.114.287,92                             | 850.702,38                           | 13,27%                                   |
| <b>Total/average</b>  | <b>984.063</b>           | <b>9.5</b>             | <b>9.702.781</b>          | <b>4.309.929</b>                         | <b>2.913.683</b>                     | <b>39,32%</b>                            |

In Italy maize-based products for human consumption create a turnover of 592 million € involving 300 of enterprises with more than 2000 employees (Tab. 3).

**Table 3. Maize industry, quantity, employers and turnover in Northern Italy, year 2005**

| <i>Main Activities</i>            | <i>Enterprises</i><br><i>n.</i> | <i>Processed</i><br><i>t</i> | <i>Employers</i><br><i>n.</i> | <i>Turnover</i><br><i>€</i> |
|-----------------------------------|---------------------------------|------------------------------|-------------------------------|-----------------------------|
| Starch industry                   | 2                               | 870.000                      | 850                           | 352.350.000                 |
| Industrial dry milling            | 20                              | 468.000                      | 265                           | 93.600.000                  |
| Small dry milling                 | 70                              | 248.500                      | 284                           | 67.095.000                  |
| <b>Total main activities</b>      | <b>92</b>                       | <b>1.586.500</b>             | <b>1.399</b>                  | <b>513.045.000</b>          |
| <b>Dependent Activities</b>       |                                 |                              |                               |                             |
| Transports                        | 128                             |                              | 250                           | 31.730.000                  |
| Maintenance                       | 50                              |                              | 150                           |                             |
| Marketing                         | 30                              |                              | 120                           |                             |
| Others                            |                                 |                              | 81                            | 47.595.000                  |
| <b>Total dependent activities</b> | <b>208</b>                      |                              | <b>601</b>                    | <b>79.325.000</b>           |
| <b>Total</b>                      | <b>300</b>                      |                              | <b>2.000</b>                  | <b>592.370.000</b>          |

In the North of Italy, where maize is intensively cultivated, the climatic conditions are particularly favourable for the development of the Fumonisin-producing fungi compared to Centre Europe (13) and every year the maize grains are contaminated by these mycotoxins. Nowadays, the quantity of Italian maize utilized for human consumption amounts to 1.5 million of tons.

According to the Reg. CENo. 856/2005, the greater part of the maize harvest of Northern Italy could no longer be utilized for human consumption.

The purpose of this document is to stress the evidence of the efforts to reduce fumonisins that are ongoing in Italy, the severe difficulty to grow maize with low content of fumonisins and to reduce it during milling and others industrial processes, face to a rather low exposure of Italian population and even more lower exposure of other European populations.

## **2 Occurrence**

Fumonisin are mainly produced by *Fusarium verticillioides* (synonym, *Fusarium moniliforme*). This mould is the most commonly reported fungal species infecting maize (40). *F. verticillioides* can be found in plant residues in almost every maize field at harvest it is associated with disease at all stages of plant development, infecting roots, stalk and kernels, but it is also present in symptomless maize plants. (31). Environmental conditions, water availability (20; 22; 32; 33), genetic background of the plant and the pathogen (3; 22; 28, 55) may all be important factors in disease

development. *F. verticillioides* in association with *F. proliferatum* and *F. subglutinans* causes the pink ear rot; pink fusariosis is prevalent in the dry and warm climates of Southern Europe, while the red fusariosis (caused by *F. graminearum*) is prevalent in years and locations characterized by frequent rainfall and low temperatures during summer and early autumn.

## 2.1 Occurrence of maize ear rot

Two separate surveys carried out in 2000-'05 and 2002-'03 in Northern Italy on more than 300 and 200 samples, respectively from maize crops confirmed the high percentage of maize kernels infected by *F. verticillioides*. In the first survey more than 50 % of maize samples showed a high level (> 60%) of kernels infected by this species (8). The second survey confirmed these results and the infection was normally higher than 40% (4; 5). Moreover, kernel infection differed among regions, with a clear west-east positive gradient at least in 2002 (44).

## 2.2 Occurrence of fumonisins

*F. verticillioides* produces several toxins, among which the fumonisins are the most significant. Since fumonisins can be detected in symptomatic and asymptomatic maize kernels, control of fumonisins contamination in maize has become a priority area in food safety research (10), and some guidelines for maximum fumonisin levels in human food and animal feeds have been issued (23). Production of fumonisins in grains before harvesting has been documented under many environmental conditions (51; 53; 9; 21; 18; 24; 42; 45). There are three main infection pathways for in planta kernel infection (38). In the first pathway, inoculum consists of macroconidia and abundant microconidia produced on crop residues and on tassels (30); these conidia are air- or splash-dispersed and infect ears through silks or wounds (26; 41). The second pathway involves the systemic development of the pathogen within the plant; systemic infection can rise from root, stalk or leaf sheath penetration, or from seed transmission (19; 34; 37). In the third pathway, conidia reach ears via spore-carrying insects, mainly the European maize borer larvae, *Ostrinia nubilalis* (27), which move inside systemically infected stalks or penetrate through bracts.

Different mycotoxins have been monitored on Italian maize. More recently surveys have been mainly conducted to determine occurrence of *Fusarium* toxins, especially fumonisins, under a variety of contexts and in different regions.

The largest recent surveys are listed below:

**Table 4. Survey No.1**

|                             |   |
|-----------------------------|---|
| <b>Area of sampling</b>     | Po plain  |
| <b>Source of samples</b>    | Commercial lots from drying units and milling plains  |
| <b>Sampling methods</b>     | According to EC Dir. 76/371   |
| <b>Methods of analysis</b>  | HPLC  |
| <b>Scientific Reference</b> | Università Cattolica di Piacenza  |
| <b>Literature Reference</b> | Pietri A. <i>et al.</i> , (2004), “ <i>Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northern Italy</i> ”, Food Additives and Contaminants 21 (5): 479-487 |
| <b>Number of samples</b>    | 503   |
| <b>Number of years</b>      | 5 (1995, 1996, 1997, 1998, 1999)  |

**Table 5. Survey No.1: concentration of Fumonisin B<sub>1</sub>**

|                          | <b>1995</b> | <b>1996</b> | <b>1997</b> | <b>1998</b> | <b>1999</b> |
|--------------------------|-------------|-------------|-------------|-------------|-------------|
| <b>Mean (ppb)</b>        | 3347        | 1324        | 3103        | 2655        | 5173        |
| <b>&lt;1000 ppb (%)</b>  | 34,7        | 45,2        | 26,6        | 28,1        | 16,1        |
| <b>1000-5000 ppb (%)</b> | 48,0        | 52,9        | 63,8        | 58,8        | 38,7        |
| <b>&gt;5000 ppb (%)</b>  | 17,3        | 1,9         | 9,6         | 13,1        | 45,2        |
| <i>N. of samples</i>     | 98          | 104         | 94          | 114         | 93          |

**Table 6. Survey No. 2**

|                             |   |
|-----------------------------|---|
| <b>Area of sampling</b>     | Po plain  |
| <b>Source of samples</b>    | a. Commercial lots from 60 drying units<br>b. Production from 44 experimental fields: network “ <i>on farm</i> ” 2003                                   |
| <b>Sampling methods</b>     | Flow-through methods from grain in movement   |
| <b>Methods of analysis</b>  | ELISA Test, fluorimetric method, validated with a HPLC ring test  |
| <b>Scientific Reference</b> | Regione Lombardia - Direzione Generale Agricoltura; CRA - Ist. Sper. per la Cerealicoltura, Sezione di Bergamo; Assincer; AIRES; Pioneer Hi-Bred Italia |
| <b>Literature Reference</b> | Verderio A. <i>et al.</i> , (2005), “ <i>La diffusione delle micotossine nelle produzioni italiane di mais</i> ”, L’Informatore Agrario 61 (10): 47-51  |
| <b>Number of samples</b>    | 1468  |
| <b>Number of years</b>      | 4 (1999, 2000, 2003, 2004)  |

**Table 7. Survey No. 2: concentration of Fumonisin B<sub>1</sub>**

|                          | <b>1999<sup>a</sup></b> | <b>2000<sup>a</sup></b> | <b>2003<sup>a</sup></b> | <b>2003<sup>b</sup></b> | <b>2004<sup>a</sup></b> |
|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>&lt;1500 ppb (%)</b>  | 49,8                    | 55,6                    | 15,8                    | 6,9                     | 22,4                    |
| <b>1500-2000 ppb (%)</b> | 24,5                    | 6,1                     | 5,5                     | 3,8                     | 12,5                    |
| <b>2000-4000 ppb (%)</b> | 18,0                    | 19,7                    | 24,5                    | 19,8                    | 61,3                    |
| <b>4000-6000 ppb (%)</b> | 5,6                     | 11,1                    | 8,7                     | 25,2                    | 2,3                     |
| <b>&gt;6000 ppb (%)</b>  | 2,2                     | 7,5                     | 45,5                    | 44,3                    | 1,5                     |
| <i>N. of samples</i>     | 323                     | 360                     | 310                     | 131                     | 344                     |

<sup>a</sup>= samples from drying units

<sup>b</sup>= samples from fields

**Table 8. Survey No. 3**

|                             |   |
|-----------------------------|---|
| <b>Area of sampling</b>     | Po Plain, Toscana   |
| <b>Source of samples</b>    | Farm field immediately pre-harvest  |
| <b>Sampling methods</b>     | Sample of 20 ears   |
| <b>Methods of analysis</b>  | HPLC  |
| <b>Scientific Reference</b> | Università di Piacenza, Milano, Udine e Pisa; CRA - Istituto Sperimentale per la Cerealicoltura   |
| <b>Literature Reference</b> | Battilani P. <i>et al.</i> (2005), “ <i>Monitoraggio della contaminazione da micotossine nel mais</i> ”, L’Informatore Agrario 61 (6): 47-49. |
| <b>Number of samples</b>    | 221   |
| <b>Number of years</b>      | 2 (2002, 2003)  |

**Table 9: Survey No. 3: concentration of Fumonisin B<sub>1</sub>**

|                          | 2002 | 2003 |
|--------------------------|------|------|
| <b>Mean (ppb)</b>        | 4797 | 5186 |
| <b>&lt;1000 ppb (%)</b>  | 20,4 | 31,5 |
| <b>1000-5000 ppb (%)</b> | 31,0 | 33,3 |
| <b>&gt;5000 ppb (%)</b>  | 48,7 | 35,2 |
| <b>N. of samples</b>     | 113  | 108  |

**Table 10: Survey No. 4**

|                             |  |
|-----------------------------|--|
| <b>Area of sampling</b>     | Po Plain   |
| <b>Source of samples</b>    | Field production from <i>Syngenta seeds</i> research network   |
| <b>Sampling methods</b>     | Flow-through sampling at harvesting  |
| <b>Methods of analysis</b>  | ELISA Test   |
| <b>Scientific Reference</b> | <i>Syngenta seeds</i> Italia   |
| <b>Literature Reference</b> | Tanzi F., (2005), “ <i>Funghi e micotossine su mais - Indagine Europea di Syngenta Seeds</i> ”, Atti della “Giornata del mais 2005”, Bergamo, Italia |
| <b>Number of samples</b>    | 586  |
| <b>Number of years</b>      | 6 (1999, 2000, 2001, 2002, 2003, 2004)   |

**Table 11. Survey No.4: concentration of Fumonisin B<sub>1</sub> + B<sub>2</sub>**

|                          | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 |
|--------------------------|------|------|------|------|------|------|
| <b>&lt;1500 ppb (%)</b>  |      | 60   | 27,4 | 9,5  | 11,0 | 24,4 |
| <b>1500-3000 ppb (%)</b> |      |      | 24,7 | 12,8 | 23,0 | 21,5 |
| <b>3000-6000 ppb (%)</b> |      |      | 17,9 | 21,8 | 30,0 | 26,1 |
| <b>&gt;6000 ppb (%)</b>  |      |      | 30,0 | 55,9 | 36,0 | 28,0 |
| <b>N. of samples</b>     | 64   | 124  | 72   | 68   | 106  | 152  |

<sup>a</sup>= average of the period 1999-2004

**Table 12. Survey No. 5**

|                             |  |
|-----------------------------|--|
| <b>Area of sampling</b>     | Piemonte   |
| <b>Source of samples</b>    | a. Commercial lots from 12 drying units<br>b. Production from 8 experimental fields<br>c. Production from 40 farm fields   |
| <b>Sampling methods</b>     | a. From drying units: Flow-through methods from grain in movement<br>b. From fields: sample of 200 ears from 5 subplots  |
| <b>Methods of analysis</b>  | HPLC   |
| <b>Scientific Reference</b> | Università di Torino   |
| <b>Literature Reference</b> | Reyneri A (2003). La presenza di micotossine nel mais coltivato in Piemonte. Regione Piemonte, pp. 32.<br>Reyneri A. et al. (2004). Impiego di tecniche agronomiche per contenere le micotossine nella granella di mais. L'Informatore Agrario, 6:45-50. |
| <b>Number of samples</b>    | 538  |
| <b>Number of years</b>      | 5 (2000, 2001, 2002, 2003, 2004)   |

**Table 13. Survey No.5: concentration of Fumonisin B<sub>1</sub>**

|                      | 2000 | 2001 | 2002 | 2003 | 2004 |
|----------------------|------|------|------|------|------|
| <1500 ppb (%)        | 78,1 | 63,1 | 22,0 | 42,4 | 68,7 |
| 1500-2000 ppb (%)    | 6,9  | 5,6  | 11,3 | 11,9 | 21,9 |
| 2000-4000 ppb (%)    | 13,8 | 18,8 | 30,8 | 27,1 | 9,4  |
| 4000-6000 ppb (%)    | 1,3  | 10,0 | 32,7 | 18,6 | 0,0  |
| >6000 ppb (%)        | 0    | 2,5  | 3,1  | 0,0  | 0,0  |
| <i>N. of samples</i> | 160  | 160  | 159  | 59   | 32   |

The sampling approach is a crucial aspect that could influence the results. The two basic approaches were the sampling from the field at harvest (Survey no.3, 4, 5) or from grain lots in storage facilities (Survey no.1, 2, 5).

Though approaches were different, data clearly confirm the high fumonisins concentrations. The results suggest that: the concentration of fumonisins in Northern Italy maize peaked in the last years (2002-2003); the majority of grain samples contains a concentration far above the level of 2000 ppb; there is a wide variability of concentrations of fumonisins content within areas and drying units that cannot be always explicated by weather conditions and field practices. Several industry surveys found very similar results (14; 15).

### 3 Research activities

Starting from the beginning of the 90's, several research projects on maize mycotoxins have been conducted in Italy in order to evaluate the diffusion and the possibilities to reduce the contaminations. More recently, an extra effort has involved an increasing number of scientific and technical institutions. Several of these projects are jointly carried out by food and agro industries.

The following list summarized some of the most recent apply projects on mycotoxins dealing with maize and cereal contaminations:

### **EU Projects**

- Early detection toxigenic *Fusarium* species and ochratoxigenic fungi in plant product (2001-04; Coordination ISPA-CNR,).
- Sustainability, product safety and quality in cereals: development of new quantitative models for risk assessment for mycotoxigenic *Fusarium* species (“RAMFIC” 2000-03, Unità ISPA-CNR).
- European mycotoxins awareness network (2000-03; Unità ISPA-CNR).
- Promoting Food Safety through a New Integrated Risk Analysis Approach for Foods IP – VI FP (SAFE FOODS) - Food-CT-2004-506446, Coordinators Dr Harry Kuiper and Hans Marvin – scientific Responsible for Italy Dr Marina Miraglia and Carlo Brera (ISS)

### **National and Interregional Projects**

- MICOCER - “Evaluation and control of mycotoxins occurrence in national cereals productions” (Interregional Project by Regione Lombardia and other 14 Regions, 2005 – 2008 with Consiglio per la Ricerca e la sperimentazione in agricoltura - CRA, Università di Torino , Università Cattolica di Piacenza -UCSC, Istituto Superiore di Sanità- ISS, Centro ricerche produzioni vegetali di Imola - CRPV, Consiglio Nazionale delle Ricerche –CNR ISPA di Bari and other partners)
- Research to reduce aflatoxin occurrence in milk and milk products “AFLARID” (Ministero delle Politiche Agricole e Forestali - MIPAF, 2004-06; with UCSC, I.S.S.; CRA, Università di Roma Tor Vergata).
- Model and integrated system for the wheat food safety “SINSIAF” (Ministero dell’Istruzione, dell’Università e della Ricerca - MIUR, 2003-2005 with CNR ISPA; UCSC Piacenza).
- Development of an integrated production system to reduce mycotoxin occurrence risk in food (MIUR-MIPAF, 2002-04; Istituto nazionale di ricerca per gli alimenti e la nutrizione - INRAN, CRA; UCSC, Università di Milano, Università di Udine, Università di Pisa).
- Prevention of chemical and mycotoxins occurrence in agro-technical and biological systems producing milk (MIPAF, 2002-04; with CRA, CNR – ISPA).
- Organic cerealiculture “BIOCER”: agro-technical and genetic actions to improve quality and quantity of hard and soft wheat, for better derivated products. (MIPAF, 2003-05; CRA, CNR ISPA).
- Interregional Research Network on cereals “SIC” (Interregional project by Regions and MIPAF 1999-2004 ; with CRA).

### **Regional projects**

Considering the great importance of maize cultivation in the Po plain, Regions in that district have activated researches and experimental projects which consider the specific local needs. Since 2005 the interregional collaboration has been coordinated by the interregional project MICOCER.

### *Lombardia*

- Individuation of maize classification instruments, including those for sanitary characteristics “PROCLAMA” - 2004-05 with CRA and Associazione interprofessionale Cereali – ASSINCER;
- Arable crop, 2002 - 2006; with Ente Regionale per ERSAF and CRA).
- Mycotoxins occurrence in the regional market of feed – TOSSMONIT – with Associazione regionale allevatori della Lombardia – ARAL.

### *Emilia Romagna*

- Development of an integrated production system to enhance maize production quality by reducing mycotoxin occurrence risk (Regione Emilia Romagna, 2004-06; with CRPV - UCSC).
- Maize agro-techniques and mycotoxins control (CRPV, UCSC Piacenza).
- Development of an integrated system to control fusariosis and mycotoxins occurrence in wheat and barley (2002-04; with CRPV, UCSC Piacenza, CRA).

### *Piemonte*

- Analysis of the relationships between climatic factors and mycotoxins production in maize. 2004-06; with Università di Torino, UCSC.
- Control of maize mycotoxins - 2004-07; with Università di Torino.

### *Veneto*

- Mycotoxins control in feed and food in Regione Veneto 2004 –2005; with Veneto Agricoltura, Università di Padova, Istituto Zooprofilattico delle Venezie.

### *Friuli Venezia Giulia*

- Mycotoxins occurrence in maize of Friuli Venezia Giulia, 2004; with ERSA.

## **Industrial projects**

- Mycotoxins distribution in maize processing (dry milling), 2003 – 2004, - Molino Favero;
- Traceability project AgriOK s.p.a.– COOP, 2003 –2004.
- Pre-harvest and harvest strategies for mycotoxins control and reduction - 2003 – 2005 Granarolo spa – Granlatte S.c.a.rl.– AgriOK s.p.a.–Università di Bologna DISTA.
- European Corn Borer management for mycotoxins reduction,- 2001 – 2005, Syngenta Crop protection– Università di Bologna, Università di Torino
- Strategies for the control of mycotoxins in maize in the Po Valley (Northern Italy), 2003 – 2005, Università di Torino, Pioneer Hi Bred Italia.
- Evaluation of strategy against *Ostrinia nubilalis* for health and production enhancement in maize cultivation. 2005-2006, Università di Torino, BASF
- Mais food grade. Production of health maize. 2004-2006, NDF Gruma Europe.

Some of these results are summarize in the following chapters dealing with field and post-harvest prevention and maize decontaminations trough the milling processes.

## **3.1 Prevention**

Several projects have been carried out in these last few years in order to reduce the growth and the diffusion of *Fusarium verticillioides*, as well as of *Fusaria* in field.

Studies on pre-harvest control strategies able to reduce toxigenic fungi development were conducted in several sites in Northern Italy. First results stress the role of hybrid selection, sowing time and density, fertilization, pest control (particularly European corn borer) and harvesting time,



while soil tillage, weed control and irrigation were less effective. The main results are summarized in Figure 2 (54; 49; 6; 50; 36; 47; 7).

**Figure 2. Effects of cultural practices on mycotoxin contamination**

|                      | FUM | DON - ZEA |
|----------------------|-----|-----------|
| Crop Rotation        |     |           |
| Management of debris |     |           |
| Seeding time         | 3   | 3         |
| Harvest time         | 3   | 3         |
| Hybrid               | 3   | 3         |
| Fertilization        | < 2 | < 2       |
| Weed control         | < 2 | < 2       |
| Insect control       | 3   | < 2       |
| Irrigation           | < 2 | < 2       |

|                         |   |     |   |     |
|-------------------------|---|-----|---|-----|
| Effect on concentration | 1 | < 2 | 3 | > 4 |
|-------------------------|---|-----|---|-----|

*F. verticillioides* is more influenced by seeding time and pest control than *F. graminearum*; therefore the good cultural practices (GAP) for fumonisins control are different to those for deoxynivalenol or zearalenone (29; 35; 16; 43; 2; 1).

Based on these results, some of the main Italian milling industries faced up to the higher fumonisins concentration on maize by applying a pre-harvest control strategies. Since 2000-2002 these industries have been progressively introducing a more detailed GAP code to the maize producers and a more effective premium price has been proposed in order to promote the farmer partnership. The essential aspects of this effort are summarized in Table 1, where 3 cases in Piemonte (A), Lombardia (B) and Veneto (C) regions are presented.

**Table 14. Pre-harvest control strategies in three milling industries (A:C)**

|   | A            | B            | C       |
|---|--------------|--------------|---------|
| Main maize products                         | flour, grits | homini grits | grits   |
| <b>GAP</b>                                  |              |              |         |
| - hybrids                                   | yes          | yes          | yes     |
| - seeding time                              | no           | yes          | yes     |
| - fertilization                             | no           | yes          | no      |
| - insect control                            | yes          | yes          | yes     |
| - irrigation                                | yes          | yes          | no      |
| - moisture at harvest                       | no           | yes          | yes     |
| <b>GMP</b>                                  | yes          | yes          | yes     |
| Rate of fumonisins reduction <sup>(1)</sup> | 1.5-2.5      | 2.0-3.0      | 2.0-2.5 |
| Premium price (€/t) <sup>(2)</sup>          | 6            | 15           | 5       |

The pre-harvest control strategies are rapidly leading to an effective separation of the maize for food from the maize for animal feeding, but the rate of the fumonisins reduction following proper GAP code is so far not always proportionate. Moreover, even if fungal growth rate and toxin production are related to the cultural practices and kernel characteristics of the hybrids, there are no doubts that an increase in the air temperature, as observed in the last decades, is particularly favourable to *F. verticillioides* and to the European Corn Borer the main pest of the maize (46). Therefore, the current fumonisins limits are, nowadays far to be reasonable achieved with the GAP codes that have been introduced in the last years though several researches have been set (cfr. 2.2) and the cultural practices are now more effective.

### **3.2 Fumonisins distribution in maize milling products**

Milling industries are facing the problem of the occurrence of mycotoxins on maize based products, and fumonisins have soon become evident as the more crucial contaminant. Development of toxigenic fungi can occur even during storage; therefore stored grains could increase mycotoxins content after harvesting. As detoxification methods are forbidden, post-harvest prevention and decontamination are to be considered of primary importance.

In order to keep the fumonisins content as low as it can be reasonably achieved, all post harvest maize chain was investigated in detail.

On one hand, since 1996, more than 1400 maize grain samples have been analysed with HPLC methods, on 16 drying and storage plants of North Italy in order to evaluate fumonisins and other mycotoxins content, in all the post harvest process: pre-storage of wet kernels before drying process, cleaning effect during all grains movement phases, conservation in silo from the delivery to the end of storage, milling operations and separations effects on final products.

On the other hand, since 2000, the distribution of fumonisins and other mycotoxins in the various maize particles and by products derived from dry milling process have been investigated. These studies were carried out in 4 milling units in Piemonte, Lombardia, Veneto ed Friuli-Venezia-Giulia Regions. Several sampling of different lots were then analysed (Table 15). This process separates grain components into various particle sizes, resulting in fractions such as grits, germ, bran, meal and flour.

The researches on drying plants have clearly demonstrated that mycotoxins accumulation is easily prevented by forced drying process and subsequent ventilation, able to reduce and uniform the bin

temperature. Furthermore, careful and repeated cleaning operations, during all grain movement phases, before and after the drying process, as well as removing of the silo-core mass and its cleaning, have significantly reduce by 10-30 % fumonisin B1 presence on grain, by removing of the lighter particles (8).

**Table 15. Researches conducted in Italy on the distribution of fumonisins and other mycotoxins in maize particles: milling industries (A:E)**

|                          | <b>A</b>                            | <b>B</b>                            | <b>C</b>                | <b>D</b>                            |
|--------------------------|-------------------------------------|-------------------------------------|-------------------------|-------------------------------------|
| Years                    | 2000-2005                           | 2005                                | 2002                    | 2004-2005                           |
| Lots (n,)                | 19                                  | 11                                  | 2                       | 46                                  |
| Sampling methods         | Dynamics sampling during processing | Dynamics sampling during processing | Following Dir. 98/57 CE | Dynamics sampling during processing |
| Methods of analysis      | HPLC - ELISA                        | ELISA                               | HPLC                    | HPLC - ELISA                        |
| Fraction analysed        |                                     |                                     |                         |                                     |
| - Unprocessed grain      | √                                   | √                                   | √                       | √                                   |
| - Germ                   | √                                   |                                     | √                       | √                                   |
| - Flour                  | √                                   | √                                   | √                       | √                                   |
| - Grits                  | √                                   | √                                   | √                       | √                                   |
| - Feed meal              | √                                   |                                     | √                       | √                                   |
| Reference <sup>(1)</sup> | 48                                  | (2)                                 | 12                      | (2)                                 |

<sup>(1)</sup> cfr. Reference in Annex 1

<sup>(2)</sup> Unpublished

The researches on drying plants have clearly demonstrated that mycotoxins accumulation is easily prevented by forced drying process and subsequent ventilation, able to reduce and uniform the bin temperature. Furthermore, careful and repeated cleaning operations, during all grain movement phases, before and after the drying process, as well as removing of the silo-core mass and its cleaning, have significantly reduce by 10-30 % fumonisin B1 presence on grain, by removing of the lighter particles (8).

Pre-storage of wet kernels before drying process is a critical phase, with a possible significant *Fusarium* toxins increase if the drying process is delayed for a long time. Pre-storage in drying units (from 6 to 36 hours before the drying process) do not increase the risk for mycotoxin accumulation in the kernels significantly (8).

Milling process has an important effect on mycotoxins reduction: grits and flour, the principal products of this process, have a lower toxins content than unprocessed grain. On the other hand by-products, such feed meal and germ are more contaminated (Table 16).

Collected data stressed the considerable presence and the large variability of these toxic metabolites on grains at delivery, the sampling and analytical difficulties to establish the concentration of

mycotoxins and the suitability of the lots to food and feed processing. Moreover, the rate of fumonisins reduction as a consequences of the milling partitioning is very unsteady. It changes on the basis of the flow diagram of the milling units and, inside the same unit, there is a variability between lots with a different content of toxins (13; 48).

**Table 16. Fumonisins distribution in maize milling products ( $\pm$  standard error)**

| Fraction analysed | Fumonisins (index value:media unprocessed maize = 100) |             |              |              |
|-------------------|--|-------------|--------------|--------------|
|                   | A  | B           | C            | D            |
| Unprocessed maize | 100  | 100         | 100          | 100          |
| Germ              | 90 $\pm$ 20  |             | 190 $\pm$ 4  | 78 $\pm$ 4   |
| Flour             | 43 $\pm$ 6   | 79 $\pm$ 10 |              | 76 $\pm$ 14  |
| Grits             |  | 42 $\pm$ 6  | 12 $\pm$ 2   | 32 $\pm$ 4   |
| Re-milled Grits   | 13 $\pm$ 2   |             | 9 $\pm$ 0    |              |
| Feed meal         | 338 $\pm$ 104  |             | 168 $\pm$ 35 | 271 $\pm$ 46 |

Generally speaking, the reduction rate in flours and grits is higher when unprocessed grains are highly contaminated, whereas it is lower when grain is less contaminated.

#### 4. Exposure assessment of Italian population to Fumonisin B<sub>1</sub>

Council Regulation (EEC) No. 315/93 of 08.02.93 laying down Community procedures for contaminants in food, is the framework of the Community action on contaminants, including mycotoxins, in food. The framework regulation provides:

- 1) that food containing a contaminant in an amount , which is unacceptable from the public health viewpoint, shall not be placed on the market;
- 2) that contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain (ALARA);
- 3) that in order to protect public health, maximum levels for specific contaminants shall be established where necessary (by specific committees);
- 4) mandatory consultation of a scientific body (EFSA) for all provisions which may have an effect on public health.

The Commission Regulation (EC) No 856/2005 of 06.06.05, in the premises reports that the Scientific Committee for Food established a Tolerable Daily Intake (TDI) of 2  $\mu$ g/kg bodyweight/day for the total of fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, alone or in combination. On point 9 of the premises, it is affirmed:

“For fumonisins, the estimated dietary intake for most population groups is far below the TDI. The dietary intake of fumonisins increases significantly when consumers only are considered. Nevertheless, the dietary intake is also for that group of consumers below the TDI. However, monitoring control results of the harvest 2003 indicate that maize and maize products can be very highly contaminated by fumonisins. It is appropriate that measures are taken to avoid that such unacceptably highly contaminated maize and maize product can enter the food chain.”

A research for the evaluation of the exposure assessment of Italian population to Fumonisin B1 has been carried out by ISS (Istituto Superiore della Sanità) in 2004, consisting in three phases (8):

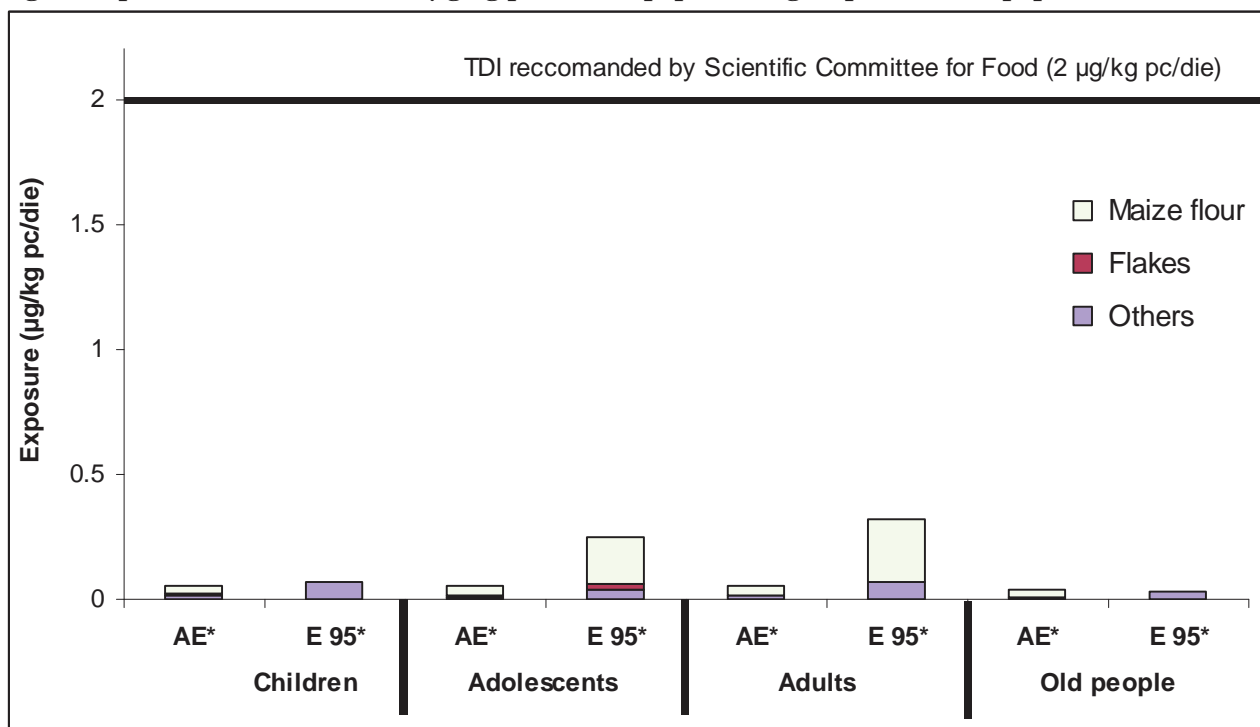
- sampling and collection of consumer data. Sampling of different food products has been carried out at major distribution centres in the whole Italian territory. Consumer data refer to a national pool performed by Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (52);
- Sample preparation and analysis. Analytical method was that proposed by Visconti et al. 2001;
- Exposure assessment. The average exposure assessment was calculated by stating the levels of mycotoxin contamination in every food and combining these data with the consumption amounts of the specified foods.

The result of the research shows that the value of exposure assessment of Italian population to Fumonisin B<sub>1</sub>, conducted with the methodology of Total Intake, is amply lower than TDI value recommended by Scientific Committee for Food in Europe (2 µg/kg pc/die) for all the population group considered (fig. 3). Moreover, research results demonstrated that the two more exposed categories are adults and adolescents, whose intake level is, however 7.9 and 6.2 times lower than TDI.

These results confirm those evidenced by Final Report of the Task 3.2.10 on "Collection of occurrence data of *fusarium* toxins in food and assessment of dietary intake by the population of EU Member States" (2003). On the summary of dietary intake, it is affirmed about fumonisins: “The average daily intakes are well below the fumonisins group TDI of 2 µg/kg body weight/day. Higher intakes were noted for young children. Cereals represent the major source of intake for fumonisins”. Moreover, the Range of average dietary intakes, calculated as percentage of the TDI-values, is much lower than those that were calculated for other *fusarium* toxins (Annex 2.)

As it can see in table 17 there's no coherence between the Tolerable Daily Intake set by the Scientific Committee and the thresholds currently proposed.

**Fig. 3. Exposure to Fumonisin B<sub>1</sub> (µg/kg pc/die) for population groups of Italian population (Ref . 11)**



\* AE: Average exposure

\* E 95: Exposure 95 percentile

The fumonisins threshold will probably expels from the food chain most of the Southern Europe maize that is relatively high on fumonisins but particularly low on other *Fusarium*-toxins

Hence, the Northern and Central Europe maize, that will probability replace the South Europe maize, will be lower on fumonisin but higher on DON and ZEA, therefore increasing the average intake of this toxins that are already closer to the TDI, because of the higher intake of ather cereals products.

**Tab. 17. Comparison among the different TDI and thresholds for *Fusarium*-toxins**

|                   | TDI<br>(µg/kg BW) | TDI<br>in µg for a<br>person of 75<br>(kg) | Final Products limits<br>currently proposed    |         | Amount di F.P. to<br>be eaten to reach<br>the TDI<br>(g) |
|-------------------|-------------------|--|--|---------|--|
|                   |                   |  |  | (µg/kg) |  |
| <b>DON</b>        | 1                 | 75   | Bread  | 500     | 150  |
|                   |                   |  | Pasta  | 750     | 100  |
| <b>ZEA</b>        | 0.2               | 15   | Bread  | 50      | 300  |
| <b>FUMONISINS</b> | 2                 | 150  | maize based<br>foods for direct<br>consumption | 400     | 375  |

## 5. Final Considerations

- Maize is the main Italian crop by volume and almost 10% of the harvest enter the food chain.
- Fumonisin are present at variable rate depending on the year and on the region considered, but on the average, most of the Italian maize is above 2000 ppb.
- Italian farmers don't have today, and it not expect they will have by 2007, the tools to keep their maize below 2000 ppb with a reasonable probability.
- The milling process does not reduce the unprocessed corn contamination consistently and equally for all the milling fractions and for all the milling diagrams. The milling industries are particularly concerned for the future of grits and flour production, which will hardly achieve the proposed maximum levels, and for the survival of typical artisanal products as polenta, with a greater involvement of the organic agriculture products;
- The DG-SANCO approach is correctly based on the precautionary principle. Nevertheless, both the European SCOOP task 3.2.10 "Collection of occurrence data on *Fusarium* toxins in food and assessment of dietary intake by the population of EU member States" presented on September 2003 and the Italian study reported in this document shows that the average intake of fumonisins is far below the TDI for most exposed consumers and even without a regolamentation.
- An Industry sector with more than 2000 employees and a turnover of 513 millions € could be unable to face the current proposed fumonisin limits.
- If the Italian food maize will enter the feed market the Italian maize producers (over 200.000 producers) could face a decrease on corn price close to 20% meaning a total loss of over 200 million €.
- We agree that very high contaminated maize lots should be kept out of the food chain but a limit of 2000 ppb will keep out of business many Italian farmers and industries without improving consumers health.

Hence

- **We ask for a better tuning of fumonisins limits and implementations time to guarantee both consumers and producers health.**

10 jannuary 2006

For the Mycotoxin Work Group  
Gianfranco Pizzolato

## Annex 1

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## Annex 2

### SCOOP TASK 3.2.10

"COLLECTION OF OCCURRENCE DATA OF *FUSARIUM* TOXINS IN FOOD AND ASSESSMENT OF DIETARY INTAKE BY THE POPULATION OF EU MEMBER STATES"

**Final Report**  
**April 2003**

**Table VI:** Range of average dietary intakes\* calculated as percentage of the TDI-values

| <b>Mycotoxin</b>             | <b>TDI<br/>µg/kg<br/>bw/day</b> | <b>Population</b> | <b>Adults</b>  | <b>Infants</b> |
|------------------------------|---------------------------------|-------------------|----------------|----------------|
| Deoxynivalenol               | 1                               | 0.8% - 33.8%      | 14.4% - 46.1%  | 11.3% - 95.9%  |
| Nivalenol**                  | 0.7                             | 4.2% - 11.1%      | 0.8% - 8.2%    | 3.7% - 22.6%   |
| T2 + HT2 toxin**             | 0.06                            | 18.3% - 250%      | 61.7% - 171.7% | 26.7% - 563.3% |
| Zearalenone**                | 0.2                             | 13.4%             | 5.3% - 14.5%   | 3% - 27.5%     |
| Fumonisin <sub>B1 + B2</sub> | 2                               | 0.8% - 13.2%      | 0.1% - 14.1%   | 22.3%          |

\* Mean food consumption and mean 1 occurrence data. For details see parts A-C.

\*\* Temporary TDI